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Abstract

Wheat allergy is an IgE-mediated disorder. Polyphenols, which are known to interact with certain proteins, could be used to reduce allergic reactions. In this study, we screened several polyphenol sources for their ability to interact with gliadins, mask epitopes and impact basophil degranulation. Polyphenol extracts from artichoke leaves, cranberries, apples and green tea leaves were examined. Of these extracts, the first three formed insoluble complexes with gliadins. Only the cranberry and apple extracts masked epitopes in dot blot assays using anti-gliadin IgG and IgE antibodies from patients with wheat allergies. The cranberry and artichoke extracts limited cellular degranulation by reducing mouse anti-gliadin IgE recognition. In conclusion, the cranberry extract is the most effective polyphenol source at reducing the immunogenicity and allergenicity of wheat gliadins.

Keywords: Allergy / Apple / Artichoke leaf / Basophils / Cranberry / Green tea leaf / Gliadins / Polyphenols / Wheat

30 Introduction

31 Wheat-based products are consumed in large quantities daily and are considered a key
32 staple food worldwide. Despite its importance in the human diet, wheat is not tolerated by a
33 number of individuals. Wheat may trigger various pathologies, such as celiac disease, gluten
34 hypersensitivity and food allergy (FA) ¹. Similar to most FAs, the incidence of FA to wheat
35 has increased during the last decade. The wheat sensitization rate is approaching 1% of the
36 global population, depending on age and location ^{2,3}.

37 Many allergens have been identified in wheat flour. Wheat proteins are separated into
38 the salt-soluble proteins albumins/globulins and the insoluble proteins called prolamins due to
39 their high proline (P) and glutamine (Q) contents. The insoluble fraction is composed of
40 gliadins and glutenins, which together form gluten. Prolamins are classified into three groups:
41 high molecular weight (HMW) prolamins, prolamins that lack cysteine (S-poor) and S-rich
42 prolamins. The latter group consists of α - and γ -gliadins and low molecular weight (LMW)
43 glutenins, with proline contents ranging from 14 to 16%, whereas S-poor prolamins are
44 mainly composed of ω -gliadins (the less abundant gliadins), which are characterized by
45 proline contents ranging from 20 to 30% ^{4,5}.

46 FA to wheat is an immune disorder that typically results from the production of
47 allergen-specific IgE by the adaptive immune system. IgE is fixed on the surface of effectors
48 cells such as mast cells or basophils. The activation of these cells is triggered by the
49 recognition of the allergen epitopes by IgE, resulting in the release of mediators such as
50 histamine and β -hexosaminidase, which induce allergic symptoms ⁶. Symptoms experienced
51 during allergic reactions vary according to the gliadin type: eczema is induced by α/β - and γ -
52 gliadins, whereas anaphylactic shock and exercise-induced anaphylaxis are triggered by ω -
53 gliadins ⁷. FA to wheat results in a significant impairment in quality of life and can lead to

potentially life-threatening reactions. To date, the only effective treatment to prevent these adverse reactions is the avoidance of wheat based food products. Unfortunately, because of the omnipresence of wheat in food, unintentional exposure to wheat allergens is frequent, even with meticulous avoidance. Given the prevalence of FA to wheat, new therapies are needed to reduce the impact of accidental exposure.

Dietary polyphenols, which are well known for their antioxidant activity, exhibit anti-tumour, anti-diabetic and anti-allergic effects^{8–10}. Polyphenols interact with many types of molecules, including lipids, carbohydrates and proteins¹¹, and some are able to form soluble or insoluble protein-polyphenol complexes¹². Insoluble protein-polyphenol complexes are responsible for haze formation in many beverages. Furthermore, the involvement of proline in polyphenol-protein interactions was reported for salivary proteins¹³. The hydrophobic nature of these interactions was revealed through experiments conducted with pure polyphenols and polypeptides¹⁴. Interactions between polyphenols and proteins may modulate food allergy disorders at both the sensitization and symptomatic levels¹⁵. Chung and Champagne reported that the binding of phenolic compounds, such as caffeic, ferulic and chlorogenic acid, to peanut allergens rendered them less allergenic¹⁶. Subsequently, polymeric cranberry polyphenols (e.g., A-type proanthocyanidins) were reported to form insoluble complexes and decrease the allergic response in an *in vivo* model. Based on these results, polyphenols may be used to create hypoallergenic peanut-based foods and have been proposed as a new tool for immunotherapy¹⁷.

Anthocyanins were recently shown to interact with gluten and gliadins by affecting their secondary structure^{18–20}. The secondary structure of a protein is critical for the recognition of epitopes by the immune system. A change in the secondary structure can alter the recognition of the epitopes by cells and therefore modulate the allergic response^{21,22}.

This study aimed to screen available polyphenol-rich plant extracts for their capacity to interact with gliadins and to reduce immune reactions by masking epitopes and subsequently limit the release of inflammatory mediators. Four plant extracts with varying phenol compositions were chosen: artichoke leaves, cranberries, apples and green tea leaves. The extracts were enriched in phenolic compounds by solid phase extraction and then used for interaction assays. The potential of the phenolic compounds to mask gliadin epitopes was measured using immunoglobulin dot blot assays (polyclonal IgG and IgE antibodies from patients with a wheat allergy and sensitized mice). The effect of the phenolic compounds on the disease-triggering properties of gliadins was determined using a mast cell degranulation test, which mimics the late phase of the allergic response.

Materials and Methods

Chemicals and reagents. All chemicals and reagents used were of analytical reagent grade. Methanol and acetonitrile were purchased from Carlo Erba reagents (Val de Reuil, France). Formic acid was obtained from NORMAPUR (VWR Prolabo, France). Acetic acid, trifluoroacetic acid, hydrochloric acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Ascorbic acid was from Fisher Scientific (Loughborough, UK). Folin-Ciocalteu Reagent, (-)-epicatechin, and chlorogenic acid were obtained from Sigma-Aldrich (Lyon, France). Alhydrogel® adjuvant 2% was obtained from InvivoGen (Toulouse, France).

Plant materials. Cranberry, apple and green tea leaf extracts were provided by NATUREX (Avignon, France) and the artichoke leaf extract was provided by EVEAR (Coutures, France).

Sample preparation. Phenolic Compound Enrichment. The plant extracts were enriched in phenolic compounds using the purification procedure described by Bernillon, *et al.*²³. Briefly,

each plant extract was purified by solid phase extraction on a C18 SepPak cartridge (Waters, Saint-Quentin-en-Yvelines, France). The cartridge was activated with 50 mL of methanol and conditioned with 0.1% formic acid (v/v). Then, 100 mg of the plant extract powder was solubilized in 25 mL of 0.1% formic acid to a final concentration of 4 mg/mL and loaded onto the cartridge. The cartridge was washed with 100 mL of 0.1% formic acid to remove sugars, organic acids, and other polar compounds. The phenolic fraction was recovered by eluting the cartridge with 50 mL of a methanol/0.1% formic acid solution (50:50). Organic solvents were removed by evaporation under reduced pressure and the concentrated aqueous fraction was freeze-dried. Powders of the phenolic compound-enriched extract (PCe) were stored in the dark at room temperature until used.

Preparation of gliadins (Glia). Glia were extracted from defatted wheat flour (cultivar Récital) using a previously described sequential procedure²⁴ and freeze-dried.

Total phenolic content. The total polyphenol content of the crude extracts and purified fractions was determined using the Folin-Ciocalteu microplate method²⁵. Briefly, 50 μ L of water, standards or crude extracts were diluted in water to a concentration of 50 μ g/mL and placed in each well along with 50 μ L of Folin-Ciocalteu reagent (1:5, v/v). Then, 100 μ L of 0.35 M sodium hydroxide was added and the absorbance was measured at 760 nm after 3 min using a Synergy HT reader (Bio-tek instruments, Colmar, France). The samples were quantified by plotting the absorbance on a calibration curve of the standard polyphenols (-)-epicatechin or chlorogenic acid.

Characterization of the phenolic compounds. Polyphenols in crude and PCe were characterized by direct reverse-phase HPLC analysis and by HPLC analysis following phloroglucinolysis reaction in order to estimate the average degree of polymerization of the global procyanidin fraction according to the method described by Kennedy and Jones²⁶.

Briefly for the phloroglucinolysis reaction, supernatants (100 μ L) or PCe powders (3 mg in 5 mL of 1% formic acid) were freeze-dried overnight. Samples were resuspended in 100 μ L of HCl (0.3 N in methanol) and 200 μ L of methanol mixture of ascorbic acid (10 g/L) and phloroglucinol (50 g/L) was added. The resulting mixture was incubated at 50°C for 30 min and then cooled on ice for 5 min. Next, 300 μ L of sodium acetate 0.2 M was added and mixed. Final mixture was filtered through a 0.45 μ m PTFE filter (Uptidisc, Interchim, France) prior to injection in the HPLC-UV/Visible-MS.

For direct HPLC analysis (without phloroglucinolysis), methanolic solution of crude and PCe extracts were directly analysed in the HPLC system described below.

The polyphenolic compounds from the crude extracts or PCe were analysed by high performance liquid chromatography coupled to UV/Visible and mass spectrometry detection using the method described by Malec *et al.*²⁷. The HPLC-DAD-MS system included a SCMA1000 vacuum membrane degasser (ThermoQuest, San Jose, CA, USA), a 1100 Series binary pump (Agilent Technologies, Palo Alto, CE, USA), and a UV6000Lp PDA detector (ThermoFinnigan, San Jose, CA, USA) coupled to an LCQ Deca ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an axial electrospray ion source used in the negative mode. A 5 μ L volume was injected into a Zorbax Eclipse XDB-C18 column (150 mm $\times$ 2.1 mm, 3.5 μ m, Agilent Technologies). Mass spectrometry data were acquired in full MS mode coupled to an MS/MS dependent scan mode, allowing for automatic acquisition of the MS/MS spectra of the most intense molecular ions detected throughout the chromatographic run. The data were processed using Xcalibur® software (version 1.2)

Glia-PCe interactions. Glia were prepared at 5 mg/mL in 0.1 M acetic acid. PCe solutions were freshly prepared in MilliQ water at concentrations ranging from 0.625 mg/mL to 20 mg/mL. Glia solutions were mixed with PCe solutions to obtain ratios ranging from 1:0.125

to 1:4 (w:w). The solutions were stirred at room temperature for 30 minutes in a Thermomixer (Thermo Fisher Scientific, France) at 1,500 rpm and centrifuged at 13,000 rpm for 5 minutes, after which the supernatants were collected. Residual Glia or PCe levels were further analyzed by SDS-PAGE or HPLC.

Electrophoresis. SDS-PAGE was performed using stain-free precast gels (12% acrylamide) from Bio-Rad (Marnes-la-Coquette, France), according to the manufacturer's recommendations. All samples were diluted 2.5-fold in Laemmli buffer (Marnes-la-coquette, Bio-Rad) prior to loading on the SDS-PAGE gel; the Bio-Rad Precision Plus Protein™ Unstained Standard was used as the ladder. After separation, the gels were exposed for 2.5 minutes under a GelDoc EZ (Marnes-la-Coquette, Bio-Rad) camera to reveal the migration of the proteins. The data were processed with Image Lab software v.5.2.1 (Bio-Rad).

Reverse-phase chromatography analysis. Glia and PCe solutions were analysed by HPLC before and after mixing. The supernatant of each Glia:PCe mixture was diluted 1:1 (v/v) in eluent A (0.1% trifluoroacetic (TFA) acid in 5% acetonitrile (ACN)) prior to HPLC. Glia and PCe solutions were analysed on an Alliance HPLC System (Waters, Saint-Quentin-en-Yvelines, France). Ten microliters of each sample were applied to a NUCLEOSIL® 300-5 C18 column (Macherey-Nagel, Hoerdtt, France) maintained at 50°C. Glia and PCe were eluted at 1 mL·min⁻¹ from eluent A to eluent B (0.08% TFA in 80% ACN) using the following gradient: 1 min in 100% eluent A, followed by a linear increase to 60% B over 30 min and a subsequent increase from 60 to 100% eluent B over 5 min. The column was then washed with 100% eluent B for 20 min. Glia and PCe were detected with a UV detector (Waters 2487, Saint-Quentin-en-Yvelines, France) at 214 and 280 nm, respectively. The data were collected and processed with Empower Software (Waters). The concentrations of Glia and phenolic compounds in PCe were calculated from the corresponding standard curves. All experiments were performed in triplicate.

174 **Immunogenicity of the Glia-PCe complexes.** *Production of mouse anti-gliadin IgE.* Three-
175 week-old BALB/cJRj mice were purchased from Janvier Labs (Le Genest-Saint-Isle, France)
176 and were fed a wheat-free diet produced by Safe-diets (Augy, France). After 3 weeks of
177 acclimation, the mice were sensitized twice (on day 0 and 14) with 10 µg of Glia adsorbed on
178 2% Alhydrogel. Fourteen days after the second sensitization, whole blood was collected via
179 cardiac puncture. The Ethics Committee in Animal Experimentation of Pays de la Loire
180 approved the experimental protocol (CEEA.2011.52; accreditation no.4478). Sera were
181 obtained by centrifugation of clotted blood, and the concentrations of gliadin-specific IgE
182 were measured using indirect fluorimetric ELISAs (F-ELISAs) using the method described by
183 Bodinier, *et al.* ²⁸.

184 *Pooling of patient sera.* Sera (#1068 and #1116) were selected from INRA BIA Biocollection
185 approved by the French Ministry of Research (authorization DC 2008-809). The sera were
186 chosen due to the reactivity of the anti-gliadin fractions (55 and 61 ng/mL of specific IgE
187 antibodies, respectively).

188 *Dot blot inhibition assay of IgG and IgE binding.* Glia solubilized in 0.1 M acetic acid were
189 spotted on a nitrocellulose membrane at 10 and 20 µg. The membranes were immersed in a
190 bath containing 100 µg/mL PCe for 60 minutes and then blocked in 4% polyvinylpyrrolidone
191 (Sigma-Aldrich, Lyon, France) overnight. After washing three times with PBS containing
192 0.05% Tween 20 (PBST), the membranes were incubated with the rabbit anti-repetitive
193 domain of gliadin IgG (PQQPYPPQQPC) ²⁹ or with IgE from pooled sera for 1 h, and then
194 washed three times with PBST. The membranes were further incubated with a 1:3,000
195 dilution of goat alkaline phosphatase (AP)-conjugated anti-rabbit-IgG (A8025, Sigma-
196 Aldrich, Lyon, France) or a 1:500,000 dilution of rabbit horseradish peroxidase (HRP)-
197 conjugated anti-human-IgE antibody (P0295, Dako, Denmark). The bound IgG and IgE

antibodies were detected with colorimetry and chemiluminescence respectively. Images were captured using LAS3000 Imaging System (Fujifilm, France)³⁰.

Basophil activation assay. The capacity of RBL-2H3 cells to degranulate and release mediators following crosslinking of their IgE-bound FcεRI by allergens reflects the response of both mast cells and basophils in allergic reaction and lead to their widespread use in degranulation studies³¹. The basophils degranulation test was performed with RBL 2H3 cells obtained from the American Type Culture Collection (ATCC Manassas, USA). Cells were cultured using the methods described by Claude, *et al.*³². Cells were seeded at 1×10^5 cells/mL and cultured at 37°C in a humidified atmosphere with 5% CO₂. Mouse sera (with IgE anti-gliadin antibodies) were pooled and tested in triplicate for their capacity to induce the degranulation of 2H3 RBL cells, as described by Gourbeyre, *et al.*³³. A 1:50 dilution of the pool of mouse sera was added to the cells for 24 h. IgE-sensitized cells were stimulated with Glia, Glia mixed with PCe or PCe only as a negative control in 2X Tyrode's buffer containing 50% deuterium oxide (D₂O, Sigma) for 45 min at 37°C. A 1:50 dilution of the pool of mouse sera without specific IgE anti-gliadin antibodies was also tested as negative control to assess non-specific degranulation. The amount of β-hexosaminidase released was measured using the methods reported by Bodinier, *et al.*³⁴. The limit of detection (LOD) was established at 10% due to the spontaneous degranulation of the untreated cells.

Results

Preparation and characterization of phenolic solutions.

Enriched phenolic solutions (PCe). Four food-grade polyphenolic sources were chosen for this study: artichoke leaf, cranberry, apple and green tea leaf extracts. Solid-phase extraction

was used to separate the polyphenolic compounds from the interfering matrices and to produce powders enriched in polyphenolic compounds that are easy to handle and soluble in water. The polyphenolic contents of the enriched extracts are summarized in Table 1. Three of the four extracts were successfully enriched in polyphenolic compounds. The artichoke leaf extract was enriched from 26 to 61%, whereas the apple and green tea leaf extracts were enhanced by 9% and 12%, respectively. The phenolic compound content of the cranberry extract (35%) was not modified by the extraction process.

Analysis of the polyphenol composition by mass spectrometry. A liquid chromatography with diode array detection and electrospray ionization tandem mass spectrometry (LC-DAD-ESI-MS/MS) method was used to identify the major phenolic compounds in the commercial extracts and their corresponding PCE powders (Figure 1). Several classes of phenolic compounds were identified in each fraction (Table 2). The green tea leaf extract was composed of flavanol monomers including (+)-catechin, (-)-epicatechin (m/z 289), (epi)gallocatechin (m/z 305), epicatechin gallate (m/z 441) and (epi)gallocatechin gallate (m/z 457). Similarly, the artichoke leaf extract was also mainly composed of chlorogenic acids, namely, caffeoylquinic acid (m/z 353), dicaffeoylquinic acid (m/z 515) and compounds from the flavone class including glycosylated luteolin and apigenin (m/z 431, 445, 447 and 461). The cranberry extract was composed of monomers such as quercetin, myricetin (m/z 301 and 317) and their glycosylated forms (m/z 433, 449, 463 and 479), as well as (+)-catechin and (-)-epicatechin (m/z 289). The cranberry extract also included procyanidin oligomers of the B-type (dimers at m/z 577) and the A-type procyanidin (from dimers at m/z 575 to tetramers at m/z 1151). Numerous monomers were also identified in the apple extract: dihydrochalcones were clearly present as phloretin (m/z 273), phloridzin (m/z 435) and phloretin xyloglucoside (m/z 567), 5-caffeoylquinic acid (m/z 353), (+)-catechin, (-)-epicatechin (m/z 289) and dimeric to tetrameric procyanidins (at m/z 577 to 1153). The average degree of polymerization of the

cranberry and apple extracts was 3.8 and 2.8, respectively. The phenol composition of the commercial extracts and their corresponding PCe powders were not qualitatively different.

Interactions between the PCe solutions and Glia.

Qualitative analysis. The capacity of polyphenols to interact with 2.5 mg/mL of Glia was studied by increasing the amount of PCe added from 0.3125 mg/mL to 10 mg/mL. With the exception of the green tea PCe, the progressive addition of polyphenols resulted in the appearance of a haze. All mixtures were centrifuged and the remaining components in the supernatant were analysed by electrophoresis (Figure 2). In the absence of PCe, Glia were characterized by bands ranging from 16 to 42 kDa. Three out of four PCe solutions interacted with Glia, inducing a decrease in the amount of soluble protein. The addition of green tea PCe did not modify the Glia levels in the soluble fraction (Figure 2A). The addition of apple PCe induced a slight decrease in the Glia levels at a ratio of 1:4 (Figure 2B). The high molecular weight bands in the gels of the Glia:apple PCe mixtures corresponded to apple PCe (data not shown). Finally, the electrophoretic profiles of the Glia remaining after the interactions with polyphenols from cranberry and artichoke leaves revealed bands characteristic of Glia until the Glia:PCe ratio reached 1:3 and 1:1, respectively (Figure 2C, 2D). The addition of more PCe resulted in the complete disappearance of Glia in these two extracts.

Quantitative analysis. A reverse-phase HPLC method was developed to separate PCe from Glia and quantify both components in a single experiment. The gradient was optimized to avoid any overlap between phenolic compounds of PCe and Glia and to establish complete separation of each of these compounds (Supplementary data 1). This procedure resulted in the

elution of all polyphenolic compounds before 20 min, whereas GliA proteins were eluted after 22 min. The PCe and GliA levels were quantified by measuring the area under their respective curves in a single analysis. For each PCe, two or three GliA:PCe ratios that showed a visible decrease in the GliA levels in the electrophoretic patterns were chosen for quantification using this method. The remaining soluble GliA were expressed as the percentage of the initial amount of GliA in the mixture, as reported in Table 3. The addition of green tea PCe only reduced the amount of GliA in solution at high PCe ratios. At a ratio of 1:4, 77.6% of the GliA were still present in the soluble fraction. Artichoke leaf PCe showed the greatest capacity to interact with GliA with 62.3% GliA observed in complexed forms at a ratio of 1:0.5 and 71.1% at a ratio of 1:1. Cranberry PCe was less effective at forming insoluble complexes with GliA at low PCe loading ratios, but at a ratio of 1:3, 78.1% of GliA were present in insoluble complexes. The same behaviour was observed with apple PCe, but the appearance of insoluble complexes required the addition of more PCe than was required for cranberry PCe. In the latter two cases, we observed a decrease in the polyphenolic polymer content in solution following the precipitation of the insoluble fractions. The supernatants obtained after the addition of cranberry and apple PCe exhibited a reduction in the DPn values for procyanidins from 3.8 to 1.9 and from 2.8 to 2.3, respectively (Supplementary data 2).

PCe masks GliA epitopes.

Dot blots. The capacity of PCe to mask GliA epitopes was evaluated using a dot blot method, first with IgG antibodies raised against the gliadin anti-repetitive domain and second with IgE antibodies from patients with a wheat allergy (Figure 3). In the absence of added PCe, GliA were well recognized by both IgG and IgE antibodies. The addition of artichoke PCe only slightly reduced the recognition of 10 µg of GliA by IgG antibodies and had no effect on the recognition of 20 µg of GliA. Cranberry and apple PCe decreased the recognition of the IgG epitope, as revealed by the lighter appearance of the spot. Cranberry PCe was more efficient,

as it prevented the recognition of the 10 μ g and 20 μ g protein spots. Apple and cranberry PCes were then tested with patient sera, and the same trend was observed. The addition of apple PCe decreased the recognition of gliadins by IgE, whereas cranberry PCe completely inhibited recognition at both Glia concentrations examined.

Rat basophil leukaemia (RBL). Three concentrated extracts were tested in a RBL model sensitized with mouse IgE (Figure 4) at various Glia:PCe ratios: apple PCe at 1:4, cranberry PCe at 1:3 and artichoke leaf PCe at 1:1. A degranulation curve was obtained for gliadins between 0.1 and 10 μ g per μ L, with a maximum degranulation of 28.2% at 1 μ g of gliadins. This concentration was chosen to compare the effects of the PCes. The addition of Glia-apple PCe did not modify the maximum degranulation (MaxD) compared to Glia alone (28.2% versus 28.5%). The other two extracts significantly decreased the amount of β -hexosaminidase up to 16.6% and 17.7% MaxD, respectively. PCe alone or the pool of sera without specific anti-gliadin IgE antibodies, which was used as a control, did not induce degranulation. These results confirm the specific action of PCe on reducing the allergic response.

Discussion

Several beneficial properties have been attributed to dietary plant phenolic compounds, including an anti-allergic potential, possibly due to their capacity to interact with proteins. Polyphenol-enriched fractions were prepared from four extracts to separate the polyphenol compounds from the residual matrix and other inactive impurities. The quantification of phenolic compound contents revealed the efficiency of this purification step for all extracts

except the cranberry extract. However, the Folin-Ciocalteu assay we used to determine the polyphenol content is known to underestimate the levels phenolic compounds in berries³⁵. The main polyphenols present in these extracts and in their respective enriched fractions were identified by LC-DAD-ESI-MS/MS. The strong similarity in the profiles obtained for each extract and its corresponding PCE suggested that the enrichment step did not induce the loss of any polyphenolic compounds. The four enriched fractions were composed of several different classes of phenolic compounds and various amounts of monomeric and polymeric polyphenols. The global composition of each extract was similar to previous reports in the literature^{36–40}, although the cranberry and apple polyphenols employed here had a lower degree of polymerization than previously described extracts^{41–44}.

Polyphenols interact with Gliadin. Polyphenols are known to interact with proteins. These interactions depend on the type and the structure of the protein and the polyphenol and are influenced by solution parameters, such as the pH, ionic strength and temperature⁴⁵. The capacities of the four polyphenolic solutions to interact with gliadins were compared in this study.

The HPLC method developed in this paper allowed the separation and quantification of both proteins and polyphenols in solution. The preparation of samples prior to injection included the addition of acetonitrile to a final concentration of 5%. The presence of this relatively non-polar solvent was expected to disrupt soluble gliadin-polyphenol complexes that are formed via hydrophobic bonds, a process that is reversible under certain conditions¹². No additional peaks were detected in the supernatant profiles compared to the profiles of the gliadins or the extracts alone. We did not explore the formation of the soluble complexes in this study; however, according to previous papers, these complexes may form in the soluble phase of gliadin-polyphenol mixtures⁴⁶.

The polyphenols tested here are divided into two groups. The first group comprises the artichoke leaf and green tea leaf extracts, in which most polyphenols occurred in monomeric or dimeric forms. The second group comprised the apple and cranberry extracts, which are enriched in condensed tannins. In the first group, the addition of artichoke polyphenols quickly led to the appearance of a haze; this phenomenon occurred only at high concentrations of green tea PCe. Small monomeric polyphenols have been shown to form soluble complexes with proteins via non-covalent interactions^{9,47,48}. However, a few small polyphenols have been shown to participate in the formation of insoluble complexes. Hasni, *et al.* reported the structural modification of casein as a function of the progressive addition of pure flavonols and determined a concentration above which insoluble complexes appeared⁴⁹. The ability of tea polyphenols to modify the structure of globular proteins and to precipitate the proteins has also been reported. Our data on wheat proteins are consistent with studies of peanut (2S albumin)⁴⁸ and milk (β -lactoglobulin) proteins⁵⁰. Although the artichoke polyphenols were also essentially monomeric, their addition induced the appearance of insoluble complexes, even at low concentrations. The rather hydrophobic nature of gliadins and the acidic conditions employed in this study might explain their propensity to precipitate upon the addition of artichoke polyphenols. Chlorogenic acid, one of the components of the artichoke leaf extract, has been shown to interact with and irreversibly insolubilize peanut proteins¹⁶.

The extracts in the second group contained a proportion of procyanidin oligomers, also known as condensed tannins, and were therefore susceptible to the formation of insoluble gliadin complexes. Even when added at low concentrations, these extracts induced the formation of insoluble complexes. The decrease in the average mean DPn of the polyphenols remaining in solution revealed that the most polymerized condensed tannins present in these two polyphenolic fractions were likely involved in the formation of insoluble gliadin complexes.

The higher DPn measured for the cranberry PCe may explain its greater capacity to complex gliadins than the apple PCe.

Interactions and immunogenicity. Gliadins are organized into a non-repetitive domain and a repetitive domain that is mainly composed of Q and P with repeat motifs such as PQQPF and QQPFP⁵¹. As summarized by Matsuo, *et al.*, epitopes involved in allergic reactions have been identified using IgE from patients with a wheat allergy. Most of these epitopes are located in the gliadin repetitive domain^{51–54}.

Allergic reactions proceed in several phases. The first phase is sensitization, which is an asymptomatic reaction that includes the production of specific IgE antibodies (due to absorbed allergens) and their binding to mast cell IgE-receptors (FcεRI). The second phase is characterized by the cross-linking of mast cell-bound IgE during a new contact with the allergen, which ultimately triggers mast cell degranulation, accompanied by the release of chemical mediators. Monomeric and polymeric phenols have been shown to interfere with the steps involving mast cells, either by inhibiting the binding of IgE to the FcεRI⁵⁵ or by interfering with allergen recognition through IgE-polyphenol interactions⁵⁶. In this study, we considered mast cells that had already been sensitized with IgE and examined the impact of gliadin:polyphenol mixtures on the degranulation process. The presence of polyphenols may also impact the immunoreactivity of gliadins; we followed this effect with IgG antibodies specific to the repetitive sequence and IgE antibodies from patients with a wheat allergy.

The artichoke PCe only slightly reduced epitope recognition by the IgG antibodies, suggesting that the complexes formed were reversible and that dissociation may occur during the washing and revealing steps. The amount of polyphenols bound to the gliadins may not have been sufficient to prevent epitope recognition. Despite this limited epitope masking capacity, the addition of artichoke PCe resulted in a decrease in the release of cell mediators, probably

due to the formation of complexes that are not recognized by IgE. In contrast, apple PCe efficiently masked the epitopes in a dot blot assay using IgG and IgE antibodies, but did not impact the gliadin response in an RBL model. Incubation of apple PCe with gliadins adsorbed on membrane induced the formation of irreversible complexes that prevent epitope recognition. Dose-dependent epitope masking may be suspected as mild recognition appears at 20 μ g of spotted gliadins. Otherwise, the inability of apple PCe to prevent RBL degranulation may be due to the presence of soluble residual free or complexed gliadins in the mixture as revealed by electrophoresis (Fig 2B). These remaining soluble gliadins may interact with RBL bound-IgE and induce degranulation. Only cranberry PCe decreased gliadin recognition by IgG and IgE and prevented the degranulation process in mast cells. Similar results were reported by Plundrich, *et al.*, who showed the potential of cranberry polyphenols to mask peanut allergen epitopes¹⁷. Although cranberry PCe has a lower DPn than the extracts used in the literature, it is efficient to mask gliadins epitopes.

Our work is consistent with previous studies of other allergens. We confirmed that among the polyphenols of different origins, only cranberry PCe reduced the immunogenicity and allergenicity of wheat gliadins. Its potency in reducing these allergic symptoms should be investigated further using *in vivo* allergy models.

Abbreviations Used: DPn, degree of polymerization; FA(s), food allergy(ies); Glia, gliadins; IgE, immunoglobulin E; IgG, immunoglobulin G; PCe, phenolic compounds enriched extracts.

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References

- (1) Ferretti, G.; Bacchetti, T.; Masciangelo, S.; Saturni, L. Celiac disease, inflammation and oxidative damage: A nutrigenetic approach. *Nutrients* **2012**, *4* (4), 243–257.
- (2) Sicherer, S. H. Food allergy. *Mt. Sinai J. Med.* **2011**, *78* (5), 683–696.
- (3) Zuidmeer, L.; Goldhahn, K.; Rona, R. J.; Gislason, D.; Madsen, C.; Summers, C.; Sodergren, E.; Dahlstrom, J.; Lindner, T.; Sigurdardottir, S. T.; et al. The prevalence of plant food allergies: A systematic review. *J. Allergy Clin. Immunol.* **2008**, *121* (5), 1210–1218.e4.
- (4) Shewry, P. R.; Tatham, A. S.; Forde, J.; Kreis, M.; Mifflin, B. J. The classification and nomenclature of wheat gluten proteins: A reassessment. *J. Cereal Sci.* **1986**, *4* (2), 97–106.
- (5) Tatham, A. S.; Shewry, P. R. The S-poor prolamins of wheat, barley and rye: Revisited. *J. Cereal Sci.* **2012**, *55* (2), 79–99.
- (6) Sicherer, S. H.; Sampson, H. A. Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. *J. Allergy Clin. Immunol.* **2014**, *133* (2), 291–307.e5.
- (7) Tatham, A. S.; Shewry, P. R. Allergens to wheat and related cereals. *Clin. Exp. Allergy* **2008**, *38* (11), 1712–1726.
- (8) Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79* (5), 727–747.
- (9) Bandyopadhyay, P.; Ghosh, A. K.; Ghosh, C. Recent developments on polyphenol–protein interactions: effects on tea and coffee taste, antioxidant properties and the digestive system. *Food Funct.* **2012**, *3* (6), 592–605.
- (10) Li, A. N.; Li, S.; Zhang, Y. J.; Xu, X. R.; Chen, Y. M.; Li, H. Bin. Resources and biological activities of natural polyphenols. *Nutrients* **2014**, *6* (12), 6020–6047.
- (11) Jakobek, L. Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chem.* **2015**, *175*, 556–567.
- (12) Siebert, K. J.; Troukhanova, N. V.; Lynn, P. Y. Nature of Polyphenol - Protein Interactions. *J. Agric. Food Chem.* **1996**, *44*, 80–85.
- (13) Luck, G.; Liao, H.; Murray, N. J.; Grimmer, H. R.; Warminski, E. E.; Williamson, M. P.; Lilley, T. H.; Haslam, E. Polyphenols, astringency and proline-rich proteins. *Phytochemistry* **1994**, *37* (2), 357–371.
- (14) Pascal, C.; Paté, F.; Cheynier, V.; Delsuc, M. A. Study of the interactions between a proline-rich protein and a flavan-3-ol by NMR: Residual structures in the natively

- 458 unfolded protein provides anchorage points for the ligands. *Biopolymers* **2009**, *91* (9),
459 745–756.
- 460 (15) Singh, A.; Holvoet, S.; Mercenier, A. Dietary polyphenols in the prevention and
461 treatment of allergic diseases. *Clin. Exp. Allergy* **2011**, *41* (10), 1346–1359.
- 462 (16) Chung, S. Y.; Champagne, E. T. Reducing the allergenic capacity of peanut extracts
463 and liquid peanut butter by phenolic compounds. *Food Chem.* **2009**, *115* (4), 1345–
464 1349.
- 465 (17) Plundrich, N. J.; Kulis, M.; White, B. L.; Grace, M. H.; Guo, R.; Burks, A. W.; Davis,
466 J. P.; Lila, M. A. Novel strategy to create hypoallergenic peanut protein-polyphenol
467 edible matrices for oral immunotherapy. *J. Agric. Food Chem.* **2014**, *62* (29), 7010–
468 7021.
- 469 (18) Mazzaracchio, P.; Kindt, M.; Pifferi, P. G.; Tozzi, S.; Barbiroli, G. Adsorption
470 behaviour of some anthocyanins by wheat gluten and its fractions in acidic conditions.
471 *Int. J. Food Sci. Technol.* **2012**, *47* (2), 390–398.
- 472 (19) Mazzaracchio, P.; Tozzi, S.; Boga, C.; Forlani, L.; Pifferi, P. G.; Barbiroli, G.
473 Interaction between gliadins and anthocyan derivatives. *Food Chem.* **2011**, *129* (3),
474 1100–1107.
- 475 (20) Tozzi, S.; Zanna, N.; Taddei, P. Study on the interaction between gliadins and a
476 coumarin as molecular model system of the gliadins-anthocyanidins complexes. *Food*
477 *Chem.* **2013**, *141* (4), 3586–3597.
- 478 (21) Waga, J. Structure and Allergenicity of Wheat Gluten Proteins – a Review. *POLISH J.*
479 *FOOD Nutr. Sci. Pol. J. Food Nutr. Sci* **2004**, *1354* (4), 327–338.
- 480 (22) Sen, M.; Kopper, R.; Pons, L.; Abraham, E. C.; Burks, A. W.; Bannon, G. A. Protein
481 structure plays a critical role in peanut allergen stability and may determine
482 immunodominant IgE-binding epitopes. *J. Immunol.* **2002**, *169* (2), 882–887.
- 483 (23) Bernillon, S.; Guyot, S.; Renard, C. M. G. C. Detection of phenolic oxidation products
484 in cider apple juice by high-performance liquid chromatography electrospray ionisation
485 ion trap mass spectrometry. *Rapid Commun. Mass Spectrom.* **2004**, *18* (9), 939–943.
- 486 (24) Battais, F.; Pineau, F.; Popineau, Y.; Aparicio, C.; Kanny, G.; Guerin, L.; Moneret-
487 Vautrin, D. A.; Denery-Papini, S. Food allergy to wheat: Identification of
488 immunoglobulin E and immunoglobulin G-binding proteins with sequential extracts and
489 purified proteins from wheat flour. *Clin. Exp. Allergy* **2003**, *33* (7), 962–970.
- 490 (25) Magalhães, L. M.; Santos, F.; Segundo, M. A.; Reis, S.; Lima, J. L. F. C. Rapid
491 microplate high-throughput methodology for assessment of Folin-Ciocalteu reducing
492 capacity. *Talanta* **2010**, *83* (2), 441–447.
- 493 (26) Kennedy, J. A.; Jones, G. P. Analysis of proanthocyanidin cleavage products following
494 acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001**, *49*
495 (4), 1740–1746.

- 496 (27) Malec, M.; Le Quéré, J. M.; Sotin, H.; Kolodziejczyk, K.; Bauduin, R.; Guyot, S.
497 Polyphenol profiling of a red-fleshed apple cultivar and evaluation of the color
498 extractability and stability in the juice. *J. Agric. Food Chem.* **2014**, 62 (29), 6944–
499 6954.
- 500 (28) Bodinier, M.; Leroy, M.; Ah-Leung, S.; Blanc, F.; Tranquet, O.; Denery-Papini, S.;
501 Wal, J. M.; Adel-Patient, K. Sensitization and elicitation of an allergic reaction to
502 wheat gliadins in mice. *J. Agric. Food Chem.* **2009**, 57 (4), 1219–1225.
- 503 (29) Bodinier, M.; Legoux, M. A.; Pineau, F.; Triballeau, S.; Segain, J. P.; Brossard, C.;
504 Denery-Papini, S. Intestinal translocation capabilities of wheat allergens using the
505 Caco-2 cell line. *J. Agric. Food Chem.* **2007**, 55 (11), 4576–4583.
- 506 (30) Lupi, R.; Denery-Papini, S.; Rogniaux, H.; Lafiandra, D.; Rizzi, C.; De Carli, M.;
507 Moneret-Vautrin, D. A.; Masci, S.; Larré, C. How much does transgenesis affect wheat
508 allergenicity? Assessment in two GM lines over-expressing endogenous genes. *J.*
509 *Proteomics* **2013**, 80, 281–291.
- 510 (31) Passante, E.; Frankish, N. The RBL-2H3 cell line: its provenance and suitability as a
511 model for the mast cell. *Inflamm. Res.* **2009**, 58 (11), 737–745.
- 512 (32) Claude, M.; Lupi, R.; Bouchaud, G.; Bodinier, M.; Brossard, C.; Denery-Papini, S. The
513 thermal aggregation of ovalbumin as large particles decreases its allergenicity for egg
514 allergic patients and in a murine model. *Food Chem.* **2016**, 203, 136–144.
- 515 (33) Gourbeyre, P.; Denery-Papini, S.; Larré, C.; Gaudin, J. C.; Brossard, C.; Bodinier, M.
516 Wheat gliadins modified by deamidation are more efficient than native gliadins in
517 inducing a Th2 response in Balb/c mice experimentally sensitized to wheat allergens.
518 *Mol. Nutr. Food Res.* **2012**, 56 (2), 336–344.
- 519 (34) Bodinier, M.; Brossard, C.; Triballeau, S.; Morisset, M.; Guérin-Marchand, C.; Pineau,
520 F.; De Coppet, P.; Moneret-Vautrin, D. A.; Blank, U.; Denery-Papini, S. Evaluation of
521 an in vitro mast cell degranulation test in the context of food allergy to wheat. *Int.*
522 *Arch. Allergy Immunol.* **2008**, 146 (4), 307–320.
- 523 (35) Lester, G. E.; Lewers, K. S.; Medina, M. B.; Saftner, R. A. Comparative analysis of
524 strawberry total phenolics via Fast Blue BB vs. Folin-Ciocalteu: Assay interference by
525 ascorbic acid. *J. Food Compos. Anal.* **2012**, 27 (1), 102–107.
- 526 (36) Wang, D.; Lu, J.; Miao, A.; Xie, Z.; Yang, D. HPLC-DAD-ESI-MS/MS analysis of
527 polyphenols and purine alkaloids in leaves of 22 tea cultivars in China. *J. Food*
528 *Compos. Anal.* **2008**, 21 (5), 361–369.
- 529 (37) Negro, D.; Montesano, V.; Grieco, S.; Crupi, P.; Sarli, G.; De Lisi, A.; Sonnante, G.
530 Polyphenol Compounds in Artichoke Plant Tissues and Varieties. *J. Food Sci.* **2012**, 77
531 (2).
- 532 (38) Vrhovsek, U.; Masuero, D.; Gasperotti, M.; Franceschi, P.; Caputi, L.; Viola, R.;
533 Mattivi, F. A versatile targeted metabolomics method for the rapid quantification of

- multiple classes of phenolics in fruits and beverages. *J. Agric. Food Chem.* **2012**, *60* (36), 8831–8840.
- (39) Sapozhnikova, Y. Development of liquid chromatography-tandem mass spectrometry method for analysis of polyphenolic compounds in liquid samples of grape juice, green tea and coffee. *Food Chem.* **2014**, *150*, 87–93.
- (40) Contreras, M. D. M.; Arráez-Román, D.; Fernández-Gutiérrez, A.; Segura-Carretero, A. Nano-liquid chromatography coupled to time-of-flight mass spectrometry for phenolic profiling: A case study in cranberry syrups. *Talanta* **2015**, *132*, 929–938.
- (41) Blumberg, J. B.; Camesano, T. A.; Cassidy, A.; Kris-Etherton, P.; Howell, A.; Manach, C.; Ostertag, L. M.; Sies, H.; Skulas-Ray, A.; Vita, J. A. Cranberries and their bioactive constituents in human health. *Adv. Nutr. An Int. Rev. J.* **2013**, *4* (6), 618–632.
- (42) Foo, L. Y.; Lu, Y.; Howell, A. B.; Vorsa, N. The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated *Escherichia coli* in vitro. *Phytochemistry* **2000**, *54* (2), 173–181.
- (43) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L. Screening of Foods Containing Proanthocyanidins and Their Structural Characterization Using LC-MS/MS and Thiolytic Degradation. *J. Agric. Food Chem.* **2003**, *51* (25), 7513–7521.
- (44) Wojdyło, A.; Oszmiański, J.; Laskowski, P. Polyphenolic compounds and antioxidant activity of new and old apple varieties. *J. Agric. Food Chem.* **2008**, *56* (15), 6520–6530.
- (45) Siebert, K. J. Effects of protein-polyphenol interactions on beverage haze, stabilization, and analysis. *J. Agric. Food Chem.* **1999**, *47* (2), 353–362.
- (46) Kusuda, M.; Hatano, T.; Yoshida, T. Water-soluble complexes formed by natural polyphenols and bovine serum albumin: evidence from gel electrophoresis. *Biosci. Biotechnol. Biochem.* **2006**, *70* (1), 152–160.
- (47) Poncet-Legrand, C.; Gautier, C.; Cheynier, V.; Imbert, A. Interactions between flavan-3-ols and poly(L-proline) studied by isothermal titration calorimetry: Effect of the tannin structure. *J. Agric. Food Chem.* **2007**, *55* (22), 9235–9240.
- (48) Vesic, J.; Stambolic, I.; Apostolovic, D.; Milcic, M.; Stanic-Vucinic, D.; Cirkovic Velickovic, T. Complexes of green tea polyphenol, epigallocatechin-3-gallate, and 2S albumins of peanut. *Food Chem.* **2015**, *185*, 309–317.
- (49) Hasni, I.; Bourassa, P.; Hamdani, S.; Samson, G.; Carpentier, R.; Tajmir-Riahi, H. A. Interaction of milk α - and β -caseins with tea polyphenols. *Food Chem.* **2011**, *126* (2), 630–639.
- (50) Kanakis, C. D.; Hasni, I.; Bourassa, P.; Tarantilis, P. A.; Polissiou, M. G.; Tajmir-Riahi, H. A. Milk γ -lactoglobulin complexes with tea polyphenols. *Food Chem.* **2011**, *127* (3), 1046–1055.

- 572 (51) Tanabe, S. IgE-binding abilities of pentapeptides, QQPFP and PQQPF, in wheat
573 gliadin. *J. Nutr. Sci. Vitaminol. (Tokyo)*. **2004**, *50*, 367–370.
- 574 (52) Matsuo, H.; Morita, E.; Tatham, A. S.; Morimoto, K.; Horikawa, T.; Osuna, H.;
575 Ikezawa, Z.; Kaneko, S.; Kohno, K.; Dekio, S. Identification of the IgE-binding
576 Epitope in ??-5 Gliadin, a Major Allergen in Wheat-dependent Exercise-induced
577 Anaphylaxis. *J. Biol. Chem.* **2004**, *279* (13), 12135–12140.
- 578 (53) Battais, F.; Mothes, T.; Moneret-Vautrin, D. A.; Pineau, F.; Kanny, G.; Popineau, Y.;
579 Bodinier, M.; Denery-Papini, S. Identification of IgE-binding epitopes on gliadins for
580 patients with food allergy to wheat. *Allergy Eur. J. Allergy Clin. Immunol.* **2005**, *60*
581 (6), 815–821.
- 582 (54) Matsuo, H.; Yokooji, T.; Taogoshi, T. Common food allergens and their IgE-binding
583 epitopes. *Allergol. Int.* **2015**, *64* (4), 332–343.
- 584 (55) Tokura, T.; Nakano, N.; Ito, T.; Matsuda, H.; Nagasako-Akazome, Y.; Kanda, T.;
585 Ikeda, M.; Okumura, K.; Ogawa, H.; Nishiyama, C. Inhibitory effect of polyphenol-
586 enriched apple extracts on mast cell degranulation in vitro targeting the binding
587 between IgE and FcεRI. *Biosci. Biotechnol. Biochem.* **2005**, *69* (10), 1974–1977.
- 588 (56) Matsuo, N.; Yamada, K.; Shoji, K.; Mori, M.; Sugano, M. Effect of tea polyphenols on
589 histamine release from rat basophilic leukemia (RBL-2H3) cells: the structure-
590 inhibitory activity relationship. *Allergy* **1997**, *52* (C), 58–64.
- 591

Figure captions

Figure 1. Reverse-phase UV/visible chromatograms ($\lambda_{\text{max}} = 280 \text{ nm}$) of the extracts after solid phase extraction.

Figure 2. SDS-PAGE of the free gliadins remaining in the supernatant after the GliA:PCe interactions. Green tea leaf (A), apple (B) cranberry (C) or artichoke leaf PCe (D). Each PCe was stirred with GliA at a different GliA:PCe ratio for 30 min and then centrifuged. The supernatants were collected and diluted in Laemmli buffer (v/v). An equal volume of each supernatant was loaded (10 μL).

Figure 3. Dot blot analysis of GliA saturated with PCe. GliA were spotted on a nitrocellulose membrane and soaked in a bath containing 100 $\mu\text{g/mL}$ PCe. Polyclonal IgG and IgE antibodies from pooled sera from patients with a wheat allergy were used to detect the free gliadin epitopes.

Figure 4. Basophil activation assay using mouse sera. RBL-2H3 cells were stimulated with gliadins alone; gliadins complexed with artichoke PCe at a 1:1 ratio, cranberry PCe at a 1:3 ratio, apple PCe at a 1:4 ratio; or each PCe alone at a concentration of 1 $\mu\text{g/mL}$, which represents the maximum degranulation. The horizontal dotted line represents the LOD (10%).

Table 1: Total phenolic content of the original plant extracts before and after enrichment.

Source	Plant extract (%)	PCe (%)
Artichoke	26.3 ± 0.6	61.3 ± 0.9^b
Cranberry	33.8 ± 1.2	34.0 ± 0.2^a
Apple	80.2 ± 1.1	89.1 ± 2.8^b
Green Tea	81.1 ± 2.4	92.8 ± 3.3^a

Determined by Folin-Ciocalteu assay as ^a(-)-epicatechin or ^bchlorogenic acid equivalent

Table 2. LC-DAD-ESI-MS/MS identification of the main phenolic compounds in each PCe (part 1).

PCe	Peak	Rt (min)	λ_{max}	(M-H)-	MS/MS	Possible identification	Standard	References
Artichoke	1	9.72	327	353	191	1-O-caffeoylquinic acid	Yes	Negro, <i>et al.</i> 2012
	2	11.4	324	353	191	3-O-caffeoylquinic acid		
	3	16.9	326	353	191	5-O-caffeoylquinic acid		
	4	18.15	326	353	191	4-O-caffeoylquinic acid	Yes	
	5	25.68	322	515	179 335 353	1,3-di-O-caffeoylquinic acid		
	6	40.58	348	447	285	Luteolin 7-O-glucoside		
	7	41.27	347	461	285	Luteolin 7-O-glucuronide		
	8	44.98	328	515	353	1,5-di-O-caffeoylquinic acid		
	9	46.08	337	431	269	Apigenin 7-O-glucoside		
	10	46.78	336	445	269	Apigenin 7-O-glucuronide		
	11	47.07	329	615	353. 453. 515	Monosuccinylidcaffeylquinic		
Cranberry	1	15	280	477	431	flavonols	Yes	Contreras, <i>et al.</i> 2015
	2	16.33	280	289	No fragment	(+)-catechin		
	3	16.88	325	353	No fragment	5-O-caffeylquinic acid		
	4	20.38	280	577	407. 425. 451	procyanidin B2	Yes	
	5	22.7	278	289	179. 205. 245	(-)-epicatechin	Yes	
	6	22.54	280	863	573. 711	procyanidin trimer A type		
	7	27.77	310	1151	739. 861. 981	procyanidin tetramer A type		
	8	31.53	359	479	316	Myricetin 3-O-galactoside		
	9	33.45	280	863	575. 711	procyanidin trimer A type		
	10	37.95	358	449	316	Myricetin pentoside		
	11	39.4	355	463	301	Quercetin hexoside		
	12	40.28	278	575	289. 423. 449	procyanidin A2		
	13	41.1	280	863	575	procyanidin trimer A type		
	14	42.27	357	433	301	quercetin 3-O-xylopyranoside		
	15	43.09	280	863	575. 711	procyanidin trimer A type		
	16	43.39	357	433	301	quercetin 3-O-arabinopyranoside		
	17	44.43	358	433	301	quercetin 3-O-arabinofuranoside		
	18	45.51	350	447	301	Methoxyquercetin pentoside		
	19	45.84	359	507	344	Syringetin-3-O-glucoside/galactoside		
	20	46.99	372	317	151. 179	Myricetin		
	21	49.88	263	583	316	Myricetin 3-O-(2"-O-p-hydroxybenzoyl)- α -rhamnopyranoside		
	22	50.83	373	301	151. 179	Quercetin		
	23	51.24	273	567	300. 445	Kaempferol 3-O- β -d-(6"-p-hydroxybenzoyl)-galactopyranoside		

Table 2. LC-DAD-ESI-MS/MS identification of the main phenolic compounds in each PCe (part 2).

PCe	Peak	Rt (min)	$\lambda_{\max}$	(M-H)-	MS/MS	Possible identification	Standard	Reference
Apple	1	14	279	577	289. 407. 425. 451	procyanidin B1		
	2	16.4	279	289	179. 205. 245	(+)-catechin	Yes	
	3	17.03	326	353	191	5-O-caffeoylquinic acid	Yes	
	4	20.5	279	577	289. 407. 425. 451	procyanidin B2	Yes	
	5	22.88	278	289	179. 205. 245	(-)-epicatechin	Yes	
	6	23.98	312	337	191	4-p-coumaroylquinic acid		Malec, <i>et al.</i> 2014
	7	26.87	279	865	577. 695	flavanol trimer		
	8	29.57	279	1153	695. 865. 1135	flavanol tetramer		
	9	39.75	357	463	301	quercetin 3-O-galactoside		
	10	40.83	279	577	289.407. 425. 451	procyanidin B5		
	11	45.53	284	567	273	phloretin-2-xyloglucoside		
	12	47.87	284	435	167 ; 273	phloridzin	Yes	
Green tea	1	9.19	270	305	175	(-)-galocatechin		
	2	14.45	270	305	175. 179. 219. 221. 261	(-)-epigallocatechin		
	3	16.47	279	289	175. 245	(+)-catechin	Yes	
	4	22.83	279	289	175. 245	(-)-epicatechin	Yes	Wang, <i>et al.</i> 2008
	5	24.02	273	457	169. 287. 305. 331	(-)-epigallocatechin-3-gallate		
	6	27.47	274	457	169. 287. 305. 331	(-)-galocatechin-3-gallate		
	7	38.15	277	441	169. 289. 331	(-)-epicatechin-3-gallate		

Table 3. HPLC measurement of the levels of free gliadins remaining in the supernatant after the Glia:PCe interactions. The initial Glia concentration was 2.5 mg/mL.

Ratio (Glia:PCe)	Remaining Glia (mg/mL)	Percent of Glia in interaction (%)
Artichoke		
1:1	0.72 ± 0.18	71.1
1:0.5	0.94 ± 0.08	62.3
Cranberry		
1:3	0.55 ± 0.06	78.1
1:2	1.45 ± 0.02	41.9
1:1	1.82 ± 0.21	27.2
Apple		
1:4	0.92 ± 0.2	63.3
1:3	2.01 ± 0.03	19.5
1:1	2.21 ± 0.11	11.5
Green tea		
1:4	1.94 ± 0.19	22.4
1:3	2.09 ± 0.3	16.6
1:1	2.23 ± 0.11	10.7

Figure 1.

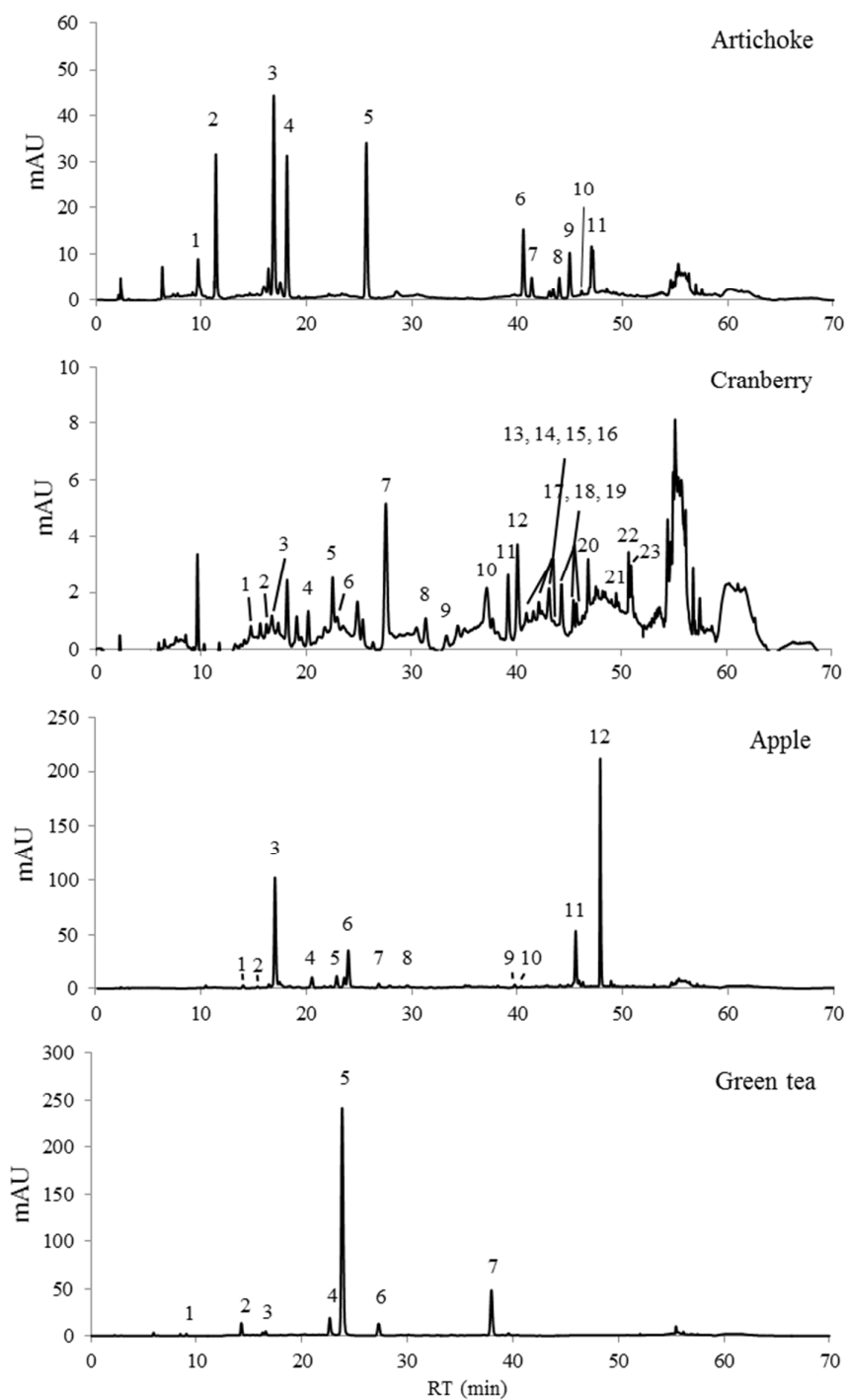


Figure 2.

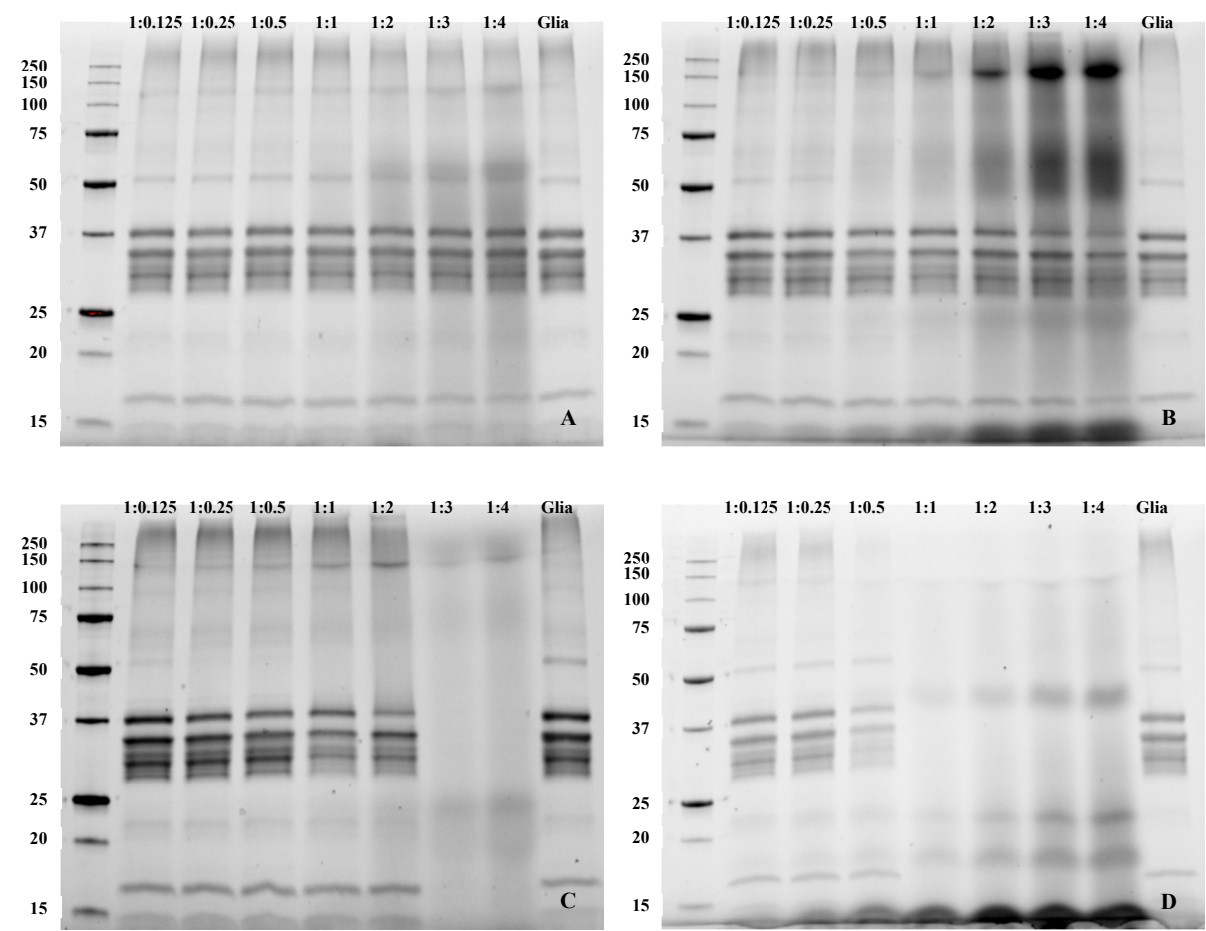


Figure 3.

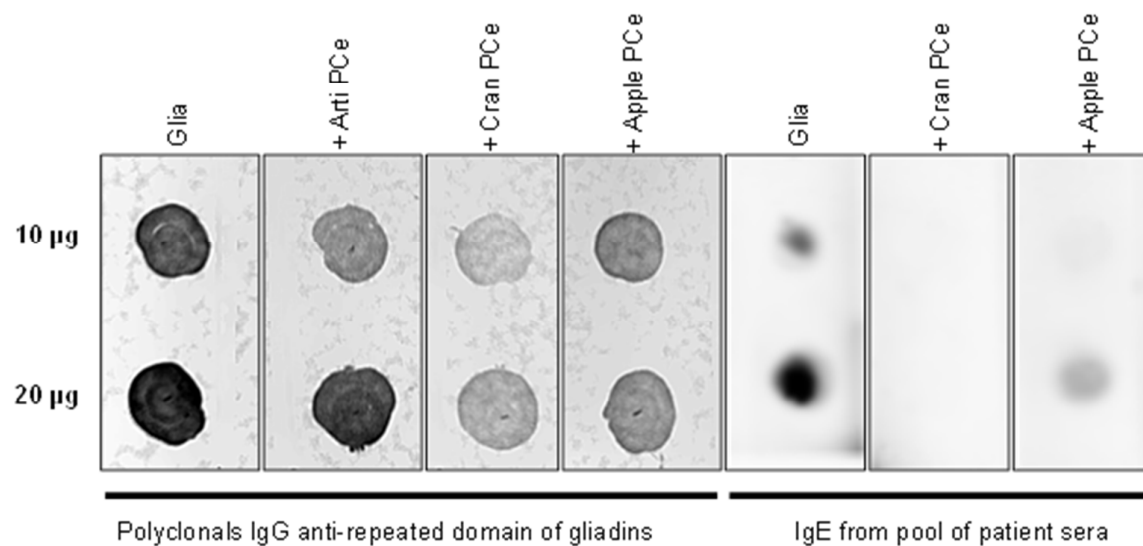


Figure 4.

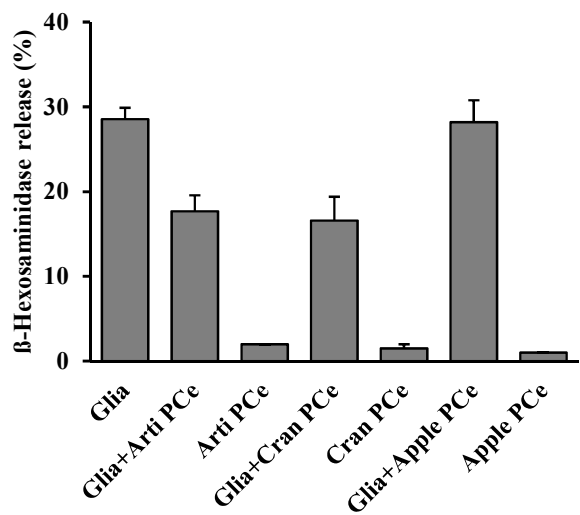


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