



Cite this: *Food Funct.*, 2017, **8**, 4760

Polyphenol-enriched berry extracts naturally modulate reactive proteins in model foods

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Healthy foods like polyphenol-rich berries and high quality edible proteins are in demand in today's functional food marketplace, but it can be difficult to formulate convenient food products with physiologically-relevant amounts of these ingredients and still maintain product quality. In part, this is because proteins can interact with other food ingredients and precipitate destabilizing events, which can disrupt food structure and diminish shelf life. Proteins in foods can also interact with human receptors to provoke adverse consequences such as allergies. When proteins and polyphenols were pre-aggregated into stable colloidal particles prior to use as ingredients, highly palatable food formulations (with reduced astringency of polyphenols) could be prepared, and the overall structural properties of food formulations were significantly improved. All of the nutritive and phytoactive benefits of the proteins and concentrated polyphenols remained highly bioavailable, but the protein molecules in the particle matrix did not self-aggregate into networks or react with other food ingredients. Both the drainage half-life (a marker of structural stability) and the yield stress (resistance to flow) of model foams made with the protein–polyphenol particles were increased in a dose-dependent manner. Of high significance in this complexation process, the reactive allergenic epitopes of certain proteins were effectively blunted by binding with polyphenols, attenuating the allergenicity of the food proteins. Porcine macrophages produced TNF- α proinflammatory cytokine when provoked with whey protein, but, this response was blocked completely when the cells were stimulated with particles that complexed whey protein with cinnamon-derived polyphenols. Cytokine and chemokine production characteristic of allergic reactions were blocked by the polyphenols, allowing for the potential creation of hypoallergenic protein–berry polyphenol enriched foods.

Received 14th June 2017,
Accepted 27th November 2017

DOI: 10.1039/c7fo00883j

rsc.li/food-function

Introduction

Health-conscious consumers today are seeking fresh, healthy, naturally functional foods that don't merely nourish the body, but can also combat health issues including cancer, cardiovascular disease, neurodegeneration and diabetes. In other words, they are seeking *nutrition* (proteins, minerals, fiber, etc.) but also *extra-nutritional compounds* (phytoactive components like proanthocyanidins, anthocyanins, and glucosinolates with recognized disease-preventative properties). These healthy nutrients and phytoactives are present in fruits, vegetables, grains, lean meats and seafood, but not necessarily at significant, health-relevant concentrations or in highly bio-

available forms.^{1,2} In addition, modern, highly mobile consumers are increasingly seeking convenience foods that simplify meal preparation or snacking options outside the home.^{3–5}

It is a particular challenge, however, to reconcile the demand for healthy foods with the concurrent demand for readily-available, quick convenience foods. Incorporation of some of the most nutritious and phytochemically-rich fruit and vegetable produce into portable convenience foods can severely compromise taste and other sensory properties, as well as the health benefits of the source material. For example, plant-derived polyphenolics, while excellent immunoprotective compounds, experience well-recognized changes in color, pH, stability, and antioxidant capacity concurrent with thermal processing.⁶ Concentrating polyphenolic compounds from fruits or vegetables into a processed food formulation (in order to deliver a biologically-significant dose of antioxidant potential) can impart an unwelcome bitter flavor to the final product.^{7–9}

Proteins are relatively large, multifaceted, and multireactive, therefore also a challenge to incorporate into desirable food product structures and textures.^{10,11} Protein molecules can interact with each other and with other ingredients (sugars,

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lipids, and charged polysaccharides) in processed foods, which may cause destabilization, including gelling in beverages, or hardening of bar formulations.¹² Natural binding reactions with protein molecules can thwart the efficacy of topical formulations, as when polyphenols and/or other active pharmaceutical agents in a skin cream bind to skin proteins, and therefore fail to reach the intended subdermal target tissues.¹³

Another example of protein reactivity, which can provoke adverse and even severe/deadly consequences, is the interaction of food proteins with human/mammalian receptors resulting in food allergy. The immune system of humans and other animals consists of two immune systems – innate and adaptive – which work together to provide immunity against infection. The cells of the innate immune system such as macrophages, dendritic cells and granulocytes use Pattern Recognition Receptors (PRRs) to detect Pathogen-Associated Molecular Patterns (PAMPs; for example, lipopolysaccharide (LPS) or flagellin) which are expressed by microbes, but not by higher life forms.¹⁴ On the other hand, the adaptive immune system, which depends on the activity of lymphocytes, is not limited by PAMP recognition and has evolved to react against any molecular structure or “antigen” (protein, carbohydrate, lipid, or nucleic acid) expressed by a microbe but not normally present within our bodies. The innate immune system reacts first, resulting in a rapid and violent response within minutes to hours designed to kill or expel the pathogen. The innate immune system also produces soluble factors (cytokines) which activate and regulate the adaptive immune response. Finally, cells in the innate system such as macrophages and dendritic cells fragment pathogen proteins and present these to the lymphocytes for their response. A number of different cellular and molecular mechanisms operate and maintain tolerance and prevent lymphocytes from reacting to antigens that we express, *i.e.*, “self-antigens”. Self-reactive lymphocytes can be deleted during maturation or their activity can be suppressed by regulatory cells.

The GI (gastrointestinal) tract presents a particularly challenging environment for immune regulation. Innate and adaptive systems must be vigilant and ready to respond to pathogens, and at the same time not react to the daily onslaught of food antigens and to the intestinal microbiota. In this context, dendritic cells take on particular significance.¹⁵ Dendritic cells surveil the GI tract, reacting to PAMPs in order to initiate a protective immune response. Dendritic cells also produce immunosuppressive factors (such as TGF- β and retinoic acid) to prevent unwanted immune responses. Finally, dendritic cells also induce differentiation of regulatory T lymphocytes, which in turn produce additional immunosuppressive soluble factors. For the most part these systems work well; immune responses are initiated against pathogens, but not against the microbiota or the antigens in food. Occasionally, however, immune responses are initiated against food proteins, resulting in a food allergy. Peanut allergy, for example, represents an immune response against peanut proteins. This response ultimately results in the production of peanut-specific IgE, which

binds to receptors on the surface of GI mast cells. Then, when a sensitized individual consumes peanut, epitopes (sectors of the peanut proteins) bind and cross-link the peanut-specific IgE, causing the mast cells to degranulate.¹⁶ Mast cell granules contain an array of bioactive substances which in turn cause the actual symptoms of the GI allergic reaction. The vast majority of food allergic reactions result from exposure to proteins of milk, egg, peanut, tree nuts, soy, wheat, and fish or shellfish.¹⁷ Food allergies may arise from errors in the regulation of the GI immune system, or, allergenic food proteins can bind PRRs on macrophages and dendritic cells initiating an immune response.^{18–20} How PRRs can discriminate between allergy-inducing and non-allergy inducing glycoproteins is not fully understood.

Recently, we exploited the natural binding affinity between edible proteins (of either plant or animal origin) and polyphenolic constituents from berries in a simple food grade strategy to produce colloidal aggregate complexes (protein–polyphenol particles).^{12,21} These particles effectively concentrated and stabilized otherwise ephemeral polyphenolic constituents, and enhanced both the bioavailability and efficacy of ingested berry polyphenols.^{22–24} We subsequently formulated protein–polyphenol particles that were able to modify protein behavior in model food systems, thereby enhancing both the *health* and *structural* functionality of some convenience foods.^{8,12} Using this strategy, peanut proteins were modified by co-aggregation with polyphenols into particles with attenuated allergenicity, which significantly reduced binding to peanut-specific IgE in plasma of peanut-allergic individuals and reduced histamine and β -hexosaminidase release from challenged, sensitized RBL-2H3 cell cultures.²⁵ Peanut protein–cranberry polyphenol particles triggered significantly less mast cell degranulation when used as an oral challenge to peanut-allergic mice, and also caused less basophil degranulation than unmodified peanut flour in an *ex vivo* assay using the blood of peanut-allergic humans. ATR-FTIR (attenuated total reflectance–Fourier transform infrared spectroscopy) analysis suggested changes in secondary protein structure, once the peanut proteins were stably bound to the berry polyphenolic compounds in the particles.¹⁶ Basic subunits of peanut allergenic proteins from the co-aggregated peanut protein–polyphenol particles proved more susceptible to hydrolysis in simulated digestion models, suggesting that these particles may also be less immunoreactive in the digestive tract.²⁶

In this manuscript, we present data demonstrating the capacity of protein–polyphenol particles to mitigate incidence of protein reactivity, in order to inhibit *both* food structural destabilization, and food allergic reactions.

Materials and methods

Protein–polyphenol particles in stabilization of food structures

Materials. Whey protein isolate (WPI) was supplied by Davisco Foods International, Inc. (Le Sueur, MN, USA, BiPro, 93.19% protein). Cranberry, muscadine, and blackcurrant

juice concentrates were gifted by Ocean Spray® (Lakeville-Middleboro, MA, USA), the Muscadine Products Corporation (Wray, GA, USA), and the New Zealand Blackcurrant Co-operative LTD (Nelson, New Zealand), respectively. NaOH, citric acid, sodium acetate, acetic acid, fructose, and glucose were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Formation of protein–polyphenol particles. Cranberry, muscadine, or blackcurrant juice concentrates were diluted with water to total phenolic (TP) concentrations of 250 $\mu\text{g g}^{-1}$ of solution, and WPI was added to achieve final protein concentrations of 10% w/w. Protein–polyphenol solutions were adjusted to pH 4 using 3 M sodium hydroxide, and mixed overnight on a stir plate set to 250 rpm to thoroughly disperse the protein. Protein–polyphenol co-aggregated particles were then pelleted by centrifugation at 7000g for 20 min at 15 °C, collected and separated from the supernatant. The protein content in the pelleted aggregate particles was measured based on nitrogen content and a conversion factor of 6.38 (% whey protein = % nitrogen $\times$ 6.38).²⁷ Nitrogen was determined using a vario MACRO cube CHN (Elementar, Mt. Laurel, NJ, USA) which uses the Dumas method.²⁸ Polyphenol content was determined using the Folin–Ciocalteu method.²⁹ Anthocyanin content was determined using the pH differential method.³⁰

Control whey protein particles (WCP) were formed using the same procedures and concentrations as described previously, except that whey protein was mixed in mock (imitation) juice liquid mixtures (same buffering capacity, pH, and sugar as cranberry, blackcurrant or muscadine grape juice, but without any of the fruit polyphenols) (Table 1). By comparing the protein–polyphenol particles to the WCP, the contribution of the polyphenols to particle properties could be isolated and assessed.

Particle size. Particle size was determined using Fraunhofer diffraction theory (Mastersizer 3000; Malvern, Worcestershire, UK). Particles were dispersed in pH 4 or 5 acetate buffers at 10% w/w protein. Buffers were composed of 0.4 M sodium acetate and acetic acid at ratios of 1:6 and 5:2, respectively. The dispersed particles were added to the sample flask until obscuration reached 10–15% before taking measurements.

Foam formation. Foams were formed and analysis of physical properties was determined according to the methods of Pernell *et al.*^{31,32} Two experiments were conducted. In one, unmodified WPI was the standard protein, and protein–poly-

phenol particles were substituted for 25, 50, 75 and 100% of the total protein in separate treatments (*i.e.*, total protein concentration was maintained at 10% w/w, but the ratio of protein–polyphenol particles/unmodified WPI was stepwise increased). The particles were stirred into foaming solutions from the wet pellet form. All foaming solutions were adjusted to pH 7 using 0.5 M NaOH. In the second experiment, whey protein isolate was mixed 1:1 with either control whey protein particles (WCP; particles made with mock juices devoid of polyphenols) or protein–polyphenol particles, adjusted to pH 3, 4 or 7 with 0.5 M NaOH, and used to form a foam. A fixed weight of 200 g of 10% w/w protein solution was whipped in a mixer (Kitchen Aid Power Mixer, Kitchen Aid, St Joseph, MI USA). After whipping, a hole in the bottom of the bowl was unstoppered, and drainage time commenced. Simultaneously, the yield stress of the foam was determined using a 10 mm diameter and 40 mm length vane fixture attached to a Brookfield 23xL VTDV-ICP viscometer (Middleboro, MA, USA).³¹

Statistical analysis. Statistical analysis was completed using Minitab statistical software (Version 16.0) to perform analysis of variance (ANOVA) using Tukey's comparison of means ($p < 0.05$) as a *post-hoc* test.

Protein–polyphenol particles in attenuation of food protein allergenicity

Protein–polyphenol particles were evaluated to determine if they were able to alter cytokine responses by antigen presenting cells. Porcine macrophages and dendritic cells were incubated with WPI alone or with whey protein–polyphenol particles prepared as described previously with polyphenol extracts from cranberry, blueberry, green tea, cinnamon, and black currant. For experiments with ovalbumin (OVA), the protein and polyphenol were administered in a non-complexed form to cultures of macrophages and dendritic cells.

Porcine blood collection and PBMC isolation. Blood collection procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University. Density gradient centrifugation of porcine blood was performed using Ficoll-Paque PLUS (GE Healthcare, Piscataway, NJ, USA) following the manufacturer's instructions for the isolation of peripheral blood mononuclear cells (PBMCs). Cell viability was assessed *via* Trypan blue exclusion.

Table 1 Composition of imitation (mock) juice solutions

	Buffer		Sugar	pH
	Acid	Base		
Imitation Cranberry Juice	0.88 M citric acid	0.88 M citrate	1.6% fructose: 8.3% glucose	3.06
Imitation Blackcurrant Juice	0.67 M citric acid	0.67 M citrate	2.6% fructose: 3.9% glucose	2.95
Imitation Muscadine Grape Juice	0.28 M tartaric acid	0.28 M tartrate	7.5% fructose: 7.5% glucose	3.20

Differentiation of blood monocytes. PBMCs (1×10^7 cells per vial) were rapidly thawed in 10 mls cRPMI medium supplemented with 2.05 mM L-glutamine (GE Healthcare, Piscataway, NJ, USA), 10% FBS, and 1% penicillin: streptomycin solution (Gemini Bio-Products, West Sacramento, CA, USA). Following centrifugation, the supernatant was removed, and cells were resuspended in 15 mL of cRPMI. Tissue culture flasks were either coated with porcine serum (Gemini Bio-Products) for dendritic cells or not pre-coated for macrophages. Dendritic cells were matured for 7 days in 80 ng mL^{-1} of recombinant porcine GM-CSF (granulocyte macrophage colony-stimulating factor; R&D Systems, Minneapolis, MN, USA) and 50 ng mL^{-1} of recombinant porcine interleukin (IL)-4 (R&D Systems).³³ For maturation of macrophages, media was supplemented with 10% L929 supernatant as a source of macrophage colony-stimulating factor.³⁴

Cytokine production by macrophages and dendritic cells. Supernatants collected from cells following 18 h of incubation were briefly centrifuged and stored at -20°C until further use. Production of the cytokines TNF- α (tumor necrosis factor- α) and IL-8 found in cell supernatants was quantified using porcine-specific Duoset® ELISA kits (R&D Systems) following the manufacturer's instructions.

Cell viability assay. After the 18 h incubation period, supernatants were collected from cells. To assess cytotoxicity, lactate dehydrogenase (LDH) production by cells was measured using the Pierce™ LDH Cytotoxicity Assay Kit (Pierce Biotechnology, Rockford, IL, USA) according to the manufacturer's instructions.

Results and discussion

Protein–polyphenol particles in stabilization of food structures

The molar ratio of protein-to-total phenolics for each protein–polyphenol particle was estimated to be 1–1.5 assuming a molecular weight of 500 Da for the average polyphenol and 18 kDa for whey proteins. The range produced was dependent on the juice source (Table 2). The degree of polyphenol binding to the protein varied depending on the specific conditions used during formation of co-aggregated particles; significantly higher polyphenol loads were achieved previously

using other, more insoluble protein sources such as soy or hemp.^{21,24}

Each juice and protein complexation process resulted in a distribution of particles between $1 \mu\text{m}$ and $100 \mu\text{m}$. The particle size tended to be smaller for particles formed at pH 4 as compared to pH 5, and it is likely that smaller particles below $1 \mu\text{m}$ were present but not detected.

Incorporation of protein–polyphenol particles into foams produced foam structures with increased stability and firmer texture. As illustrated in Fig. 1A and B, both the drainage half-life (a marker of structural stability) and the yield stress (resistance to flow) of model foams increased in a dose-dependent manner when whey protein–cranberry polyphenol particles were incorporated into the foam structure as increasingly larger proportions of the total protein content. Similar stabilization of foams occurred when other fruit polyphenol sources were used to create the protein–polyphenol particles (data not shown).

While incorporation of protein–polyphenol particles increased the stability and altered the texture of model foams, foams made with control whey protein-only particles, (prepared using the polyphenol-free imitation juice mixtures; WCP), did not exhibit the same attributes over a range of pH values (Fig. 2A and B). These results indicated that improvements in the physical attributes of foams made with protein–polyphenol particles were not simply an effect of adding particles in general, but were related to the composition of the protein–polyphenol particles.

Incorporation of these whey protein–polyphenol aggregate particles into model bar formulations resulted in a reduction of bar hardening reactions typically encountered in high protein bars.³⁵ Sensory panels reported a reduction in hardness and toothpacking when whey protein–cranberry polyphenol particles were incorporated into bar formulations, and this descriptive analysis was supported by a reduction in creep recovery when the model bars were evaluated with a rheometer. Bars that contained protein–polyphenol particles maintained a lower creep recovery (higher degree of fluidity) than bars produced with the same concentrations of unmodified whey proteins, and an increase in maximum compliance (J_{max}), a parameter that is inversely related to firmness. Interestingly, bars made with higher concentrations of polyphenols in the aggregate particles (2 mg TP per g dry aggregate particle) remained significantly more pliable (higher J_{max}) than bars made with aggregates containing half that TP level.³⁵ Protein–polyphenol aggregate particles used to make the bars were significantly less dispersible (larger particle size) than unmodified whey proteins, or particles made with proteins alone.

Protein–polyphenol particles attenuate responses to allergenic food proteins

The interaction of allergy-inducing food proteins with host macrophages and dendritic cells is a key step in the establishment of food allergies. Macrophages and dendritic cells perform antigen processing and presentation, and produce

Table 2 Dry particle composition (mg g^{-1}) of protein–polyphenol particles produced with cranberry, blackcurrant, and muscadine grape juices. TP = total phenolics (gallic acid equivalent), ANC = anthocyanins (cyanidin-3-O-glucoside equivalent). Numbers in a column followed by a different letter were statistically different ($p < 0.05$), Tukey's *post-hoc* test

	Particle TP (mg g^{-1})	Juice ANC (mg mL^{-1})	Particle ANC (mg g^{-1})	Particle protein (mg g^{-1})
Cranberry	0.40 ^a	0.23 ^a	0.14 ^b	853 ^a
Blackcurrant	0.60 ^b	0.58 ^b	0.19 ^b	903 ^b
Muscadine grape	0.56 ^b	0.68 ^c	0.09 ^a	843 ^a

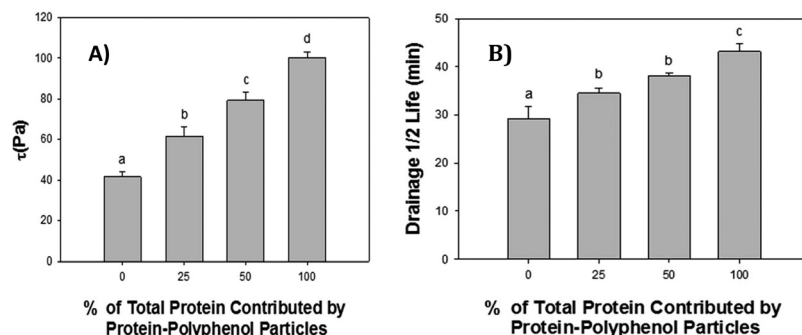


Fig. 1 A. Yield stress (τ) and B. Drainage half-life (indicating stability) of foams. Foaming solutions were control (0% protein–polyphenol particles, 100% whey protein isolate) or treatments, containing 25, 50, or 100% of the protein as particles containing polyphenols. All solutions in were at pH 7 and contained 10% w/w protein and polyphenols from cranberry juice. Bars with different lowercase letters are significantly different ($p < 0.05$).

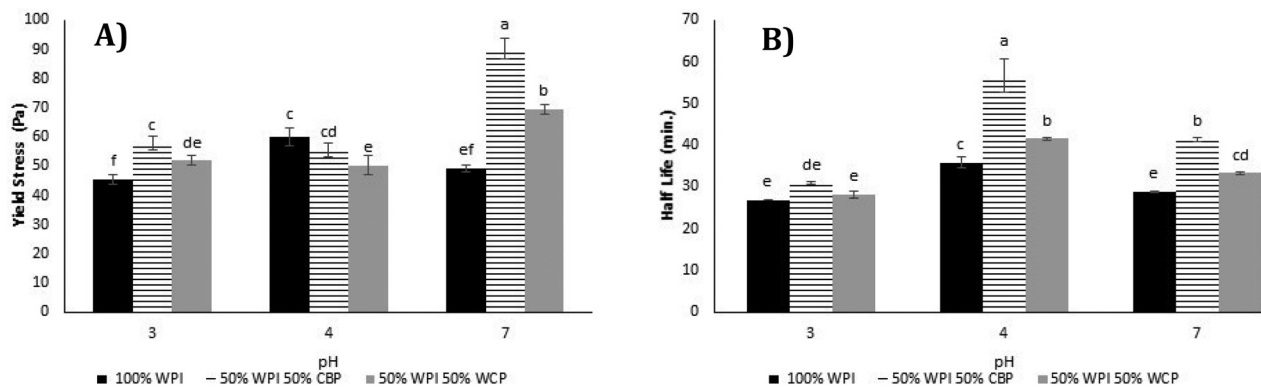


Fig. 2 Yield stress (τ) and B. Drainage half-life of foams at three different pH levels. WPI = whey protein isolate. CBP = protein–cranberry polyphenol particles. WCP = particles made with mock (imitation) juice (devoid of polyphenols). Foaming solutions were control (100% WPI), 50% WPI/50% CBP, or 50% WPI/50% WCP. All solutions in were at pH 7 and contained 10% w/w protein and polyphenols from cranberry juice. Bars with different lowercase letters are significantly different ($p < 0.05$).

immunomodulatory cytokines. In a series of experiments, we tested whether plant-derived polyphenols presented in protein–polyphenol particles could inhibit cytokine production from macrophages and dendritic cells responding to food allergenic proteins. Peripheral blood mononuclear cells were isolated from swine and differentiated *in vitro* to either macrophages or dendritic cells. We found that porcine macrophages produced TNF- α proinflammatory cytokine, when stimulated with WPI, and that this response was blocked completely when the cells were stimulated with particles that complexed WPI with cinnamon-derived polyphenols (Fig. 3A). Similar results were found with porcine dendritic cells (Fig. 3B). Co-aggregated particles were formed with WPI and polyphenol extracts from cinnamon, cranberry, or blueberry. As shown in Fig. 3C, the same suppressive effect was noted when the protein OVA was added to cultures of porcine macrophages along with polyphenols (at the same concentration) in a non-complexed form. Finally we considered whether the lack of macrophage or dendritic cell response to the complex might result from a cytotoxic effect. All extracts and complexes were tested using an LDH cytotoxicity assay and the results of a typical experiment using cinnamon extract are shown in Fig. 3D, which confirms

that cytotoxicity toward porcine macrophages was not observed at any of the concentrations tested.

The results shown in Fig. 3 demonstrate that plant derived polyphenols can suppress cytokine responses from macrophages and dendritic cells and therefore might be useful to reduce immune responses to allergenic proteins. The suppressive effect noted could stem from a “blocking” effect, where the polyphenol coupled to the protein prevents the protein from binding the appropriate receptors on macrophages or dendritic cells. Also, or alternatively, this effect could stem from the independent action of the polyphenols on additional cell surface receptors, *e.g.*, receptors not binding the allergenic protein. The polyphenol-mediated inhibition of the OVA response noted in Fig. 3C could arise from such an effect. To more fully explore this hypothesis, porcine macrophages and dendritic cells were treated simultaneously with bacterial LPS, which stimulates cytokine production through toll-like receptor (TLR)-4, and uncomplexed plant polyphenols. The results of these experiments, with polyphenol fractions from cranberry (CB), blueberry (BB), green tea (GT), cinnamon (CN), and black currant (BC) are summarized in Fig. 4. With macrophages (Fig. 4A and B) and dendritic cells (Fig. 4C and D) poly-

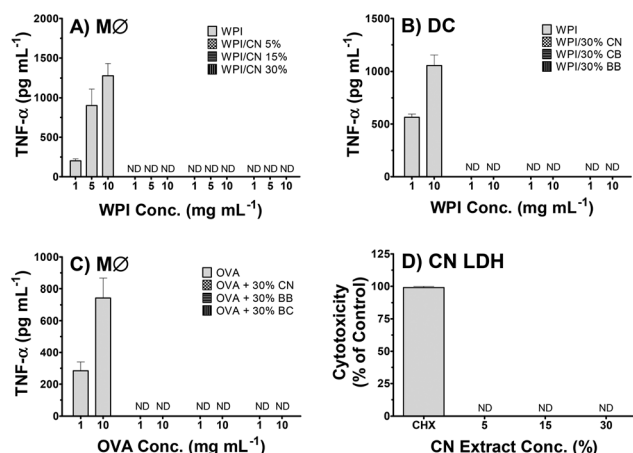


Fig. 3 Inhibition of TNF- α production by polyphenols and protein: polyphenol complexes. Porcine macrophages (A, C, and D) and dendritic cells (B) were differentiated *in vitro* then treated overnight with whey protein isolate (WPI) or ovalbumin (OVA) alone or in the presence of polyphenol fractions from cinnamon (CN), blueberry (BB), or black currant (BC). Protein and polyphenol were added in pre-complexed form (as colloidal particles; A and B) or separately but simultaneously to the cultures (C). In C, polyphenol concentrations for extracts were CN (0.72 mg mL⁻¹), BB (1.32 mg mL⁻¹), and BC (0.93 mg mL⁻¹). Measurements of porcine TNF- α in culture supernatants were performed by ELISA. Panels A–C show means $\pm$ SEM from three independent experiments. A representative overnight lactate dehydrogenase (LDH) assay is shown in panel D, where cycloheximide (CHX) is used as the positive control. Not detectable (ND).

phenol fractions again completely inhibited production of TNF- α and the chemokine CXCL8. It is likely therefore, that while the polyphenols in a complex with proteins or lipopoly-saccharides may block receptor-dependent recognition of that compound (in this case, blocking recognition of LPS by TLR-4), polyphenols also exert independent suppressive effects on these cell types. Polyphenols are well known for their ability to affect macrophage function and more specifically the TLR-4 pathway.³⁶ (It should also be noted that polyphenol concentrations in Fig. 4 were 1/3 of those used in Fig. 3C). The mechanisms underlying this effect, and the recognition of allergenic proteins needs to be explored further.

Conclusions

Both structural and health functionality of foods are enhanced when proteins and polyphenols are delivered in the form of protein–polyphenol particles. The binding of polyphenols to proteins effectively blunts the typically reactive properties of proteins, which can otherwise trigger destabilizing events. Model food structures like foams showed greater stability (resistance to drainage) and behaved more like solids when formulated with protein–polyphenol particles. In addition, protein–polyphenol particles suppressed production of TNF- α from macrophages and dendritic cells stimulated with allergenic proteins, and both particles and separately-administered polyphenol extracts suppressed cytokine and chemokine production from LPS-stimulated macrophages and dendritic cells, indicative of potential applications in hypoallergenic food products that do not provoke food protein allergy.

Conflicts of interest

MAL and NP have conducted research on commercialization of related technology. All other authors declare no conflict of interest.

Acknowledgements

Financial support from USDA-CSREES-AFRI-002010 (Transdisciplinary Graduate Training Program in Functional Foods, Bioactive Food Components, and Human Health) and Plants for Human Health Seed Grant Initiative (2015) are gratefully acknowledged. Thanks to Mr Charles Warlick and Ms. Lorie Beale for technical assistance in some of these experiments.

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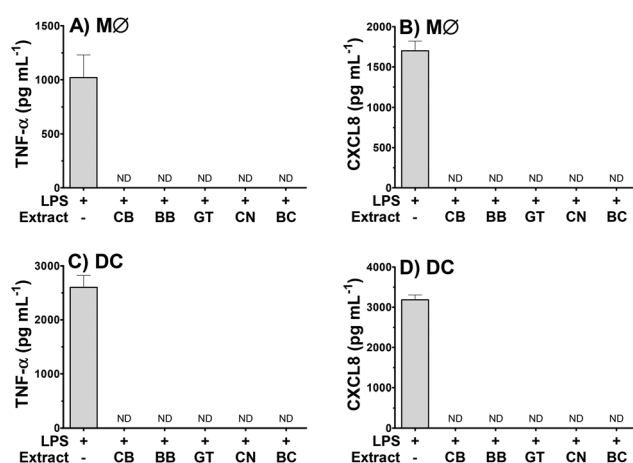


Fig. 4 Suppression of lipopolysaccharide (LPS)-induced cytokine and chemokine production by plant polyphenols. Porcine macrophages (A and B) and dendritic cells (C and D) were incubated overnight with 1 μ g mL⁻¹ LPS from *S. minnesota* R595. Polyphenol fractions from cranberry (CB, 0.34 mg mL⁻¹), blueberry (BB, 0.44 mg mL⁻¹), green tea (GT, 0.33 mg mL⁻¹), cinnamon (CN, 0.24 mg mL⁻¹), and black currant (BC, 0.31 mg mL⁻¹) were added as indicated. Supernatants were collected and levels of TNF- α and CXCL8 were measured by ELISA. Values shown are means $\pm$ SEM from three independent experiments. Not detectable (ND).

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