

Protein-bound *Vaccinium* fruit polyphenols decrease IgE binding to peanut allergens and RBL-2H3 mast cell degranulation *in vitro*

Nathalie J. Plundrich^a, Rishipal R. Bansode^b, E. Allen Foegeding^c, Leonard L. Williams^b, Mary Ann Lila^{a*}

^aPlants for Human Health Institute, Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, North Carolina Research Campus, 600 Laureate Way, Kannapolis, NC 28081, USA. ^bCenter for Excellence in Post-Harvest Technologies, North Carolina Agricultural and Technical State University, North Carolina Research Campus, 500 Laureate Way, Kannapolis, NC 28081, USA. ^cDepartment of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, 400 Dan Allen Dr., Raleigh, NC 27695, USA.

*Corresponding author: Dr. Mary Ann Lila, 600 Laureate Way, North Carolina Research Campus, Kannapolis, NC 28081, USA; mlila@ncsu.edu; Tel.: +1 704 250 5400; Fax: +1 704 250 5409

Electronic Supplementary Information

RBL-2H3 mast cell degranulation upon co-exposure with plant extracts alone

The method used is described in sections 2.8 – 2.10 of the original research article. Polyphenol concentrations tested were the same as present in the peanut protein-cranberry or blueberry polyphenol complexes containing 5 - 40% polyphenols.

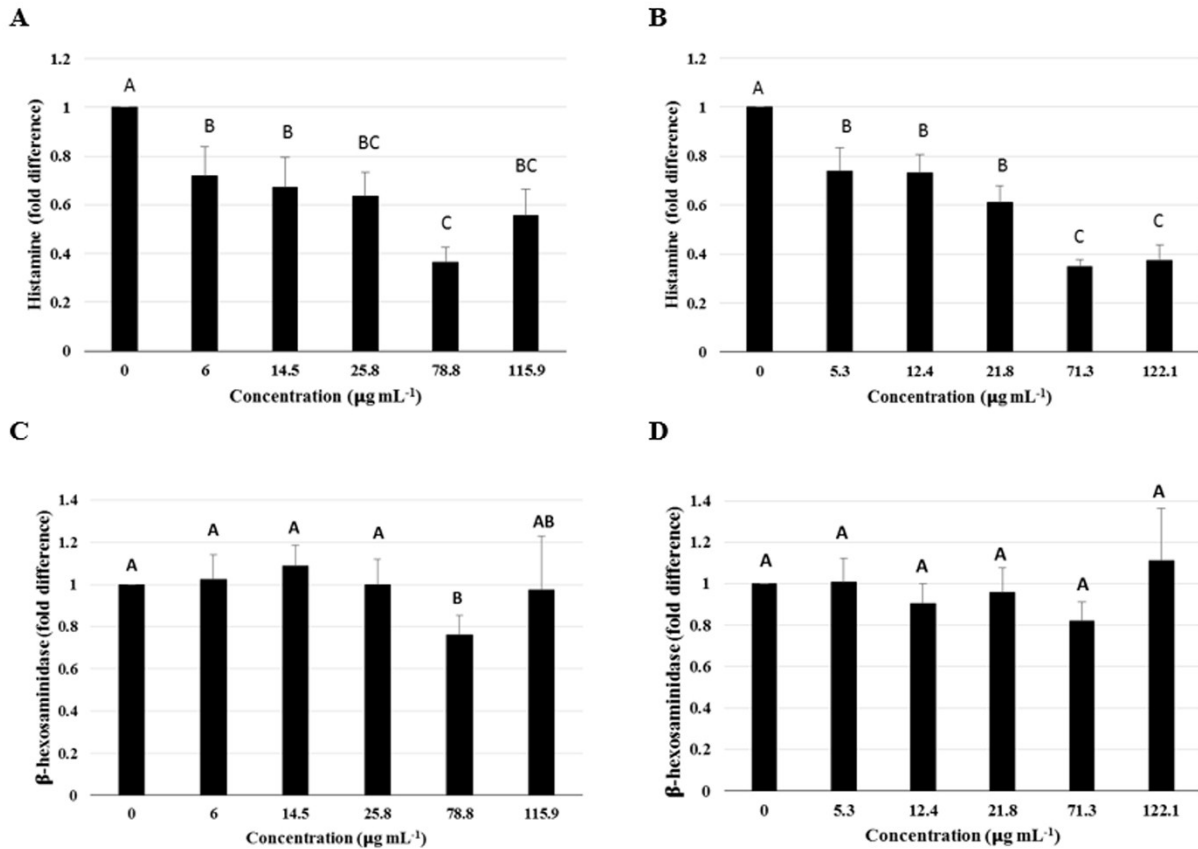


Fig. 1S. Histamine (A and B) and β -hexosaminidase (C and D) release following cranberry (A and C) or blueberry pomace extract (B and D) co-exposure with DNP-BSA in RBL-2H3 cells sensitized with anti-DNP IgE and ionomycin. Polyphenol concentrations tested were the same as present in the peanut protein-cranberry or blueberry polyphenol complexes containing 5 - 40% polyphenols. Controls (treatment 0 $\mu\text{g mL}^{-1}$) were cells which only received ionomycin and PBS 1x. Data shown are means of six replicates with SE; values with different letters are significantly different at $p < 0.05$. DNP, dinitrophenyl; BSA, bovine serum albumin, PBS; phosphate buffered saline.

Measurement of cell viability using the XTT assay

RBL-2H3 cells in Eagle's minimum essential medium (EMEM; Gibco, ThermoFisher Scientific, Waltham, MA, USA) and 15% FBS (fetal bovine serum) in EMEM (v/v) (Gibco, ThermoFisher Scientific, Waltham, MA, USA) were seeded at a concentration of 2×10^5 cells/well in a 24-well plate. The cells were incubated in a humidified incubator (37 °C; 5% CO₂) for at least 24 h. The cells were serum starved in 1% FBS containing EMEM (v/v) and were treated with peanut protein-polyphenol complexes (containing 5-40% polyphenols) and incubated for 3 h in a humidified incubator (37 °C; 5% CO₂). The effect of polyphenols alone on cell viability was also tested in a separate experiment. After incubation, the toxicity was assessed using XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2*H*-Tetrazolium-5-Carboxanilid) reagent as per manufacturer's protocol (ATCC, Manassas, VA, USA). Two hundred μL of activated XTT solution was added to each well and subsequently incubated for an additional 2 h at 37 °C and 5% CO₂. The plate was then removed from the incubator and immediately measured for absorbance at 475 nm using a Synergy 2 microplate reader (BioTek, Winooski, VT, USA).

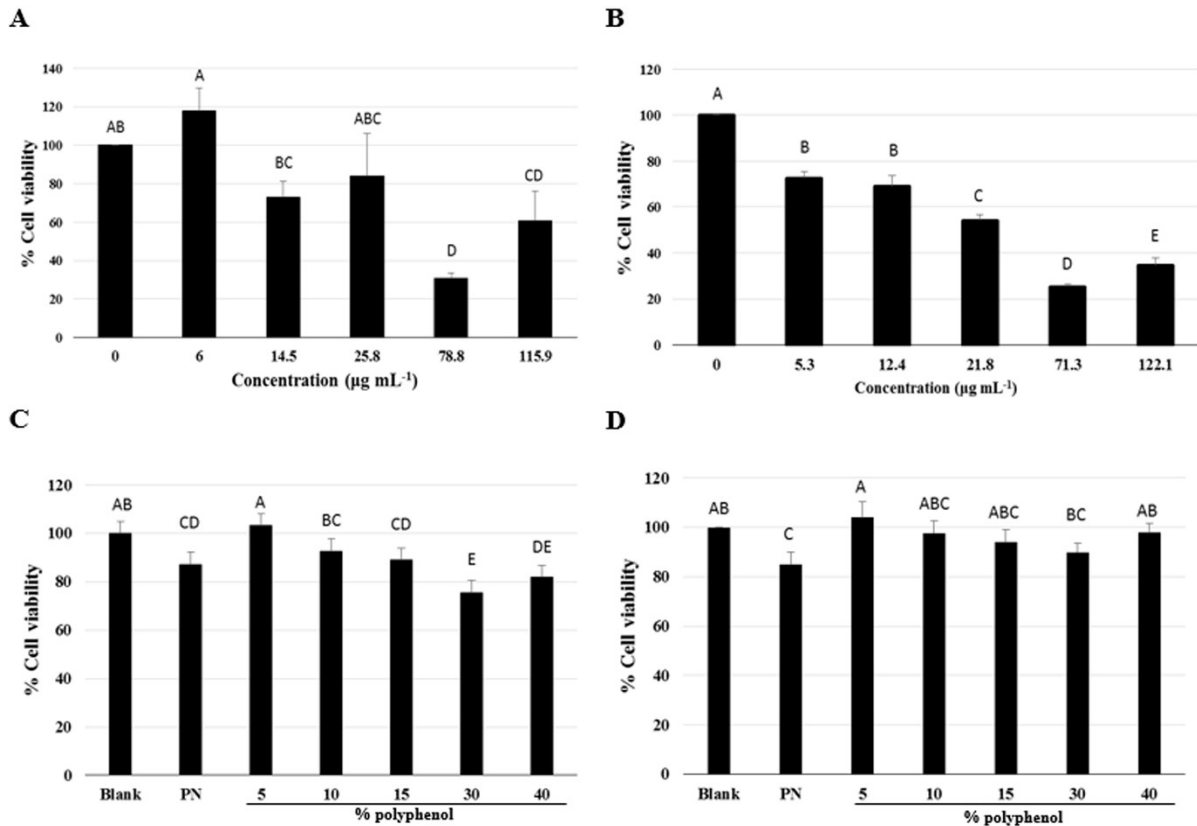


Fig. 2S. Cell viability (%) following cranberry (A) or blueberry (B) pomace extract co-exposure with DNP-BSA in RBL-2H3 cells sensitized with anti-DNP IgE and ionomycin. Polyphenol concentrations tested were the same as present in the peanut protein-cranberry or blueberry polyphenol complexes containing 5 - 40% polyphenols. Controls (treatment 0) were cells which only received ionomycin and PBS 1x. Cell viability (%) following peanut protein- cranberry (C) or blueberry (D) polyphenol complex (5-40% polyphenols) or unmodified peanut flour (PN) co-exposure with DNP-BSA in RBL-2H3 cells sensitized with anti-DNP IgE and ionomycin. Controls were cells which only received PBS 1x (blank). Data shown are means of six replicates with SE; values with different letters are significantly different at $p < 0.05$. DNP, dinitrophenyl; BSA, bovine serum albumin, PBS; phosphate buffered saline.