Composition and biological properties of soluble post-gastrointestinal digestion fractions of common dry edible beans

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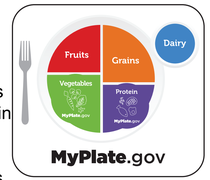
Introduction

- Dry edible beans are inexpensive source of **quality protein.**
- Approximately 25.85 million cwt of dry edible bean were produced in 2022 (USA).
- The **USDA MyPlate** food guidance system includes beans, peas, and lentils in the vegetable and protein foods groups.
- Bioactive peptides and polyphenols, found in beans have been shown to improve health via an array of actions such as **anti-hypertensive**, **anti-inflammatory**, and **anti-atherosclerotic** activities.
- There are not enough literatures to compare digestibility and bioactive compounds of various bean varieties after gastrointestinal digestion.



Objectives

- Determine **in-vitro digestibility** of the selected bean varieties and evaluate anti-oxidant and anti-inflammatory properties in an intestinal cell model.
- Determine free **amino acids, peptide and phytochemical profile** after simulated gastrointestinal digestion.

Materials & Methods

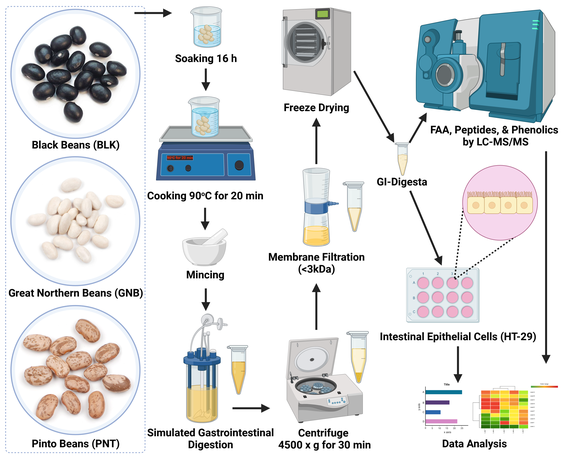
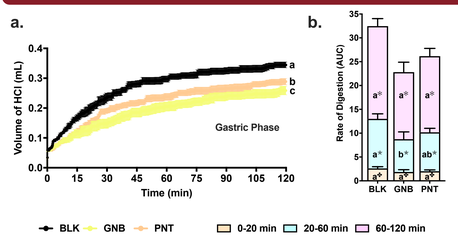


Fig. 1. Experiment overview. Preparation of dry edible beans for the simulated gastrointestinal (GI) digestion, freeze drying of soluble peptide fraction (<3kDa), physiochemical analysis by LC-MS/MS, and bioactivity measurement of the GI-digesta in an in-vitro system.

Results



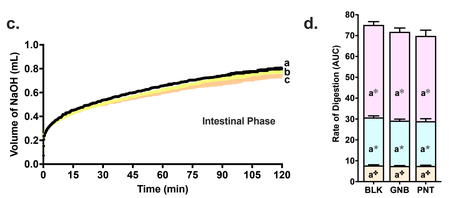


Fig. 2. Rate of digestion. In vitro digestibility of proteins under simulation digestion conditions using pH-stat method (function of pH change) during the gastric **(a, b)** and intestinal **(c, d)** phases over the course of 120 minutes. Values not sharing a common letter are significantly different (a, b, c: between beans; ✣✳✲: within beans). Repeated measures two-way ANOVA followed by Tukey's multiple comparisons test, $P < 0.05$, $n=6$.

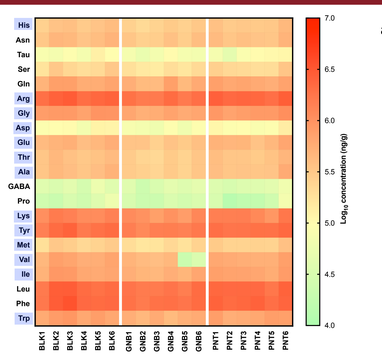


Fig. 5. Heatmap of Free Amino Acids (FAA). Logarithmic concentration of individual amino acid measured by LC-MS/MS and expressed as ng/g of lyophilized bean digesta. Values not sharing a common letter are significantly different. One-way ANOVA followed by Tukey's multiple comparisons test. The abundance of amino acids highlighted in blue is significantly different among the three beans, $P < 0.05$, $n=6$.

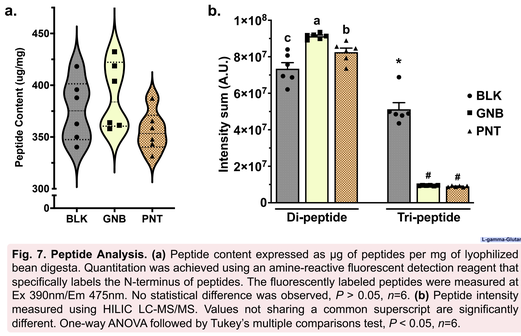


Fig. 7. Peptide Analysis. (a) Peptide content expressed as µg of peptides per mg of lyophilized bean digesta. Quantitation was achieved using an amine-reactive fluorescent detection reagent that specifically labels the N-terminus of peptides. The fluorescently labeled peptides were measured at Ex 390nm/Em 475nm. No statistical difference was observed, $P > 0.05$, $n=6$. **(b)** Peptide intensity measured using HILIC LC-MS/MS. Values not sharing a common superscript are significantly different. One-way ANOVA followed by Tukey's multiple comparisons test, $P < 0.05$, $n=6$.

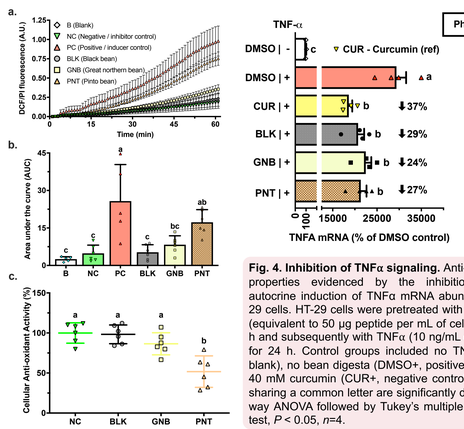
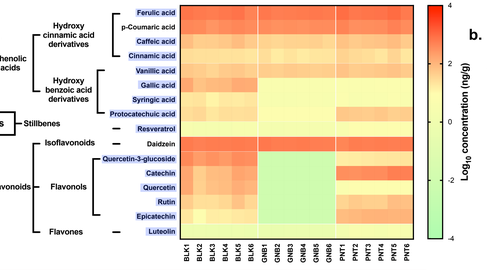


Fig. 4. Inhibition of TNFα signaling. Anti-inflammatory properties evidenced by the inhibition of TNFα autocrine induction of TNFα mRNA abundance in HT-29 cells. HT-29 cells were pretreated with bean digesta (equivalent to 50 µg peptide per mL of cell media) for 2 h and subsequently with TNFα (10 ng/mL of cell media) for 24 h. Control groups included no TNFα (DMSO-, blank), no bean digesta (DMSO+, positive control), and 40 mM curcumin (CUR+, negative control). Values not sharing a common letter are significantly different. One-way ANOVA followed by Tukey's multiple comparisons test, $P < 0.05$, $n=4$.

Fig. 3. AAPH induced oxidative stress in HT-29. Black (BLK), Great Northern (GNB), and Pinto (PNT) bean digesta (equivalent to 50 µg peptides per mL of cell media) lowered AAPH-induced oxidative stress in human intestinal epithelial HT-29 cells. **(a)** DCF/propidium iodide fluorescence (A.U.) **(b)** Area under the curve (AUC), and **(c)** Cellular antioxidant activity. Cellular antioxidant activity (CAA) was expressed as % of negative/inhibitor control (NC), which included 25 µM of Quercetin, B refers to blank, which was made with no inducer, and PC refers to positive control, which included 600µM of AAPH in the reaction. Values not sharing a common letter are significantly different. One-way ANOVA followed by Tukey's multiple comparisons test, $P < 0.05$, $n=6$.



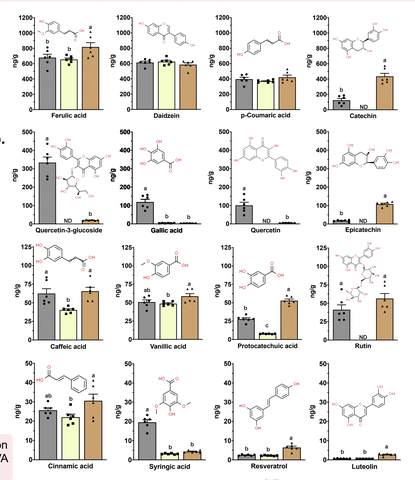


Fig. 6. Heatmap of phytochemicals. (a) Logarithmic concentration of the identified phytochemicals measured by LC-MS/MS and expressed as ng/g of lyophilized bean digesta clustered by its classification. The abundance of phenolic compounds highlighted in blue is significantly different among the three beans, $P < 0.05$, $n=6$.

(b) Individual phytochemicals concentration. Values not sharing a common superscript are significantly different (ND = not detected). One-way ANOVA followed by Tukey's multiple comparisons test, $P<0.05$, $n=6$.

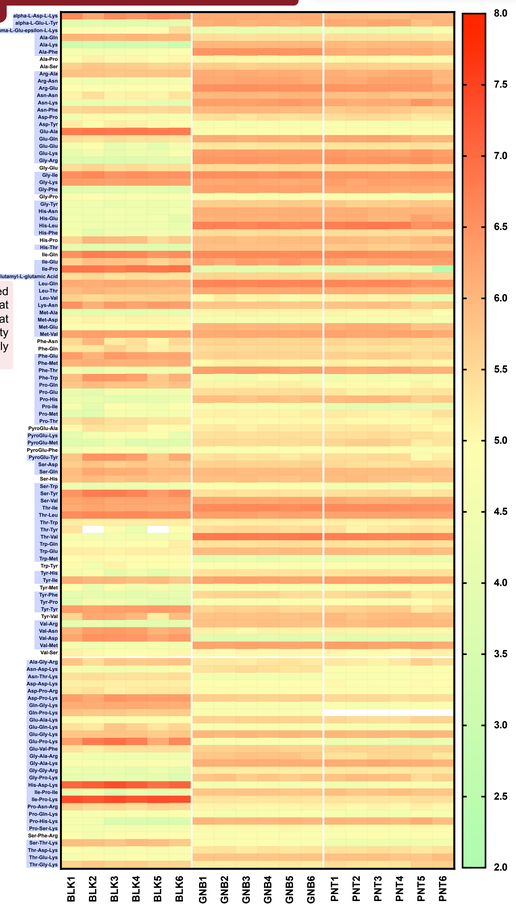


Fig. 8. Peptide distribution. Logarithmic concentration of di and tri peptide residues intensity measured via HILIC LC-MS/MS in the lyophilized bean digesta (n=6). The abundance of peptides highlighted in blue is significantly different among the three beans $P < 0.05$, $n=6$.

Conclusions

- Differences in the rate of digestion indicates **different proteolysis pattern** suggesting differences in storage protein structure.
- **Black and pinto beans are proportionally richer in flavonols**, and hydroxy benzoic acid derivatives compared to great northern bean.
- All bean digesta **ameliorated TNF-α signaling** over DMSO control in HT-29 cell model.
- All bean digesta were **effective in reducing AAPH induced oxidative stress** in HT-29 cell model.

References

- Chen, Y., Zhang, H., Liu, R., Mats, L., Zhu, H., Pauls, K. P., Deng, Z., & Tsao, R. (2019). Antioxidant and anti-inflammatory polyphenols and peptides of common bean (*Phaseolus vulga* L.) milk and yogurt in Caco-2 and HT-29 cell models. *Journal of Functional Foods*, *53*, 125– 135.
- Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants and Redox Signaling*, *18*(14), 1818–1892.
- U.S. dry edible bean production 2001-2022 by M. Shahbandeh, Jan 30, 2023
- USDA MyPlate Dietary Guidelines for Americans, 2020-2025

Acknowledgments

SYSTEM SCIENCE
Nebraska Dry Bean Commission

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