Developing Staple Food Crops for Improved Nutritional Quality: Characterization of Compounds in Bean Seed Coats That Inhibit and Enhance Fe Absorption

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Objectives

Previous research in our laboratory using Caco-2 cultured human intestinal cells, right, showed that some individual polyphenols can promote iron uptake, while others inhibit iron uptake. This current work examined interactions between specific polyphenols to gain understanding of their relative power to promote or inhibit iron uptake. We also studied the effects of serial removal of inhibiting polyphenols from a mix of polyphenols that simulate the polyphenolic composition of black bean seed coats.

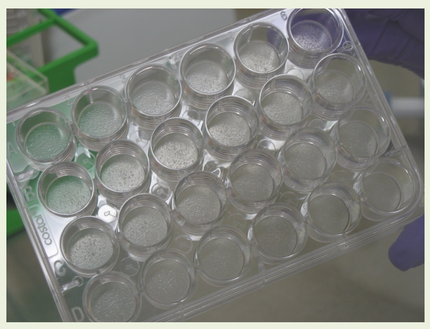
Materials and methods

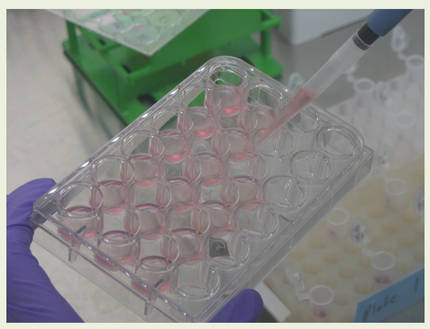
Black bean seed coat extraction. To one gram of dried and ground black bean seed coats, 5 mL of methanol:water (50:50) was added. The slurry was vortexed for one minute, placed in a sonication water bath at 40C for 10 minutes, vortexed again for one minute, and centrifuged at 4000 x g for 15 minutes. The supernatant was filtered with a 0.2 µm syringe filter and stored for later use in a -20C freezer.

Caco-2 assays. Individual polyphenols (Sigma; Myricetin 3-glucoside: Extrasynthese, Genay, France) were solubilized in DMSO to 1.6 mM and diluted with pH 2 medium to achieve 400 µM stock solutions. Maximum DMSO concentration applied to Caco-2 monolayers was 1.25%. $FeCl_3$ stock solutions were prepared from 1000 µg/mL standard (High-Purity Standards, Charleston, SC, USA).

Caco-2 assays were performed as described previously (Glahn, et al., 1998 J. Nutr. 128: 1555-1561) with minor modifications. Aliquots (200 µL) of pH 2 solution containing polyphenol and $FeCl_3$ were pipetted into 1 mL of Modified Eagle Medium solution. One half mL of this combined solution was applied directly to Caco-2 cells in 24 well plates. Plates were placed in a 37C incubator overnight. Cells were then lysed and measured for ferritin concentration by immunoassay.

LC-MS analysis. Black bean seed coat extracts were analyzed by LC-MS with an Acquity UPLC coupled to a Xevo G2 QTOF mass spectrometer (Waters Corp). For LC analysis, 5 µL samples of extract were injected and passed through a BEH Shield RP18 1.7 µm 2.1 x 100 mm column (Waters) at 0.5 mL/min. Mobile phase consisted of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B). Polyphenols were eluted using linear gradients of 86.7 to 84.4% A in 1.5 min, 84.4 to 81.5% A in 0.2 min, 81.5 to 77% A in 2.8 min, 77 to 55% A in 0.5 min, 55 to 46% A in 1 min, 46 to 86.7% A in 0.2 min and a 0.8 min hold at 86.7% A for a total 7 min run time. ESI mass spectrometry was performed in negative ionization mode with a scan speed of 5/s in the mass range from 50 to 1200 Da. Lock-mass correction was used, with leucine enkephalin as the external lock-mass standard. LC-MS data were analyzed by MassLynx software. Individual polyphenols in black bean seed coat samples were tentatively determined by mass using MarkerLynx software and their identities were confirmed by comparison of LC retention times with authentic standards.





Caco-2 cell cultures in assay plates

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Identification of Black Bean (*Phaseolus vulgaris* L.) Polyphenols That Inhibit and Promote Iron Uptake by Caco-2 Cells

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ABSTRACT: In nutritional studies, polyphenolic compounds are considered to be inhibitors of Fe bioavailability. Because they are presumed to act in a similar manner, total polyphenols are commonly measured via the Folin−Ciocalteu colorimetric assay. This study measured the content of polyphenolic compounds in white and black beans and examined the effect of individual polyphenols on iron uptake by Caco-2 cells. Analysis of seed coat extracts by LC-MS revealed the presence of a range of polyphenols in black bean, but no detectable polyphenols in white bean. Extracts from black bean seed coats strongly inhibited iron uptake. Examination of the eight most abundant black bean seed coat, non-anthocyanin polyphenols via Caco-2 cell assays showed that four (catechin, 3,4-dihydroxybenzoic acid, kaempferol, and kaempferol 3-glucoside) clearly promoted iron uptake and four (myricetin, myricetin 3-glucoside, quercetin, and quercetin 3-glucoside) inhibited iron uptake. The four inhibitors were present in 3-fold higher total concentration than the promoters (143 ± 7.2 vs 43.6 ± 4.4 µM), consistent with the net inhibitory effect observed for black bean seed coats. The ability of some polyphenols to promote iron uptake and the identification of specific polyphenols that inhibit Fe uptake suggest a potential for breeding bean lines with improved iron nutritional qualities.

KEYWORDS: *polyphenols, iron bioavailability, Caco-2 cells, catechin, myricetin*

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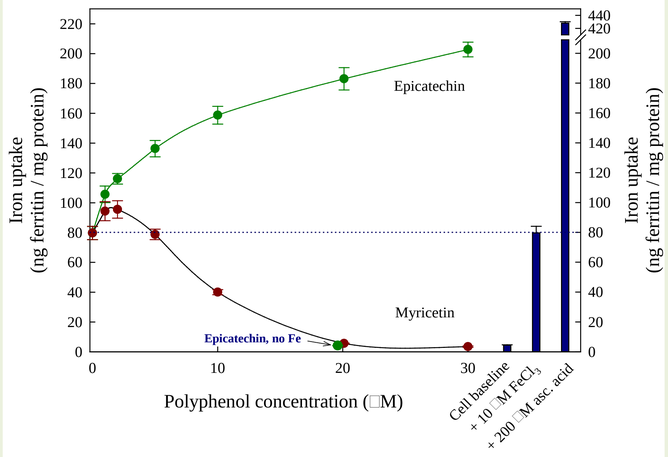
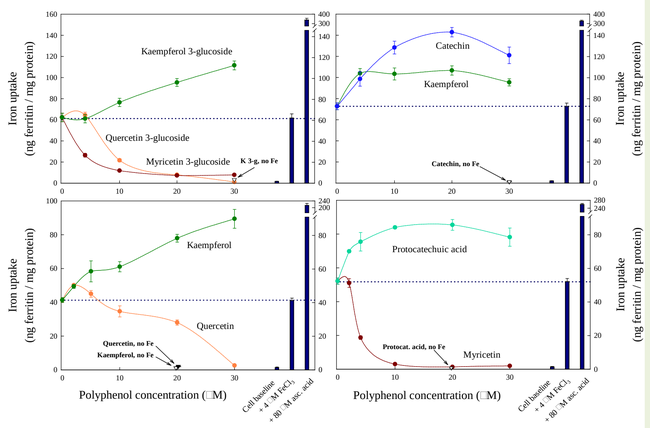
Iron uptake in response to varying concentrations of epicatechin and myricetin



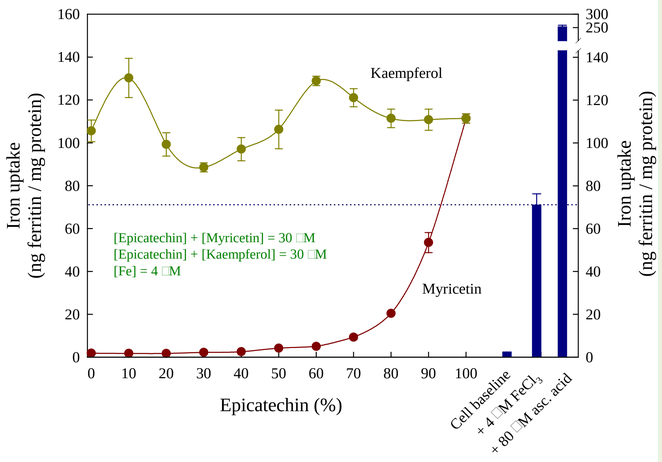
Myricetin inhibited and epicatechin promoted iron uptake. Dotted line represents iron uptake with no polyphenol present.

Iron uptake in response to varying concentrations of eight black bean polyphenols



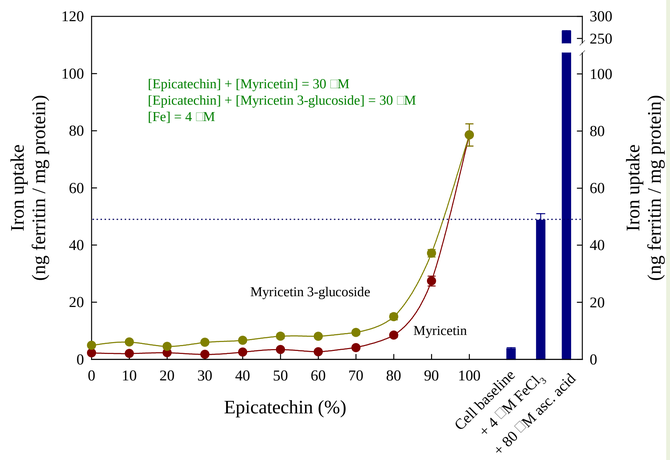
Myricetin 3-glucoside, quercetin 3-glucoside, quercetin and myricetin inhibited, and catechin, kaempferol 3-glucoside, kaempferol and protocatechuic acid promoted iron uptake. Dotted line represents iron uptake with no polyphenol present.

Iron uptake in response to varying ratios of epicatechin to myricetin, and epicatechin to kaempferol



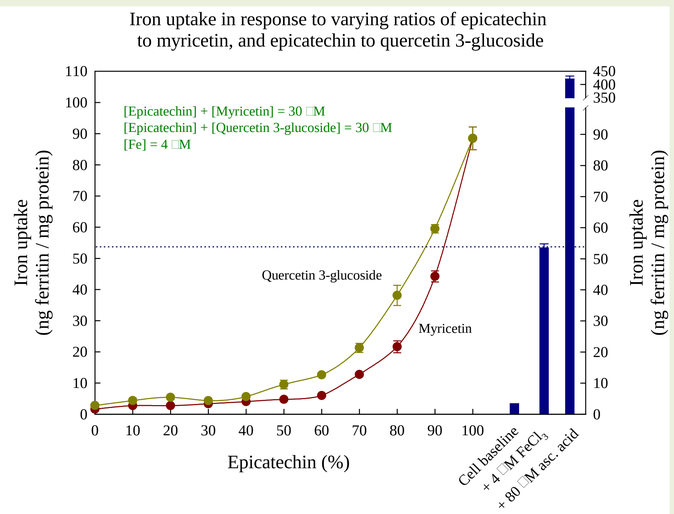
Application of epicatechin and kaempferol promoted iron uptake at all concentration combinations. Dotted line represents iron uptake with no polyphenol present.

Iron uptake in response to varying ratios of epicatechin to myricetin, and epicatechin to myricetin 3-glucoside



When applied in combination with myricetin or myricetin 3-glucoside, epicatechin did not overcome iron uptake inhibition until it was present at greater than 90% of total concentration. Dotted line represents iron uptake with no polyphenol present.

Iron uptake in response to varying ratios of epicatechin to myricetin, and epicatechin to quercetin 3-glucoside



When applied in combination with quercetin 3-glucoside, epicatechin did not overcome iron uptake inhibition until it was present at greater than 80% of total concentration. Dotted line represents iron uptake with no polyphenol present.

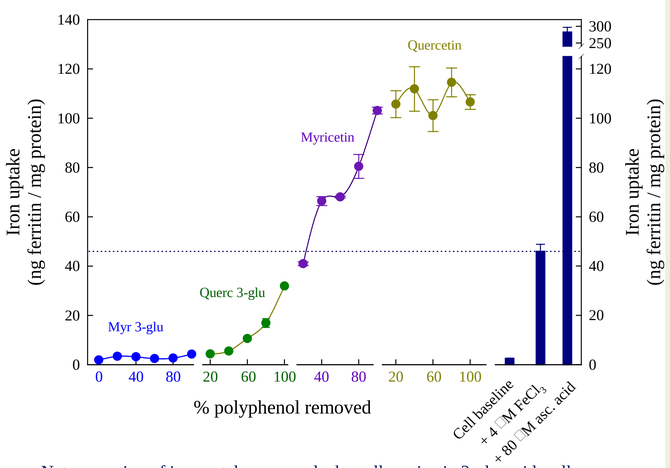
Concentrations of Polyphenols Present in Extracts from Black Bean Seed Coats

Polyphenol	Mean ± SD (µM)	inhibitor / promoter of iron uptake
Myricetin 3-*O*-glucoside	100.6 ± 5.1	inhibitor
Quercetin 3-*O*-glucoside	33.2 ± 1.6	inhibitor
Kaempferol 3-*O*-glucoside	17.6 ± 0.9	promoter
Protocatechuic acid	14.3 ± 2.5[a]	promoter
Catechin	10.2 ± 0.9[a]	promoter
Myricetin	7.4 ± 0.4	inhibitor
Quercetin	1.8 ± 0.1	inhibitor
Kaempferol	1.5 ± 0.1	promoter

[a] Quantified by MS ion intensity. Other concentrations were determined by integration of UV absorption peak areas.

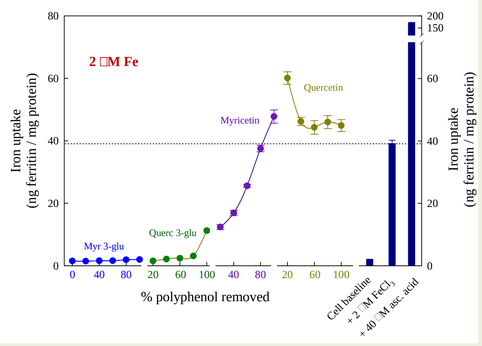
Total concentration of inhibitors was greater than the total of promoters (143.0 µM vs. 43.6 µM).

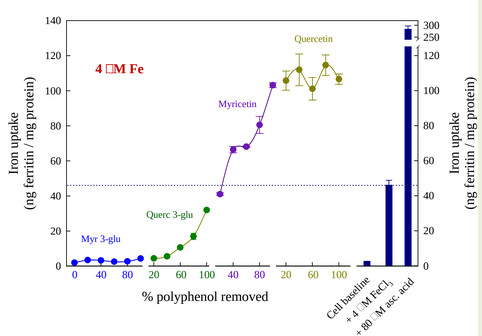
Iron uptake in response to serial removal of the inhibitors myricetin 3-glucoside, quercetin 3-glucoside, myricetin and quercetin from a mixture of eight black bean seed coat polyphenols

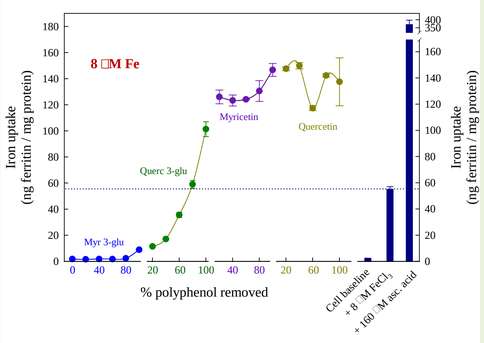


Net promotion of iron uptake occurred when all myricetin 3-glucoside, all quercetin 3-glucoside and 40% of myricetin was removed from a simulated mixture of eight black bean polyphenols. Dotted line represents iron uptake with no polyphenol present.

Iron uptake in response to serial removal of myricetin 3-glucoside, quercetin 3-glucoside, myricetin and quercetin from a mixture of eight black bean seed coat polyphenols; three Fe concentrations







At higher iron concentrations, lesser amounts of inhibiting polyphenols needed to be removed to increase iron uptake. Dotted line represents iron uptake with no polyphenol present.

Conclusions

- Specific polyphenols in black bean seed coats can inhibit or promote Fe uptake.
- Inhibiting polyphenols such as myricetin are more powerful at decreasing Fe uptake than promoters such as epicatechin are at increasing Fe uptake.
- Removal of inhibiting polyphenols from a mixture of polyphenols that simulate those in black bean seed coats can produce a net increase in Fe uptake.
- Results suggest that alteration of polyphenol composition is a potential strategy to develop bean lines with improved nutritional qualities.