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Article

The Fast Cooking and Enhanced Iron Bioavailability Properties of the Manteca Yellow Bean (*Phaseolus vulgaris* L.)

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Abstract: The common dry bean (*Phaseolus vulgaris* L.) is a nutrient dense food produced globally as a major pulse crop for direct human consumption, and is an important source of protein and micronutrients for hundreds of millions of people across Latin America, the Caribbean and Sub-Saharan Africa. Beans require large amounts of heat energy and time to cook, deterring consumers worldwide from purchasing beans. In regions where consumers rely on expensive fuelwood for food preparation, the yellow bean is often marketed as fast cooking. A Yellow Bean Panel (YBP) was assembled to explore the cooking time and health benefits of the five major seed types within the yellow bean market class (Amarillo, Canary, Manteca, Mayocoba, Njano) over two field seasons. This study shows how the Manteca yellow bean possess a fast cooking phenotype, which could serve a genetic resource for introducing fast cooking properties into a new generation of dry beans with cooking times < 20 minutes when pre-soaked and < 80 minutes unsoaked. Nutritional evaluation revealed fast cooking yellow beans have high iron retention (>80%) after boiling. An *in vitro* digestion/Caco-2 cell culture bioassay revealed a strong negative association between cooking time and iron bioavailability in the YBP (r values > -0.73). When either pre-soaked or left unsoaked the highest iron bioavailability scores were measured in the fast cooking Manteca genotypes providing evidence that this yellow market class is worthy of germplasm enhancement through the added benefit of improved iron quality after cooking.

Keywords: *Phaseolus vulgaris* L., yellow beans, Manteca, cooking time, iron, bioavailability, polyphenols.

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1. Introduction

Dry beans (*Phaseolus vulgaris* L.) are a nutrient dense food produced globally as a major pulse crop for direct human consumption. Biofortification efforts over the last decade focused primarily on developing new varieties of beans with increased iron concentrations adapted to thrive in Latin American and Sub-Sahara Africa [1-3]. The premise of iron biofortification is that more dietary iron will be available for absorption, thus alleviating iron deficiencies in regions where beans are a dietary staple [3,4]. Despite their capacity to be a rich source of iron, polyphenols in seed coats, high concentrations of phytate and thick cotyledon cell walls limit the bioavailability of iron from beans [5-9].

Cooking time is an additional factor that limits obtaining nutrients from beans, by simply discouraging bean consumption. Long cooking times deter consumers from purchasing dry beans worldwide; especially in nations where energy needed for cooking is often expensive or scarce. Nearly three billion people in the world depend on traditional biomass, such as fuelwood or charcoal, as their main source of energy for cooking [10-12]. Regions where fuelwood is the primary source of energy are also the main areas with populations at risk for iron deficiencies, such as Sub-Sahara Africa, Central America or the Caribbean [13,14]. The problem is aggravated by widespread

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deforestation in these same nations, leading to dwindling stocks of fuelwood, and placing the burden of collecting cooking provisions principally on rural families [15-17]. The behavioral responses to fuelwood shortages in these communities are a significant impasse for using the bean as a biofortified crop to improve the nutritional well-being and food security of their inhabitants [18-20]. Research by Brouwer et al. demonstrated that as the scarcity of fuelwood increased, households of central Malawi would often postpone, or even omit energy-demanding beans from their meals and replace them with foods that required less fuelwood to cook [21,22].

There is great need for a fast cooking bean, which can positively impact consumers by reducing fuelwood needs, while simultaneously boosting the iron quality of meals [23]. The Andean Common Bean Diversity Panel (ADP) was assembled as a genetic resource of Andean, as well as Middle American *P. vulgaris* germplasm to help accelerate the production of new dry bean varieties in Sub-Saharan Africa. Currently, there are over 500 landraces, cultivars and breeding lines in the ADP that are being characterized to develop the next generation fast cooking, nutritional improved and biotic/abiotic resistant varieties (http://arsftfbean.uprm.edu/bean/) [24]. After a germplasm screening of the ADP for atmospheric cooking times in boiling water, fast cooking dry beans were identified, becoming palatable in half the time as their market class counterparts [25,26]. The fast cooking trait was discovered to be very rare among the large collection of bean genotypes in the ADP [25]. Of the only five genotypes in the ADP with fast cooking properties, two were 'Manteca' yellow beans named Cebo and Mantega. They were collected in 2010 from marketplaces located in the central Crystal Mountains of Angola (Tim Porch, USDA-ARS, Mayaguez, Puerto Rico; personal communication). The Manteca is a pale lemon colored seed native to Chile, where traditional knowledge describes the Manteca as an "easy-to-digest" bean with low flatulence [27-29].

An excellent opportunity to reduce the cooking time and improve the iron bioavailability of dry beans lies within the yellow bean market class [26]. A vast number of shades and tones distinguish the yellow bean as a unique food crop, with 'eye-catching' appeal in world marketplaces. While only a minor market class produced and sold in the United States, yellows are an important crop in Mexico, South America, and Sub-Saharan Africa with a long history of domestication. Originating from the Peruano coast, over the millennium the yellow bean has diversified into a wide landscape of seed types, shapes and sizes; facilitating their adaption into the traditional meals of communities worldwide [30]. At least a dozen different types of yellow beans are grown and sold throughout Latin America [30]. Yellow beans are also important in Africa, especially in Angola, Mozambique, Uganda, Tanzania and Zambia. Their popularity has been increasing in recent years and they often fetch the highest prices at the marketplace [31-33]. Notwithstanding their appeal to the modern day consumer, common bean breeding programs can also benefit from focusing on how yellow beans might distinguish themselves – nutritionally - from other bean market classes.

The aim of this study was to examine the cooking quality, iron nutrition and iron absorption properties of the yellow bean market class. A Yellow Bean Panel was assembled to compare white and red mottled varieties with distinct cooking and nutritional profiles against five yellow seed types (Amarillo, Canary, Manteca, Mayocoba, Njano) that would be recognized by consumers in the marketplaces of Africa, the Americas or the Caribbean [25,26,34]. The Yellow Bean Panel was evaluated for cooking time and seed iron density over the course of two field seasons at the Montcalm Research Farm located near Entrican, Michigan. Beans from the Yellow Bean Panel were either soaked overnight or left unsoaked prior to cooking. An *in vitro* digestion/Caco-2 cell culture model was also used to measure iron bioavailability after cooking either the pre-soaked or unsoaked beans from the panel.

2. Materials and Methods

2.1. The Yellow Bean Panel

The Yellow Bean Panel (YBP) is a collection of 18 *P. vulgaris* genotypes selected to represent the five major seed types of the yellow bean market class with geographic origins from East and South Africa, as well as North and South America. The seed types include Manteca (pale yellow),

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Mayocoba (Peruano), Canary (bright yellow), Amarillo (yellow-orange) and Njano (yellow-green). A summary describing the collection sites, sources and cultivation status (gene pool) of the YBP genotypes are presented in **Table 1**. Photographs of the YBP arranged from the lightest to darkest colored seed types are shown in **Figure 1**. The landraces Ervilha (Manteca) and Canario (Canary) were both collected from the Instituto de Investigação Agronómica located in the Huambo province of Angola. The landraces Cebo and Mantega Blanca (Manteca); Canario Cela (Canary); Chumbo (Njano); as well as the Middle American landrace, Amarelo (Amarillo) were all collected from the public marketplaces of Cuanza Sul province in Angola (Tim Porch, USDA-ARS, Mayaguez, Puerto Rico; personal communication). The Njano, PI527538 was collected from Burundi in 1985. Genetic diversity analysis with SNP markers indicates this landrace is from the Andean gene pool and is likely a member of race Nueva Granada. The Njano and Soya Njano are preferred seed types grown in Eastern Africa [35] and are widely accepted for their agronomic performance, plant architecture and high yields (Susan Nchimbi-Msolla, Sokoine University of Agriculture; personal communication). Cultivars Uyole 98 and Uyole 04 were released in 1999 and 2004 by the Tanzanian National breeding program, renowned for their high yields, disease resistance, fast cooking properties and excellent ratings for palatability [36].

Table 1. Description, Collection Sites, Source, Cultivation Status and Center of Domestication (COD) of the Eighteen Genotypes that Characterize the Yellow Bean Panel (YBP).[1]

Seed Type	Genotype	Collection Site	Source	Cultivation	COD
White	PI527521	Burundi	US GRIN	Landrace	Andean
White	Blanco Fanesquero	Ecuador	INIAP	Variety	Andean
Manteca	Ervilha	IIA	Huambo, Angola	Landrace*	Andean
Manteca	Cebo	marketplace	Cela, Angola	Landrace*	Andean
Manteca	Mantega Blanca	marketplace	Kibala, Angola	Landrace*	Andean
Mayocoba	CDC-Sol	Canada	Unv. of Saskatchewan	Variety	Andean
Mayocoba	ACC Y012	Canada	Alberta	Variety	Andean
Mayocoba	Y11405	United States	Michigan State Unv.	Breeding Line	Andean
Mayocoba	DBY28-1	United States	Oregon State Unv.	Breeding Line	Andean
Canary	Canario	IIA	Huambo, Angola	Landrace*	Andean
Canary	Canario, Cela	marketplace	Cela, Angola	Landrace*	Andean
Amarillo (*lt.*)	Uyole 04	Tanzania	Tanzania Breeding	Variety	Andean
Amarillo (*dk.*)	Uyole 98	Tanzania	Tanzania Breeding	Variety	Andean
Amarillo (*dk.*)	Amarelo	marketplace	Cela, Angola	Landrace*	MA
Njano	Chumbo	marketplace	Cela, Angola	Landrace*	Andean
Njano	PI527538	Burundi	US GRIN	Landrace	Andean
Red Mottled	JB178	Dominican Rep.	CIAS	Variety	Andean
Red Mottled	PR0737-1	Puerto Rico	Unv. of Puerto Rico	Variety	Andean

[1]The YBP consists of medium to large Andeans ranging from 40 - 65 g/100 seed, and a small Middle American (MA) averaging 30g/100 seed. Genotypes are arranged from the lightest to the darkest seed types. *Not verified as landraces; accessions collected from provinces located in Angola, Africa. IIA, Instituto de Investigação Agronómica; US GRIN, U.S. Germplasm Resources Information Network; INIAP, Instituto Nacional de Investigaciones Agropecuarias; CIAS, Centro de Investigación Agricolas del Suroeste. (*lt.*) light yellow; (*dk.*) dark yellow.

The North American Mayocoba seed types include CDC-Sol, which was released in 2013 and developed by the Crop Development Centre, University of Saskatchewan, Saskatoon, Saskatchewan [37]. This Canadian yellow is moderately resistance to Anthracnose (race 73), early maturing and maintains its bright yellow color after storage [37]. AAC Y012 is an early maturing, high yielding yellow bean with partial field resistance to white mold, developed at the Agriculture and Agri-Food Canada (AAFC) Research and Development Centre located in Lethbridge, Alberta [38]. Y11405 is an advanced breeding line of the Michigan State University Dry Bean Breeding program. Y11405 is a North American adapted yellow bean with desirable end-use quality traits, such as a bright

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"highlighter" yellow seed coat and a consumer preference in seed size (James D. Kelly, Michigan State University; personal communication). DBY28-1 is a bean common mosaic virus (BCMV) and beet curly top virus (BCTV) resistance sister line to the early maturing yellow bean variety named 'Patron,' which is a joint release of Oregon State University and the University of Idaho (James R. Myers, Oregon State University; personal communication). Four non-yellow *P. vulgaris* controls are also part of the YBP, which include a white bean landrace collected from Burundi (PI527521) and a white bean variety from Ecuador (Blanco Fanesquero). The other two controls include the red mottled JB178, a high yielding disease resistance variety released by the Dominican Republic in 1998 [39] and PR0737-1, a high yielding virus resistant red mottled line released jointly in 2013 by the University of Puerto Rico, USDA-ARS and the Haiti National Program [40]. The non-yellow controls were selected based upon their unique fast or slow cooking properties, which were measured from past investigations [25,26,34].

Figure 1. High-resolution photographs depicting the eighteen genotypes of the Yellow Bean Panel (YBP) arranged in order from lightest to darkest seed coat color. To compare differences in seed sizes, all photographs were taking to scale under standardized lighting conditions.

2.2. Field Design and Storage Conditions

All YBP genotypes were planted in a Randomized-Complete-Block Design with 2 field replicates at the Michigan State University, Montcalm Research Farm near Entrican, MI in 2015 and 2016. Experimental units for each genotype consisted of two rows 4.75 meters long with 0.5 meter spacing between rows. Each experimental unit was separated by a cv. Red Hawk broader row. The soil type is Eutric Glossoboralfs (coarse-loamy, mixed) and Alfic Fragiorthods (coarse-loamy, mixed, frigid). Rainfall was supplemented with overhead irrigation as needed. Recommended practices were followed for fertilization, weed and pest control. Seed were harvested upon maturity by hand pulling the entire experimental unit and threshing with a Hege 140 plot harvester (Wintersteiger, Utah). Immediately after harvest, bean seeds from each field replicate were hand sorted to eliminate any external material and any immature, wrinkled, discolored or damaged seeds. Sorted seed (moisture content 14 – 20%) were placed into dark storage under ambient conditions (20 – 22^{0}C, 50 – 60% relative humidity RH) at standard atmospheric pressure for six weeks. At this time, subsets of 100 randomly selected seeds from each field replicate were evaluated for cooking time, iron analysis and iron bioavailability.

2.3. Moisture Equilibration, Cooking Time Determination and Sample Preparation

To equilibrate moisture content after six weeks of storage, seeds were placed into paper envelopes and stored at room temperature until seed reached a moisture content range of 10 - 12% [41]. Prior to cooking, moisture-equilibrated bean seeds were either left unsoaked or soaked in distilled water (1:8 weight/weight) for 12 hours at room temperature. Cooking time was determined using a Mattson pin drop cooking device [42,43] fitted into a 4 L stainless steel beaker containing 1.8 L of boiling distilled water heated over a Waring SB30™ portable burner. Cooking time was standardized as the number of minutes required for 80% of 25 piercing tip rods (70 gram, 2 mm

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diameter) to pass completely through each seed under a low-steady boil at 100oC. Once removed from boiling water, cooked seeds were cooled for 10 min at room temperature. For serving size determinations (defined as a half cup; 89 grams wet weight) the number of cooked seed to fill a quarter cup (44.5 grams, wet weight) was recorded, then doubled. Raw whole seed and their cooked whole seed counterparts were frozen at -80°C before freeze-drying (VirTis Research Equip. Gardiner, NY). To create a homogenous mixture of each genotype for chemical analysis, pre-weighed lyophilized raw seed and lyophilized cooked seed were ground into a fine powder with a Kinematica Polymix® analytical mill (PX-MFC 90D, New York, USA) fitted with a 0.5 mm sieve followed by storage in sealed, opaque polypropylene plastic containers at 20°C. A schematic illustrating the processing and cooking of the YBP is shown in **Figure 2.**

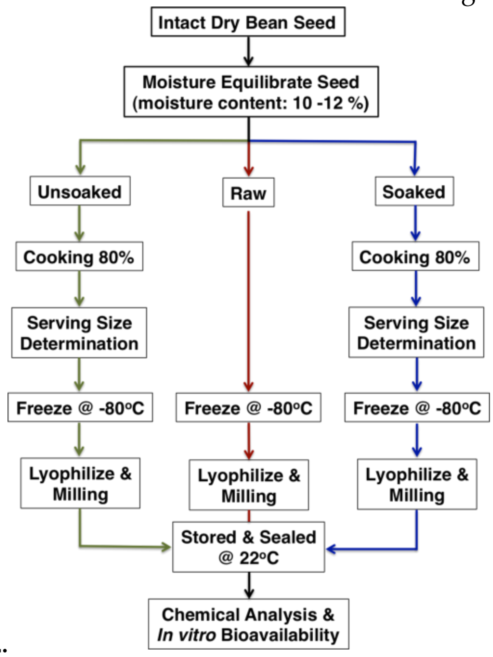

Figure 2. Flow diagram illustrating how cooking time is measured for bean seeds and how raw/cooked seed are processed for nutritional analysis and bioavailability assays.

2.4. Iron Analysis

For iron analysis, 500 mg of lyophilized powder from raw and cooked seed was pre-digested in boro-silicate glass tubes with 3 mL of a concentrated ultra-pure nitric acid and perchloric acid mixture (60:40 v/v) for 16 hours at room temperature. Samples were then placed in a digestion block (Martin Machine, Ivesdale, IL) and heated incrementally over 4 hours to a temperature of 120°C with refluxing. After incubating at 120°C for 2 hours, 2 mL of concentrated ultra-pure nitric acid was subsequently added to each sample before raising the digestion block temperature to 145°C for an additional 2 hours. The temperature of the digestion block was then raised to 190°C and maintained for at least ten minutes before samples were allowed to cool at room temperature. Digested samples were re-suspended in 20 mL of ultrapure water prior to analysis using ICP-AES (inductively coupled plasma-atomic emission spectroscopy; Thermo iCAP 6500 Series, Thermo Scientific, Cambridge, United Kingdom) with quality control standards (High Purity Standards, Charleston, SC) following every 10 samples. Yttrium purchased from High Purity Standards (10M67-1) was used as an internal standard. To ensure batch-to-batch accuracy and to correct for matrix inference, all samples were digested and measured with 0.5 µg/mL of Yttrium (final concentration). The concentration of iron is expressed as the number of micrograms per gram of a lyophilized/milled powder that represents a homogeneous mixture of either 50 raw or 50 cooked seed for each YBP genotype.

2.5. Iron Content, Serving-Size, Dietary Reference Intake and Retention Values

To account for the intrinsic differences in seed sizes between the two field seasons and the extrinsic losses of seed mass during the cooking process, iron content was calculated for each

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genotype as the number of milligrams in 100 raw or 100 cooked seed. Iron contents are used to calculate serving size densities, by accounting for the number of cooked seed needed to fill a fixed serving volume [44]. The USDA National Nutrient Database for Standard Reference (https://ndb.nal.usda.gov/ndb/) defines one serving of beans as a half of a cup, which equates to 89 grams of cooked, drained and cooled whole seed (wet weight). Nutritional impact between the different genotypes of the YBP can be measured using the National Academy of Science's Dietary Reference Intake (DRI) that is met with each serving of cooked seed [45]. Many initiatives sponsored by the U.S. Agency for International Development (USAID), U.S. State Department and World Health Organization (WHO) are focused on improving the health of vulnerable populations at risk to malnutrition, mainly women and children [46]. Therefore, the DRI values calculated in this study are based on the daily needs of an active adult female 19 – 50 years of age with a BMI ≤ 24 kg/m^2 and an Estimated Energy Requirement (EER) of 2,025 kcal/day [45]. Retention percentages were determined by comparing the total iron content between 100 raw and 100 cooked seeds. Iron content, serving size densities, DRI percentages and retention values are calculated according to the following formulas:

iron content = [iron concentration in lyophilized powder (mg/g)] x [average weight of lyophilized powder that represents 100 raw or cooked whole seeds (g /100 seed)] (1)

$$\textbf{serving size} = \frac{[\text{iron content (mg/100 seed)}] \times [\text{number of seed per serving (half cup)}]}{[\text{100 seed}]} \quad (2)$$

$$\textbf{\% DRI} = \frac{\text{milligrams iron per serving (mg/half cup)}}{\text{milligrams iron required per day (mg/day)}} \times [100\%] \quad (3)$$

$$\textbf{retention} = \frac{\text{cooked iron content (g/100 seed)}}{\text{raw iron content (g/100 seed)}} \times [100\%] \quad (4)$$

2.6. Iron Bioavailability: in vitro Digestion/Caco-2 Cell Bioassay

A 500 mg sample of lyophilized powder from cooked seed were subject to an *in vitro* digestion/Caco-2 cell culture model for the determination of iron bioavailability as described previously in Glahn et al., 1998 [47]. Iron uptake is measured as the increase in Caco-2 cell ferritin production (ng ferritin per milligram of total cell protein) following a simulated gastric and intestinal digestion, most recently described in Glahn et al., 2017 [48]. Iron bioavailability is expressed as a percentage score of Caco-2 cell ferritin formation that is relative to a control cooked/lyophilized/milled navy bean (cv. Merlin). The navy bean control is run with each assay to index the ferritin/total cell protein ratios of the Caco-2 cells over the course of experimentation. Baseline ferritin values for the Caco-2 cells averaged 3.9 ± 1.6 ng/mg protein (mean ± SD) for 10 experiments spanning 3 months. Ferritin values for the Merlin navy bean control averaged 15 ± 4.7 ng/mg protein (mean ± SD). Ferritin values for the white bean control PI527521 averaged 14 ± 4.4, and the ferritin values for a blank digest with 66 µM $FeCl_3$ averaged 64 ± 17 ng/mg protein (mean ± SD). The iron concentration of the cooked navy bean control over the course of experimentation averaged 76 ± 1.9 µg/g (mean ± SD).

2.7. Statistical Analysis

All statistical analyses were conducted using SAS 9.2 (SAS Institute Inc. Cary N.C.). Mean separations for genotypes were determined using the Proc MIXED procedure with the model including genotype (18 levels) and field season (2 levels) as fixed effects and field replicates (2 levels) as a random effect; followed by a Tukey *post hoc* test. Pearson correlation coefficients were calculated to determine the associations between measured variables and cooking time of the YBP. Differences with *P* values of ≤ 0.05 were considered statistically significant.

3. Results

3.1. Cooking Times and Cooking Classifications of the YBP Subsection

The cooking times of the eighteen YBP genotypes after soaking are listed in **Table 2**. The genotypes are ranked in **Table 2** from fastest to slowest in one of three cooking classes: fast (<20 minutes), moderate (20 – 35 minutes) or slow (>35 minutes). Cooking time rank of all eighteen genotypes in YBP remained the same between the 2015 and 2016 field seasons (reported as combined means in **Table 2**). Year interactions ($P = 0.257$), as well as genotype x year interactions ($P = 0.899$) were not significant. A wide variation ($P < 0.0001$) in cooking times were measured among the yellow beans after soaking, ranging from 18 - 19 minutes for the three Manteca seed types (Ervilha, Cebo, Mantega) to 69 minutes for the Middle American Amarelo (**Table 2**). Significant variations ($P < 0.0001$) in cooking times were also measured between the yellow beans that were not soaked prior to cooking, ranging from 76 - 79 minutes for the three Manteca landraces (Ervilha, Cebo, Mantega) to 126 minutes for Amarelo (**Table 3**). Unsoaked YBP genotypes listed in **Table 3** are ranked from fastest to slowest in one of three cooking classes: fast (<80 minutes), moderate (80 – 110 minutes) or slow (>110 minutes). Year interactions and genotype x year interactions for cooking time were not significant among the unsoaked beans, and cooking time ranks were similar between the two field seasons. There was a strong relationship between the cooking times of the pre-soaked genotypes and the cooking times of the unsoaked genotypes in the YBP ($r = 0.848$, $P < 0.0001$). The cooking classifications of unsoaked genotypes, however, were not necessarily the same as pre-soaked genotypes (**Tables 2 & 3**).

Table 2. Cooking Times of Pre-Soaked Genotypes in the Yellow Bean Panel.[1]

Genotype (*Seed Type*)	Cooking Time (min)[2]	Cooking Class
Blanco (*white*)	16^{k}	fast
PI527521 (*white*)	18^{k}	fast
Ervilha (*Manteca*)	18^{jk}	fast
Cebo (*Manteca*)	19^{jk}	fast
Mantega (*Manteca*)	19^{jk}	fast
Uyole 04 (*lt. Amarillo*)	22^{ij}	moderate
Chumbo (*Njano*)	24^{hi}	moderate
Uyole 98 (*dk. Amarillo*)	26^{fgh}	moderate
JB178 (*Red Mottled*)	26^{gh}	moderate
ACC Y012 (*Mayocoba*)	28^{efg}	moderate
Canario, Cela (*Canary*)	29^{efg}	moderate
CDC-Sol (*Mayocoba*)	30^{def}	moderate
DBY28-1 (*Mayocoba*)	31^{de}	moderate
Y11405 (*Mayocoba*)	33^{d}	moderate
Canario (*Canary*)	38^{c}	slow
PI527538 (*Njano*)	39^{c}	slow
PR0737-1 (*Red Mottled*)	59^{b}	slow
Amarelo (*dk. Amarillo*)	69^{a}	slow

[1]Values are combined means of two field replicates per genotype for field seasons 2015 and 2016. Means sharing the same subscript are not significantly different at $P \leq 0.05$. [2]Raw seed were soaked in distilled water for 12 hours prior to determining the number of minutes to reach 80% cooking time with an automated Mattson pin-drop device, then categorized top to bottom from the fastest to slowest cooking entry.

Table 3. Cooking Times of Unsoaked Genotypes in the Yellow Bean Panel.[1]

Genotype (*Seed Type*)	Cooking Time (min)[2]	Cooking Class
Blanco (*white*)	76^{kl}	fast
PI527521 (*white*)	76^{jkl}	fast
Ervilha (*Manteca*)	76^{l}	fast

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Cebo (*Manteca*)	76^{l}	fast
Mantega (*Manteca*)	79^{ijk}	fast
Uyole 04 (*lt. Amarillo*)	82^{hij}	moderate
Chumbo (*Njano*)	83^{h}	moderate
Uyole 98 (*dk. Amarillo*)	83^{hi}	moderate
JB178 (*Red Mottled*)	95^{g}	moderate
Canario, Cela (*Canary*)	101^{f}	moderate
Y11405 (*Mayocoba*)	101^{f}	moderate
DBY28-1 (*Mayocoba*)	108de	moderate
PI527538 (*Njano*)	108^{e}	moderate
Canario (*Canary*)	112^{cd}	slow
ACC Y012 (*Mayocoba*)	113^{bc}	slow
CDC-Sol (*Mayocoba*)	116^{b}	slow
PR0737-1 (*Red Mottled*)	124^{a}	slow
Amarelo (*dk. Amarillo*)	126^{a}	slow

[1]Values are combined means of two field replicates per genotype for field seasons 2015 and 2016. Means sharing the same subscript are not significantly different at $P \leq 0.05$. [2]Raw seed were left unsoaked prior to determining the number of minutes to reach 80% cooking time with an automated Mattson pin-drop device, then categorized top to bottom from the fastest to slowest cooking entry.

3.2. Iron Density of the YBP

Tables 4 & 5 show the milligrams (mg) of iron provided in one serving of cooked beans from pre-soaked and unsoaked genotypes of the YBP organized from the fastest to slowest cooking. Iron DRI percentages for an adult female met with each serving of cooked beans are also shown in **Tables 4 & 5**. The measurements used to determine the serving densities of iron in the soaked and unsoaked genotypes of the YBP, including the concentrations, contents and retention values of iron between the raw and cooked seed are presented in **Supplementary Tables 1 – 5**. Genotype, year interactions as well as genotype x year interactions for iron densities in the pre-soaked beans of the YBP were significant ($P < 0.0001$) after cooking. Serving densities ranged from 1.70 mg (9% of DRI) to 2.63 mg (15% of DRI) among the yellow beans across the 2015 and 2016 field seasons (**Table 4**). High serving densities of iron (14 – 16% of DRI) were measured in both the red mottled varieties JB178 and PR0737-1 in 2015 and in 2016. The yellow breeding line Y11405 had the highest serving density of iron among the yellow beans (14 – 15% of DRI) for both field seasons (**Table 4**). There was no relationship between the cooking times and the iron densities of pre-soaked genotypes in the YBP for either the 2015 ($r = 0.221$, $P = 0.299$) and 2016 ($r = -0.134$, $P = 0.533$) field seasons.

The milligrams (mg) of iron provided in one serving of cooked beans from unsoaked genotypes of the YBP are shown in **Table 5**. Genotype, year interactions and genotype x year interactions for iron densities among the unsoaked bean samples were significant ($P < 0.0001$). **Table 5** shows the serving densities of iron ranged from 1.39 mg (8% of DRI) to 2.50 mg (14% of DRI) among the yellow bean landraces and varieties in both the 2015 and 2016 field season. The highest serving densities of the iron (2.35 – 2.50 mg; 13 – 14% of DRI) were measured in the red mottled variety JB178 and the yellow breeding line Y11405 (**Table 5**). There was no relationship between the cooking times and the iron densities of the unsoaked genotypes for field seasons 2015 ($r = 0.127$, $P = 0.556$) and 2016 ($r = 0.393$, $P = 0.058$).

Table 4. Cooked Seed Iron Density of Pre-Soaked Genotypes in the Yellow Bean Panel Organized by Cooking Class.[1]

		One Serving Size (half cup)			
		2015		2016	
Genotype (*Seed Type*)	**Cooking Class**	Iron (mg)[2]	% DRI[3]	Iron (mg)	% DRI
Blanco (*white*)	fast	1.95^{def}	11	2.28^{bcde}	13

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PI527521 (*white*)	fast	2.13^{cd}	12	2.32^{bcd}	13
Ervilha (*Manteca*)	fast	2.02^{de}	11	2.30^{bcde}	13
Cebo (*Manteca*)	fast	1.75^{fg}	10	2.02^{gh}	11
Mantega (*Manteca*)	fast	2.06^{cd}	11	2.29^{bcde}	13
Uyole 04 (*lt. Amarillo*)	moderate	1.84^{efg}	10	2.16^{defg}	12
Chumbo (*Njano*)	moderate	1.98^{de}	11	2.25^{cdef}	12
Uyole 98 (*dk. Amarillo*)	moderate	1.85^{efg}	10	2.06^{fhg}	11
JB178 (*Red Mottled*)	moderate	2.71^{a}	15	2.89^{a}	16
ACC Y012 (*Mayocoba*)	moderate	1.84^{efg}	10	2.10^{efg}	12
Canario, Cela (*Canary*)	moderate	2.24^{c}	12	2.30^{bcde}	13
CDC-Sol (*Mayocoba*)	moderate	1.82^{efg}	10	1.95^{gh}	11
DBY28-1 (*Mayocoba*)	moderate	1.73^{g}	10	2.02^{gh}	11
Y11405 (*Mayocoba*)	moderate	2.63^{ab}	15	2.49^{b}	14
Canario (*Canary*)	slow	1.98^{de}	11	2.14^{defg}	12
PI527538 (*Njano*)	slow	1.71^{g}	10	1.87^{h}	10
PR0737-1 (*Red Mottled*)	slow	2.49^{b}	14	2.45^{bc}	14
Amarelo (*dk. Amarillo*)	slow	1.70^{g}	9	2.02^{gh}	11

[1]Values are means of two field replicates per genotype, measured for field seasons 2015 and 2016. Means sharing the same subscript in each column are not significantly different at $P \leq 0.05$. [2]Average grams of iron measured in a half cup (89g, wet weight) of cooked drained whole seed that were first soaked in distilled water for 12 hours prior to determining the number of minutes to reach 80% cooking time. [3]Percent of daily reference intake met for iron (18 mg) of an adult female (19-50 years) measured in each serving of cooked whole seed.

3.3. Iron Retention Values of the YBP

The content and retention values for iron in 100 raw and 100 cooked seed of the YBP are presented in Supplementary **Tables 4 & 5**. Genotype, year interactions as well as genotype x year interactions for iron retention after cooking the pre-soaked and unsoaked genotypes of the YBP were significant ($P < 0.0001$). After soaking and cooking the YBP, iron retention values ranged from 77 – 91% across the 2015 and 2016 field seasons (**Supplementary Table 4**). High retention values for iron (83 – 91%) were measured in the three fast cooking Manteca yellow beans (**Supplementary Table 4**), and there was a significant relationship between the cooking times of the YBP and retention of iron in both the 2015 ($r = -0.659$, $P = 0.0001$) and 2016 ($r = -0.572$, $P = 0.003$) field seasons.

Iron retention values in the unsoaked and cooked YBP genotypes ranged from 71 – 85% across the 2015 and 2016 field seasons (**Supplementary Tables 5**). Higher retention values for iron (80 – 84%) were measured in the fast cooking Manteca yellows when compared to the slow cooking yellow beans (**Supplementary Tables 5**). There was a strong relationship between the retention of iron and the cooking times of the eighteen unsoaked YBP genotypes in 2015 ($r = -0.789$, $P < 0.0001$) and 2016 ($r = -0.729$, $P < 0.0001$).

3.4. Iron Bioavailability of the YBP

The results illustrated in **Figure 3** and listed with mean separations in **Supplementary Table 6** show significant variations ($P < 0.0001$) in the percentage scores of iron bioavailability after cooking the pre-soaked genotypes of the YBP. Year interactions as well as genotype x year interactions for iron bioavailability in pre-soaked/cooked beans of the YBP were significant ($P < 0.0001$). In 2015, iron bioavailability scores as a percent of the navy bean control ranged from as low as 19% in the slow cooking Middle American, Amarelo to a high of 107% in the fast cooking Manteca landrace, Ervilha (**Figure 3A**). Similar variations in iron bioavailability among the YBP genotypes were also measured in 2016, ranging from 22% in Amarelo to 136% in Cebo, the fast cooking Manteca landrace (**Figure 3B**). When compared to the other moderate and slow cooking genotypes in the YBP, the fast cooking white bean controls and Manteca landraces had significantly higher iron bioavailability scores (**Figure 3**). Iron bioavailability was strongly correlated with the cooking times of pre-soaked YBP genotypes in 2015 ($r = -0.814$, $P < 0.0001$) and 2016 ($r = -0.737$, $P < 0.0001$). Iron bioavailability

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scores were low in red mottled varieties JB178 and PR0737-1, ranging from only 29 – 45% across the 2015 and 2016 field seasons (**Figure 3**).

Significant variations ($P < 0.0001$) in iron bioavailability were also measured after cooking the unsoaked genotypes of the YBP (**Figure 4; Supplementary Table 7**). Year interactions and genotype x year interactions were significant ($P < 0.0001$) with iron bioavailability scores ranging from a low of 20% in the slow cooking Amarelo to as high as159% in the fast cooking Mantega Blanca across the 2015 and 2016 field seasons (**Figure 4**). For the unsoaked and cooked genotypes in the YBP, the highest iron bioavailability scores were measured in the fast cooking three Manteca landraces, while the lowest scores for iron bioavailability were measured in the slow cooking red mottled PR0737-1 and Middle American yellow Amarelo (**Figure 4**). There was a strong relationship between the cooking times and iron bioavailability of unsoaked YBP genotypes in 2015 (r = - 0.726, $P < 0.0001$) and 2016 (r = - 0.788, $P < 0.0001$).

Table 5. Cooked Seed Iron Density of Unsoaked Genotypes in the Yellow Bean Panel Organized by Cooking Class.[1]

		One serving size (half cup)			
		2015		2016	
Genotype (*Seed Type*)	**Cooking Class**	Iron (mg)[2]	% DRI[3]	Iron (mg)	% DRI
Blanco (*white*)	fast	2.07^{cd}	11	2.24^{bcd}	12
PI527521 (*white*)	fast	1.98^{de}	11	2.17^{bcdef}	12
Ervilha (*Manteca*)	fast	2.19^{bc}	12	2.20^{bcde}	12
Cebo (*Manteca*)	fast	1.62^{ij}	9	2.00^{efgh}	11
Mantega (*Manteca*)	fast	1.85^{ef}	10	2.01^{efgh}	11
Uyole 04 (*lt. Amarillo*)	moderate	1.68^{fghij}	9	2.12^{defg}	12
Chumbo (*Njano*)	moderate	1.83^{efg}	10	1.95^{ghi}	11
Uyole 98 (*dk. Amarillo*)	moderate	1.79^{fgh}	10	1.98^{fghi}	11
JB178 (*Red Mottled*)	moderate	2.43^{a}	13	2.49^{a}	14
Canario, Cela (*Canary*)	moderate	2.25^{b}	12	2.14^{defg}	12
Y11405 (*Mayocoba*)	moderate	2.50^{a}	14	2.35^{ab}	13
DBY28-1 (*Mayocoba*)	moderate	1.65^{hij}	9	1.90^{hi}	11
PI527538 (*Njano*)	moderate	1.60^{j}	9	1.79^{i}	10
Canario (*Canary*)	slow	2.01^{d}	11	2.04^{efgh}	11
ACC Y012 (*Mayocoba*)	slow	1.68^{ghij}	9	1.89^{hi}	10
CDC-Sol (*Mayocoba*)	slow	1.77^{fghi}	10	1.83^{hi}	10
PR0737-1 (*Red Mottled*)	slow	2.11^{bcd}	12	2.28^{abc}	13
Amarelo (*dk. Amarillo*)	slow	1.56^{j}	9	1.39^{j}	8

[1]Values are means of two field replicates per genotype, measured for field seasons 2015 and 2016. Means sharing the same subscript in each column are not significantly different at $P \leq 0.05$. [2]Average grams of iron measured in a half cup (89g, wet weight) of cooked drained whole seed that were left unsoaked prior to determining the number of minutes to reach 80% cooking time. [3]Percent of daily reference intake met for iron (18 mg) of an adult female (19-50 years) measured in each serving of cooked whole seed.

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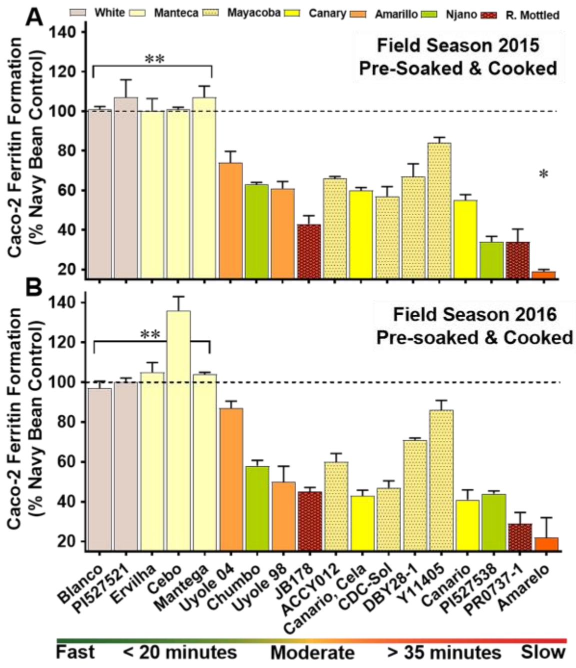

Figure 3. Iron bioavailability scores of pre-soaked and cooked whole seed genotypes in the YBP for field season 2015 (**A**) and field season 2016 (**B**). Values are means (± SD) of two field replicates per genotype. Genotypes are categorized on the x-axis by cooking class, ranked from the fastest cooking genotype to slowest cooking entry. *Significantly lower ($P \leq 0.05$) iron bioavailability score when compared to the other YBP entries. **Significantly higher ($P \leq 0.05$) iron bioavailability scores compared to the other YBP genotypes.

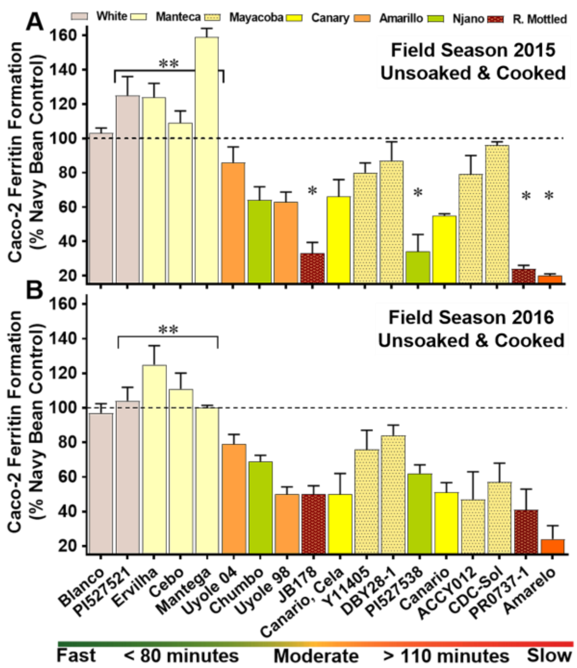

Figure 4. Iron bioavailability scores of pre-soaked and cooked whole seed genotypes in the YBP for field season 2015 (**A**) and field season 2016 (**B**). Values are means (± SD) of two field replicates per genotype. Genotypes are categorized on the x-axis by cooking class, ranked from the fastest cooking genotype to slowest cooking entry. *Significantly lower ($P \leq 0.05$) iron bioavailability score when compared to the other YBP entries. **Significantly higher ($P \leq 0.05$) iron bioavailability scores compared to the other YBP genotypes.

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4. Discussion

4.1. The YBP is a Model to Explore the Health Benefits Yellow Beans

The YBP includes a diverse set of landraces, varieties and breeding lines within the yellow bean market class. The model takes into consideration how different cultures around the world traditionally prepare beans for cooking: by either soaking or not soaking prior to boiling [49,50]. The two white beans from Burundi and Ecuador were selected as non-yellow controls because of their fast cooking properties. They serve as a benchmark for being the fastest cooking genotypes in the Andean Diversity Panel [25]. The two red mottled beans from the Caribbean were selected as non-yellow controls because of their ability to acquire high concentrations of iron at the Montcalm Research Farm in Michigan. They also have contrasting fast (JB178) and slow (PR0737-1) cooking properties [26]. White and red mottled beans are on opposite ends of the iron bioavailability spectrum for dry beans [51,52], creating the ideal framework for evaluating the iron quality of the different yellow beans in the YBP.

Information on dry bean nutrition is most often reported on raw seed, which is first milled into a powder, then dried to remove moisture [53-55]. This study is unique because the nutritional evaluation was conducted after cooking, allowing for the genotypic differences in nutrient retention to be expressed in the model. Raw seed analysis of the dry bean does not take into consideration the genetic variability in 1) the loss of total seed mass during cooking process, 2) the retention of nutrients after cooking and 3) the size of hydrated seed in a fixed volume for the calculation serving size density [26, 56-58]. Minerals in dry beans are particularly sensitive to long cooking times [26,43]. Even under the standardized conditions of this study, the losses of iron in the yellow beans were not trivial after cooking. Retention values below 75% for iron were measured in the slowest cooking genotypes of the YBP, especially when the cooking times are extended in the unsoaked seed (**Supplementary Tables 4 & 5**).

For breeding programs, advancing new traits into the next generation of food crops depends on access to a large collection of diverse germplasm [59]. Although beneficial alleles can be introduced between different the market classes of *P. vulgaris* (e.g. white bean crossed to a red mottled), common bean breeding programs focus on crosses within a market class because of the challenge to maintain the appropriate combination of genes for seed size, shape and color [55,60]. The YBP model shows there is wide diversity in consumer friendly traits to explore within the yellow bean market class. To increase the consumption and health promoting properties of beans worldwide, consumer targeted traits, such as fast cooking times and boosted nutritional value are now being considered in addition to the new cultivar's strong agronomic performance [46,61].

4.2. The Manteca Yellow Bean: A Genetic Resource for the New Generation of Fast Cooking Andean Beans

The three Manteca landraces collected from Angola had fast cooking times when either soaked or left unsoaked for both the 2015 and 2016 field season (**Tables 2 & 3**). Two previous studies have also identified the Manteca as a fast cooking yellow bean when grown at the Montcalm Research Farm, cooking in less than 25 minutes under a set of standardized storage and soaking conditions over the course of the 2012 – 2013 field seasons [25,26]. With a set of nearly 5000 polymorphic SNPs, Nei genetic distance [62] on 206 genotypes of Andean Diversity Panel revealed a phylogenetic relationship between the Manteca landraces and other fast cooking beans, including the white bean control PI527521 from Burundi and a fast cooking cranberry bean (G23086) from Malawi [25]. The genetic relatedness of these genotypes suggests a common genetic control for the fast cooking phenotype. Their origins are from regions in Africa where fuelwood is the major source of energy for cooking, which could explain why farmers valued and maintained the fast cooking trait within these landraces [25]. What impact the environment might play on the genetic expression of the fast cooking phenotype is still under investigation.

Specific genetic mechanisms that control the cooking time of *P. vulgaris* have yet to be identified. How different morphological features of a bean seed influence cooking time could be the clue to what underlying genetic mechanisms might be involved. The surface area and shape of the seed, as well as the thickness and chemical composition of the seed coat can affect the water uptake and the cooking time of dry beans [63-65]. The expression of flavonol glycosides, anthocyanins and condensed tannins in seed coats not only leverages color, but also contribute to the hydration and cooking properties of dry beans [66]. Previous research shows there is a strong positive correlation (r = 0.77) between cooking time and seed tannin content in dry beans [67]. More recent research demonstrates after soaking and boiling, fast cooking beans have higher soluble dietary fiber concentrations when compared to their slow cooking counterparts from yellow, cranberry, red mottled and light red kidney market classes [34]. These findings suggest the physical and chemical composition of the fast cooking dry bean may be unique, and might have a common genetic architecture.

4.3. Iron Nutrition Benefits of the Fast Cooking Manteca Yellow Bean

Environmental factors, such as precipitation, drought stress and soil characteristics affect the mineral concentrations of dry beans [55,68]. The iron nutrition of the YBP was diverse, and there was a significant year and genotype x year interaction. There was no relationship between the cooking times of the genotypes in the YBP and the intrinsic concentrations of iron in their raw seed. The amount of iron retained after cooking, however, was strongly associated with cooking time in the YBP. Although the Manteca landraces did not have high iron concentrations in their raw seed when compared to other yellow and red mottled genotypes in the YBP, their fast cooking properties contribute to an improved nutritional value through the benefit of high iron retention during the cooking process (**Supplementary Tables 4 & 5**).

There was a large genotype and genotype x year interaction for iron bioavailability in the YBP, with many of the yellows performing just as poorly as the low iron bioavailable red mottled controls (**Figures 3 & 4**; **Supplementary Tables 6 & 7**). The iron bioavailability of YBP was independent of iron concentrations in raw and cooked seed. A strong relationship was detected between cooking time and iron bioavailability in the YBP. The light colored and faster cooking Uyole 04 outperformed the darker orange Amarillo's (Uyole 98, Amarelo); suggesting that a darker seed coat color may be contributing to lower iron bioavailability [6,51,52]. The same observation was previously demonstrated in a separate cooking model for dry beans that examined fast, moderate and slow cooking genotypes from four different market classes of economic importance in Africa, the Americas and the Caribbean [26]. The evidence is building that breeding for fast cooking times may have the added benefit of improving the iron absorption properties in dry beans. Whether pre-soaked or left unsoaked the fast cooking Mantecas distinguish themselves from the other yellow seed types in the YBP with the highest iron bioavailability scores measured in both the 2015 and 2016 field seasons. Not soaking the Manteca yellow beans prior to boiling did not negatively impact their iron bioavailability scores (**Figure 4, Supplementary Table 7**). This is an important feature of the Manteca to note, because many cultures in Africa, Latin America and the Caribbean do not soak their beans before cooking because it alters the flavor [49,50].

4.4. Mysteries of the Manteca

New questions arise in understanding how the alleged digestibility of the Mantecas might be related to their high iron bioavailability. The antidotal clam of the 'easy-to-digest' Manteca bean was first investigated by British agriculture scientist Colin Leaky (1933-2018), who noticed the more expensive Manteca in the markets of Chile in the late 1970's, lauded by traders as "beans for the rich man's table" [28]. A decade earlier, Dr. Leaky was challenged by nutritionists in Uganda to help improve the nutrient quality of meals by breeding a more digestible bean for babies to tolerant as a first food [69]. Leakey was successful in releasing Prim (named after the saying "Prim and Proper") a modern Manteca variety with low-flatulence and excellent flavor [69,70]. Indeed, there is evidence to support the Manteca yellow bean may have a unique nutritional profile compared to other beans: with less dietary fiber, less indigestible protein and starch, but with similar concentrations of oligosaccharides [29,34, 70-72]. Manteca beans are also free of proanthocyanins and condensed tannins - classes of compounds shown to reduce protein digestibility and iron absorption [5,73,74].

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Secondary metabolites in beans, such as phytate and certain polyphenolic compounds can inhibit the absorption of iron [5,6,75]. Yellow beans with the Prim heritage are believed to carry a recessive allele that shifts the polyphenolic pathway in seed coats away from tannin and proanthocyanin synthesis towards the accumulation of kaemperfol derived flavonoids, primarily keampferol-3-glucoside [27,73]. Iron uptake assays with Caco-2 cells have recently demonstrated that kaemperfol and kaemperfol-3-glucoside are actually promoters of iron absorption. In contrast, polyphenols expressed in the seed coats of red or black beans, such as quercetin or myricetin act as strong inhibitors to iron absorption [6,75]. As an example to support these findings, the Canary colored yellow beans in the YBP (Canario, Canario, Cela) expresses a dominant form of this allele in their seed coats, opening the biosynthetic pathway for the production of iron inhibitory polyphenols, such as procyanidins and quercetin 3-glucoside [76,77]. For both the 2015 and 2016 field seasons, the two Canary genotypes (Canario, Canario, Cela) had higher iron concentrations in their cooked seed (**Supplementary Tables 2 & 3**), but had significantly lower iron bioavailability scores when compared to the Manteca landraces Ervilha, Cebo and Mantega (**Figures 3 & 4**). The secret of improved iron bioavailability in the Manteca may be revealed by the unique polyphenolic pattern expressed in their seed coats. Detailed studies examining the polyphenolic profile and how they might be related to the different iron bioavailability properties of the yellow, white and red mottled genotypes in the YBP are currently being conducted.

4.5. A New Horizon for the Yellow Bean: Convenience, Nutrition and Taste

A sustainable public breeding effort is under way to increase the global production and health benefits of the common dry bean through pre breeding and germplasm enhancement. The propose of this study was to explore the different yellow bean market classes for promising phenotypes that can be added to the next generation of dry beans. The Yellow Bean Panel was assembled to explore the unique traits that would distinguish the yellow bean from other dry beans at the marketplace or grocery store. The hope is the yellow bean can be used to encourage more bean consumption by appealing to the consumers through traits not given a priority in other bean market classes, such as fast cooking time for convenience, improved iron quality for nutrition and a delicious taste when prepared traditionally in boiling water. The vision of the modern day yellow bean is one of cooking in the same amount of time as starchy grains or vegetables while maintaining its exceptionally nutritious content after cooking.

The Manteca yellow bean is certainly a prize of the Andean gene pool, providing the blueprint for a modern day yellow variety to reach its potential as a food crop desired by consumers for convenience, nutrition and taste. This is not the first time Manteca beans have interested bean breeders and food scientists [29, 70]. Following in steps of the great Colin Leakey, this study provides evidence that the Manteca is a nutritionally viable target for germplasm enhancement through the added benefit of fast cooking times and improved iron bioavailability. Manteca beans formulated into bean-based diets for a long-term *in vivo* feeding trial is the next step in evaluating the iron benefits of this market class beyond the current *in vitro* assessment presented in this study.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, **Supplementary Table 1**, raw seed iron concentrations. **Supplementary Tables 2 – 5**, iron concentrations, contents and retention values for pre-soaked and unsoaked genotypes in the YBP after cooking. **Supplementary Tables 6 – 7**, iron bioavailability scores for pre-soaked and unsoaked genotypes in the YBP after cooking.

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Abbreviations

The following abbreviations were used in this manuscript: ADP, Andean Diversity Panel; DRI, Dietary Reference Intake; *P. vulgaris*, *Phaseolus vulgaris* L. YBP, Yellow Bean Panel.

References

1. Blair, M.; Izquierdo, P. Use of the advanced backcross-QTL method to transfer seed mineral accumulation nutrition traits from wild to Andean cultivated common beans. *Theor. Appl. Genet.* **2012**, *125*, 1015-1031.
2. Blair, M.; Monserrate, F.; Beebe, S.; Restrepo, J.; Flores, J. Registration of high mineral common bean germplasm lines NUA35 and NUA56 from the red-mottled seed class. *J. Plant Regist.* **2010**, *4*, 55-59.
3. Bouis, H.; Saltzman, A. Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Glob. Food Secur.* **2017**, *12*, 49-58.
4. Petry, N.; Boy, E.; Wirth, J.; Hurrell, R. Review: The potential of the common bean (Phaseolus vulgaris) as a vehicle for iron biofortification. *Nutrients* **2015**, *7* (2), 1144-1173.
5. Petry, N.; Egli, I.; Zeder, C.; Walczyk, T.; Hurrell, R. Polyphenols and phytic acid contribute to the low iron bioavailability from common beans in young women. *J. Nutr.* **2010**, *140* (11), 1977-1982.
6. Hart, J.; Tako, E.; Kochian, L.; Glahn, R. Indentification of black bean (Phaseolus vulgaris L.) polyphenols that inhibit and promote iron uptake by caco-2 cells. *J. Agric. Food Chem.* **2015**, *63* (25), 5950-5956.
7. Glahn, R.; Tako, E.; Cichy, K.; Wiesinger, J. The cotyledon cell wall and intracellular matrix are factors that limit iron bioavailability of the common bean (Phaseolus vulgaris). *Food Funct.* **2016**, *7* (7), 3193-3200.
8. Hughes, J.; Swanson, B. Microstructural changes in maturing seeds of the common bean (Phaseolus vulgaris L.). *Food Struct.* **1985**, *4* (2), 2.
9. Dhital, S.; Bhattarai, R.; Gorham, J.; Gidley, M. Intactness of cell wall structure controls the in vitro digestion of starch in legumes. *Food Funct.* **2016**, *7*, 1367-1379.
10. IEA World energy outlook 2006, International Energy Agency, Paris, France, Agency, I. E., Ed. International Energy Agency, Paris, France, **2006**.
11. WEC World energy insight: powering up the south through energy poverty alleviation, by Suleiman J Al-Herbish, World Energy Council, London, Council, W. E., Ed. World Energy Council, London, **2011**.
12. Nijhuis, M., When cooking kills. *Natl. Geogr.* **2017**, 232 (3), 76-81.
13. de Benoist, B.; Mclean, E.; Egli, I.; Cogswell, M. *Worldwide prevalence of anaemia 1993-2005: WHO global data on anaemia*; WHO Press, World Health Organization, **2008**.
14. WHO World Health Organization, Global Health Atlas, Map library, World: Population using solid fuels (%), **2013**. http://apps.who.int/globalatlas/ (accessed on 06/24/17).
15. Adkins, E.; Oppelstrup, K.; Modi, V. Rural household energy consumption in the millennium villages in Sub-Saharan Africa. *Energ. Sustain. Develop.* **2012**, *16* (3), 249-259.
16. Bandyopadhyay, S.; Shyamsundar, P.; Baccini, A. Forests biomass use and poverty in Malawi. *Ecol. Econ.* **2011**, *70* (12), 2461-2471.
17. FAO. Global forest resources assessment 2010. *Food and Agriculture Organisation*: Rome, Italy. **2010**.
18. FAO. Forests for improved nutrition and food security. *Food and Agriculture Organization*: Rome, Italy. **2011**.
19. Makungwa, S.; Epulani, F.; Woodhouse, I. Fuelwood supply: A missed essential component in a food security equation. *J. Food Secur.* **2013**, *1* (2), 49-51.
20. Pinstrup-Anderson, P.; Pandya-Lorch, R. Food security and sustainable use of natural resources: A 2020 vision. *Ecol. Econ.* **1997**, *26*, 1-10.
21. Brouwer, I.; den Hartog, A.; Kamwendo, M.; Heldens, M. Wood quality and wood preferences in relation to food preparation and diet composition in Central Malawi. *Ecol. Food Nutr.* **1996**, *35* (1), 1-13.
22. Brouwer, I.; Hoorweg, J.; VanLiere, M. When households run out of fuel: responses of rural households to decreasing fuelwood availability, Ntcheu District, Malawi. *World Develop.* **1997**, *25*, 255-266.
23. Rebello, C.; Greenway, F.; Finley, J. Whole grains and pulses: A comparison of the nutritional and health benefits. *J. Agric. Food Chem.* **2014**, *62*, 7029-7049.

Peer-reviewed version available at *Nutrients* **2018**, *10*, 1609; doi:10.3390/nu10111609

24. Cichy, K.; Porch, T.; Beaver, J.; Cregan, P.; Fourie, D.; Glahn, R.; Grusak, M.; Kamfwa, K.; Katuuramu, D.; McClean, P.; Mndolwa, E.; Nchimbi-Msolla, S.; Pastor-Corrales, M.; Miklas, P. A Phaseolus vulgaris diversity panel for Andean bean improvement. *Crop Sci.* **2015,** *55* (5), 2149-2160.
25. Cichy, K.; Wiesinger, J.; Mendoza, F. Genetic diversity and genome wide association analysis of cooking time in dry bean (Phaseolus vulgaris L.). *Theor. Appl. Genet.* **2015**, *128* (8), 1555-1567.
26. Wiesinger, J.; Cichy, K.; Glahn, R.; Grusak, M.; Brick, M.; Thompson, H.; Tako, E. Demonstrating A Nutritional Advantage to the Fast Cooking Dry Bean (Phaseolus vulgaris L.). *J. Agric. Food Chem.* **2016**, *64* (45), 8592-8603.
27. Bassett, M. J. The Seedcoat Color Genotype of Prim'and the Manteca and Coscorrón Market Classes of Common Bean. *HortSci.* **1999**, *34* (2), 336-337.
28. Leakey, C. L. Breeding on the C and J and B loci for modification of bean seedcoat flavonoids with the objective of improving food acceptability. *Annu. Rep. Bean Improv. Coop. (USA)* **1992**.
29. Leakey, C.; Hosfield, G.; Dubois, A. Mantecas, a new class of beans (Phaseolus vulgaris) of enhanced digestibility. *Proceedings of the 3rd European Conference on Grain Legumes*, Valladolid, Spain, **1998**; pp 336-337.
30. Voysest, O. *Yellow beans in Latin America*; *Report* 0084-7747. Centro Internacional de Agricultura Tropical (CIAT): Cali, Colombia, **2012**.
31. Buruchara, R.; Chirwa, R.; Sperling, L.; Mukankusi, C.; Rubyogo, J. C.; Mutonhi, R.; Abang, M. Development and delivery of bean varieties in Africa: the Pan-Africa Bean Research Alliance (PABRA) model. *Afr. Crop Sci. J.* **2011**, *19* (4), 227-245.
32. Sichilima, T.; Mapemba, L.; Tembo, G. Drivers of dry common beans trade in Lusaka, Zambia: A trader's perspective. *Sustain. Agric. Res.* **2016**, *5* (2), 15-25.
33. United Nations. A value chain analysis of the dry bean sub-sector in Uganda : Development of Inclusive Markets in Agriculture and Trade (DIMAT) Project. United Nations Development Programme Uganda Issuing Body: Kampala, Uganda, **2012**.
34. Hooper, S.; Wiesinger, J.; Echeverria, D.; Thompson, H.; Brick, M.; Nchimbi-Msolla, S.; Cichy, K. The carbohydrate profile of a dry bean (Phaseolus vulgaris L.) panel encompassing broad genetic variability for cooking time. *Cereal Chem.* **2017**, *94* (1), 135-141.
35. Sones, D. Soya Njano is the bean for home consumption. In *Our Blog: The Inside Story*, Africa Soil Health Consortium http://africasoilhealth.cabi.org/2015/09/29/soya-njano-is-the-bean-for-home-consumption/ (accessed 07/28/17).
36. Hillocks, R.; Madata, C.; Chirwa, R.; Minja, E.; Nchimbi-Msolla, S. Phaseolus bean improvement in Tanzania, 1959-2005. *Euphytica* **2006**, *150* (1-2), 215-231.
37. Canadian Food Insepection Agency (CFIA). CDC Sol. http://www.inspection.gc.ca/english/plaveg/pbrpov/cropreport/bea/app00007688e.shtml (accessed 05/15/18).
38. Balasubramanian, P.; Chatterton, S.; Conner, R. AAC Y012 and AAC Y015 yellow dry bean. *Canad. J. Plant Sci***.** **2017**, *97*, 340-343.
39. Arnaud-Santana, E.; Nin, J.; Saladin, F.; Bodoy-Lutz, G.; Beaver, J.; Coyne, D.; Steadman, J. Registration of 'JB-178' red mottled bean. *Crop Sci.* **2000**, *40*, 857-858.
40. Prophete, E.; Demosthenes, G.; Godoy-Lutz, G.; Porch, T.; Beaver, J. Registration of PR0633-10 and PR0737-1 red mottled dry bean germplasm lines with resistance to BGYMV, BCMV, BCMNV and common bacterial blight. *J. Plant Regist.* **2014**, *8*, 49-52.
41. Morris, H.; Wood, E. Influence of moisture content on keeping quality of dry beans. *Food Technol.* **1956**, *10* (5), 225-229.
42. Wang, N.; Daun, J. Determination of cooking times of pulses using an automated Mattson cooker apparatus. *J. Sci. Food Agric.* **2005**, *85*, 1631-1635.
43. Wang, N.; Hatcher, D.; Tyler, R.; Toews, R.; Gawalko, E. Effect of cooking on the composition of beans (Phaseolus vulgaris L.) and chickpeas (Cicer arietinum L.). *Food Res. Int.* **2010**, *43* (2), 589-594.
44. Marinangeli, C.; Curran, J.; Barr, S.; Slavin, J.; Puri, S.; Seaminathan, S.; Tapsell, L.; Patterson, C. Enhancing nutrition with pulses: defining a recommended serving size for adults. *Nutr. Rev.* **2017**, *75* (12), 990-1006.
45. NAS *Dietary reference intakes: the essential guide to nutrient requirements*; National Academy of Sciences: National Academies Press Washington, DC, **2006**.

46. USAID Feed the Future. *The U.S. government's global food security research strategy*. http://www.feedthefuture.gov (accessed 04/08/17).
47. Glahn, R.; Lee, O.; Yeung, A.; Goldman, M. Caco-2 Cell ferritin formation predicts nonradiolabeled food iron availability in an in vitro digestion/Caco-2 Cell culture model. *J. Nutr.* **1998**, *128* (9), 1555-1561.
48. Glahn, R.; Tako, E.; Hart, J.; Haas, J.; Lung'aho, M.; Beebe, S. Iron bioavailability studies of the first generation of iron-biofortified beans released in Rwanda. *Nutrients* **2017**, *9* (787), 10.3390/nu9070787.
49. Borchgrevink, C. Culinary perspective of dry beans and pulses. In *Dry Beans and Pulses Production, Processing and Nutrition*, Uebersax, M. S. Ed. John Wiley & Sons, Inc.: **2013**; pp 313-334.
50. Castellanos, J.; Guzmán, M.; Jiménez, A.; Mejia, C.; Ramos Muñoz, J.; Gallegos Acosta, J.; Hoyos, G. Preferential habits of consumers of common bean (Phaseolus vulgaris L.) in Mexico. *Archivos latinoamericanos de nutricion* **1997**; Vol. 47, pp 163-167.
51. Tako, E.; Glahn, R. White beans provide more bioavailable iron than red beans: Studies in poultry (Gallus gallus) and an in vitro digestion/Caco-2 model. *Int. J. Vit. Nutr. Res.* **2010**, *80* (6), 416-429.
52. Ariza-Nieto, M.; Blair, M.; Welch, R.; Glahn, R. Screening of iron bioavailability patterns in eight bean (Phaseolus vulgaris L.) genotypes using the Caco-2 cell in vitro model. *J. Agric. Food Chem.* **2007**, *55* (19), 7950-7956.
53. Beebe, S.; Viviana Gonzalez, A.; Rengifo, J. Research on trace minerals in the common bean. *Food Nutr. Bull.* **2000**, *21* (4), 387-391.
54. Blair, M.; Izquierdo, P.; Astudillo, C.; Grusak, M. A legume biofortification quandary: variability and genetic control of seed coat micronutrient accumulation in common beans. *Front. Plant Sci.* **2013**, *4* (275), 1-14.
55. McClean, P.; Moghaddam, S.; Lopez-Millan, A.; Brick, M.; Kelly, J.; Miklas, P.; Osorno, J.; Porch, T.; Urrea, C.; Soltani, A.; Grusak, M. Phenotypic diversity for seed mineral concentration in North American dry bean germplasm of Middle American ancestry. *Crop Sci.* **2017**, *57*, 3129-3144.
56. Barampama, Z.; Simard, R. Oligosaccharides, antinutritional factors and protein disgestibility of dry beans as affected by processing. **1994**, *J. Food Sci.* (4), 833-838.
57. Pujola, M.; Farreras, A.; Casanas, F. Protein and starch content of raw, soaked and cooked beans (Phaseolus vulgaris L.). *Food Chem.* **2007**, *102* (4), 1034-1041.
58. Saha, S.; Singh, G.; Mahajan, V.; Gupta, H. Variability of nutritional and cooking quality in bean (Phaseolus vulgaris L) as a function of genotype. *Plant Foods Hum. Nutr.* **2009**, *64*, 174-180.
59. McCouch, S.; et al. Feeding the future. *Nature* **2013**, *499*, 23-24.
60. Kelly, J.; Kolkman, J.; Schneider, K. Breeding for yield in dry bean (Phaseolus vulgaris L.). *Euphytica* **1998**, *102*, 343-356.
61. PABRA. Transforming agriculture for better incomes and diets in Africa. Pan-Africa Bean Research Alliance Press Release. http://www.pabra-africa.org (accessed 03/23/18).
62. Nei, M.; Tajima, F.; Tateno, Y. Accuracy of estimated phylogenetic trees from molecular data. *J. Mol. Evol.* **1983**, *19*, 150-170.
63. Deshpande, S.; Cheryan, M. Water uptake during cooking of dry beans (Phaseolus vulgaris L.). *Plant Food Hum. Nutr. (Formerly Qualitas Plantarum)* **1986**, *36* (3), 157-165.
64. Santos, G.; Ribeiro, N.; Maziero, S. Evaluation of common bean morphological traits identifies grain thickness directly correlated with cooking time. *Pesquisa Agropecuária Tropical* **2016**, *46* (1), 35-42.
65. Agbo, G.; Hosfield, G.; Uebersax, M.; Klomparens, K. Seed microstructure and its relationship to water uptake in isogenic lines and a cultivar of dry beans (Phaseolus vulgaris L.). *Food Struct.* **1987**, *6* (1), 12.
66. Beninger, C.; Hosfield, G. Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from Phaseolus vulgaris L. seed coat color genotypes. *J. Agric. Food Chem.* **2003**, *51* (27), 7879-7883.
67. Elia, F.; Hosfield, G.; Kelly, J.; Uebersax, M. Genetic analysis and interrelationships between traits for cooking time, water absorption, and protein and tannin content of Andean dry beans. *J. Amer. Societ. Hortl. Sci.* **1997**, *122* (4), 512-518.
68. Pereira, H.; Del Peloso, M.; Bassinello, P.; Guimaraes, C. Gentic variability for iron and zinc content in common bean lines and interaction with water availability. *Genet. Mol. Res. J.* **2014**, *13*, 6773-6785.
69. Kingsbury, N. *Hybrid the history and science of plant breeding*. The University of Chicago Press: Chicago and London, **2009**.
70. Leakey, C. L. Progress in developing tannin-free dry phaseolus beans. *Ann. Rep. Bean Improv. Cooper. (USA)* **2000**, *43*, 18-20.

71. Hosfield, G.; Bennink, M.; Beninger, C.; Engleright, R.; Ospina, M. Variability for Starch Digestibility in Dry Bean (Phaseolus vulgaris L.). *HortSci.* **1998**, *33* (3), 472.
72. Engleright, R.; Beimiriki, M.; Hosfield, G. Determination of total dietary fiber, indigestible starch, and indigestible protein in dry bean, Phaseolus vulgaris L *Ann. Rep. Bean Improv. Cooper. (USA)* **1999**, *42* (42), 123-124.
73. Beninger, C.; Hosfield, G.; Nair, M. Flavonol Glycosides from the Seed Coat of a New Manteca-Type Dry Bean (Phaseolus vulgaris L.). *J. Agric. Food Chem.* **1998**, *46* (8), 2906-2910.
74. Ozdal, T.; Capanoglu, E.; Altay, F. A review on protein–phenolic interactions and associated changes. *Food Res. Int.* **2013**, *51* (2), 954-970.
75. Hart, J.; Tako, E.; Glahn, R. Characterization of polyphenol effects on inhibition and promotion of iron uptake by caco-2 cells. *J. Agric. Food Chem.* **2017**, *65* (16), 3285-3294.
76. Bassett, M.; Lee, R.; Otto, C.; McClean, P. Classical and Molecular Genetic Studies of the Strong Greenish Yellow Seedcoat Color in `Wagenaar' and `Enola' Common Bean. *Journal of the American Society for Horticultural Science* **2002**, *127* (1), 50-55.
77. Beninger, C.; Hosfield, G.; Bassett, M. Flavonoid Composition of Three Genotypes of Dry Bean (Phaseolus vulgaris) Differing in Seedcoat Color. *J. Amer. Societ. Hortl. Sci.* **1999**, *124* (5), 514-518.