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Yellow bean (*Phaseolus vulgaris* L.) germplasm with less dietary fiber have shorter cooking times and more bioavailable iron

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ABSTRACT

Some yellow-colored market classes of dry bean (*Phaseolus vulgaris* L.) are valued by consumers as an easy-to-digest, fast cooking alternative to darker colored red and black beans, which in comparison generally have longer cooking times and reduced iron bioavailability. There is evidence that the cooking time of yellow beans is linked to the dietary fiber content and may also contribute to nutrient digestibility and bioavailability. Therefore, 52 fast-, moderate-, and slow-cooking yellow beans with diverse iron bioavailability from five market classes (Amarillo, Canario, Green-yellow, Manteca, and Mayocoba) were selected for total dietary fiber (TDF) analysis. TDF was measured as insoluble (IDF) + soluble (SDF) + oligosaccharides (OLIGO) using method AOAC2011.25. Wide variations in the concentrations of IDF (16.0–23.1%), SDF (1.6–7.7%), OLIGO (1.5–3.4%), and TDF (20.6–31.3%) were detected among the yellow beans with various cooking times. Lower concentrations of IDF in yellow beans were associated with shorter cooking times and higher iron bioavailability. The larger sized Andean yellow beans had more SDF than Middle American. One Mayocoba breeding line from Puerto Rico, PR1146-124, had 42% less OLIGOs than average, and may be useful for breeding low-flatulence beans for consumer acceptability. Fast cooking yellow beans provide the same SDF and OLIGO concentrations as yellow beans with longer cooking times but have the added benefit of shorter cooking times (convenience) and provide more bioavailable iron after cooking.

1. Introduction

Yellow beans (*Phaseolus vulgaris* L.) are a nutritious pulse crop produced in many regions of Mexico, South America, and Africa (Wortmann et al., 1998; Voysest, 2012; Mishili et al., 2011). With over a dozen market classes worldwide, yellow beans are sold under a number of names, which include Amarillo, Azufrado, Canario, Manteca, Mayocoba, Njano, and Peruano. Regional preferences for yellow beans have generated a large diversity of seed coat colors, which range from an intense 'highlighter' yellow to several shades of orange and green (Sadohara et al., 2022). Some yellow beans have unique polyphenolic profiles in their seed coats, which accumulate little to no red- or blue-colored pigments and have low concentrations of condensed tannins (Hart et al., 2020; Beninger et al., 1998). Condensed tannins in the seed coats of pinto, red, and black beans are associated with longer cooking times, reduced protein digestibility and less bioavailable iron (Hart et al., 2020; Elia et al., 1997; Petry et al., 2010; Wiesinger et al., 2021). Therefore, pale-colored yellow beans express unique culinary traits that are otherwise lost in dark brown, red, or black colored dry beans, including shorter cooking times and improved iron bioavailability (Wiesinger et al., 2018).

There is evidence to suggest that the culinary traits of yellow beans are also related to their dietary fiber (DF) compositions (Wiesinger et al., 2018; Hooper et al., 2016, 2021; Leakey, 2000). As an important non-nutritive component of food, DF has numerous health benefits including the prevention and management of cardiometabolic diseases and some types of cancer. Pulses such as dry beans have high concentrations of DF compared to foods prepared from cereals and starchy root vegetables (Murphy et al., 2008; Chen et al., 2016; Li et al., 2002). There is some information on the DF concentrations of yellow beans collected

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from diverse world markets (Hooper et al., 2016, 2021; Wiesinger et al., 2016), but the relationship between DF and the fast cooking times and high iron digestibility that distinguish yellow beans from other market classes need further examination.

An analytical method for measuring DF in foods was developed by McCleary et al. (2009) to comply with the 2009 CODEX definition of total dietary fiber (TDF) as the combination of 1) insoluble dietary fiber (IDF), 2) soluble dietary fiber (SDF) and 3) non-digestible oligosaccharides (OLIGO). This integrated method was accepted as AOAC method 2011.25 after inter-laboratory evaluations (McCleary et al., 2009; V McCleary et al., 2012). SDF is water-soluble and is fermented by the gut microbiota, whereas IDF is fermented by the gut microbiota to a lesser extent depending on its chemical constituents. Both SDF and IDF have the potential to add fecal bulk and reduce transit time in the colon (Dai and Chau, 2017). OLIGOs include raffinose family oligosaccharides that escape digestion because of the absence of galactosidase in the mammalian intestinal tract; however, the oligogalactans are metabolized by the gut microflora (Singh et al., 2017). Along with other fermentable components of DF, OLIGOs are considered to be one of the causes of flatulence when pulse consumption is not a routinized eating behavior (Thompson, 2019). Nonetheless, fermetable components of DF, inlcuding OLIGOs also contribute to the long-term health benefits of consuming beans (Didinger and Thompson, 2020).

Using the AOAC2011.25 method, a wide genetic variability in IDF, SDF, OLIGO, and TDF have been reported in the Middle American Diversity panel accessions (Brick et al., 2022; Moghaddam et al., 2018); however, this research did not include yellow beans. Middle American and Andean gene pools exist in common bean due to separate domestication events in Mexico and in the Andes, causing the two gene pools to differ both genetically and phenotypically (Gepts et al., 1986). In addition, Andean beans ($n = 4$) had higher SDF than the Middle American beans ($n = 21$) in a diverse population of bean cultivars/lines (Kleintop et al., 2013). The small sample size used by Kleintop et al. (2013) calls for more investigation on the DF compositions in Andean beans. Yellow beans come from both Andean and Middle American origins (Sadohara et al., 2022), making them an interesting market class for the evaluation of DF.

Previous reports demonstrated that fast-cooking Manteca yellow beans have less resistant starch and lower concentrations of DF (insoluble and soluble) when compared to kidney beans (Bassett et al., 2021; Wiesinger et al., 2019). Manteca yellow beans were once lauded by vendors in Chile and continue to be sold in multiple countries in Africa for being “easy-to-digest” (Leakey et al., 1998; Leakey, 2001). Although these observations warrant further investigation, a direct comparison of TDF (IDF + SDF + OLIGO) concentrations between a broad mix of Manteca and other fast- or slow-cooking yellow beans has never been conducted. Understanding the relationship between TDF and the cooking time of dry beans could implement new breeding targets that enhance the digestibility of fast cooking bean varieties, while at the same time, preserving their nutritional value (Wiesinger et al., 2018). Therefore, a 295-accession Yellow Bean Collection (YBC) with diverse cooking times (17–123 min) was assembled (Sadohara et al., 2022).

The purpose of this study was to understand the relationship between cooking time and the nutritional attributes of beans by measuring the TDF (AOAC, 2011.25), iron concentration, and iron bioavailability of yellow beans classified into either fast- (<20 min), moderate-(20–30 min), or slow- (>30 min) cooking classes. Yellow bean genotypes from the YBC were selected from five market classes representing the major bean-consuming regions of the world (Amarillo, Canario, Green-yellow, Manteca, and Mayocoba). In addition, a group of fast- and slow-cooking beige, brown, red, and white beans (with diverse iron bioavailability) were also included as reference controls in this study. The reference controls are needed to compare the cooking times, iron concentrations, iron bioavailability, and TDF concentrations of non-yellow market classes produced under the same field conditions and cooked in the same manner as the yellow beans evaluated in this study.

2. Materials and methods

2.1. Plant materials

Out of 295 YBC accessions, a panel of 52 yellow bean (*P. vulgaris*) genotypes were selected for DF measurements to represent the five major market classes of yellow beans, which include the orange-colored Amarillo dark (dk), the yellow-orange colored Amarillo light (lt), the dark yellow colored Canary, the pale-yellow Manteca (Mantequilla), and bright yellow Mayocoba (Peruano). The yellow beans in this panel were also selected from three cooking time classes (fast <20 min; moderate 20–30 min; and slow >30 min). Green-yellow was included as part of the Canary market class in Fig. 1, Fig. S1, and Fig. S2. Eight non-yellow bean controls were selected as field references; such that diversity in cooking class, origin, and seed color also are preserved among the non-yellow bean controls (Table S1). The controls included two fast- (W616488, G17913), two moderate- (Merlin, Al 146), and four slow-cooking classes (Red Hawk, Incomparable TZ-27, PR0737-1, G10994). The seed coat colors of the controls were: brown for W616488 and Incomparable TZ-27; beige for G17913 and G10994; white for Merlin (Navy) and Al 146 (Great Northern); dark red for Red Hawk (Dark Red Kidney); and red mottled for PR0737-1. Gene pools and races within gene pools (Andean, Jalisco/Durango (Middle American), Mesoamerica (Middle American), and Admix) among the 52 yellow beans was previously determined by using Genotyping-By-Sequencing SNPs generated via sequencing 295 YBC accessions (Sadohara et al., 2022).

2.2. Production sites and storage conditions

The 52 yellow beans and eight field control genotypes were grown at the Michigan State University Montcalm Research Station located near Entrican, Michigan (43°21.2′N, 85°10.6′W) in 2018. The field design was a randomized controlled block design with two field replications, as described in detail by Sadohara et al. (2022) (Sadohara et al., 2022). Bean seeds were harvested at maturity and stored under room temperature while drying, then stored in a cold storage (4 °C, 75% relative humidity) until subjected to cooking time measurements.

2.3. Cooking time determination and sample preparation

The cooking time was determined for each of the field replications by using the automated Mattson Cooker apparatus, which is equipped with 25 pins (Wang and Daun, 2005). After confirming that seeds were equibrillated to 10–14% moisture during cold storage, thirty seeds were taken per field replication and soaked in distilled water for 12 h. The 30 seeds were weighed before soaking and used to calculate 100-seed weight. The soaked seeds were drained and placed underneath 25 pins before cooking in distilled water on an electronic burner. Water level was maintained as a constant, such that plenty of boiling water covers the seeds during cooking. When the seeds were sufficiently soft, each pin penetrates the seed beneath it, which was recorded by the Mattson Cooker software. We defined cooking time as the time it took for 80% of the pins (20 pins) to drop. Cooked seeds were drained, cooled to room temperature and frozen at −80 °C prior to freeze-drying (Virtis Research, Garniner, NY, USA). Lyophilized beans were milled into a fine powder with a Kinematica Polymix® analytical hammer mill (PX-MFC 90D, Bohemia, NY, USA) fitted with a 0.5 mm sieve. Milled samples were stored in sealed polypropylene containers at room temperature in preparation for mineral, iron bioavailability, and TDF analysis.

2.4. Iron analysis

For iron analysis, 0.5 g of each cooked, lyophilized, and milled bean samples were predigested in boro-silicate glass tubes with 3 mL of a concentrated ultra-pure nitric acid and ultra-pure perchloric acid mixture (60:40 v/v) for 16 h at room temperature. Samples were then

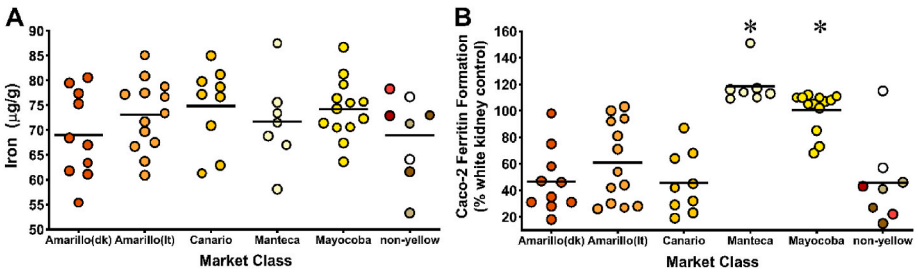

Fig. 1. Dot plots illustrating (**A**) the iron concentrations and (**B**) the iron bioavailability of 52 yellow bean entries from major yellow market classes, as well as eight non-yellow field control genotypes. Each dot represents the combined means of two field replicates, each measured in duplicate. Iron concentrations are measured as micrograms per gram of soaked, cooked, lyophilized and milled whole beans (dry weight). Iron bioavailability is measured as Caco-2 cell ferritin formation after exposure to an *in vitro* digestion of soaked, cooked, lyophilized, and milled whole beans. Values are expressed as the percentage of Caco-2 cell ferritin formation (ng ferritin/mg total cell protein) relative to a white kidney bean reference standard that is run with each assay (*cv*: Snowdon). *Significantly ($\alpha = 0.05$) higher iron bioavailability when compared to the other yellow and non-yellow bean market classes.

placed in a digestion block and heated incrementally over 4 h to a temperature of 120 °C with refluxing. After incubating at 120 °C, 2 mL of ultrapure nitric acid was added to each sample before raising the digestion block temperature to 145 °C for an additional 2 h. To evaporate the remaining acid, the digestion block was raised to 190 °C for at least 10 min before allowing samples to cool to room temperature. Digested samples were re-suspended in 20 mL of ultra-pure water prior to analysis using ICP-AES (inductively coupled plasma-atomic emission spectroscopy; Thermo iCAP 6500 Series, Thermo Scientific, Cambridge, United Kingdom). Quality control standards (High Purity Standards, Charleston, SC, USA) were measured following every 20 samples. All samples were digested and measured with 0.5 µg/mL of Yttrium (final concentration) from High Purity Standards (10 M67-1) as an internal standard to ensure batch-to-batch accuracy and to correct for matrix interference during digestion.

2.5. Iron bioavailability (Caco-2 cell bioassay)

An established *in vitro* digestion/Caco2 cell culture model of the human intestinal epithelial barrier was used to assess the iron bioavailability of all 60 cooked yellow and non-yellow beans selected for evaluation in this study (Glahn, 2022).

2.5.1. Cell culture

Caco-2 cells (obtained at passage 24; American Type Culture Collection, Gaithersburg, MD, USA) were seeded at a density of 50,000 cells/cm^2 in 6-well collagen coated plates (Costar, Cambridge, MA, USA) at passage 28–38. Cells were grown for 13 days before each bioassay at 37°C in an incubator with a 5% CO_2 air atmosphere (constant humidity) using Dulbecco's modified Eagle's medium (DMEM; GIBCO, Grand Island, NY, USA) supplemented with 25 mM HEPES (pH 7.2), 10% (v/v) fetal bovine serum (GIBCO) and 1% antibiotic-antimycotic solution (ZellShield®, Minerva Biolabs, Germany). The medium was changed every 2 days. Twenty-four hours prior to each bioassay, the culture medium was replaced with iron-free Minimum Essential Medium (MEM [pH 7]; GIBCO) supplemented with 10 mM PIPES (piperazine-N,N′-bis-[2-ethanesulfonic acid]), 1% antibiotic-antimycotic solution, hydrocortisone (4 mg/L), insulin (5 mg/L), selenium (5 µg/L), triiodothyronine (34 µg/L) and epidermal growth factor (20 µg/L). A fresh 1 mL aliquot of MEM (pH 7) covered the cells during each experiment.

2.5.2. Caco-2 cell bioassay

The bioassay was performed according to the detailed methods described in Glahn et al. (1998) (Glahn et al., 1998) and Glahn (2022) (Glahn, 2022). The bioassay works according to the following principle: in response to increases in cellular iron concentrations after exposure to an *in vitro* digestion of a cooked, lyophilized and milled bean sample, Caco-2 cells produce more ferritin protein, therefore, iron bioavailability was determined as the increase in Caco-2 cell ferritin production expressed as a ratio to total Caco-2 cell protein (ng ferritin per mg of total cell protein) after exposure to a digested food sample. Ferritin was measured by enzyme-linked immunoassay (Human Ferritin ELISA kit, FRR31-K01, Eagle Biosciences Inc., Nashua, NH, USA) and total cell protein concentrations were quantified using the Bio-Rad DC™ protein assay kit (Bio-Rad Laboratories Inc., Hercules, CA, USA).

To confirm the responsiveness of the Caco-2 bioassay, each experiment was run with several quality controls. These include a blank-digest, which is only the physiologically balanced saline and the gastrointestinal enzymes. The blank-digest is used to ensure there is no iron contamination in the bioassay. Ferritin values of Caco-2 cells exposed to the blank-digest averaged 2.74 ± 0.83 ng ferritin/mg cell protein (mean ± Standard Deviation; SD) over the course of ten cell culture experiments. A cooked, lyophilized, and milled white kidney bean (cultivar name: Snowdon) is run with each assay as a reference standard to index the ferritin/cell protein ratios of the Caco-2 cells after being exposed to a digested food matrix of cooked beans. Ferritin values from Caco-2 cells exposed to the cooked white kidney bean reference standard averaged 10.89 ± 0.47 ng ferritin/mg cell protein across all ten experiments. The responsiveness of the Caco-2 cells is monitored using a cooked, lyophilized and milled white kidney bean pasta that was processed with or without the addition of 2 mM ascorbic acid. Ferritin values in Caco-2 cells exposed to a digest of the white kidney bean pasta with and without ascorbic acid over the course of ten experiments averaged 35.46 ± 3.24 and 104.39 ± 7.29 ng ferritin/mg cell protein, respectively.

2.6. DF analysis

IDF, SDF, and OLIGO in the cooked, lyophilized, and milled bean samples were measured by using the AOAC.2011.25 with details described by Kleintop et al. (2013) (Kleintop et al., 2013). In brief, bean samples were subjected to amylolysis by α-amylase for 16 h, followed by proteolysis by protease for 30 min. Residues were collected by vacuum filtration on Celite® to determine the IDF content after adjusting for ash and protein content of the residue. The filtrate was dried, and SDF was precipitated by ethanol and collected by vacuum filtration with Celite® the second time. The SDF contents were determined after adjusting for the ash and protein contents of the residue. OLIGO (raffinose, stachyose, and verbascose) were collected in the filterate that passed through the filter and quantified by high-performance anion-exchange chromatography.

2.7. Statistical analyses

Statistical analyses and mean separations were performed with SAS 9.4 software (SAS Institute, Cary, NC, USA) using the proc mixed command for the analysis of variance with each of the traits: cooking time, iron concentrations, iron bioavailability, SDF, IDF, OLIGO, and TDF. Genotype was designated as a fixed effect, followed by a Tukey-Kramer

post-hoc test. Dot plots illustrating the traits measured in each of the 52 yellow and 8 non-yellow bean genotypes across the five market classes were developed in Graphpad Prism8 (GraphPad Software, La Jolla, CA, USA).

Correlation coefficients and *p*-values were calculated by using the cor.test function in base R (R Core Team, 2017). A linear model was built with the market class or the cooking class as a fixed effect and each of the traits as a response variable. Analysis of variance was significant ($\alpha = 0.05$) for all the traits, thus the Least Significant Difference (LSD) was calculated for each trait using the agricolae package in R (de Mendiburu, 2021). Estimated marginal means (emmeans) of the market classes or the cooking classes were estimated using the emmeans package in R (V Lenth, 2021). Log- or square-transformations were used if normality and/or heteroscedasticity of the residuals of the linear model was violated and was improved after a transformation. The emmeans were back-transformed after mean separation was done by using the multcomp package (Hothorn et al., 2008). Scatter plots were generated by using the ggplot2 package (Wickham, 2016).

3. Results

3.1. Fast-, moderate-, and slow-cooking yellow beans selected for TDF analysis

Table 1 shows the overall cooking time, seed weight, iron concentrations, iron bioavailability, IDF, SDF, OLIGO, and TDF of the 60 yellow and non-yellow beans selected for TDF analysis. Previous research discovered fast cooking varieties and landraces in every major market class of the 295 yellow beans surveyed from the YBC (Sadohara et al., 2022). Table 2 shows the ranges of cooking times within each cooking class (fast, moderate, and slow) of the 52 yellow beans selected from the YBC. Table S1 lists each genotype's gene pool, origin, and market class (color pattern), as well as their corresponding cooking time and cooking classification. The results presented in Table 2 and Table S1 show the wide range of cooking times within each market class of yellow and non-yellow beans selected for the DF analysis. By design, the estimated marginal means (emmeans) for cooking time between the market classes were not significantly different because each market class had fast-, moderate-, and slow-cooking genotypes (Table 3). Fast-, moderate-, and slow-cooking genotypes were not different in mean 100-seed weight.

3.2. Iron concentrations and iron bioavailability

The iron concentrations and iron bioavailability of the fast-cooking yellow and non-yellow genotypes in each market class are illustrated in Fig. 1. The iron concentrations and iron bioavailability of each of the 60 genotypes are shown in Table S1. The iron concentrations of the 60 genotypes ranged from 53.3 to 87.5 (μg/g), (Fig. 1A–Table 1). The iron concentrations observed in this study were within the range of those for the genotypes in the Andean Diversity Panel: 41–99 (μg/g), where bean samples were cooked with the same method (Katuuramu et al., 2018, 2021). Interestingly, the iron concentrations did not differ among the cooking classes (Table 2) or market classes (Table 3), indicating a wide diversity in iron concentrations within yellow beans (Fig. 1A). The genotypes showed large variability in iron bioavailability, ranging from 14.5% to 151.0% (relative to a white bean reference) (Fig. 1B–Table 1). Manteca and Mayocoba market classes had higher means (117.9% and 100.2%, respectively) of iron bioavailability than the other market classes (Fig. 1B–Table 3).

3.3. DF concentrations and their correlations with cooking time and iron bioavailability

The IDF and SDF of the fast cooking yellow and non-yellow genotypes in each market class are visualized in Fig. S1, and OLIGO and TDF in Fig. S2. IDF ranged from 16.0 to 23.1% with an overall mean of 18.6%; SDF ranged from 1.6 to 7.7% with an overall mean of 5.7%; OLIGO ranged from 1.5 to 3.4% with an overall mean of 2.6%; and TDF ranged from 20.6 to 31.3% with an overall mean of 26.9% (Table 1). Table 4 shows the correlation coefficients between cooking time, iron concentration and bioavailability, and the DF components. Cooking time was positively correlated with IDF and TDF for the 52 yellow bean genotypes ($r = 0.333$, $p = 0.001$; and $r = 0.349$, $p < 0.001$, respectively), and this correlation was also significant for all the 60 genotypes including non-yellows ($r = 0.360$, $p < 0.0001$; and $r = 0.396$, $p < 0.0001$, respectively) (Table 4). The fast-cooking yellow beans had lower IDF and TDF than the moderate- and slow-cooking beans, but SDF and OLIGO were not different among the cooking classes; thus, the lower TDF of the fast-cooking yellow beans are due to lower IDF (Fig. 2, Table 2, Fig. S3). Among the market classes, IDF were not different, but SDF of Manteca and Mayocoba was lower than Amarillo (dk) and Amarillo (lt) (Fig. S1). Iron bioavailability was negatively correlated with cooking time and IDF for the 52 yellow bean genotypes ($r = -0.294$, $p = 0.002$; and $r = -0.212$, $p = 0.031$, respectively), and this correlation also held for the 60 genotypes including non-yellow beans ($r = -0.302$, $p = 0.001$ for iron bioavailability and cooking time; and $r = -0.200$, $p = 0.029$ for iron bioavailability and IDF) (Table 4). This was consistent with the observation that the fast-cooking genotypes had higher iron bioavailability (Table 2). Iron bioavailability was positively correlated with 100-seed weight ($r = 0.365$, $p < 0.001$, Table 4), but the correlation was weaker ($r = 0.177$, $p = 0.053$, Table 4) when non-yellow genotypes were included.

Table 1
Summary statistics of cooking time, iron concentrations, iron bioavailability, and dietary fiber (AOAC2011.25) of all 60 yellow and non-yellow bean genotypes selected for analysis from the Yellow Bean Collection.

	Cooking time	100-seed weight	Iron	Iron bioavailability	Dietary fiber[c]			
	(minutes)	(g)	(μg/g)[a]	(% white kidney control)[b]	IDF (%)	SDF (%)	OLIGO (%)	TDF (%)
Range	14.6–68.2	19.4–69.4	53.3–87.5	14.5–151.0	16.0–23.1	1.6–7.7	1.5–3.4	20.6–31.3
Overall mean	27.3	40.4	72.2	69.2	18.6	5.7	2.6	26.9
Coefficient of variation (%)	35.7	26.7	10.8	52.1	8.5	19.2	13.1	7.1
LSD value[d]	8.2	4.2	10.3	17.6	2.4	1.5	0.7	1.8

IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF + SDF + OLIGO).

[a] Iron was measured in soaked, cooked, drained, lyophilized, and milled whole beans (% dry weight).

[b] Iron bioavailability was determined after exposure to an *in vitro* digestion of soaked, cooked, lyophilized, and milled whole beans. Values are expressed as the percentage of Caco-2 cell ferritin formation (ng ferritin/mg total cell protein) relative to a white kidney bean reference standard that was run with each assay (*cv*: Snowdon).

[c] Dietary fiber concentrations are measured as grams per 100 g of soaked, cooked, drained, lyophilized, and milled whole beans (% dry weight).

[d] Least Significant Difference (ANOVA *p*-value <0.005 for all the traits).

Table 2
Range of cooking times and estimated marginal mean values (emmeans) for cooking time, iron concentrations, iron bioavailability, and dietary fiber components (AOAC, 2011.25) of fast-, moderate-, and slow-cooking yellow beans. Values are emmeans ± SE except for the ranges. Emmeans sharing the same letter in each column are not significantly different (α = 0.05).

Cooking class		Cooking time (minutes)		100-seed weight	Iron	Iron bioavailability	Dietary fiber			
	N[a]	Range	Emmean	(g)	(μg/g)	(% white kidney control)	IDF (%)	SDF (%)	OLIGO (%)	TDF (%)
Fast (<20 min)	17	14.6–20.8	18.4 ± 0.5 a	38.2 ± 1.6 NS[b]	72.6 ± 1.4 NS	90.1 ± 5.9 b	17.7 ± 0.3 a	5.5 ± 0.2 NS	2.6 ± 0.1 NS	25.6 ± 0.3 a
Moderate (20–30 min)	19	21.0–29.5	24.1 ± 0.7 b	41.5 ± 1.6	74.6 ± 1.4	64.7 ± 5.5 a	18.9 ± 0.3 b	6.0 ± 0.2	2.6 ± 0.1	27.5 ± 0.3 b
Slow (>30 min)	16	31.1–68.2	37.6 ± 1.1 c	37.5 ± 1.7	70.5 ± 1.5	64.0 ± 6.0 a	18.8 ± 0.3 b	5.9 ± 0.2	2.5 ± 0.1	27.0 ± 0.3 b

Cooking time was log-transformed, and SDF was square-transformed before emmeans calculation and multiple comparisons of each cooking class using Tukey's honestly significant test (α = 0.05). Emmeans listed in the table are back-transformed.
See the footnote of Table 1 for detailed descriptions of the traits.
IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF + SDF + OLIGO).
[a] Number of genotypes.
[b] Not significant.

Table 3
Range of cooking times and estimated marginal mean values (emmeans) for cooking time, iron concentrations, iron bioavailability, and dietary fiber components (AOAC, 2011.25) of different market classes of yellow and non-yellow beans. Values are emmeans ± SE except for the ranges. Emmeans sharing the same letter in each column are not significantly different (α = 0.05).

Market Class		Cooking time (minutes)		100-seed weight	Iron	Iron bioavailability	Dietary fiber			
	N[a]	Range	Emmean	(g)	(μg/g)	(% white kidney control)	IDF (%)	SDF (%)	OLIGO (%)	TDF (%)
Amarillo dk. (*orange*)	10	16.0–38.2	27.7 ± 2.1 NS[b]	36.1 ± 2.0 ab	69.0 ± 1.9 NS	46.4 ± 5.4 a	18.9 ± 0.4 NS	5.1 ± 0.2 ab	2.5 ± 0.1 ab	26.5 ± 0.4 a
Amarillo lt. (*orange-yellow*)	13	16.4–68.2	25.1 ± 1.6	30.4 ± 1.7 a	73.0 ± 1.6	60.6 ± 4.8 a	19.0 ± 0.3	5.1 ± 0.2 a	2.6 ± 0.1 ab	26.6 ± 0.4 a
Canario	7	19.8–36.6	27.3 ± 2.4	41.4 ± 2.4 bc	73.6 ± 2.2	41.9 ± 6.5 a	18.1 ± 0.5	6.1 ± 0.2 bc	2.6 ± 0.1 ab	26.8 ± 0.5 ab
Green-yellow	2	22.8–26.0	24.3 ± 4.0	47.3 ± 4.4 bc	79.2 ± 4.2	56.5 ± 12.2 a	17.2 ± 0.9	6.4 ± 0.4 abc	2.8 ± 0.2 ab	26.4 ± 1.0 ab
Manteca	7	18.9–36.5	23.6 ± 2.1	48.1 ± 2.4 c	71.7 ± 2.2	117.9 ± 6.5 b	18.1 ± 0.5	6.5 ± 0.2 c	2.6 ± 0.1 ab	27.3 ± 0.5 ab
Mayocoba	13	14.6–43.6	23.8 ± 1.5	43.1 ± 1.7 bc	74.2 ± 1.6	100.2 ± 4.8 b	18.2 ± 0.3	6.3 ± 0.2 c	2.4 ± 0.1 a	26.9 ± 0.4 ab
Non-yellow (*white, beige, brown, red*)	8	20.2–47.9	28.9 ± 2.4	48.5 ± 2.2 c	68.9 ± 2.1	45.6 ± 6.1 a	19.6 ± 0.4	6.1 ± 0.2 bc	2.8 ± 0.1 b	28.5 ± 0.5 b

Cooking time was log-transformed, and SDF and TDF were square-transformed before emmeans calculation and multiple comparisons of each market class using Tukey's honestly significant test (α = 0.05). Emmeans listed in the table are back-transformed.
See the footnote of Table 1 for detailed descriptions of the traits.
IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF + SDF + OLIGO).
[a] Number of genotypes.
[b] Not significant.

4. Discussion

4.1. Cooking time measurements of yellow beans

Cooking time among the 60 selected genotypes ranged from 14.6 to 68.2 min with an overall mean of 27.3 min after 12 h-soak (Table 1). When interpreting the cooking time distributions listed in Table 1, Table 2, Tables 3 and it is important to note that these beans were purposely selected for their fast-, moderate-, and slow-cooking traits to evaluate how DF, iron concentrations, and iron bioavailability differed among the three cooking classes. The cooking times in this study should also be interpreted knowing that the beans were stored under ideal storage conditions, soaked in distilled water for 12 h before cooking, and boiled in distilled water under a supervised heating source, resulting in the shortest possible cooking times for each genotype. Moreover, storing beans in high-humidity and high-temperature conditions or using mineral-containing water for cooking could result in longer cooking times, which could make the differences in the cooking times between the fast- and slow-cooking genotypes more drastic. In this study, cooking times were measured on pre-soaked beans in boiling distilled water; however, it should be noted that beans are often cooked unsoaked in many cultures and require significantly longer cooking times. Nevertheless, pre-soaked cooking times of beans can be a predictor of unsoaked cooking times due to the high correlation between them (Cichy et al., 2019; Mendoza et al., 2018).

4.2. Yellow beans are a rich source of iron, but their market classes differ in iron bioavailability

Iron bioavailability was higher in the fast-cooking beans despite the similar iron concentrations of the three cooking classes (Table 2), supporting the findings of previous studies that demonstrate the

Table 4
Correlations between dietary fiber, cooking time, and iron bioavailability.[a].

	Cooking time		Iron Bioavailability	
	Pearson's r	*p*-value	Pearson's r	*p*-value
Yellow market classes only (n = 52)				
Cooking time	–	–	−0.294	0.002[b]
100-seed weight	−0.127	0.198	0.365	<0.001[b]
Iron concentration	−0.174	0.077	0.034	0.734
IDF	0.333	0.001[b]	−0.212	0.031[b]
SDF	0.103	0.300	0.186	0.059
Insoluble/Soluble Ratio	−0.039	0.691	−0.116	0.240
OLIGO	−0.109	0.270	0.000	0.999
TDF	0.349	<0.001[b]	−0.073	0.459
All market classes (n = 60)				
Cooking time	–	–	−0.302	0.001[b]
100-seed weight	−0.084	0.360	0.177	0.053
Iron concentration	−0.226	0.013[b]	0.054	0.561
IDF	0.360	<0.0001[b]	−0.200	0.029[b]
SDF	0.153	0.097	0.139	0.128
Insoluble/Soluble Ratio	−0.056	0.542	−0.091	0.325
OLIGO	−0.086	0.349	−0.091	0.319
TDF	0.396	<0.0001[b]	−0.113	0.219

[a] Correlation coefficients and associated *p*-values comparing the relationship between dietary fiber (% dry weight), iron concentrations (μg/g), cooking time (minutes) and iron bioavailability (% of white kidney control) of soaked, cooked, drained, lyophilized, and milled whole beans. IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF + SDF + OLIGO).
[b] *p*-values ≤0.05 are considered statistically significant.

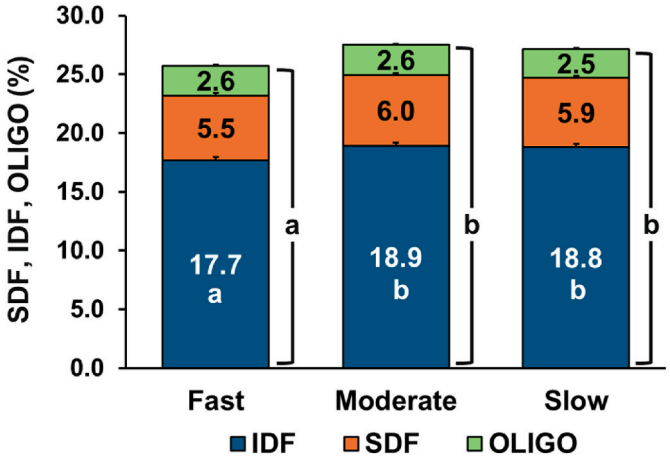

Fig. 2. The estimated marginal mean values (emmeans) of dietary fiber (AOAC2011.25) components for each cooking class of the 52 yellow bean accessions. Standard errors for the emmeans are shown as error bars. Mean differences in IDF and Total Dietary Fiber (IDF + SDF + OLIGO) between the cooking classes are shown with alphabets. SDF and OLIGO were not statistically different among the cooking classes (α = 0.05). IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose).

relationship between fast-cooking yellow beans and iron bioavailability (Wiesinger et al., 2018, 2019). The higher means of iron bioavailability for Manteca and Mayocoba beans were an interesting comparison with a previous study that measured iron bioavailability of selected yellow beans from breeding programs in the U.S. and Canada, and found that four Mayocoba genotypes, which had moderate cooking time (28–33 min), had lower iron bioavailability (~50–80% of white bean reference) (Wiesinger et al., 2018). The four Mayocoba genotypes were not included in the present study, but it suggests that genetic variability exists in iron bioavailability within Mayocoba type. Genetic diversity in iron bioavailability within yellow beans with various shades of yellow was also reported in a study with 275 genotypes from the YBC, which included 56 Mayocoba genotypes (Izquierdo et al., 2024). The highest iron bioavailability was measured in genotypes from the Manteca class (Fig. 1B) as described previously (Hart et al., 2020; Wiesinger et al., 2018). The pale-yellow Manteca beans have no condensed tannins in their seed coats, which improves the performance of iron delivery in the upper small intestine during digestion (Hart et al., 2020; Beninger et al., 1998; Wiesinger et al., 2019).

4.3. DF compositions of yellow beans compared to other market classes and gene pools after cooking

A preceding study measured the TDF concentrations in 261 Middle American bean genotypes of various seed types using the AOAC 2011.25 method, and reported overall mean concentrations of 14.1% for IDF, 7.65% for SDF, 4.39% for OLIGO, and 26.2% for TDF (Moghaddam et al., 2018). Similar values were reported by Brick et al. (2022) (Brick et al., 2022) with 275 Middle American bean genotypes using the same AOAC 2011.25 method. These two studies reported lower IDF (~14%) than the current study (~19%). The previous two studies cooked beans by autoclaving (i.e., pressure-cooking to emulate the commercial canning process), whereas in the current study, bean samples were pre-soaked, drained, boiled in distilled water (under atmospheric pressure), and drained to reproduce traditional stovetop home cooking conditions. Past research has shown that pressure-cooking resulted in a more pronounced decrease in cellulose and hemicellulose, the main constituents of IDF (Singh et al., 2017), than boiling under atmospheric pressure (Rehinan et al., 2004). This seems to explain the lower IDF concentrations in pressure-cooked beans when compared to the boiled beans of this study. In addition, discarding soaking and cooking water seems to have resulted in lower OLIGO (2.6%) in this study than the previous two studies (4.39%), where the soaking water was used as cooking water and was retained after pressure-cooking. OLIGOs are known to decrease by about 50% upon pre-soaking and cooking due to leaching – if the soaking and cooking water is discarded (Kosson and Bakowski, 1986; Barampama and Simard, 1993; Thirunathan and Manickavasagan, 2019). DF measurements should be conducted with bean samples cooked with an appropriate method for their target regions because typical soaking (or the lack thereof) and cooking methods differ among regions worldwide (Borchgrevink, 2012).

The Andeans had higher SDF than Middle Americans (Table 5), corroborating the findings by Kleintop et al. (2013) (Kleintop et al., 2013) that four Andean genotypes had a higher mean of SDF in cooked seeds than 21 Middle American genotypes. The current study had a more balanced sample size with the Middle American genotypes further separated into two races (seven Jalisco/Durango, and 13 Mesoamerica) and 21 Andeans. TDF and OLIGO concentrations were not different between gene pools; therefore, it is possible that Andean and Middle American genotypes differ in the composition of SDF and IDF after cooking. Seed size may be part of the reason, as Andean beans have larger seeds in general, which makes the cotyledon (that is solubilized upon cooking) percentage relatively larger than Middle American beans. In this study, Andean yellow beans had the largest mean seed size, followed by Jalisco/Durango, and then Mesoamerica (Table 5), and 100-seed weight was significantly correlated with SDF ($r = 0.433$, $p < 0.0001$). Further research could be useful to investigate the implications of the gene pool-wise differences in TDF components for bean breeding.

Moghaddam et al. (2018) (Moghaddam et al., 2018) also found that newer cultivars of Middle American race Durango had higher IDF than old cultivars observed since 1997 as a result of breeding. A speculation is that beans with higher IDF might have been indirectly selected because beans that retain their shape without splits after canning were selected. Higher IDF is associated with thicker seed coat and longer cooking times (Bassett et al., 2021), which lead to seed coat intactness after canning, which is one of the most important quality traits in dry bean breeding in the U.S. (Miklas et al., 2022). It would be of interest to compare new and old cultivars of Andean origin for DF components.

Table 5
Range of cooking times and estimated marginal mean values (emmeans) for cooking time, iron concentrations, iron bioavailability, and dietary fiber components (AOAC, 2011.25) from different gene pools and races of yellow beans. Values are emmeans ± SE except for the ranges. Emmeans sharing the same letter in each column are not significantly different (α = 0.05).

Gene pool		Cooking time (minutes)		100-seed weight	Iron	Iron bioavailability	Dietary fiber			
	N[a]	Range	Emmean	(g)	(μg/g)	(% white kidney control)	IDF (%)	SDF (%)	OLIGO (%)	TDF (%)
Admix	11	14.6–43.6	26.6 ± 1.9 NS[b]	39.4 ± 1.1 b	71.1 ± 1.8 NS	75.4 ± 7.1 b	18.2 ± 0.4 NS	6.6 ± 0.2 c	2.4 ± 0.1 NS	27.2 ± 0.4 NS
Andean	21	18.7–38.2	25.0 ± 1.3	47.0 ± 1.0 c	75.3 ± 1.3	85.4 ± 5.1 b	18.1 ± 0.3	6.1 ± 0.1 bc	2.6 ± 0.1	26.7 ± 0.3
Jalisco/ Durango	7	19.5–35.2	24.5 ± 2.2	35.5 ± 1.2 b	70.1 ± 2.2	75.9 ± 8.9 ab	19.4 ± 0.4	5.6 ± 0.3 ab	2.7 ± 0.1	27.6 ± 0.5
Mesoamerica	13	16.0–68.2	25.2 ± 1.7	27.0 ± 0.7 a	71.2 ± 1.6	48.6 ± 6.5 a	18.8 ± 0.3	4.7 ± 0.2 a	2.5 ± 0.1	26.1 ± 0.4

Cooking time was log-transformed, and SDF and TDF were square-transformed before emmeans calculation and multiple comparisons of each genepool categories using Tukey's honestly significant test (α = 0.05). Emmeans listed in the table are back-transformed.
See the footnote of Table 1 for detailed descriptions of the traits.
IDF: insoluble dietary fiber; SDF: soluble dietary fiber; OLIGO: total oligosaccharides (raffinose + stachyose + verbascose); TDF: total dietary fiber (IDF + SDF + OLIGO).
[a] Number of genotypes.
[b] Not significant.

4.4. Lower IDF concentrations are associated with shorter cooking times in dry beans

Lower IDF in fast-cooking beans found in this study (Fig. 2) was consistent with the finding by Bassett et al. (2021), which showed that cooking time was positively correlated with insoluble cotyledon cell wall isolate and IDF in whole seeds for 12-h pre-soaked beans (Bassett et al., 2021). Bean cooking is a hydrothermal process where seeds imbibe water allowing for cell swelling, while pectin in the middle lamella that cements adjacent cells is solubilized, leading to cell separation and bean softening (Shomer et al., 1990; Bernal-Lugo et al., 1997). Therefore, higher insoluble cotyledon cell wall components, contributing to higher IDF in the whole seeds, are likely to lead to a longer cooking time required for cell wall solubilization and cell separation. Similarly, whole cooked seeds of fast-cooking genotypes had higher SDF than their slow-cooking counterparts for three out of four market classes including yellow bean (Hooper et al., 2016). Evidence is accumulating that high SDF and low IDF are important for short cooking times in beans. Yellow beans could be used as a genetic resource for progressing research on the relationship between TDF and cooking time and for improving DF in beans. In this study, the fast cooking group had 6% less TDF than the moderate- or slow-cooking counterparts (Table 2). However, beans in general have higher TDF than cereal grains (Rainakari et al., 2016), and beans with 6% less TDF would still deliver the health benefits of dietary fiber.

4.5. Lower IDF concentrations are associated with higher iron bioavailability in yellow beans

The correlations between IDF, cooking time, and iron bioavailability among the yellow beans supported previous findings that pale-colored, fast-cooking beans generally exhibit high iron bioavailability (Wiesinger et al., 2016, 2018). The relationship between the IDF and iron bioavailability of yellow beans is more evident due to the general presence of the yellow-colored, iron absorption-promoting Kaempferol compounds in their seed coats (Hart et al., 2020). In contrast, these correlations are more difficult to detect in dark colored brown, red, and black beans because of the overwhelming effect anthocyanins and condensed tannins can have on the physicochemical properties of beans after cooking, exhibiting uniformly longer cooking times and low iron bioavailability regardless of their TDF concentrations (Hart et al., 2020; Elia et al., 1997; Wiesinger et al., 2019, 2021; Hooper et al., 2016). The high correlation between seed weight and iron bioavailability (r = 0.365, $p < 0.001$) was mainly because Manteca and some Mayocoba beans are large-seeded and had high iron bioavailability, but this correlation did not hold ($r = 0.177$, $p = 0.053$) with non-yellow controls due to the large-seeded brown, red, and beige beans having low iron bioavailability (Table 4, Fig. S4), reiterating that iron bioavailability should be considered market class specific.

4.6. Variations in the concentrations of OLIGO and their implications to breeding

Wide variations in OLIGO were detected in both yellow and non-yellow beans (Table 1, Fig. S2A). In particular, several Mayocoba genotypes were low in OLIGOs (Fig. S2A, Table S1). OLIGOs are considered to be responsible for flatulence after bean consumption along with other soluble fiber components (Granito et al., 2001; Martínez-Villaluenga et al., 2008). Thus, beans with decreased OLIGO levels would likely be favorable to consumers who suffer from digestive discomfort after eating oligosaccharide-rich foods like beans (Thompson, 2019). The low OLIGO Mayocoba genotypes (PR1146-124 and PR1146-138) may be a useful genetic resource for reduced flatulence beans. They are both breeding lines from the University of Puerto Rico with virus and leafhopper resistance (Beaver et al., 2016). No OLIGO measurements were previously available for these lines. Conversely, OLIGOs are important molecules in plant survival – OLIGOs play an important part in plant defense mechanisms against certain diseases, cold temperatures, and water stress. In addition, OLIGOs are beneficial to human health as prebiotics (Martínez-Villaluenga et al., 2008; ElSayed et al., 2014; Elango et al., 2022). For human health, OLIGOs are fermented in the colon and act as substrate for the production of short-chain fatty acids, which are found to have various anti-tumor and gut health-promoting effects (Gill et al., 2018). Given the potential benefits of OLIGOs, breeders would want to pay close attention to possible tradeoffs for reducing OLIGOs. On the other hand, beans with low OLIGO concentrations could attract new consumers who were once wary of eating beans due to their poor digestibility (Thompson, 2019). This study showed no relationship between the concentrations of OLIGOs, cooking time, and iron bioavailability in yellow beans (Table 4), indicating the health benefits of consuming OLIGOs from cooked beans can still be achieved when selecting new bean varieties with short cooking times and improved iron bioavailability.

5. Conclusions

This study demonstrates that the TDF compositions of fast cooking yellow beans across multiple market classes are unique to slower cooking yellow beans. The results show that lower concentrations of IDF and TDF are associated with shorter cooking times in yellow beans, such that faster cooking beans have about 6% less TDF than their slower cooking counterparts. These observations lend support to the hypothesis that lower IDF concentrations are indicative of thinner seed coats, a larger seed size (e.g. Andean gene pool), and thinner cotyledon cell walls in beans with shorter cooking times. These same features also appear to increase the iron bioavailability of yellow beans; especially in pale-yellow Manteca and bright yellow Mayocoba beans that have very low concentrations of condensed tannins, which can inhibit the absorption of iron. Interestingly, OLIGO concentrations were not associated with the cooking time or iron bioavailability of yellow beans. However, some fast-cooking Mayocoba beans have low OLIGOs, which creates a unique opportunity for breeders to balance the health-promoting effects of consuming beans with the ease in digestibility. Easy-to-prepare and easy-to-digest yellow beans could be an innovative food option to help address the DF needs of busy consumers.

CRediT authorship contribution statement

Rie Sadohara: data collection, Formal analysis, Writing – original draft, Writing – review & editing. **Jason A. Wiesinger:** conceive experiment, data collection, Formal analysis, Writing – original draft, Writing – review & editing. **Henry J. Thompson:** data collection, Funding acquisition, Writing – review & editing. **Raymond P. Glahn:** Funding acquisition, Writing – review & editing. **Karen Cichy:** conceive experiment, Funding acquisition, Writing – review & editing.

Data availability

The original data used in this study are available for download at: https://github.com/RieSadohara/YBC_Fiber/tree/main.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Karen Cichy reports financial support was provided by National Institute of Food and Agriculture. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100942.

Abbreviations

DF	Dietary Fiber
IDF	Insoluble Dietary Fiber
MA	Middle American
OLIGO	Oligosaccharides
SDF	Soluble Dietary Fiber
TDF	Total Dietary Fiber
YBC	Yellow Bean Collection

Data availability

Data is available in supplemental files associated with this manuscript.

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