



Effect of extrusion on folic acid concentration and mineral element dialyzability in Great Northern beans (*Phaseolus vulgaris* L.)



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ABSTRACT

Great Northern beans (GNB) contain appreciable magnesium (Mg), potassium (K), phosphorus (P), and iron (Fe), together with the heat-labile vitamin, folate, and the anti-nutritional compound phytate. Thus, the objective was to increase dialyzability of essential mineral elements while degrading phytate and minimizing destruction of folate through extrusion of GNB. Extrusion resulted in significant ($p < 0.05$) increases in dialyzability of Mg, P, K, and Fe by as much as 50%, 30%, 5%, and 79%, respectively, while decreasing cadmium (Cd) dialyzability. Screw speed (SS) had a significant quadratic effect on dialyzability of all elements. Low MC resulted in a significant reduction (46%) in phytate, although this was accompanied by as much as 24% destruction of folate. In conclusion, low barrel temperature, medium MC and high SS were identified as the optimum conditions to maximize essential mineral element dialyzability and folate retention while minimizing phytate and dialyzable Cd.

1. Introduction

Despite the availability of food together with fortification, supplementation, and enrichment, there are several micronutrients, including magnesium (Mg), potassium (K), choline, calcium (Ca), iron (Fe), and vitamins A, D, E, and C, that are under-consumed in the US (U.S. Department of Health and Human Services, 2015). Additionally, while folate deficiency is nearly non-existent in the US, the major source of folate is fortified, processed food, which can have a negative connotation (Odewole et al., 2013). Individuals with chronic moderate deficiencies of these essential nutrients can develop conditions like hypertension, coronary heart disease, diabetes, and metabolic syndromes that are common in the US (Long & Romani, 2015). Thus, it is important to promote the consumption of food sources that are naturally rich in under-consumed micronutrients to tackle nutrient deficiencies and encourage healthy eating habits.

Typically, dry beans (*Phaseolus vulgaris* L.), such as Great Northern beans (GNB), are valued from a nutritional standpoint for their high protein and dietary fiber contents. However, they are also good sources (i.e., > 10% of the US Daily Value) of folate and the essential minerals Mg, Fe, K, and phosphorus (P) (Office of Dietary Supplements and National Library of Medicine, 2018; U.S. Department of Agriculture, 2018), making dry beans an excellent source of many under-consumed nutrients in the US diet.

However, the availability of these nutrients for absorption is not only dependent on their concentrations, but also on factors like anti-nutritional compounds, processing techniques, and physicochemical state of the nutrient (Fairweather-Tait, 1993). For example, phytate is a naturally occurring compound present in grains and legumes, including GNB, and can form insoluble complexes with essential elements and reduce their absorption (Thompson, 1993). Different processing techniques like germination, pressure-cooking, and extrusion have been shown to reduce phytate, but the extent of reduction depends on the raw material and processing technique (Nergiz & Gokgoz, 2007).

Unfortunately, the techniques adapted for reducing these anti-nutritional factors and increasing mineral element bioavailability can adversely affect other important nutrients like the heat labile folates. Additionally, while heavy metals are typically not abundant in GNB, the same processes that increased bioavailability of essential mineral elements may also increase bioavailability of toxic heavy metals like cadmium (Cd) (Watzke, 1998). Thus, it is important to identify processing techniques that can enhance the bioavailability of essential mineral elements while minimizing loss in important labile vitamins and reducing bioavailability of heavy metals. One such processing technique may be extrusion.

Extrusion has shown promising results in improving the bioavailability of mineral elements, and reducing anti-nutritional compounds in legumes and cereal products (Alonso, Rubio, Muzquiz, & Marzo, 2001;

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Hazell & Johnson, 1989). However, no research has simultaneously focused on the effect of extrusion on vitamins and bioavailability of essential mineral elements and heavy metals in dry beans.

Bioavailability is a complex process that is defined as the amount of an ingested nutrient that is absorbed and is available for physiological function while bio-accessibility is the amount of an ingested nutrient that has the potential to be absorbed and utilized (Etcheverry, Grusak, & Fleige, 2012). *In vitro* dialyzability measures the proportion of the total elements that diffuse through a membrane during food matrix digestion (Miller, Schrickler, Rasmussen, & VanCampen, 1981) and is commonly employed to measure the bio-accessibility of mineral elements. This method can also be used as a screening tool to assess if a certain process would have an effect on bioavailability when moved to more complex models.

Thus, the objective of this study was to determine the effect of extrusion on degradation of folate and dialyzability of Mg, Fe, K, P, Fe, and Cd from GNB flour and identify conditions that achieve maximum dialyzability of the essential mineral elements while minimizing dialyzability of heavy metals and degradation of folate. The results from this study can serve as the basis for increasing bioavailability of essential nutrients from processed GNB flour to promote dry beans in the diet.

2. Materials and methods

2.1. Materials

Great Northern beans (GNB) were obtained from FNJ Inc. (Alta Loma, CA, USA) and milled using a pilot scale hammer mill (20SSHMBD, C.S. Bell, Tiffin, OH, USA) with screen size of 0.7 mm. The flour was analyzed for moisture, fat, and ash using approved methods (AACC International, 2018). Protein content was analyzed using a nitrogen analyzer (FP 528, Leco, St. Joseph, MI, USA) with a protein factor of 6.25. Total starch content was analyzed using a total starch assay kit (K-TSTA, Megazyme, Bray, Ireland) following the KOH format. GNB flour was stored at 4 °C until extrusion.

2.2. Experimental design

The effect of extrusion on GNB flour was studied by varying three extrusion factors: barrel temperature [Temp (90–140 °C)], feed moisture content [MC (17–25%)], and screw speed [SS (156–250 rpm)], while keeping other factors such as feed rate and screw configuration constant. The levels of these factors were determined based on preliminary trials. The commonly used central composite rotatable design (CCRD) was used, except Temp was not randomized during the experiment due to equipment operating constraints. Thus, experiments were conducted in increasing order of Temp while randomizing MC and SS. Temp was tested at three fixed levels: –1, 0, +1 (i.e., 90, 115 and 140 °C), respectively. In order to maintain orthogonality of the experiment, the design space was rotated by 45°, which resulted in new axial ($\pm a = 1.43$) and factorial ($\pm b = 0.70$) points for MC ($+a = 25\%$; $-a = 17\%$; $+b = 22.5\%$; $-b = 19\%$) and SS ($+a = 250$ rpm; $-a = 156$ rpm; $+b = 225$ rpm; $-b = 180$ rpm). The new values for MC and SS were calculated based on the ratio of block error variance and experimental error obtained during preliminary experimentation (Draper & John, 1998). Based on the design, extrusion was carried under 18 experimental conditions (MC, SS, Temp): $\pm a, 0, 0$ (2 runs); $0, \pm a, 0$ (2 runs); $\pm b, \pm b, \pm 1$ (8 runs); and $0, 0, 0$ (6 runs) (Table 1).

2.3. Extrusion process

To adjust the MC of GNB flour, batches (2 kg) representing each experimental run were blended in an upright mixer (H-600-D, Hobart, Troy, OH, USA) at medium speed with the required water to obtain the

target moisture content according to the experimental design. The samples were then sealed in polyethylene bags and tempered for 16 h at 4 °C. The GNB flour was then fed into the extruder barrel using a single screw volumetric feeder (FW 40 Plus, C. W. Brabender, Hackensack, NJ, USA) set at a constant delivery rate of 76 g/min.

A laboratory scale co-rotating conical twin screw extruder with mixing zones was used for extrusion (CTSE-V, C.W. Brabender, Hackensack, NJ, USA). The specifications of extruder and operating conditions used were the same as described in Gulati, Weier, Santra, Subbiah, and Rose (2016).

The extrudate sample for each experimental condition was collected after a stable temperature and torque reading was observed. The collected samples were dried in a belt drier (4800 series Wenger, Sabetha, KS, USA) at 100 °C for 10 min and ground using cyclone sample mill (UDY, Fort Collins, CO, USA) with a screen size of 1 mm. The ground extruded samples were stored at 4 °C until analysis.

2.4. Phytic acid content

Phytic acid in GNB flour and extrudates was quantified as phytate phosphorus using 2,2'-bipyridine as described (Haug & Lanstzsch, 1983), with slight modifications. Briefly, phytic acid was extracted from the sample (250 mg) using 0.2 N HCl (10 mL) overnight at 4 °C with gentle shaking. The contents were centrifuged and the supernatant was used for analysis after dilution with distilled water (25 mL). For unprocessed flour, 0.25 mL of the diluted supernatant was mixed with 0.75 mL of 0.2 N HCl; for extrudates, 0.5 mL of the diluted supernatant was mixed 0.5 mL of 0.2 N HCl. One mL of 415 μM $\text{Fe}(\text{NH}_4)(\text{SO}_4)_2$ (Sigma-Aldrich, St. Louis, MO, USA) was then added to diluted extracts and tubes were placed in a boiling water bath for 30 min. The tubes were cooled immediately and contents centrifuged. One mL of the supernatant was mixed with 1.5 mL of 2, 2'-bipyridine. The color was measured at 530 nm. The samples were quantified by means of external calibration using sodium phytate dodecahydrate (Sigma-Aldrich, 71649) which contained 19% phytate phosphorus as measured with inductive coupled plasma mass spectrometry (ICP-MS) as described later (Section 2.7).

2.5. Folic acid content

Total folates were measured using the standard microbiological assay (*L. casei* subsp. *Rhamnosus*, ATCC no. 7469) with the tri-enzyme extraction technique (AACC method 86-47; DeVries, Keagy, Hudson, & Rader, 2001). The analysis was conducted on replicated sample submissions by a commercial lab (NP Analytical Laboratories, St. Louis, MO, USA).

2.6. *In vitro* digestion

In order to measure the dialyzability of elements, bean flour was digested under the *in vitro* conditions described by Luten et al. (1996), with some modifications. The modifications were to reduce sample weights and volumes to fit in a 48-well dialysis plate format (Rapid Equilibrium Dialysis Plate, MWCO 8 K Dalton, ThermoScientific, 90006, Waltham, MA, USA). For digestion, 20 mg of sample was weighed in the sample chamber and mixed with 0.2 mL of pepsin (284 units/mg, P7000, Sigma-Aldrich) solution (50 mg/mL in 50 mM HCl). The plate was covered with a sealing tape (15036 ThermoScientific), and incubated at 37 °C for 2 h with gentle shaking at 125 rpm. Pepsin digestion was stopped by adding 0.25 mL of dialysis buffer (0.1 M NaHCO_3) in the buffer chamber and the mixture incubated for 55 min under previously described conditions. The amount of dialysis buffer added was pre-determined by titrating the gastric mixture with dialysis buffer until the pH reached 6. Meanwhile a pancreatin-bile solution (0.4 g pancreatin; 2.5 g bile salts) was prepared in 10 mL of 0.1 M NaHCO_3 . Given the low solubility of pancreatin, the solution was centrifuged and

Table 1
Experimental design used for extrusion processing of Great Northern bean (GNB) flour.^A

Run	Coded values				Actual values				
	Temp (°C)	SS (rpm)	MC (%)	T (°C)	SS (rpm)	MC (%)	T (°C)	SS (rpm)	MC (%)
1	-1	b	b	-1	0.7	0.7	90.0	225	23.1
2	-1	-b	-b	-1	-0.7	-0.7	90.0	180	18.9
3	-1	-b	b	-1	-0.7	0.7	90.0	180	23.1
4	-1	b	-b	-1	0.7	-0.7	90.0	225	18.9
5	0	0	0	0	0.0	0.0	115	203	21.0
6	0	0	0	0	0.0	0.0	115	203	21.0
7	0	-a	0	0	-1.43	0.0	115	156	21.0
8	0	0	0	0	0.0	0.0	115	203	21.0
9	0	a	0	0	1.43	0.0	115	250	21.0
10	0	0	0	0	0.0	0.0	115	203	21.0
11	0	0	0	0	0.0	0.0	115	203	21.0
12	0	0	a	0	0.0	1.43	115	203	25.3
13	0	0	0	0	0.0	0.0	115	203	21.0
14	0	0	-a	0	0.0	-1.43	115	203	16.7
15	1	b	b	1	0.7	0.7	140	225	23.1
16	1	-b	-b	1	-0.7	0.7	140	180	23.1
17	1	-b	b	1	-0.7	-0.7	140	180	18.9
18	1	b	-b	1	0.7	-0.7	140	225	18.9

^A Temp, barrel temperature; MC, feed moisture content; SS, screw speed.

0.05 mL of the supernatant was added to each sample chamber, covered with sealing tape and incubated for 2 h. After digestion, the dialysis buffer (0.07–0.08 mL) was collected from the buffer chamber and used to quantify dialyzable elements. In order to avoid element contamination from enzymes, both pepsin and pancreatin enzyme solutions were dialyzed using centrifugal filter devices (Centriprep 10 K, Merck Millipore, Burlington, MA, USA). Enzyme powders were suspended in 15 mL of HCl for pepsin or 15 mL of NaHCO₃ for pancreatin and placed in the outer chamber of the device. The device was then centrifuged four times at 3000 g for 30 min each at 4 °C to remove contaminating elements. Between each centrifugation, additional buffer was added to the outer chamber to restore the original volume. In preliminary testing, there was no significant reduction in activity of these enzymes before or after dialysis.

2.7. Total and dialyzable element concentrations

The total concentration of mineral elements in GNB bean flour and extrudates were quantified after wet ashing. Briefly, 500 mg of sample was digested using 4 mL of concentrated nitric acid at 100 °C for 1 h. The tubes were cooled to room temperature, mixed with 4 mL of hydrogen peroxide (30%) and heated for 1.5 h on a heating block set at 125 °C. A second volume of hydrogen peroxide was then added and tubes were heated at 150 °C until the sample dried. The dried samples were re-suspended in 10 mL of 1% nitric acid, mixed, and used for element analysis (Guttieri, 2014).

Element analysis in unprocessed wet ashed samples and dialysis buffer collected after digestion and dialysis (Section 2.6) was performed using inductively coupled plasma mass spectrometry (7500cx, Agilent Technologies, Santa Clara, CA, USA) operating in kinetic discrimination mode with helium gas at 5 mL/min and 50 ppb Ga as an internal standard. Sample (40 µL) was injected using a micro peripump, and 2% nitric acid was used for rinsing between runs. Each sample was analyzed in duplicate. The method was optimized for the analysis of Mg, Fe, P, K, and Cd, as well as lithium, boron, sodium, sulfur, calcium, vanadium, chromium, manganese, cobalt, nickel, copper, zinc, arsenic, selenium, and molybdenum. Only the results for Mg, Fe, P, K, and Cd are discussed in this paper since GNB has greater than 20% DV of these essential elements and Cd is a heavy metal of concern. The results were calculated in mg/kg sample for model fitting. For presentation of the data, units were converted to the percentage of total elements that were dialyzable compared with unprocessed GNB flour.

2.8. Data analysis

The data were analyzed using JMP statistical software (JMP version 10.0.0, SAS institute, Cary, NC, USA). The following second order model was fitted for each response variable:

$$Y_{ijk} = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2j} + \beta_3 x_{3k} + \beta_{11} x_{1i}^2 + \beta_{22} x_{2j}^2 + \beta_{33} x_{3k}^2 + \beta_{12} x_{1i} x_{2j} + \beta_{13} x_{1i} x_{3k} + \beta_{23} x_{2j} x_{3k}$$

where, Y was the response (folic acid, phytic acid, total and dialyzable Mg, K, P, Ca, Fe and Cd) for *i*th level of x_1 (Temp); *j*th level of x_2 (MC) and *k*th level of x_3 (SS). The models were checked using ANOVA (F-test), the lack-of-fit test, and adjusted R² values. Results of extruded samples were compared with unprocessed GNB flour using Dunnett's multiple comparison test at a significance level of 0.05, with extrudate results averaged over low, medium and high MC, SS and Temp settings separately. Correlations among response variables were calculated using Pearson's method and further used to conduct principal components analysis (PCA).

To predict extrusion conditions that would result in the highest folic acid and dialyzable Mg, P, K, and Fe together with the lowest phytic acid and dialyzable Cd, a simultaneous optimization technique was used, which makes use of a desirability function (Myers & Montgomery, 2009). All responses were given equal weight in the optimization process. The desirability function (*d_i*) ranges from 0 to 1 and was constructed for each response with targets to minimize phytic acid content and Cd dialyzability while maximizing folic acid and dialyzable Mg, P, K, and Fe. Overall desirability was calculated using the following equation:

$$D = (d_1 d_2 \dots d_m)^{1/m}$$

where, *m* = number of responses (7).

3. Results and discussion

3.1. Composition of Great Northern bean flour

The GNB flour used for extrusion had the following composition (mean of 3 replicates ± standard deviation, wet basis): 14.1 ± 0.9% moisture; 21.6 ± 0.2% protein; 1.2 ± 0.1% crude fat; 40.1 ± 0.9% starch; 2546 ± 65 mg/kg Mg; 6379 ± 85 mg/kg P; 19178 ± 855 mg/kg K; 58.6 ± 3.4 mg/kg Fe; 0.029 ± 0.014 mg/kg Cd; 1430 µg/kg folic acid; 4.33 ± 0.07 mg/g phytic acid.

Table 2
Regression coefficients for each response surface equation and model fit parameters.^A

Parameter ^a	Phytic acid	Folic acid	Dialyzability				
			Mg	P	K	Fe	Cd
Intercept	6.44	36.8	3235	12,469	15,574	47.6	−0.08
SS	−0.01	−1.14	−15.44	−35.8	−26.9	−0.03	0.0003*
MC	0.15*	16.5*	79.50	−451	959	−2.10	0.005*
Temp	0.03*	−0.24	−21.74	−25.9	−146	−0.28	−0.0001
SS ²	0.00016	0.002	0.08*	0.22*	0.15	0.001*	−9.6e−8
SS*MC	0.00	0.02	−0.71*	−1.67	−1.64	−0.02*	−1.6e−5
MC ²	0.00095	−0.47*	−0.13	13.3	−18.5	0.09*	−0.00004
SS*Temp	0.000085	0.001	−0.02	−0.15	0.06	−0.0005	3.4e−7
MC*Temp	0.001*	0.02	0.32	1.07	−0.02	0.01	5.8e−7
Temp ²	0.00012	−0.002	0.08*	0.13	0.56	0.0004	7.6e−8
LOF	NS	NS	NS	NS	NS	NS	NS
Adjusted R ²	0.86	0.83	0.85	0.75	0.20	0.75	0.79
F ratio	12.8*	10.3*	11.8*	6.58*	0.83	5.26*	3.46*

^A Temp, barrel temperature; MC, feed moisture content; SS, screw speed; GNB, Great Northern bean; Mg, Magnesium; P, Phosphorus; K, Potassium; Fe, Iron; Cd, Cadmium LOF, lack of fit; NS, not significant; *significant at $p \leq 0.05$.

3.2. Effect of processing parameters on GNB composition

3.2.1. Model diagnostics

A non-significant lack-of-fit result for all the dependent variables indicated that fitted second order models were appropriate for all the responses (Table 2). Further, all responses, except dialyzable K, had an adjusted R² greater than 0.70, which suggested that the model could explain more than 70% variation in the data. A significant F-ratio for these responses indicated that different extrusion conditions affected the response. For K, a non-significant F-ratio showed that there was no effect of individual extrusion conditions on K dialyzability.

3.2.2. Phytic acid

Extrusion dramatically reduced the concentration of phytic acid in GNB flour by as much as 46% (Fig. 1); however, among the extrusion variables, linear effects of MC and Temp and their interaction significantly affected the extent of change during extrusion (Table 2). Higher values of both MC and Temp resulted in higher measured concentration of phytic acid and thus less degradation. On the other hand, lower MC conditions resulted in reduced phytic acid concentration (Fig. 1).

A similar effect of extrusion on reduction of phytic acid in legumes and other bean varieties has been reported by other researchers (Alonso, Aguirre, & Marzo, 2000; Batista, Prudencio, & Fernandes, 2010). The effect of extrusion on phytate is mainly linked to thermal

hydrolysis of reactive phosphate esters (Sandberg, Andersson, Carlsson, & Sandstrom, 1987). The dephosphorylated phytate no longer possesses the detrimental chelating effects of the native compound. Samples at high feed MC would be exposed to less severe conditions due to the lubricating action of water, resulting in less degradation of phytate, as seen in our study. By the same logic we would expect high Temp to have a severe destructive effect on phytic acid; however, we found that an increase in barrel temperature resulted in less degradation. Similar results were shown when the phytic acid content was averaged over low, med and high conditions of MC, SS and Temp (Fig. 1), although high Temp runs also showed greater variability in the data. We observed a significant interaction between MC and Temp, with increasing values of both resulting in higher phytic acid. This suggests that high feed MC had a greater protective effect on phytate than the destructive effect of high temperature.

3.2.3. Folic acid

Feed MC had a significant quadratic effect on folic acid in GNB, while other extrusion variables had no effect (Table 2). Higher MC resulted in more folate (less degradation) than low moisture conditions (Fig. 2). The observed range of folate in processed GNB were similar to previous reports for other beans (Hefni, Shalaby, & Witthoft, 2014). As mentioned earlier, the observed folate concentrations in extrudates were significantly lower than the folate concentration in GNB flour, which can be explained by the sensitivity of the vitamin to heat,

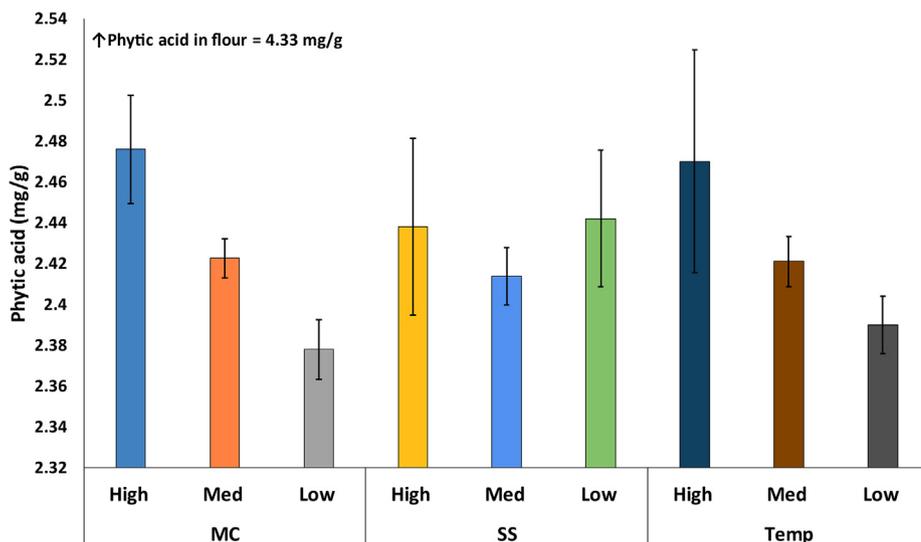


Fig. 1. Phytic acid in extruded samples averaged over low, medium and high conditions of moisture content (MC, %), screw speed (SS, rpm), and barrel temperature (Temp, °C); low and high conditions $n = 5$, medium conditions $n = 8$ with standard error bars plotted; the decrease in phytic acid was a significant for all extrusion conditions when compared with unprocessed Great Northern bean flour ($\alpha = 0.05$).

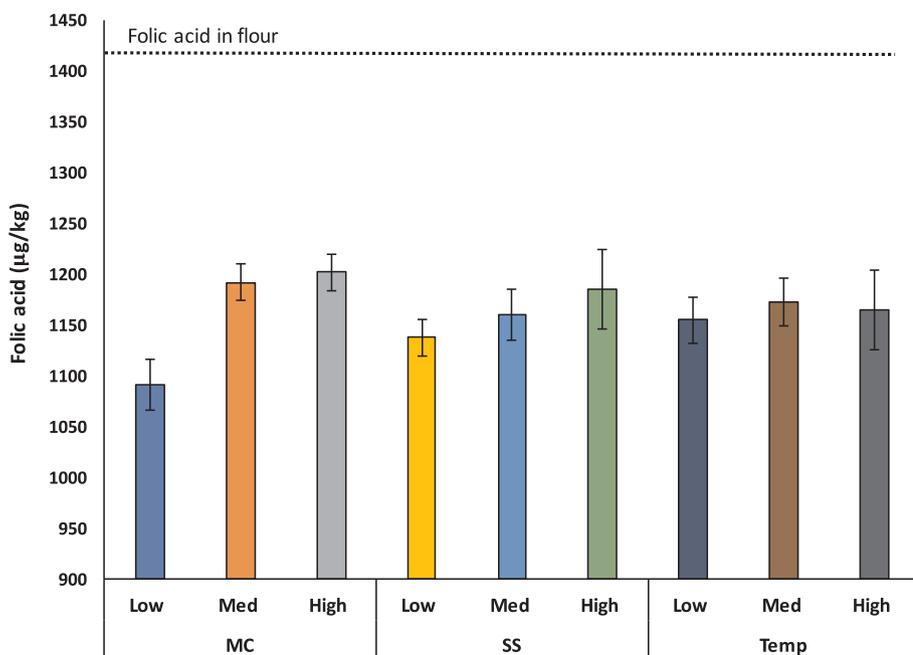


Fig. 2. Folic acid in extruded samples averaged over low, medium and high conditions of moisture content (MC, %), screw speed (SS, rpm), and barrel temperature (Temp, °C); low and high conditions $n = 5$, medium conditions $n = 8$ with standard error bars plotted; the decrease in phytic acid was a significant for all extrusion conditions when compared with unprocessed Great Northern bean flour ($\alpha = 0.05$).

pressure, and shear (Dozier, 2002). The loss in folate during extrusion has been reported by other researchers for different folate-rich grains with thermal degradation and shear identified as the main cause (Ramos-Diaz et al., 2016). The significant effect of MC found in our study can be linked to the protective effect of moisture inside the extruder barrel such that at low moisture conditions the exposure of folate to high temperature resulted in greater thermal degradation. Folate is sensitive to a wide range of processing techniques, but based on our study and previous reports the loss in folate is moderate during extrusion when compared to other techniques like roasting and autoclaving (Kariluoto et al., 2006) or just simple cooking (Xue et al., 2011). Charlton and Ewing (2007) showed that there is complete destruction of folate when exposed to temperatures over 95 °C in other processing techniques; however, in our study at the maximum temperature of 140 °C we observed only moderate loss in folate (except for 1 sample processed at the lowest MC resulting in 24% loss). In fact, MC appeared to be more important to folate retention than Temp. This suggests that extrusion is a complex process and with optimized conditions one can achieve only moderate loss in folate even after processing.

3.2.4. Mineral element dialyzability

Extrusion significantly increased the dialyzability of Mg, P, K, and Fe when compared with unprocessed flour (Fig. 3). While only 19%, 16%, 56%, and 2% of Mg, P, K, and Fe was dialyzed respectively, from the flour, the percent increased up to 38%, 22%, 60%, and 9% for Mg, P, K, and Fe upon extrusion. The maximum improvement in dialyzability was observed for Mg (50% improvement) and Fe (78% improvement) upon extrusion. On the other hand, extrusion greatly reduced the dialyzability of the heavy metal Cd when compared to unprocessed flour: 4% Cd dialyzed from unprocessed GNB flour and as low as 0.4% dialyzed from extruded flour. SS had a significant linear or quadratic effect on the dialyzability of all the elements except K which was not affected by any individual extrusion parameter, as discussed (Table 2). After SS, feed MC had a significant impact on element dialyzability with a quadratic effect and negative interaction with SS for Fe, a negative interaction with SS for Mg, and a linear impact on Cd dialyzability. Temp had a significant effect only on Mg dialyzability.

Several researchers have reported an increase in either apparent absorption, dialyzability or bio-accessibility of these elements after extrusion (Alonso et al., 2001; Hazell & Johnson, 1989), while some reported no change (Drago, Velasco-Gonzalez, Torres, Gonzalez, &

Valencia, 2007). Most of these reports focused on limited extrusion conditions and linked the improvement in availability of these elements to thermal degradation of phytate that is known to form complexes with Fe. In our study, we found a significant effect of SS on dialyzability of elements, which suggests that apart from thermal degradation of interfering substances like phytates, shear during extrusion may facilitate other changes in GNB flour that increase element dialyzability. Alonso et al. (2000) showed that there was a significant improvement in apparent absorption of Mg, P and Fe for an extruded sample enriched with amino acids due to enhanced function of enterocytes with respect to mineral intestinal uptake (Welters, Dejong, Dutz, & Heineman, 1999). Additionally, lignin and other fiber fractions are known to form insoluble complexes with divalent cations (Lestienne, Caporiccio, Besancon, Rochette, & Treche, 2005) and extrusion is known to reduce the lignin fraction in bean fiber while changing the distribution of soluble and insoluble dietary fibers resulting in an improved absorption of divalent cations like Mg and Ca (Sumargo, 2016).

High MC and SS had a significant impact on the amount of dialyzable Cd with higher values increasing Cd dialyzability. Surprisingly, this is opposite to the effect observed for dialyzability of the essential mineral elements. The potential reason for this could be the competition between Cd and other divalent cations to dialyze through the membrane used in the *in vitro* dialyzability test. However, *in vivo* studies have shown that absorption of Cd from food is diminished if the amount of other divalent cations is high (Larsson & Piscator, 1971; Reeves & Chaney, 2008). As mentioned, thermal degradation of phytates or increased soluble dietary fiber during extrusion as can significantly increase the dialyzability of other essential elements, which may result in lower Cd absorption. Since the observed effect of extrusion on Cd dialyzability not been reported before and thus requires further investigation.

Coupling high MC and high SS typically does not result in a desirable puffed product, suggesting an unlikely scenario for higher Cd dialyzability. The effects of processes like baking and milling on Cd concentration (Cubadda, Raggi, Zanasi, & Carcea, 2003; Guttieri, Seabourn, Liu, Baenziger, & Waters, 2015) and processing like microwave cooking on Cd dialyzability (Wang, Duan, & Teng, 2014) have been reported before, but there is no report on the effect of extrusion processing on dialyzability or bio-accessibility of Cd. In our study, we found that lower MC and SS would result in lower Cd dialyzability.

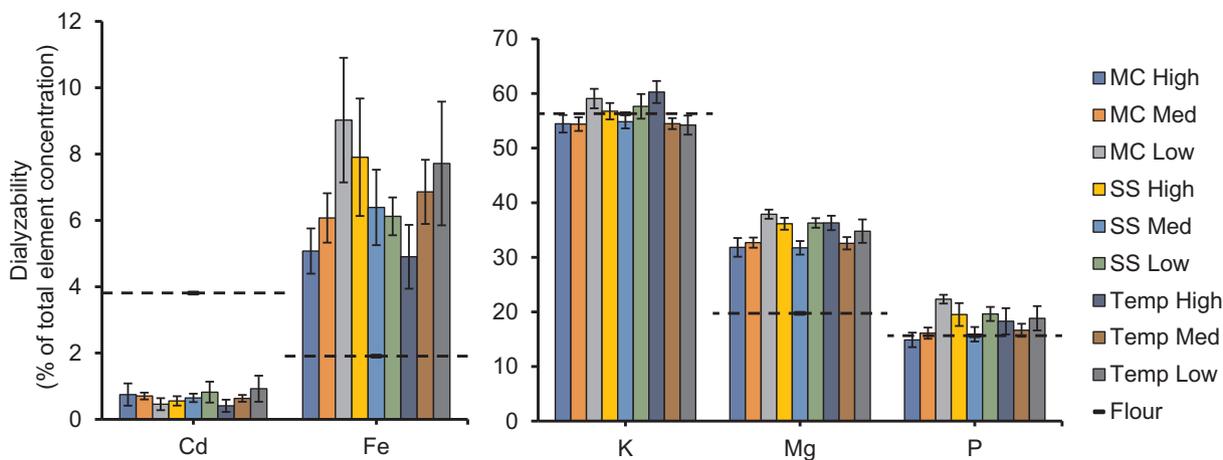


Fig. 3. Percentage of total elements (Mg, Magnesium; P, Phosphorus; K, Potassium; Fe, Iron; Cd, Cadmium) that were dialyzable in extruded samples averaged over low, medium and high conditions of moisture content (MC, %), screw speed (SS, rpm), and barrel temperature (Temp, °C); low and high conditions n = 5, medium conditions n = 8 with standard error bars plotted; dotted lines represent the percent dialyzed from unprocessed GNB flour, *there was a significant for all extrusion conditions when compared with unprocessed Great Northern bean flour ($\alpha = 0.05$).

3.2.5. Multivariate analysis

Fig. 4A shows the correlations between phytic acid, folic acid, and total and dialyzable element concentrations in extrudates. Phytic acid was not correlated with any other variable, while folic acid was negatively correlated with dialyzable P. There was no association between the total concentration of a certain element and how much was dialyzed. However, there were correlations among the total amount of all serum elements (Mg, K and P) and among dialyzable concentrations of

these elements, which suggests that changes during extrusion were consistent for these elements. Total and dialyzed fractions of the heavy metal Cd were not correlated with any variable.

Since there was no correlation between the total and dialyzable element concentrations, and the objective of our study was to have a better understanding of processing conditions that affect dialyzable element concentrations, all responses except total element concentrations were used for principal component analysis (Fig. 4B). The first two components explained 67% of the variation in the data. The essential elements (Mg, K, P, and Fe) had high positive loadings on Component 1 and were grouped together, while phytic acid and folic acid were negative on Component 1 and positive on Component 2. Low MC conditions combined with either high or low SS seemed to have maximum effect on dialyzability of these elements, while folic acid and phytic acid were retained under high MC. Cd could be considered separate from all, with almost no loading on Component 1 and high positive loading on Component 2. The dialyzability of Cd was enhanced by high MC and low SS, which, as discussed, are unlikely extrusion conditions as it results in an unacceptable product from a physical standpoint.

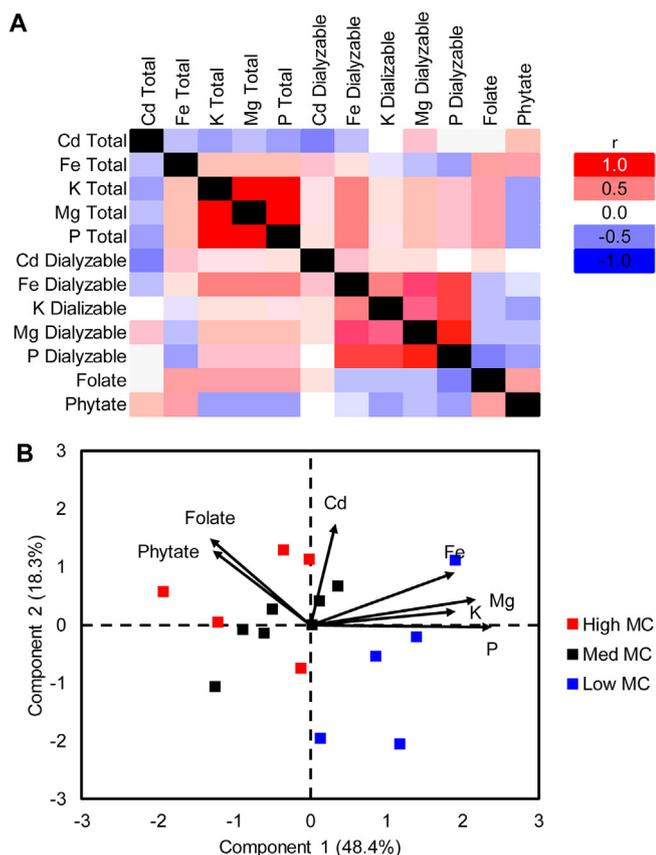


Fig. 4. Multivariate analysis on extruded samples: A) correlation matrix; B) biplot using principal component analysis (PCA) on correlations; Mg, Magnesium; P, Phosphorus; K, Potassium; Fe, Iron; Cd, Cadmium; MC, moisture content (%); SS, screw speed (SS, rpm); Temp (barrel temperature, °C).

3.3. Optimization

Based on the results of model fitting and analysis, extrusion parameters have differential effects on phytic acid, folic acid, and dialyzability of elements from GNB flour. Because some of these components are desirable and others are undesirable, it was relevant to identify extrusion conditions that yield the best results in terms of measured responses. With the criteria of minimizing the phytic acid content and Cd dialyzability while maximizing all other responses, a desirability function was constructed using the simultaneous optimization technique. This gave a maximum desirability of 0.87 for the following extrusion conditions: Temp: 100 °C; MC: 20.7%; SS: 245 rpm. This suggests a low Temp, medium feed MC, and high SS are ideal conditions to achieve maximum destruction in phytic acid while achieving minimum loss in folate and high element dialyzability in GNB flour.

4. Conclusion

Extrusion resulted in up to 85% reduction in phytates in GNB flour while moderately affecting folate concentration. Low MC conditions resulted in maximum reduction of these compounds. Extrusion significantly improved the dialyzability of all the mineral elements (Mg, P, K and Fe) while significantly reducing the dialyzability of the heavy metal Cd when compared with dialyzed amounts from unprocessed

flour. SS was the major extrusion variable causing the changes in dialyzable elements which was followed by feed MC. Low barrel temperature, medium MC and high SS were identified as extrusion conditions that resulted in maximum dialyzability while minimum destruction to folate. The study suggests that extrusion had a marked influence on availability of mineral elements in GNB and the results can form basis for bio-availability studies for GNB or other dry bean varieties.

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