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Proceeding Paper

Characterization of Dietary Fiber Extracts from Corn (*Zea mays* L.) and Cooked Common Bean (*Phaseolus vulgaris* L.) Flours and Evaluation of Their Inhibitory Potential against Enzymes Associated with Glucose and Lipids Metabolism In Vitro †

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Abstract: This research aimed to characterize dietary fiber (DF)-aqueous extracts from corn (commercial and Northwestern White Population) and common bean (cv. Bayo Madero) flour blends, evaluating their inhibitory effect on enzymes involved in glucose/lipid metabolism in vitro. Insoluble fiber showed the highest content of total phenolics, chlorogenic and ellagic acids being the main identified phenolics. Soluble fiber displayed the α-glucosidase and pancreatic lipase inhibitions (20–25%). The results suggested that corn/bean DF is a functional ingredient to potentially alleviate obesity and type II diabetes.

Keywords: corn (*Zea mays* L.); common bean (*Phaseolus vulgaris* L.); α-amylase; α-glucosidase; antioxidant capacity; dietary fiber; nixtamalization; phenolic compounds; pancreatic lipase

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1. Introduction

Inadequate dietary patterns lead to the development of chronic non-communicable diseases, where obesity and type II diabetes occupy a relevant place in Mexico [1]. Since both conditions are associated with glucose and lipid metabolism, targeting these pathways and participating enzymes might be a proper way to prevent and alleviate their development [2].

It has been reported that bioactive compounds from cereals such as corn (*Zea mays* L.) and legumes such as common bean (*Phaseolus vulgaris* L.) flours could target some of the enzymes involved in glucose and lipids metabolism [3]. For instance, common bean proteins have been found to inhibit α-amylase and improve insulin resistance in vivo, decreasing postprandial glucose and glycosylated hemoglobin. As antilipidemic agents, common beans have been associated with body weight regulation, lipid-lowering effects, and appetite and satiety control, mainly for their content of phenolic compounds and dietary fiber [4].

Since common beans and corn are usually consumed together in Latin America, providing bioactive compound-rich ingredients based on these raw materials could deliver nutritionally rich, hypolipidemic, and glucose-lowering effects. The biological properties of food products combining corn and beans have demonstrated their ability to provide benefits against obesity and type II diabetes [5]. Previously, our research group has characterized the anti-lipidemic [5], anti-inflammatory [6,7], and chemoprotective effect [8] of whole

nixtamalized Northwestern White Population corn and Bayo Madero beans. However, most of their bioactive compounds are retained in the non-digestible fraction, mainly composed of dietary fiber and phenolic compounds [9]. As these matrices have shown the potential for the elaboration of corn/bean snacks [9] and tortillas [10], the dietary fiber extracts from these materials could be used as a functional ingredient with technological potential. Hence, this research aimed to obtain and characterize the antioxidant dietary fiber extract from blends of nixtamalized corn (*Zea mays* L.) and cooked common bean (*Phaseolus vulgaris* L.) flours and to evaluate their inhibitory potential on enzymes associated with glucose and lipids metabolism in vitro.

2. Materials and Methods

2.1. Biological Material and Preparation of the Flours

Commercial (A: MASECA brand flour, Mexico) and Northwestern White Population (B) corn (*Zea mays* L.) flours and common bean (C) (*Phaseolus vulgaris* L., cv. Bayo Madero) seeds were used for the preparation of the flours. The corn flours were subjected to alkaline cooking or nixtamalization, as previously reported, using commercial lime ($Ca(OH)_2$). The resulting nixtamalized corn was milled, dehydrated, and screened through a 0.85 mm mesh [9]. For the common bean flours, the beans were cooked for 2.5 h with an excess of water (1:4 common beans:water proportion), dehydrated (40 °C, 16 h), ground, and screened (0.425 mm mesh) [9]. The corn and common bean flours were blended in 70:30 and 80:20 proportions of corn and common bean flours, resulting in four experimental mixtures: 7030AC, 7030BC, 8020AC, and 8020BC.

2.2. Aqueous Extraction of Dietary Fiber (DF)

The aqueous extraction was conducted following the procedure of Benitez et al. [11] and following the traditional dietary fiber extraction from the official methods of analysis of AOAC, without using enzymes. Briefly, the samples (10 g) were mixed with deionized water (40 mL) and incubated (60 °C, 60 min; 100 °C, 30 min). The water-insoluble residues (the insoluble dietary fiber or IDF) were washed with distilled water and ethanol at 60 °C (80% *v*/*v*), filtered (Whatman No. 4), and dried (60 °C, 24 h). An equal volume of ethanol (80% *v*/*v*) was added to the filtrate and was left for 12 h. Then, two ethanol (80% *v*/*v*) washes were conducted, followed by acetone and filtration (Whatman No. 42) (soluble dietary fiber of SDF).

2.3. Extraction and Quantification of Total Phenolic Compounds and Individual Phenolics from DF Fractions

Methanolic extracts from raw materials and extracted fibers were prepared as previously reported [9]. The total phenolic compounds were determined using the Folin–Ciocalteu method [12]. High-performance liquid chromatography coupled with a diode array detection (HPLC-DAD) method was used [13] to detect and quantify individual phenolic compounds. An Agilent 1100 System (Agilent Technologies, Palo Alto, CA, USA) was used, and the phenolic compounds were separated in a Zorbax Eclipse XDB-C18 column (Agilent Technologies) at a flow rate of 1 mL/min, 35 ± 0.6 °C, using standard curves from HPLC-grade standards of caffeic, chlorogenic, ellagic, ferulic, and gallic and flavonoids such as (+)-catechin and epigallocatechin gallate. The results were reported as the microgram equivalents of each phenolic compound/g dry sample.

2.4. Inhibition Potential of DF Fractions against Enzymes Linked to Glucose and Lipids Metabolism

The screening of the DF's ability to inhibit enzymes linked to glucose and lipids metabolism was tested based on the residual α-amylase [11], α-glucosidase [14], and pancreatic lipase inhibition [11].

For the residual α-amylase inhibition, type IV B α-amylase from a porcine pancreas solution (13 U/mL dissolved in 0.02 M sodium phosphate buffer, 250 µL, Sigma-Aldrich, St. Louis, MO, USA) was mixed with 250 µL of either the sample (raw materials or mixture

fiber flours) or the positive control (1 mM acarbose, Sigma-Aldrich) at 25 °C for 10 min. Then, 250 µL potato starch solution (1% soluble starch solution, dissolved in sodium phosphate buffer and boiled for 10–15 min) was added and incubated for 10 min. Afterwards, 0.5 mL dinitrosalicylic acid was added, and the solutions were boiled at 92 °C for 5 min and diluted with 5 mL distilled water. The absorbance was read at 520 nm. The results (%) were expressed as the inhibition against the positive control (acarbose).

Regarding α-glucosidase inhibition, both the sample and the positive control (acarbose) (50 µL) were mixed with 100 µL of α-glucosidase solution (1 U/mL, dissolved in 0.1 M sodium phosphate buffer, pH 6.9, Sigma-Aldrich) and incubated at 22 °C for 10 min. Then, 50 µL *p*-nitrophenyl-alpha-D-glucopyranoside (5 mM, dissolved in 0.1 M sodium phosphate buffer pH 6.9) was added and incubated at 22 °C for 5 min. The absorbance was read at 405 nM, and the results were expressed as the inhibition (%) against the positive control (Acarbose).

For the lipase inhibition, 0.1 g of the sample or the control (olive oil) was mixed with 2 mL olive oil, 10 mL PBS solution (0.1 M, pH: 7.2), and 2 mL pancreatic lipase solution (0.75 mg/mL, Sigma-Aldrich), and the solutions were incubated in a water batch (37 °C, 1 h). Then, the samples were placed in boiling water (92 °C) to stop the reaction, and the amount of released fatty acids was quantified with 0.05 M NaOH titration. The inhibitory activity was expressed against the control (olive oil).

2.5. Statistical Analysis

The results were expressed as the means ± SD of at least two independent experiments in triplicate. An ANOVA analysis was conducted, followed by a post-hoc Tukey-Kramer test, establishing the significance at $p < 0.05$ using the JMP v. 16.0 software (SAS Institute, Cary, NC, USA).

3. Results and Discussion

3.1. Dietary Fiber Contents from Raw Materials and Blends

The total dietary fiber (DF) was extracted using an aqueous method from the corn and common bean flours and their blends. The Northwestern White Population (B) corn presented the highest total dietary fiber content, followed by the commercial corn (A) and the cooked common bean flour (C) (10.65–15.76%). The 7030BC and 8020BC mixtures showed the highest fiber content, which can be explained by the higher fiber yields from their raw materials (16.30–16.67%). Despite the obtained fiber contents, the values were significantly lower than the reported DF values for the same corn and common bean varieties using the traditional enzymatic method [10,13]. However, the IDF values (raw materials: 10.40–12.77%, blends: 10.63–15.04%) were higher ($p < 0.05$) than those of the enzymatically extracted fibers, agreeing with previous reports using this extraction method [11].

3.2. Total Phenolic Compounds of DF Fractions

Depending on the sample, the raw materials (RM), and the fiber fractions (IDF and SDF), the content of total phenolics significantly varied ($p < 0.05$) (Figure 1A). The total phenolics associated with the SDF displayed the highest content in the RM, while those from the fiber fractions in the blends exhibited the highest values. The results were plotted based on chromatograms from the representative phenolics of each sample (Supplementary Figure S1).

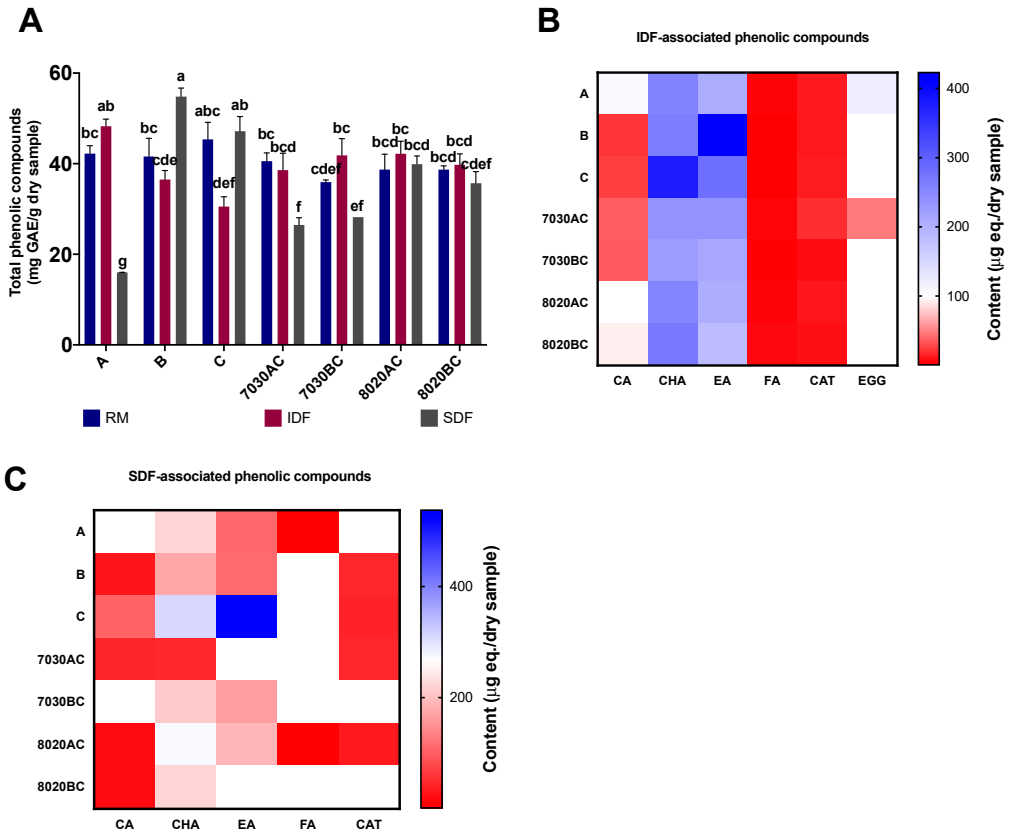


Figure 1. Content of total phenolic compounds (**A**); and individual phenolics detected from IDF (**B**) and SDF (**C**) by HPLC-DAD. The results are expressed as the means ± SD of two independent experiments in triplicate. Different letters express significant differences ($p < 0.05$) by Tukey-Kramer's test. Fiber samples: A: nixtamalized Northwestern White Population corn flour; B: commercial (MASECA®) nixtamalized corn; C: cooked common bean flour; CA: caffeic acid; CHA: chlorogenic acid; EA: ellagic acid; FA: ferulic acid; CAT: (+)-catechin; EGG: epigallocatechin gallate; GAE: gallic acid equivalents; IDF: insoluble dietary fiber; RM: raw materials; SDF: soluble dietary fiber.

The results obtained for the raw samples agreed with previous reports of the total phenolics for the same food matrices [15]. An overall higher amount of individual phenolics was presented in the IDF (Figure 1B), which could explain the higher proportion of IDF in the RM, while the SDF generally accounts for 1/3 of the DF [16]. Moreover, the richness of the phenolics to the IDF and SDF (Figure 1C) can be explained by the aromatic rings and hydrophilic groups from the phenolic compounds forming strong binding to the polysaccharides and proteins from the cell walls of the food matrices, making its extraction more difficult [17]. The IDF fractions mainly contained chlorogenic and ellagic acids (Figure 1B), while the SDF (Figure 1C) primarily showed caffeic and ellagic acid as the most abundant phenolics, quantified from representative chromatograms (Supplementary Figure S1). It has been reported that ferulic and gallic acid are the major free phenolic compounds from corn, while chlorogenic, p-coumaric, (+)-catechin, and rutin are the major bound phenolic compounds. Nixtamalization has a negative effect in ferulic acid, which explains its low content in the fiber fractions [18]. The concentrations of phenolics for the RM mixtures agree with a previous report from our research group [9]. However, reports indicate that ferulic, isoferulic, vanillic, syringic, and p-coumaric acids are the primary phenolics from corn IDF. In contrast, hydroxybenzoic acid derivatives are the major phenolics from the IDF and SDF fractions of cooked common beans [19]. The differences could be explained by the thermal process and the agronomic variety.

3.3. Inhibitory Activity of DF Fractions against Glucose and Lipid Metabolism Enzymes In Vitro

There were no differences in the α-amylase inhibition for both fiber fractions, but the SDF fractions showed overall higher values of α-glucosidase and pancreatic lipase inhibition (Table 1). Since phytochemicals exhibit low α-amylase inhibition but strong α-glucosidase inhibition, they are useful properties that could be used to reduce postprandial glycemia with minimum adverse effects [20]. Although the observed results for α-amylase inhibition were lower than those reported for raw corn and common beans (20–85% and 55%, respectively) [20], our results consider cooked matrices to be used as ingredients, indicating a more realistic effect from the matrices in the way they are commonly consumed.

Table 1. Inhibitory activity of the IDF and SDF fractions over α-amylase, α-glucosidase, and pancreatic lipase (%).

Sample	α-Amylase Inhibition (%)	α-Glucosidase Inhibition (%)	Lipase Inhibition (%)
		IDF	
A	22.0 ± 5.1 ^{a}	48.7 ± 8.0 ^{cde}	41.6 ± 1.5 ^{e}
B	18.7 ± 1.0 ^{a}	54.0 ± 9.1 ^{bcde}	42.4 ± 3.3 ^{e}
C	20.9 ± 1.2 ^{a}	74.1 ± 3.6 ^{ab}	43.2 ± 3.0 ^{de}
7030AC	18.3 ± 1.4 ^{a}	57.1 ± 3.6 ^{bcd}	42.6 ± 4.5
7030BC	17.5 ± 3.1 ^{a}	48.0 ± 3.5 ^{cde}	55.8 ± 3.0 ^{abcd}
8020AC	18.9 ± 2.3 ^{a}	61.3 ± 7.3 ^{abcd}	57.9 ± 7.4 ^{ab}
8020BC	15.0 ± 2.3 ^{a}	41.9 ± 1.5 ^{def}	43.7 ± 0.7 ^{cde}
		SDF	
A	21.4 ± 1.9 ^{a}	80.2 ± 0.9 ^{a}	46.8 ± 2.2 ^{bcde}
B	16.1 ± 2.6 ^{a}	21.5 ± 2.4 ^{f}	41.8 ± 1.1 ^{e}
C	21.7 ± 0.7 ^{a}	50.1 ± 1.8 ^{cde}	46.6 ± 0.4 ^{bcde}
7030AC	20.7 ± 0.1 ^{a}	69.3 ± 5.7 ^{abc}	56.3 ± 0.7 ^{abc}
7030BC	19.6 ± 0.8 ^{a}	44.9 ± 3.3 ^{de}	60.8 ± 0.4 ^{a}
8020AC	14.6 ± 1.6 ^{a}	35.2 ± 4.8 ^{ef}	56.6 ± 4.1 ^{ab}
8020BC	16.4 ± 0.6 ^{a}	50.2 ± 9.4 ^{cde}	59.7 ± 3.3 ^{a}

The results are expressed as the mean ± SD of two independent experiments in triplicate. Different letters express significant differences ($p < 0.05$) by Tukey-Kramer's test.

The values for α-glucosidase inhibition were higher than those for the raw matrices (25–45%) [21], while there are no reports for the specific fiber fractions from the nixtamalized corn and cooked common beans. To our knowledge, there are no reports of pancreatic lipase inhibition from thermal-treated corn and common beans. Nonetheless, the importance of these food products in inhibiting this enzyme suggest an important mechanism avoiding the related production of free fatty acids that could be easily absorbable at the small intestine, contributing to obesity development. Dietary fibers have been largely associated with lipase inhibition due to the viscosity increase in the stomach at low pH values or the ability of pectin components to protonate active serine and histidine residues from the lipase, inactivating the enzyme [22]. Although certain health-promoting potentials of these dietary fiber extracts are presented in this research, additional experiments exploring the interactions of the main phenolics derived from the fibers with each enzyme (e.g., fluorescence-guided inhibition and molecular docking analyses) and the results from other biological activities will better refine the potential of these ingredients to offer health benefits. Moreover, the technological feasibility of the fibers should also be explored, and the in vitro and in vivo evaluation of these fibers as food ingredients might also provide more insights into their biological behavior in food matrices and biological systems.

4. Conclusions

The results suggest the feasibility of using aqueous fiber extracts from nixtamalized corn and cooked common bean as functional ingredients delivering phenolic compounds with the ability to inhibit critical enzymes linked to glucose and lipids metabolism. Since

no enzymes are used in the fiber extraction, these fiber-rich ingredients could be achieved through a low-cost manufacturing process. Due to the biological potential of these extracts, further research exploring their technological properties and experiments at the in vitro and in vivo levels are justified.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/Foods2021-11049/s1, Figure S1: Representative chromatograms (λ: 280 nm and λ: 320 nm) from (**A**) insoluble dietary fiber (IDF) from Nixtamalized Northwestern White Population corn flour (A); (**B**) soluble dietary fiber (SDF) from A; (**C**) IDF from commercial (MASECA®) nixtamalized corn (B); (**D**) SDF from B; (**E**) IDF from cooked common bean flour (C); (**F**) SDF from C; (**G**) IDF from 7030AC mixture; (**H**) SDF from 7030AC; (**I**) IDF from 7030BC; (**J**) SDF from 7030BC; (**K**) IDF from 8020AC; (**L**) SDF from 8020AC; (**M**) IDF from 8020BC; (**N**) SDF from 8020BC. 1: Ellagic acid; 2: chlorogenic acid; 3: caffeic acid; 4: ferulic acid; 5: (+)-catechin; (6) epigallocatechin gallate.

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