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The Effects of CO_2 Injection and Barrel Temperatures on the Physiochemical and Antioxidant Properties of Extruded Cereals

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ABSTRACT: The effects of CO_2 injection and barrel temperatures on the physiochemical and antioxidant properties of extruded cereals (sorghum, barley, oats, and millet) were studied. Extrusion was carried out using a twin-screw extruder at different barrel temperatures (80, 110, and 140°C), CO_2 injection (0 and 500 mL/min), screw speed of 200 rpm, and moisture content of 25%. Extrusion significantly increased the total flavonoid content (TFC) of extruded oats, and β-glucan and protein digestibility (PD) of extruded barley and oats. In contrast, there were significant reductions in 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, PD of extruded sorghum and millet, as well as resistant starch (RS) of extruded sorghum and barley, and total phenolic content (TPC) of all extrudates, except extruded millet. At a barrel temperature of 140°C, TPC in extruded barley was significantly increased, and there was also an increase in DPPH and PD in extruded millet with or without CO_2 injection. In contrast, at a barrel temperature of 140°C, the TPC of extruded sorghum decreased, TFC of extruded oats decreased, and at a barrel temperature of 110°C, PD of extruded sorghum without CO_2 decreased. Some physical properties [expansion ratio (ER), specific length, piece density, color, and water absorption index] of the extrudates were significantly affected by the increase in barrel temperature. The CO_2 injection significantly affected some physical properties (ER, specific length, piece density, water solubility index, and water absorption index), TPC, DPPH, β-glucan, and PD. In conclusion, extruded barley and millet had higher potential for making value added cereal-based foods than the other cereals.

Keywords: cereals, physiochemical properties, antioxidant properties, extrusion, CO_2 injection

INTRODUCTION

Cereal grains are generally used as the major raw materials for extruded snack foods due to their good expansion characteristics and high starch content. Sorghum and millet are important cereals in many parts of Africa, Asia, and the semi-arid tropics worldwide. Sorghum is widely used for animal feed and human food, and its bioactive components might increase its use in foods (1). In recent years, millets have received attention, mainly because of their high fiber content, which can be provided in convenient forms to consumers (2). Barley is also a widely consumed cereal among the ancient cereal crops. Almost 80~90% of barley production is for animal feed and malt, but now barley is gaining renewed interest as an ingredient for production of functional foods due to their concentration of bioactive compounds such as β-glucans and tocols (3,4). Oats are one of the most valuable cereal grains since they contain a variety of natural health-promoting components such as β-glucans.

Extrusion is one of the most important food processing techniques widely used for production of various types of food products such as cereal-based snack foods and ready-to-eat breakfast cereals (5). During extrusion, biopolymers formed by gelatinization of starch and denaturation of proteins, have several effects on the physicochemical properties of the extrudates (6). During extrusion, the CO_2 injection enables the formation of an expanded structure, where the CO_2 functions as a blowing agent instead of steam in traditional extrusion. During the CO_2 injection process, nucleated bubbles are expanded by hydrodynamic effects, and CO_2 diffuses into the growing bubbles from the surrounding melt (7). The diffusion driven by concentration difference provides more controlled expansion than puffing by water flash-off.

In terms of the physical properties, the extent of expansion of the extrudate is important as it influences the porous structure (8). Besides, changes in hydration prop-

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erties such as water absorption index (WAI) and water solubility index (WSI) have been reported to affect both the ability of feed to mix with digestive enzymes and the general behavior of feed in the digestive tract of monogastric animals (9), and to affect extrudate stability in water which is an important property in fish and aquaculture feeds.

Starch is an important component in cereals. Extrusion cooking is known to influence the yield of resistant starch (RS) in foods (10). The RS content of native flours of hull-less barley (waxy and regular), in general, decreased by extrusion cooking, but not significantly (11). The retention of cereal protein quality and quantity during processing is important for dietary reasons. Fapojuwo et al. (12) observed that extrusion temperature improved *in vitro* protein digestibility of 2 low tannin sorghum varieties. Ejeta et al. (13) reported that the digestibility values for the cooked pearl millet varieties were higher than that of sorghum and was comparable to that of maize. Die temperature had a significant effect on β-glucan from barley flour and barley-grape pomace extrudates (14). On the other hand, Yao et al. (15) reported that changing the extrusion temperature or moisture content did not affect β-glucan from oat. The barrel temperature and CO_2 injection significantly affected the physical properties of extruded germinated wheat and barley and increased β-glucan in extruded germinated wheat (16). In view of the antioxidant properties, extrusion cooking decreased the antioxidant activity and total phenolics of barley, barley-tomato pomace, and barley-grape pomace extrudates (14). Researchers reported the reduction of total phenolic content by extrusion in oat cereals and oat extrudates (17,18). While cereal extrusion of wheat and maize has been studied extensively (19), the cereals investigated in this research have been studied by some researchers.

According to the observations mentioned above, it was of interest to study the selected 4 cereals extruded at different conditions. Therefore, this research was carried out to determine the effects of CO_2 injection and barrel temperatures on the physiochemical and antioxidant properties of extruded cereals.

MATERIALS AND METHODS

Materials

Sorghum, barley, oats, and millet grains were purchased at a local market in Korea and ground to flour for use in this experiment. The moisture content was calculated using the Association of the Official Analytical Chemists (20) drying method in which the sample (3 g) was dried in an oven at 135°C for 1 h and cooled for 30 min. The moisture contents of sorghum, barley, oats, and millet were 7.73, 8.46, 6.81, and 9.19%, respectively.

Extrusion process

Extrusion was performed in a twin-screw extruder (Incheon Machinery Co., Incheon, Korea) equipped with a 32-mm diameter at a length to diameter ratio of 23:1. The extrusion conditions were CO_2 injection of 500 mL/min, different barrel temperatures (80, 110, and 140°C), and die diameter of 3 mm. The screw configuration is shown in Fig. 1. The moisture content (25%) and screw speed (200 rpm) were fixed. After extrusion, the samples were dried in an oven at 55°C for 8 h and then ground to powder using a stainless blender. The grounded samples were passed through a 600 μm sieve and stored in plastic bags at room temperature for analysis.

Physical properties

***Expansion ratio (ER) and specific length*:** The ER was determined as the diameter of extrudates divided by the diameter of the dye (3 mm). The specific length was evaluated as the length of extrudates divided by the weight of extrudates (21). Ten measurements were taken for each sample.

***Piece density*:** The piece density of the extrudates was determined by the millet seed displacement method. The extrudates (2~5 g) were placed in the 125 mL cup and then filled with millet seeds. The cup with extrudates and millet seeds were weighed. The piece density was obtained by using the following equation. Triplicates were taken for each sample.

$$Pe=\frac{M\times Pm}{M+M_0-M_1}$$

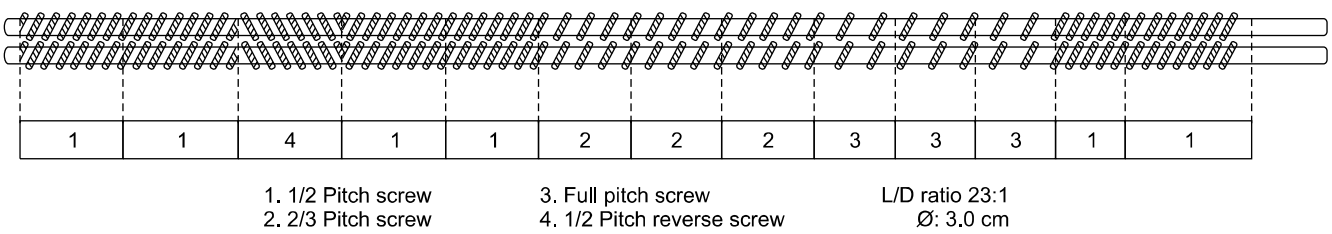

Fig. 1. Screw configuration of the twin-screw extruder.

where Pe is piece density of extrudate (g/cm^3), Pm is piece density of millet (g/cm^3), M is mass of extrudate (g), M_0 is mass of millet in tube (g), and M_1 is mass of extrudate and millet in tube (g).

***Color*:** A colorimeter (Minolta JP/CR-300, Konica Minolta, Tokyo, Japan) was used to determine the color of ground extruded samples as lightness (L^*), redness (a^*), and yellowness (b^*). In the colorimeter, the color was calibrated against the standard white (L^*=97.42, a^*=−0.19, and b^*=−1.71). The lightness was denoted by the L^* value (from white 100 to black 0). The redness and yellowness was denoted by a^* values (from green to red) and the b^* values (from blue to yellow), respectively. Each sample was measured in triplicates.

***WAI and WSI*:** WAI and WSI were determined by the method of Anderson et al. (22). The sample (3 g) was mixed with 25 mL of distilled water in a centrifuge tube, which was vigorously agitated in a laboratory shaker until complete dispersion and then placed in a shaker (110 rpm) for 30 min at 30°C. The suspension was centrifuged at 3,000 *g* for 20 min. WAI was expressed as the weight precipitated per gram of sample. The supernatant was evaporated in an oven at 105°C until dry, and the WSI was the weight of dry solids in the supernatant represented as a percentage of the sample's original weight.

Chemical properties

***Protein digestibility (PD)*:** The PD was determined by a modified method from Mertz et al. (23). The sample (200 mg) was suspended with 35 mL of pepsin solution (1.5 g of enzyme/1,000 mL of 0.084 N HCL). These samples were digested at 37°C for 2 h in a shaker at 150 rpm. After digestion, 2 mL of 2 M NaOH was added to stop enzyme activity. These solutions were centrifuged at 3,000 *g* for 15 min. After centrifugation, the supernatant was decanted, and the residue was washed with 10 mL of 0.1 M phosphate buffer. As described above, the residue was washed 2 times. The undigested residues were dried at 30°C in an oven until dried. These samples were used to analyze the total protein content using the ninhydrin based method (24). The percentage of PD was calculated by subtracting the undigested protein from the total protein, divided by the total protein, and multiplied by 100 (25).

***β-Glucan*:** The β-glucan content was determined according to the procedure of the mixed-linkage beta-glucan assay kit (Megazyme International Ireland, Bray, Wicklow, Ireland). One milliliter of aqueous ethanol (50% v/v) was added to each sample (0.5 g). Then, 5.0 mL of sodium phosphate buffer (20 mM, pH 6.5) was added to the solution tubes and then vortexed. The tubes were incubated in a boiling water bath for 2 min and then vortexed to prevent the formation of a lump of gel material. The tubes were heated again for 3 min in the boiling water bath and cooled to 40°C. Lichenase (0.2 mL) was added to each tube and incubated at 40°C for 1 h. The volume was adjusted to 30 mL with distilled water and then thoroughly mixed the contents of the tubes. These tubes were centrifuged at 1,000 *g* for 10 min. Aliquots (0.1 mL) were taken from each tube and transferred to blank reaction tubes with 0.1 mL of sodium acetate buffer (50 mM, pH 4.0) and the reaction tubes with 0.1 mL of β-glucosidase (0.2 U) in 50 mM acetate buffer (pH 4.0). These tubes were incubated at 40°C for 15 min. After that, 3.0 mL of glucose oxidase/peroxidase (GOPOD) reagent (glucose determination reagent) was added to each tube and incubated at 40°C for 20 min. The absorbance was measured at 510 nm using a spectrophotometer. D-glucose was used as the standard for determination of β-glucan. The content of β-glucan was calculated by the following equation:

$$\beta\text{-Glucan (\% w/w)} = \triangle A \times \frac{F}{W} \times 27$$

where △A is absorbance after β-glucosidase treatment (reaction) minus reaction blank absorbance, F is a factor for the conversion of absorbance values to μg of glucose, and W is the calculated dry weight of the sample (mg).

***RS*:** The RS contents were measured according to the procedure of the RS assay kit (Megazyme International Ireland). The sample (100 mg) was treated with 4 mL of pancreatic α-amylase solution made by the mixture of pancreatic α amylase and amyloglucosidase, 300 U/mL) and incubated in a shaking water bath at a rate of 200 strokes/min at 37°C for 16 h. After incubation, 4 mL of ethanol (99% v/v) was added to stop the reaction and centrifuged at 3,000 *g* for 10 min. The supernatant was carefully decanted, and the residue was mixed with 8 mL of 50% ethanol followed by centrifugation at 3,000 *g* for 10 min. The 50% ethanol-washing step was repeated once more. Two milliliters of KOH was added to the pellets, followed by stirring in an iced water bath for 20 min. At that point, 8 mL of 1.2 M sodium acetate buffer (pH 3.8) was added and stirred on a magnetic stirrer. Then, 0.1 mL of amyloglucosidase (3,300 U/mL) was immediately added and mixed well. The incubation process was done in a water bath at 50°C for 30 min followed by centrifugation at 3,000 *g* for 10 min. After centrifugation, 0.1 mL of aliquots (in duplicate) were taken from the supernatant and treated with 3 mL of GOPOD reagent. Samples were incubated at 50°C for 20 min. Absorbance was measured at 510 nm against the reagent blank using a spectrophotometer. D-glucose standard was used in this determination. The content of RS was calculated by the following equation:

$$RS\ (\%) = \triangle E \times \frac{F}{W} \times 9.27$$

where $\triangle E$ is absorbance (reaction) read against the reagent blank, F is a factor for the conversion of absorbance values to μg of glucose, and W is dry weight of sample.

Antioxidant properties

***Total phenolic content (TPC)*:** The TPC was determined according to the method of Slinkard and Singleton (26). The sample (1 g) was extracted with 10 mL of 80% methanol. The centrifugation was done at 3,000 *g* for 30 min after incubation at room temperature for 2 h. The supernatant (300 μL), taken from extraction, was mixed with 1.5 mL of 10 % (v/v) Folin-Ciocalteu reagent. This solution was vortexed and allowed to react for 5 min. After the reaction, 1.5 mL of Na_2CO_3 solution (60 g/L) was added to this solution and then was incubated again at room temperature for 2 h. The absorbance of the sample solution was measured at 765 nm using a spectrophotometer. The concentration of TPC was determined as milligrams of gallic acid equivalent (GAE) per gram of dry sample using an equation obtained from the gallic acid standard curve.

***Total flavonoid content (TFC)*:** The TFC was measured according to the method of Ebrahimzadeh et al. (27). The sample (1 g) mixed with 10 mL of 80% methanol was extracted at room temperature for 2 h and then centrifuged at 3,000 *g* for 30 min. The supernatant (0.5 mL) was taken from the extracted sample each, and then mixed with 1.5 mL of methanol and 0.1 mL of 10% aluminum chloride. Then, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water were added to this sample. The sample was incubated at room temperature for 30 min. The absorbance was measured at 415 nm using a spectrophotometer. Milligrams of quercetin per gram of dry sample, using an equation obtained from the quercetin standard curve, were used as the concentration for determination of TFC.

***1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity*:** The DPPH radical scavenging activity was determined according to the method of Brand-Williams et al. (28). The extraction of the sample (1 g) was done with 80% methanol at room temperature for 2 h and centrifuged at 3,000 *g* for 30 min. The DPPH solution was made at a rate of 0.0024 g of DPPH in 100 mL of methanol. Then, 3.9 mL of DPPH solution was added to 0.1 mL of the supernatant. After incubation at room temperature for 30 min in the dark, the absorbance was read at 515 nm. The DPPH radical scavenging percentage was determined with the following equation:

$$\text{Radical scavenging activity}\ (\%) = \frac{A_o - A_i}{A_o} \times 100$$

where A_o is absorbance of control and A_i is absorbance of sample.

Statistical analysis

Data were analyzed using the SPSS program (Version 12.0.1, SPSS Inc., Chicago, IL, USA). Duncan's least significant difference (LSD) tests were used to analyze the differences between mean values of the treatments. Significant differences were determined at $P<0.05$.

RESULTS AND DISCUSSION

Physical properties

***ER and specific length*:** As shown in Table 1, the ER values of extrudates ranged from 1.23 to 2.70. Depending on the sample and the injection of CO_2, various trends were observed between the barrel temperature and ER. Extruded millet without CO_2 injection and extruded oats with CO_2 injection showed significant increases in ER with increasing barrel temperature. Extruded oats without CO_2 injection at 140°C also showed a significant increase in ER as the barrel temperature increased. Huth et al. (29) reported similar results that increased barrel temperature leads to a significant increase in ER of extruded barley. When the barrel temperature increased from 80 to 110 °C, ER of extruded sorghum and extruded barley significantly increased and then significantly decreased when extruded at 140°C. In extruded millet with CO_2 injection, there was a significant decrease from 1.51 to 1.23 as the temperature increased from 80 to 140°C ($P<0.05$). It was found that if the temperature is higher than the critical temperature, expansion decreases due to excessive softening and potential structural degradation of the starch melt, which becomes unable to withstand the high vapor pressure (30). The CO_2 injection significantly increases ER of extruded sorghum, extruded barley, and extruded millet at 80°C. On the other hand, CO_2 injection decreased ER of extruded sorghum and extruded barley at 140°C, and extruded millet (110°C and 140°C).

In this study, when the barrel temperature increased, the specific length significantly increased in all extrudates without CO_2 injection at 140°C ($P<0.05$). There was a significant increase in specific length of all extrudates with CO_2 injection except for the extruded sorghum with CO_2 injection as the barrel temperature increased. The decrease in expansion and the increase in specific length have been attributed to increased dextrinization and weakening of the structure (31).

***Piece density*:** The result of piece density in this study is shown in Table 1. Piece density has been linked to the

Table 1. Effects of CO_2 injection and barrel temperatures on the physical properties of extruded cereals

Sample	CO_2 injection	Temperature (°C)	ER	Specific length (m/kg)	Piece density (g/cm^3)	Color[1)]			WSI (%)	WAI (g/g)
						*L**	*a**	*b**		
Sorghum	Raw	—	—	—	—	81.38^{d}	2.27^{f}	10.21^{i}	3.45^{h}	1.12^{m}
	Non-CO_2	80	1.27^{ij}	69.15^{ghi}	0.57^{ab}	62.73^{m}	7.69^{d}	16.18^{cdef}	9.08^{abc}	1.98^{kl}
		110	2.41^{b}	77.89^{fg}	0.22^{ij}	63.38^{m}	7.94^{c}	16.14^{cdef}	9.14^{abc}	1.71^{l}
		140	2.15^{d}	93.99^{d}	0.20^{ij}	58.67^{n}	9.27^{a}	15.92^{ef}	7.97^{bcd}	2.42^{hij}
	CO_2	80	1.67^{f}	69.13^{ghi}	0.40^{def}	66.74^{l}	7.29^{e}	16.61^{cdef}	10.97^{a}	2.32^{ijk}
		110	2.28^{bcd}	73.32^{fgh}	0.24^{hij}	65.78^{l}	7.53^{d}	16.05^{def}	9.69^{ab}	2.06^{jkl}
		140	1.58^{fg}	69.13^{ghi}	0.38^{def}	63.24^{m}	8.19^{b}	15.63^{f}	5.82^{efg}	2.30^{ijk}
Barley	Raw	—	—	—	—	91.25^{a}	0.74^{n}	5.28^{j}	5.63^{fg}	0.95^{m}
	Non-CO_2	80	1.85^{e}	34.63^{lm}	0.57^{ab}	74.87^{gh}	1.43^{ijk}	13.36^{g}	6.89^{efg}	4.74^{bc}
		110	2.70^{a}	42.93^{kl}	0.27^{ghi}	74.91^{gh}	1.66^{gh}	13.93^{g}	5.51^{fg}	2.71^{hi}
		140	2.39^{bc}	66.10^{hij}	0.21^{ij}	74.35^{h}	1.70^{gh}	14.34^{g}	5.77^{efg}	3.38^{ef}
	CO_2	80	2.40^{bc}	31.30^{1m}	0.45^{cd}	74.07^{hi}	1.73^{g}	14.19^{g}	7.50^{cdef}	4.54^{c}
		110	2.66^{a}	46.10^{k}	0.25^{hij}	75.35^{gh}	1.68^{gh}	13.82^{g}	6.47^{defg}	2.79^{gh}
		140	1.87^{e}	108.24^{c}	0.25^{hij}	79.50^{e}	0.81^{n}	11.41^{h}	7.70^{cde}	3.14^{fg}
Oats	Raw	—	—	—	—	87.89^{b}	0.33^{o}	9.62^{j}	5.41^{g}	0.96^{m}
	Non-CO_2	80	1.23^{j}	75.10^{fgh}	0.47^{cd}	73.88^{hij}	1.56^{ghi}	16.95^{cde}	2.95^{hij}	3.31^{ef}
		110	1.27^{ij}	83.35^{ef}	0.44^{cdf}	75.30^{gh}	1.30^{kl}	16.86^{cde}	2.72^{hij}	3.47^{ef}
		140	1.66^{f}	107.35^{c}	0.35^{efg}	75.35^{gh}	1.05^{m}	16.52^{cdef}	3.13^{hij}	3.37^{ef}
	CO_2	80	1.25^{j}	78.31^{fg}	0.51^{bc}	73.97^{hij}	1.52^{hij}	17.25^{c}	2.63^{hij}	3.52^{ef}
		110	1.41^{hi}	91.25^{de}	0.38^{def}	76.13^{g}	1.14^{lm}	16.11^{cdef}	2.57^{hij}	3.65^{e}
		140	1.57^{fg}	115.16^{bc}	0.25^{hij}	74.70^{gh}	1.00^{m}	16.68^{cdef}	2.82^{hij}	3.40^{ef}
Millet	Raw	—	—	—	—	83.80^{c}	0.78^{n}	17.19^{cd}	3.31^{hi}	0.95^{m}
	Non-CO_2	80	1.33^{ij}	62.11^{ij}	0.60^{a}	73.89^{hij}	1.36^{jk}	21.79^{a}	1.19^{k}	2.71^{hi}
		110	1.67^{f}	58.07^{j}	0.35^{efg}	72.67^{ijk}	1.37^{ijk}	22.05^{a}	1.40^{hij}	4.04^{d}
		140	2.26^{cd}	96.75^{d}	0.17^{j}	72.09^{k}	1.29^{kl}	22.37^{a}	2.66^{hij}	4.94^{b}
	CO_2	80	1.51^{gh}	57.78^{j}	0.45^{cd}	72.50^{jk}	1.53^{hij}	22.61^{a}	1.27^{ij}	3.31^{ef}
		110	1.32^{ij}	119.75^{b}	0.33^{fgh}	80.66^{de}	0.12^{p}	19.19^{b}	2.57^{hij}	4.92^{bc}
		140	1.23^{j}	146.63^{a}	0.27^{ghi}	77.95^{f}	0.35^{o}	19.34^{b}	2.97^{hij}	6.04^{a}

Different letters (a-p) in the same column are significantly different at $P<0.05$.
ER, expansion ratio; WSI, water solubility index; WAI, water absorption index.
[1)] *L**, lightness; *a**, redness; *b**, yellowness.

ER in describing the degree of puffing in extrudates. The piece density in all extrudates ranged from 0.17 to 0.60 g/cm^3. The increasing barrel temperatures significantly decreased piece density of extruded oats with CO_2 injection and extruded millet without CO_2 injection ($P<0.05$). When the barrel temperature was increased from 80 to 110°C, the reduction of piece density was shown in extruded sorghum and extruded barley with or without CO_2 injection. The raising of barrel temperature increases the degree of superheating of water in the extruder and encourages bubble formation and decrease in melt viscosity resulting in a reduction in the piece density (32). Huth et al. (29) also have reported a similar result that increased barrel temperature leads to a significant decrease in the piece density and an increase in ER of extruded barley. The CO_2 injection showed a significant increase in piece density of extruded sorghum and extruded millet at 140°C. However, a significant decrease in piece density with CO_2 injection was shown in extruded sorghum, extruded barley, and extruded millet at 80°C and extruded oats at 140°C.

***Color*:** Table 1 shows a significant decrease in lightness of extruded sorghum with or without CO_2 injection at 140°C when the barrel temperature increased. Under the CO_2 injection, the lightness of extruded barley at 140°C and extruded millet at 110°C had significant increases.

In extruded sorghum with or without CO_2 injection, a significant increase in redness was observed as the barrel temperature increased. When the barrel temperature increased from 80 to 110°C, the redness of extruded barley without CO_2 injection showed a significant increase and extruded oats with CO_2 injection showed a significant decrease in redness. Moreover, a significant decrease in redness was observed in extruded oats without CO_2 injection and extruded millet with CO_2 injection.

The yellowness of extruded millet with CO_2 injection (from 80 to 110°C) and extruded barley with CO_2 injection at 140°C was significantly decreased as the barrel temperature was increased. The slight increases in yellowness of extruded barley without CO_2 injection and extruded millet without CO_2 injection were observed, but not significant. Increase in redness and yellowness of the extrudates may have resulted from caramelization and Maillard reactions that take place during the extrusion

process. The changes in yellowness during extrusion cooking of yellow maize were induced by the effects of two different reactions: the non-enzymatic browning and pigment destruction. Some of the carotenoids might have been damaged by the thermal treatment and some browning might have been responsible for the color loss (33). In this study, the CO_2 injection did not favor the Maillard reaction in the redness of extruded sorghum, extruded barley at 140°C, extruded oats and extruded millet, as well as the yellowness of all extrudates at higher temperatures (110°C and 140°C) and extruded barley at 140°C.

***WAI and WSI*:** The WAI is a physico-chemical parameter that has been reported to indicate the hydrolytic breakdown of starch during extrusion (34), and the swelling behavior of the starch components (9).

There were significant increases in WAI of all extrudates after extrusion ($P<0.05$), compared to their raw samples (Table 1). Increasing the barrel temperature had significant increases in WAI of extruded millet with or without CO_2 injection and extruded sorghum without CO_2 injection at 140°C. The highest value of WAI (6.04 g/g) was in extruded millet with CO_2 injection at 140°C. Singh et al. (35) has reported that protein denaturation, starch gelatinization and swelling of the crude fiber, which occurred during extrusion, could all be responsible for the increased WAI of extrudates. In contrast, when the temperature increased from 80 to 110°C, extruded barley with or without CO_2 injection showed a significant decrease in WAI. Ng et al. (36) reported that increasing the die temperature during the extrusion of onion waste decreased WAI and increased WSI. In extruded millet, WAI with CO_2 injection was significantly increased and was higher than that of extruded millet without CO_2 injection.

WSI has been reported to represent the extent of soluble polysaccharides released from the grain in excess water (9). After extrusion, the significant increase of WSI was shown in extruded sorghum (Table 1). There was a significant decrease in WSI of extruded oats and extruded millet compared to their raw samples. The WSI ranged from 1.19 to 10.97%. The significant increase of WSI (1.19 to 2.66%) was shown especially in extruded millet (without CO_2 injection) with increasing temperature from 80 to 140°C. On the other hand, extruded sorghum with CO_2 injection showed a significant decrease (from 10.97 to 5.82%) due to higher barrel temperature ($P<0.05$). Singkhornart et al. (16) reported that a decrease in the macro-molecular degradation during extrusion with the increase in barrel temperatures could be responsible for the decreased WSI of extrudates.

Table 2. Effects of CO_2 injection and barrel temperatures on the chemical properties of extruded cereals

Sample	CO_2 injection	Temperature (°C)	PD (%)	β-Glucan (% w/w)	RS (g/100 g)
Sorghum	Raw	—	81.58^{b}	0.10^{h}	0.69^{b}
	Non-CO_2	80	40.77^{e}	0.21^{h}	0.25^{cdefgh}
		110	30.79^{f}	0.11^{h}	0.24^{cdefgh}
		140	56.55^{d}	0.27^{h}	0.35^{bcdefgh}
	CO_2	80	42.33^{e}	0.26^{h}	0.48^{bcde}
		110	34.37^{ef}	0.07^{h}	0.43^{bcdefg}
		140	65.50^{c}	0.06^{h}	0.52^{bc}
Barley	Raw	—	64.85^{c}	2.92^{f}	1.06^{a}
	Non-CO_2	80	84.17^{b}	4.35^{cde}	0.16^{cdefgh}
		110	86.90^{b}	4.61^{bc}	0.28^{cdefgh}
		140	86.85^{b}	4.54^{bcd}	0.22^{cdefgh}
	CO_2	80	86.19^{b}	4.92^{ab}	0.19^{cdefgh}
		110	85.27^{b}	4.99^{ab}	0.30^{bcdefgh}
		140	98.87^{a}	5.24^{a}	0.34^{bcdefgh}
Oats	Raw	—	65.76^{c}	2.07^{g}	0.45^{bcdef}
	Non-CO_2	80	83.15^{b}	3.95^{e}	0.06^{fgh}
		110	83.57^{b}	4.08^{cde}	0.09^{efgh}
		140	83.75^{b}	3.98^{de}	0.10^{defgh}
	CO_2	80	83.86^{b}	4.14^{cde}	0.11^{defgh}
		110	83.59^{b}	4.10^{cde}	0.10^{defgh}
		140	89.99^{b}	4.19^{cde}	0.35^{bcdefgh}
Millet	Raw	—	83.70^{b}	0.05^{h}	0.50^{bcd}
	Non-CO_2	80	52.57^{d}	0.08^{h}	0.22^{cdefgh}
		110	66.30^{c}	0.09^{h}	0.11^{cdefgh}
		140	84.46^{b}	0.06^{h}	0.02^{h}
	CO_2	80	55.49^{d}	0.08^{h}	0.38^{bcdefgh}
		110	80.46^{b}	0.12^{h}	0.12^{cdefgh}
		140	90.10^{b}	0.16^{h}	0.03^{gh}

Different letters (a–h) in the same column are significantly different at $P<0.05$.
Each sample was measured in triplicates.

Chemical properties

***PD*:** As shown in Table 2, the highest value (83.7%) of PD was shown in millet among the tested raw cereals. After extrusion, extruded barley and extruded oats showed a significant increase in PD compared to their raw materials. Our result was in accordance with the report of Abd El-Hady and Habiba (37). After extrusion, extruded sorghum showed a significant reduction in PD followed by the extruded millet. This may be due to their highly hydrophobic nature and inaccessibility of the protein bodies to enzymatic attack (25).

Increasing barrel temperature significantly improved the PD of extruded millet with or without CO_2 injection and extruded barley with CO_2 injection (140°C). A similar influence of extrusion temperature on *in vitro* PD of extruded 2 low tannin sorghum varieties have been reported by Fapojuwo et al. (12). In addition, extrusion cooking, especially under the highest barrel temperature (140°C), resulted in higher PD in all extrudates with or without CO_2 injection, especially in extruded barley (98.87%). The increase in PD in wheat flour has been reported by Singh et al. (38). This indicates that a higher temperature enhances the degree of inactivation of protease inhibitors, causing an increase in PD. In extruded

sorghum with or without CO_2 injection, PD was significantly decreased at 110°C and then increased at 140°C. This could be due to the formation of either a disulfide-bound protein coat produced by proteins surrounding the protein body or an anterior "toughening" of the periphery of the protein body because of disulfide bond formation (25).

Extrudates with CO_2 injection showed higher values in PD than the ones that were not injected with CO_2. The dissolution of CO_2 in the aqueous phase of the feed during the CO_2 injection process is expected to decrease pH. Onyango et al. (39) also reported that a low pH promotes structural changes and denaturation of storage proteins and increase their accessibility to proteolytic enzymes.

***β-Glucan*:** The β-glucan content in all extrudates ranged from 0.06 to 5.24% (w/w). Extrusion can significantly influence the β-glucan content of extruded barley and extruded oats ($P<0.05$) compared to their raw materials. Similarly, Singkhornart et al. (40) reported that the increase in β-glucan content was observed in extruded whole germinated wheat. It could be that the insoluble β-glucan becomes water-extractable by high shear force and temperature during the extrusion process. In addition, our results are in agreement with the report on extruding waxy barley, whole barley grains, or barley bran which shows a significant increase in the content of soluble dietary fiber (41). This effect is mainly attributed to an increase in solubility of β-glucan.

By contrast, there was no significant increase in the β-glucan content of extruded sorghum and extruded millet compared to their raw materials. Moreover, changing the barrel temperature did not affect the β-glucan content of all extrudates. This was in agreement with Yao et al. (15), who reported that the impact of extrusion temperature and moisture content did not affect the β-glucan concentration of oat. The maximum value (5.24%) of β-glucan content was shown in extruded barley at 140°C with CO_2 injection. The effect of CO_2 injection on β-glucan concentration was shown in extruded barley (80°C and 140°C) with a significant increase.

***RS*:** RS is the starch or the product of starch degradation that escapes digestion in the small intestine of healthy individuals and may be completely or partially fermented in the colon (42).

As shown in Table 2, the RS content of extruded sorghum and extruded barley was significantly decreased after extrusion ($P<0.05$) compared to their respective raw materials. The RS value of all extrudates ranged from 0.02 to 0.52% (g/100 g). Under higher temperatures, RS in all extrudates except extruded millet without CO_2 injection increased with no significance.

Our results are in agreement with the report of Faraj et al. (11) that starch fragmentation readily occurs at higher temperatures at the specified moisture content leading to the formation of amylose chains (with reduced degree of polymerization) that could be incorporated into the crystalline structure of RS_3. In contrast, the decrease of RS was shown in extruded millet without CO_2 injection due to the increase in barrel temperature. It could be due to the low amylose content, which was also reported by Vasanthan et al. (43). The effect of CO_2 injection was observed in all extrudates, but was deemed not significant.

Antioxidant properties

***TPC*:** While extrusion cooking showed a significant decrease in TPC of extruded sorghum ($P<0.05$), extruded barley and extruded oats, a slight increase was shown in extruded millet compared to their raw materials (Table 3). The TPC losses in this study were consistent with previous reports, showing a decrease in TPC of oat cereals and oat extrudates by 24～46 and 50%, respectively (17,

Table 3. Effects of CO_2 injection and barrel temperatures on the antioxidant properties of extruded cereals

Sample	CO_2 injection	Temperature (°C)	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH
Sorghum	Raw	–	32.69^{a}	2.89^{cdefg}	38.91^{a}
	Non-CO_2	80	20.8^{c}	2.84^{defg}	9.65^{defg}
		110	19.54^{c}	3.11^{abcdef}	9.15^{fg}
		140	14.85^{ef}	3.48^{abc}	11.22^{bcde}
	CO_2	80	24.11^{b}	2.58^{efgh}	12.89^{b}
		110	20.53^{c}	2.61^{defgh}	11.94^{bc}
		140	17.35^{d}	2.96^{bcdefg}	11.40^{bcd}
Barley	Raw	–	15.38^{e}	1.84^{jkl}	4.69^{j}
	Non-CO_2	80	7.46^{kl}	1.59^{l}	4.56^{j}
		110	8.98^{jk}	2.45^{ghij}	4.56^{j}
		140	11.66^{hi}	2.46^{ghij}	5.32^{ij}
	CO_2	80	8.67^{jkl}	1.76^{kl}	5.72^{ij}
		110	10.11^{ij}	2.32^{ghijk}	4.42^{j}
		140	14.65^{efg}	2.52^{efghi}	6.19^{ij}
Oats	Raw	–	20.45^{c}	2.86^{cdefg}	10.04^{cdefg}
	Non-CO_2	80	14.22^{efg}	3.65^{a}	8.42^{gh}
		110	13.28^{fgh}	3.24^{abcd}	9.45^{efg}
		140	12.74^{gh}	2.50^{fghi}	10.41^{cdef}
	CO_2	80	14.28^{efg}	3.56^{ab}	8.20^{gh}
		110	13.29^{fgh}	3.15^{abcde}	10.12^{cdefg}
		140	13.29^{fgh}	2.37^{ghijk}	10.04^{cdefg}
Millet	Raw	–	6.79^{l}	1.58^{l}	8.20^{gh}
	Non-CO_2	80	7.34^{kl}	1.69^{l}	5.24^{ij}
		110	7.49^{kl}	1.98^{hijkl}	5.54^{ij}
		140	8.61^{jkl}	2.39^{ghijk}	6.87^{hi}
	CO_2	80	8.12^{jkl}	1.91^{ijkl}	5.10^{ij}
		110	9.53^{j}	2.10^{hijkl}	5.69^{ij}
		140	9.83^{ij}	2.38^{ghijk}	8.42^{gh}

Different letters (a-l) in the same column are significantly different at $P<0.05$.
The concentration of total phenolic content (TPC) and total flavonoid content (TFC) were determined as milligrams of gallic acid equivalent (GAE) and quercetin equivalent (QE) per gram of dry sample, respectively.
Each sample was measured in triplicates.

18). The observed increase in TPC was consistent with Nayak et al. (44), who reported that TPC of extruded purple potato flour mix increased compared with the non-extruded product.

The TPC content of all extrudates ranged from 7.34 to 24.11 mg GAE/g (Table 3). The highest TPC value (24.11 mg GAE/g) was shown in extruded sorghum. Increasing barrel temperatures (140°C) caused the significant decrease in TPC of extruded sorghum with CO_2 injection and extruded sorghum without CO_2 injection. It has been stated that high temperatures during extrusion can alter molecular structure of phenolic compounds and either reduce their chemical reactivity or decrease their extractability due to a certain degree of polymerization causing the loss of antioxidant properties (17). In contrast, there was a significant increase in TPC in extruded barley with or without CO_2 injection at 140°C due to a higher barrel temperature. An increase was shown in extruded millet with or without CO_2 injection but was not significant. This may be due to the release of total phenolics from the cell wall matrix (45) caused by harsh thermal processing. Generally, TPC of all extrudates was slightly affected by injection of CO_2 during extrusion. The CO_2 injection significantly affected TPC of extruded sorghum (80°C and 140°C), extruded millet at 110°C, and extruded barley at 140°C within the same conditions. These results may be attributed to the cooling effect of the CO_2 (46) and its relatively low chemical reactivity.

***TFC*:** Flavonoids have generated interest because of their broad effects in promoting health, most of which are related to their antioxidant properties and their synergistic effects with other antioxidants (47).

Among the cereals, extruded sorghum and extruded barley had a decrease in TFC in comparison with their raw samples. Shimelis and Pakshit (48) reported that extrusion cooking has been used to bring numerous chemical changes such as gelatinization of starch, denaturation of protein, losses of lipid, and inactivation of enzymes resulting in a reduction of flavonoid compounds. On the other hand, a significant ($P<0.05$) increase in TFC was shown in extruded oats ($P<0.05$). Decreasing TFC in extruded oats with or without CO_2 injection (at 140°C) was observed due to increasing barrel temperatures. This may be attributed to the thermal destruction of flavonoids, as flavonoids are reported to be heat sensitive (49). In contrast, a slight increase was observed in extruded sorghum, extruded barley and extruded millet (with or without CO_2 injection) when the barrel temperature during extrusion increased. The increase in TFC due to CO_2 injection was only observed in extruded barley (80°C and 140°C) and extruded millet (80°C and 110°C), respectively (Table 3).

***DPPH radical scavenging activity*:** The DPPH free-radical assay was used to estimate the antioxidant activity by its ability to attract hydrogen atoms from polyphenols. The degree of discoloration indicates the scavenging potential of the sample total antioxidant capacity (50).

The significant decrease in DPPH radical scavenging activity was observed in extruded sorghum and extruded millet compared to their raw samples (Table 3). The DPPH value (38.91%) of sorghum was highest in raw cereals and significantly decreased to 9.65% after extrusion. This result was in agreement with that reported by Altan et al. (14). When the barrel temperature was increased, an increase was observed in DPPH radical scavenging activity of all extrudates except extruded sorghum with CO_2 injection. When the barrel temperature increased from 80°C to 110°C, a slight decrease was found in extruded sorghum without CO_2 injection and in extruded barley with CO_2 injection, and an increase was observed for both at 140°C. The significant increase was shown in extruded millet with CO_2 injection, when the barrel temperature increased from 110°C to 140°C. Similarly, Sharma et al. (6) also explained that increased DPPH radical scavenging activity of barley extrudates was due to the formation of brown color pigments from Maillard browning reaction during extrusion. A change in the extrusion temperature and moisture could have lead to the formation of different amounts of Maillard browning products (51). The significant effect of CO_2 injection on DPPH was observed in the sorghum extrudate at 80°C and 110°C, respectively.

Extrusion conditions in this study induced significant changes in some physical properties of all extrudates. Under a higher barrel temperature, extruded barley with or without CO_2 injection at 140°C showed a significant increase in TPC. The significant increase in DPPH of millet without CO_2 injection at 140°C and PD of extruded sorghum with or without CO_2 injection, extruded barley with CO_2 injection at 140°C, and extruded millet with or without CO_2 injection was due to higher barrel temperatures. The CO_2 injection had a significant effect in some physical properties (ER, specific length, piece density, color, and WAI). Moreover, the CO_2 injection caused a positive effect on some antioxidant properties (TPC and DPPH) and chemical properties (β-glucan and PD). Results from this study showed that millet and barley have higher potential for cereal-based extruded foods than other cereals.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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