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RESEARCH PAPER

Effect of Extrusion Processing on Phenolics, Flavonoids and Antioxidant Activity of Millets

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Abstract

The study was aimed to evaluate the effect of different extrusion treatments on total phenolic content, total flavonoids and antioxidant activity of finger millet and sorghum. The flours were subjected to eight different extrusion treatments at varying feed moisture, die head temperature and screw speed. Statistical analysis revealed that the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (FRAP, DPPH, TEAC) of extruded finger millet and sorghum flours was reduced significantly ($p < 0.05$) over their native counterparts. However, extrusion at high feed moisture, low temp and high screw speed (HM/LT/HS) retained considerable percentage of bioactives. Maximum retention of TPC, TFC and FRAP was observed as: 54 %, 78 % and 57 % in finger millet respectively and 87%, 89% and 86% in sorghum, respectively. High retention of bioactives in extruded millet flours demonstrated their enormous potential for the development of phenolic and antioxidant rich ready-to-eat snacks.

Keywords: Extrusion, finger millet, sorghum, phenolics, flavonoids, antioxidant activity

The current demand for novel functional foods together with the increasing trend of lifestyle diseases has dramatically driven a new market for the whole grains, millets, legumes and psuedocereals. Millets are considered as nutraceutical grains and offer enormous potential to qualify as functional foods; yet underutilized. Among millets, finger millet (*Eleusine coracan*), sorghum (*Sorghum bicolor L.*) are gluten-free grains high in dietary fibre, protein, micronutrients (calcium, iron, magnesium, zinc) and phytochemicals; known to possess antioxidant activity, anti-celiac, anti-carcinogenic, anti-diabetic and cholesterol reducing properties (Dykes and Rooney, 2006; Shobana *et al*. 2009). Gluten-free nature and unique bioactive principles from millets would be of special interest in development of healthy, nutritious and convenient products.

Unprocessed/native millet flours have poor functional properties, making them unsuitable for use in certain products. Thus, processing of these flours is essential in order to improve the quality of end product so that they can be effectively consumed in daily diet (Martinez *et al*. 2015). Extrusion is high temperature short time cooking process and has been used extensively to modify the functionality of flours by the way of gelatinization and degradation of starch, solubilization of dietary fibre, protein denaturation and inactivation of anti-nutritional factors (Martinez *et al*. 2014).

Plethoras of publications are available on physicochemical and sensory properties of extruded flours (Filli *et al*. 2013; Geetha *et al*. 2014; Omwamba and Mahungu, 2014). However, there remains scarcity of information that describe the effects of extrusion on bioactive compounds and antioxidant activity of millets. Furthermore, the available literature showed that extrusion may enhance or reduce the levels of bioactives (Sharma *et al*. 2012; Nayak *et al*. 2011). Thus, a systematic study on effect of extrusion variables is warranted, to understand the role of factors associated with retention or degradation of phenolics compounds and total antioxidant activity. Against this background, the study was designed to evaluate the effects of different extrusion treatments on total phenolic content, total flavonoids and antioxidant activity of millets.

Materials and methods

Flour preparation

Finger millet (moisture: 8.99%, ash: 3.51%, protein: 6.88%; crude fat: 1.57%; crude fibre: 3.43%, carbohydrate: 75.62%) and sorghum (moisture: 5.94%, ash: 2.64%, protein: 8.45%; crude fat: 3.90%; crude fibre: 4.11%, carbohydrate: 74.96%) were purchased from local commercial suppliers. The grains were ground separately in hammer mill and sieved through 30 mesh screen. Prior to extrusion, flours was pre-conditioned to a pre-determined moisture content by spraying with a calculated amount of water and mixing continuously at medium speed in a blender. The moistened flour was allowed to stay overnight in polyethylene pouches to equilibrate at room temperature.

Extrusion

Pre-conditioned flours were subjected to eight pre-designed extrusion treatments based on preliminary trials (as shown in Table 2). Extrusion was performed using Brabender Lab-Compounder KETSE 40/20 twin screw extruder (Germany) with length to diameter (L/D) ratio of 40:1. A die with a single circular opening (3 mm), equipped with a rotary die face cut er (speed of 150 rpm) was used. The expelled hot extrudates were dried (60°C) to 5-6% moisture (wb) and ground to obtain flours with size < 500 μm. Subsequently, flours were analyzed for their phenolic compounds, and antioxidant activity. Unextruded flours served as control.

Analysis

Proximate Composition

Proximate composition of flours was determined using AACC International methods (AACC, 2000): 44-15A (moisture), 08-01(ash), 46-08 (protein), 30-10 (crude fat), 32-10 (crude fibre). Carbohydrate was calculated by difference.

Total phenolic content

Total (free and bound) phenolic content of flours was estimated spectrophotometrically using Folin–Ciocalteu reagent (FCR) (Singleton *et al*. 1999). Results were expressed as Gallic acid equivalent (mg FE/100 g dw).

Total flavonoid content

Total flavonoid content of flours was measured by a colorimetric assay developed by Zhishen, *et al*. (1999) and results expressed as Quercetin equivalent (mg QE/100g dw).

Antioxidant activity

Antioxidant activity of flours was determined by three assays namely; FRAP (Ferric Reducing Ability of Plasma), DPPH (1,1-diphenyl-2-picrylhydrazyl) and TEAC (Trolox Equivalent Antioxidant Capacity) and results expressed as Trolox equivalent (μmol TE/100 g dw). FRAP assay was performed according to the method of Benzie and Strain (1996). DPPH radical scavenging activity was estimated according to the procedure of Brand-Williams *et al*. (1995). TEAC was measured using the modified method described by Pellegrini *et al*. (2003).

Statistical analysis

All the experiments were performed in triplicates and data are presented as mean values. Analysis of Variance (ANOVA) was performed to identify significant differences among the effects of various extrusion treatments, using SAS (9.4) sof ware. Further, data were subjected to Tukey's HSD test at a significance level of P<0.05 for pair-wise comparison of treatment effects for each parameter.

Results and discussion

Proximate composition of raw (unextruded) flours

Results showed significant ($p < 0.05$) variations in proximate composition of millet flours (Table 1). Proximate composition is one of the important nutritional values for food to be considered as ingredients in food products.

Low moisture and fat range of millet flours indicated their good shelf life and long term storage stability without spoilage food (Onwuka, 2005). Moisture and fat content are the key to preservation and storage of food. Both millets showed high crude fibre (3.43-4.11%), indicating higher content of non-digestible carbohydrate and lignin; which help enhancing digestibility and aids absorption of glucose, thus modulate glucose release in blood (David *et al*. 2014). Protein content of finger millet and sorghum were 6.88 and 8.45, respectively. The high carbohydrate content of millets was expected as they are generally known to be good source of calories. Study suggested that millets have good nutritional quality and thus can be used as functional ingredient with high health benefits. Our results have been well supported by earlier reports (David *et al*. 2014; Udachan *et al*. 2012).

Effect of different extrusion treatments on total phenolics content

Whole grain cereals, millets and legumes are rich source of dietary fiber, minerals, vitamins and phenolic compounds known to possess strong antioxidant activity and health promoting capacity (Van-Hung, 2016). Most of the reports on plant phenolics are in context to fruits and vegetables and there is scarce information on millets.

Table 1: Proximate composition of millet flours

	Moisture content (% wb)	Ash content(%)	Protein content (%)	Crude fat (%)	Crude fibre (%)	Carbohydrate (%)
Finger millet	8.99[a]	3.23[a]	6.88[b]	1.57[b]	3.43[b]	75.62[a]
Sorghum	5.94[b]	2.64[b]	8.45[a]	3.90[a]	4.11[a]	74.96[b]
Standard error	0.381	0.104	0.731	0.189	0.582	2.762

Means with different superscript letter within same column are significantly different ($p < 0.05$)

Table 2: Different extrusion treatments

Treatments	Feeder Speed (rpm)	Moisture Content (%wb)	Screw Speed (rpm)	Temperature (°C)					
				Z1	Z2	Z3	Z4	Z5	Z6 (Die)
T1:LM/LT/LS	20	10	200	30	50	90	100	110	120
T2:LM/LT/HS	20	10	400	30	50	90	100	110	120
T3:LM/HT/LS	20	10	200	30	50	90	100	180	180
T4:LM/HT/HS	20	10	400	30	50	90	100	180	180
T5:HM/LT/LS	20	20	200	30	50	90	100	110	120
T6:HM/LT/HS	20	20	400	30	50	90	100	110	120
T7:HM/HT/LS	20	20	200	30	50	90	100	180	180
T8:HM/HT/HS	20	20	400	30	50	90	100	180	180

L: low; H: high; M: moisture; T: temperature; S: screw speed.

Processing operations can significantly alter phenolic compounds which have direct implications on bioactive properties and their potential health benefits. Native finger millet exhibited 1.9 times higher TPC (966.32 mg FE/100g dw) than sorghum (506.21 mg FE/100g dw) (Table 3). Extrusion caused significant ($p<0.05$) decrease in TPC. The values (mg FE/100g) in extruded flours ranged from 360.64-525.77 (finger millet) and 173.02-441.99 (sorghum), depicting overall maximum retention of 54-87%. Highest and lowest TPC retention was registered at T6 (HM/LT/HS) and T3 (LM/HT/LS), respectively for both flours. Stabilizing effect of high feed moisture on TPC, especially at low temperature was observed. Taking the case of finger millet, treatment T6 (HM/LT/HS) had 16.07% more retention over T2 (LM/LT/HS). Similar trend was observed in sorghum. On the other hand, high temperature under similar circumstances caused still higher destruction of TPC; illustrating an obvious detrimental effect of high temperature processing. Generally, high feed moisture protects phenolics from degradation thus maintaining their stability; however it may lead to flavour loss at high temperature. An adverse impact of moisture at high temperature may be accredited to more destructive nature of moist heat over dry heat (Sharma *et al*. 2012). Increase in screw speed resulted in increased TPC retention, irrespective of moisture and temperature, thus elucidating its positive effect on TPC. This can be accounted for dual effect of high shear and low residence time. High shearing and friction caused degradation of cell walls, breakdown of conjugated or bound phenols, thus released free phenolic acids (Nayak, 2011). Further, reduced residence time caused minimum thermal degradation, thus high retention of phenolics (Mora-Rochin *et al*. 2010).

Loss in the TPC of flours under extrusion is expected to occur due to heat-liable nature of phenolic compounds. Extrusion reduces the chemical reactivity or extractability of phenolics as a result of oxidative degradation, formation of complex acids or structural alteration of phenolics (Altan *et al*. 2009). Similar reduction in phenolics has also been reported earlier in extrusion of barley (Sharma *et al*. 2012), oat flour (Viscidi *et al*. 2004) and bean–corn blend (Delgado-Licon *et al*. 2009). The authors explained that the effect of extrusion on phenolic compounds could be strongly dependent on the type and nature of material being extruded.

Effect of different extrusion treatments on total flavonoids content

Flavonoids consist of a large group of polyphenolic

Table 3: Effect of extrusion treatments on total phenolic content (TPC) and total flavonoid content (TFC)

Treatments	TPC (mg QE/100g dw)		TFC (mg QE/100g dw)	
	Finger millet	Sorghum	Finger millet	Sorghum
Control	966.32^{a}	506.21^{a}	214.67^{a}	21.18^{a}
T1: LM/LT/LS	370.51^{c}	185.12^{e}	121.61^{b}	11.97^{cb}
T2: LM/LT/HS	400.33^{c}	349.51^{c}	155.94^{ab}	15.17^{cab}
T3: LM/HT/LS	360.64^{c}	173.02^{e}	111.89^{b}	11.23^{cb}
T4: LM/HT/HS	448.79^{bc}	433.64^{b}	110.39^{b}	8.75^{c}
T5: HM/LT/LS	401.72^{c}	401.66^{cb}	132.28^{b}	14.92^{cab}
T6: HM/LT/HS	525.77^{b}	441.99^{ab}	167.22^{ab}	18.87^{ab}
T7: HM/HT/LS	374.39^{c}	275.27^{d}	126.33^{b}	13.57^{cab}
T8: HM/HT/HS	404.67^{c}	407.22^{cb}	118.44^{b}	11.62^{cb}
Standard error	20.672	13.885	13.953	1.566

Means with same superscript letter within same column are not significantly different ($p > 0.05$)

compounds and recent interest in flavonoids has been stimulated by the potential health benefits arising from the antioxidant, anti-carcinogenic, antiallergic, anti-inflammatory and gastroprotective properties (Kumar and Pandey, 2013). However, influences of extrusion process on the content and stability of millet flavonoids are unknown. In this context, total flavonoid content (TFC) of flours was determined and is presented in Table 3.

Native finger millet was found to have very high TFC (214.67 mg QE/100g), which was about 10 times higher than that of sorghum. Extrusion caused a significant ($p<0.05$) decrease in TFC of both the flours. Irrespective of extrusion treatments, however, a considerable percentage of TFC was retained. Per cent retention ranged from 51-73% in extruded finger millet and 41-89% in extruded sorghum. Results demonstrate fairly good thermostability of flavonoids against extrusion cooking. For both the flours, maximum and minimum TFC retention was observed for T6 (HM/LT/HS) and T4 (LM/HT/HS). Irrespective of temperature and screw speed, high feed moisture had positive effect on TFC retention. High screw speed increased TFC retention at low temperature; whereas same caused decrease in retention at high temperature. In general, the effect of temperature was adverse, resulting in more destruction of flavonoids at high temperature. For finger millet, we observed high positive correlation between the TFC and TPC ($R^2 = 0.88$). This is expected as flavonoids are a part of phenolic family. However, TFC and TPC had moderate correlation ($R^2 = 0.52$) in case of sorghum and could be due to difference in nature of flavonoids and phenolic compounds and their sensitivity to thermal processing (Tiznado *et al*. 2013).

Flavonoids are heat sensitive and liable to thermal destruction. Thermal stability (retention/degradation) of flavonoids during thermal processing is a combined effect of different factors such as nature of matrix, kind of processing or temperatures used (Moussa-Ayoub *et al*. 2015). Our results are in good agreement with those previously reported for thermal processing of buckwheat and sweet potato (Im *et al*. 2003; Huang *et al*. 2006). A considerable retention of TFC in flours selected in our study can be justified by the findings of Rohn *et al*. (2007) who reported that high temperature application at dry conditions (i.e. roasting at 180°C) caused severe degradation to quercetin diglucoside isolated from onions, forming quercetin monoglucoside as an intermediate product and aglycone quercetin as final product. The lat er was the main product, but remained stable during further roasting.

In contrast, an increase of 4.1-8.2% in TFC has been reported in extruded chickpea (Tiznado *et al*. 2013). This increase could be related to Maillard reaction products (MRPs) formed during extrusion or due to the denaturation of cell wall components at high temperature, leading to release of bound flavonoids. The flavonols content of extruded cranberry-pomace-corn starch mixture have also been reported to increase by 30-34%; which was explained by enhanced extraction of the compounds due to disruption of the food matrix upon extrusion (White *et al*., 2010).

Effect of different extrusion treatments on antioxidant activity

Antioxidant activity (AOX) quantifies the ability of a complex biological material to scavenge free radicals and evaluates reducing property related to their health promoting effects. Three complementary *in-vitro* assays, namely FRAP (Ferric Reducing Ability of Plasma), DPPH (1,1-diphenyl-2-picrylhydrazyl) and TEAC (Trolox Equivalent Antioxidant Capacity) were used to determine the AOX of flours. FRAP assay is used to determine the ability of the antioxidant to reduce Fe^{3+} to Fe^{2+}, whereas, DPPH and ABTS radicals assess free radical scavenging effects through the donation of hydrogen ions.

Results of AOX are summarized in Table 4. Finger millet presented higher AOX than sorghum. FRAP activity (μmol TE/g dw) of native finger millet and sorghum was 29.34 and 15.55, respectively, depicting 2 fold variability. Similar trend was obsereved for DPPH and TEAC values. Extrusion caused significant ($p < 0.05$) decrease in AOX of both flours. The percent reduction (FRAP) in respective flours was: finger millet (43-62%) and sorghum (13-69%). Maximum and minimum AOX retention was registered at T6 (HM/LT/HS) and T3 (LM/HT/LS) respectively. The effect of extrusion variables on AOX was in line with the trend of total phenolic content (TPC) as previously explained in section 4.1. The higher the TPC, higher AOX, further confirmed through positive correlation ($R^2 = 0.89$ to 0.99) between them. It is a well-known fact that phenolics compounds are the major contributors to the total antioxidant activity.

Our results are in accordance with the earlier reports discussed here. Similar reduction in AOX after extrusion has been reported in barley (Altan *et al*. 2009), whole grains (Yang *et al*. 2014) and green banana flour (Sarawong *et al*. 2014). However, several researchers have reported enhanced antioxidant activity consequent to extrusion processing in barley, lentil, corn-pumpkin blends and purple potato-dry pea blend (Sharma *et al*. 2012; Morales *et al*. 2015; Rocha-Guzman *et al*. 2012; Nayak *et al*. 2011). This has been attributed to the formation of novel MRPs (particularly melanoidins); extensively known for their antioxidant properties. Under certain situations where the net degradation effect of antioxidants exceeds that of the formation of MRPs, the reduction in AOX is apparent. This may be applicable to our findings also.

Conclusion

Finger millet exhibited higher bioactive compounds (phenolics, flavonoids) and antioxidant activity (FRAP, DPPH, TEAC) than sorghum. Extrusion cooking caused a significant loss in bioactives and antioxidants activity and can be attributed to the combined effect of moisture, temperature and screw speed. Highest and lowest retention of aforesaid properties was registered in treatment T6 (HM/LT/HS); hence designated as most 'mild' treatment amongst all. Flours extruded at high moisture, low temperature and high screw speed retained considerable percentage of phenolics, flavonoids and antioxidants; thus offers an interesting healthy ingredient for the development of phenolic and antioxidant rich ready-to-eat snacks.

Table 4: Effect of different extrusion treatments on antioxidant activity

Treatments	Antioxidant activity					
	FRAP(μmol TE/g dw)		DPPH (μmol TE/g dw)		TEAC (μmol TE/g dw)	
	Finger millet	Sorghum	Finger millet	Sorghum	Finger millet	Sorghum
Control	29.34[a]	15.55[a]	60.69[a]	29.76[a]	23.19[a]	10.64[a]
T1: LM/LT/LS	12.08[b]	5.86[d]	30.17[fe]	11.89[dc]	8.96[ef]	4.20[de]
T2: LM/LT/HS	12.90[b]	9.99[cb]	34.49[de]	19.65[b]	10.57[edf]	6.14[cd]
T3: LM/HT/LS	11.27[b]	4.79[d]	27.24[f]	9.96[d]	8.32[f]	3.03[e]
T4: LM/HT/HS	14.86[b]	13.33[ab]	43.71[bc]	26.11[a]	13.92[bc]	8.45[cab]
T5: HM/LT/LS	13.05[b]	12.85[ab]	37.53[dc]	25.04[a]	11.16[edc]	7.59[cb]
T6: HM/LT/HS	16.71[b]	13.48[ab]	48.02[b]	27.99[a]	16.37[b]	9.81[ab]
T7: HM/HT/LS	12.58[b]	7.76[cd]	30.24[fe]	15.75[bc]	9.29[ef]	4.84[de]
T8: HM/HT/HS	13.20[b]	13.04[ab]	40.79[dc]	25.94[a]	12.50[dc]	8.22[cab]
Standard error	1.356	0.754	1.417	0.998	0.566	0.544

Means with same superscript letter within same column are not significantly different ($p > 0.05$);
FRAP: Ferric reducing ability of plasma; DPPH: 1, 1-diphenyl-2 picrylhydrazyl;
TEAC: Trolox Equivalent Antioxidant Capacity.

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