Extrusion Conditions Affect the Functional and Antioxidant Properties of Sorghum, Barley and Horse-Gram Based Snack

[1]Shalini G. Rudra, [1]Neetu Jakhar, [1]Jyoti Nishad, [1]Neha Saini, [1]Sangita Sen, [3]Rakesh Bhardhwaj, [2]Sarika Jaiswal, [3]Poonam Suneja, [1]Sukhbir Singh and [1]Charanjit Kaur
[1]Institute of Indian Agricultura Research New Delhi, Division of Food Science and Post Harvest, Technology, 110 012 New Delhi, India
[2]Indian Agricultural Statistics Research Institute, India
[3] National Bureau of Plant Genetic Resources, India

Key words: Sorghum, extrusion, barley, phenolics, antioxidant activity, functionality

Corresponding Author:
Shalini G. Rudra
Institute of Indian Agricultura Research New Delhi, Division of Food Science and Post Harvest, Technology, 110 012 New Delhi, India

Page No.: 7-17
Volume: 13, Issue 2, 2015
ISSN: 1684-8462
Journal of Food and Technology
Copy Right: Medwell Publications

Abstract: Sorghum, barley and horse-gram are an excellent source of bioactives (phenolics, minerals, β-glucan) with documented health effects. Effect of four different extrusion variables, viz. high temperature, high moisture (HTHM, 120°C, 20%); high temperature, low moisture (HTLM, 120°C, 14%); low temperature, high moisture (LTHM, 100°C, 20%) and low temperature and low moisture (LTLM, 100°C,14%) on three composite blends from sorghum, barley and horse-gram was examined in the present study. Extrusion processing significantly ($p<0.05$) affected the physico-chemical and functional properties viz. Expansion Ratio (ER), Bulk Density (BD), hydration properties, total phenolics, antioxidant activity and texture of extrudates. Free phenolics and antioxidant activity in extrudates processed at HTLM increased appreciably by 25-40 and 16-52%, respectively in comparison to un-extruded blends. Thus, tailoring extrusion conditions can help increase total phenolics and antioxidant activity of snacks. Extrudates developed from sorghum-barley-horsegram (45:45:10) produced acceptable snacks with high antioxidant activity and sensory quality.

INTRODUCTION

The serious health implications of ‘Western diet’ characterized by high fat, sugar and refined carbohydrate is prompting consumers to a dramatic switch over to traditional whole and coarse cereal grains for higher nutrition and health. Millets offer an outstanding opportunity to administer healthy components to the consumers. In this context, they are rightly termed as ‘nutri-cereal’s and are an excellent source of dietary fibre, phytochemicals and micro-nutrients. Although, millets have been an integral part of traditional Indian diet but in recent years, growing awareness about their health benefits is prompting consumers for increased consumption. Moreover, there has been increased budgetary support by Indian government and incentives are being provided to farmers to increase production.

Extrusion technology is a versatile and a promising technology which has been widely used to develop corn and rice based extruded snacks. In past few years, there has been increasing interest to develop millet (pearl-millet, sorghum, ragi) based snacks to offer diverse products with added functionality (Chakraborty *et al*., 2011; Sawant *et al*., 2013). Extrusion is a hydrothermal treatment which improves functionality by way of improved digestibility of starch and protein, soluble fibre content, pre-gelatinized starch, hydration capacity, gelling properties, expansion and softer product texture and sensory quality (Wolf *et al*., 2010; Zhang *et al*., 2013). Extruded products from millets are innovative and healthier than conventional rice-corn as they are low in fat, high in mineral, fibre and "health-promoting" phytochemicals and possess antioxidant and free-radical scavenging properties (Dykes and Rooney, 2006).

Sorghum (*Sorghum bicolor* L.) Moenchis is nutritionally comparable to other cereals and is a valuable source of health functional ingredients including resistant starch and phenolic compounds (Dykes and Rooney, 2006). Resistant starch is a probiotic functional food component that resists hydrolysis by enzymatic digestion in the small intestine; undergoes complete or partial fermentation in the colon to produce beneficial short-chain fatty acid and stimulate healthy gut microflora (Young *et al*., 2004; Le Leu *et al*., 2005). The consumption of resistant starch in place of digestible starch helps reduce postprandial glycaemia and insulinaemia, thus lowering the risk of type 2 diabetes. Hence, products containing sorghum as an ingredient could act as a vehicle for increased dietary intake of heath promoting phenolic compounds, reducing the risk of chronic diseases.

Barley is a rich source of tocols, including tocopherols and tocotrienols and dietary fiber (β-glucans) which are known to reduce serum LDLcholesterol through their antioxidant action. Total dietary fibre ranges from 11-34% and soluble dietary fibre from 3-20%. Recently there has been renewed interest in barley due to its potential health benefits. The effectiveness of barley in lowering blood cholesterol (Behall *et al*., 2004) and glycemic index is well documented. The recent approval of soluble barley β-glucan health claims by the FDA for lowering blood cholesterol level could further boost food product development from barley.

Horse-gram (*Dolichos biflorus* L.) commonly known as'kulthi' is a traditional unexploited tropical grain legume and well known for its hardness, adaptability to poor soil and adverse climatic conditions. It is a cheapest source of protein, an excellent source of iron, molybdenum and calcium and has anti diabetic and anti-calcifying properties (Thirukkumar and Sindumathi, 2014). Based on above considerations, combining bioactive principles from sorghum, barley and horse-gram seemed a good proposition for development of a healthy extruded snack. Optimizing blend formulations and extrusion conditions can significantly improve the functionality of extrudates. Keeping in view the above, the objective of the present study was to evaluate the effect of selected extrusion variableson sorghum, barley and horse-gram composite blend and for developing novel extruded snack with high functional and antioxidant properties.

MATERIALS AND METHODS

Composite flour preparation: Sorghum, barley and horse-gram were purchased from local commercial suppliers, grounded separately in hammer mill and sieved through a 500 μm screen. The proximate composition of sorghum, barley and horse gram flour are presented in Table 1. Three composite blend formulations with varying ratios of sorghum, barley were: SB_1 (60:30), SB_2 (45:45), SB_3 (30:60) with constant ratio of horse-gram (10%) in each blend. The above said blends were subjected to several preliminary tests without jamming the extruder and four set of extrusion variables viz, high temperature, high moisture (HTHM, 120°C, 20%); high temperature, low moisture (HTLM, 120°C, 14%); low temperature, high moisture (LTHM, 100°C, 20%) and low temperature and low moisture (LTLM,100°C, 14%) were optimized. Preconditioning was carried by spraying with a calculated amount of water and mixing continuously at medium speed in a blenderto reach final moisture content (14 and 20%). The feed material was transferred to polyethylene pouches and then allowed to stay for 2 h to equilibrate at room temperature prior to extrusion. Pre-conditioning ensured uniform mixing and hydration and to minimize variability in the state of feed material. Moisture content of composite mixes was determined by hot air oven method AOAC (1990).

Extrusion process: Extrusion was performed in a laboratory-scale co-rotating twin screw extruder (M/S. BTPL, Kolkata, India). The extruder specifications were barrel bore diameter (30 mm), screw length (450 mm) and die opening (3 mm). The extruder had self-wiping system for easy cleaning of the machine. Screw speed was set up at 350 rpm and equipped with 3 mm restriction die or nozzle to extruder. Constant feeding rate was kept throughout the experiments. The expelled hot extrudates were fed directly into a tray drier, maintained at 60°C and dried to 5-6% moisture level and subjected to various analysis.

Preparation of extract: For extraction of total phenolics, 5 g of homogenized samples were extracted

Table 1: Proximate composition of raw materials (sorghum, barley and horse gram flour) used for extrusion

Parameters	Sorghum	Barley	Horse (g)	SB_1	SB_2	SB_3
Carbohydrates (g/100 g)	73±8.12	77±1.33	57±6.12	72.6±6.080	75±6.23	73.8±7.0200
Fat (g/100 g)	3.2±0.07	1.2±0.02	ND	2.28±0.09	1.98±0.1	1.68±0.060
Proteins (g/100 g)	11.9±0.06	9.5±0.17	20.56±0.09	12.63±0.13	11.47±0.09	11.18±0.021
Dietary fibre (g/100 g)	2.3±0.05	8.2±0.11	12.17±0.29	5.06±0.18	5.94±0.025	6.83±0.140
Calcium (mg)	26±1.2	29±1.41	287±38.62	53.6±7.190	53.9±6.21	54.2±5.9800
Iron (mg)	4.3±0.13	2.5±0.092	7±0.33	3.95±0.16	3.76±0.12	3.49±0.075

SB_1 = Sorghum:Barley::60:30; SB_2 = Sorghum:Barley::45:45 SB_3 = Sorghum:Barley::30:60

twice with 30 mL of ethanol (80%) by stirring and sonicating for 30 min in the dark. The homogenate was then centrifuged for 15 min at 10,000 g at 4°C (Eppendorf, Westbury and USA). The supernatant was then vacuum concentrated at 40°C in a rota-evaporator (GenevacSp scientific Chennai, India) and stored at -20°C. The concentrated sample was used as sample extract for estimation of total phenolics and hydrophilic antioxidant activity.

Free and bound phenolics: Total phenolics were estimated spectrophotometrically using Folin-Ciocalteu Reagent (FCR) (Singleton *et al*., 1999). To 100 μL of sample extract (80% ethanol), 2.9 mL of deionised water 0.5 mL of Folin-Ciocalteu reagent and 2.0 mL of 20%Na_2CO_3 solution were added. The mixture was allowed to stand for 60 min and absorption was measured at 760 nm against a reagent blank in UV vis spectrophotometer (Varian Cary 50). For conjugated phenolics, sample (5 g) were thoroughly crushed and homogenized in 10 mL of 80% ethanol and hydrolyzed with 20 mL of 4N NaOH and neutralized with HCl and extracted six times with ethyl acetate. The ethyl extract fraction was reduced to dryness at 45°C and volume made up with methanol. Results were expressed as ferulic acid equivalent (FAE mg/100 g dw).

Antioxidant activity

Cupric Reducing Antioxidant Capacity (CUPRAC): The cupric ion reducing antioxidant capacity of extrudates was determined according to the method of Apak *et al*. (2004). Briefly, according to the protocol 0.1 mL of sample extract was mixed with 1 mL each of $CuCl_2$ solution (1.0×10-2 mol L^{-1}), neocuproine alcoholic solution (7.5×10-3 mol L^{-1}) and NH_4Ac (1 mol L^{-1}, pH 7.0) buffer solution and 1 mL of water to make the final volume 4.1 mL. After 30 min, the absorbance was recorded at 450 nm against the reagent blank. Standard curve was prepared using different concentrations of Trolox. The results were expressed as μmol TE g^{-1} using molar absorptivity of Trolox as 1.67×104 L/mol/cm.

Bulk density: Bulk density was estimated according to the Zhu *et al*. (2010) method where a cylindrical steel container (Vc = 1 L) was filled with extrudates and their weight was recorded. BD of the extrudates was calculated as:

$$BD = \frac{W_{Sample}}{Vc}$$

Where:

W_{sample} = The weight of extrudates (g)
V_c = The volume of the steel cylinder

The average of 10 measurements was reported (Asare *et al*., 2004).

Expansion Ratio (ER): ER is the ratio of the cross-sectional area of extrudateto the cross-sectional area of die orifice and was calculated as follows:

$$ER = \frac{d^2}{d_{die}^2}$$

Where:

d = The extrudate diameter (average of 10 determinations)
d_{die} = The die diameter as measured using a verniercalliper (Zhu *et al*., 2010)

Water Absorption Index (WAI) and Water Solubility Index (WSI): The WAI and WSI were determined by the modified method of Kaur *et al*. (2002). Extruded samples were ground in a laboratory blender (Philips, India) and sifted through a 500 screen sieve prior to testing. The ground sample (2.5 g) was immersed in 25 mL of distilled water at room temperature, continuously stirred for 30 min and centrifuged at 3000 g for 10 min. The supernatant liquid was poured into a tared evaporating dish.The remaining gel was weighed and WAI was calculated as:

$$WAI = \frac{W_{sediment}}{W_{dry\,solid}}$$

Where:

$W_{sediment}$ = The weight of gel
$W_{dry\ solid}$ = The weight of drysample

The WSI was determined from the amount of dry solids recovered by evaporating the supernatant and calculated as follows:

$$WSI = \frac{W_{dissolved\,solid}\ \text{in supernatant}}{W_{dry\,solid}} \times 100$$

Where:

$W_{\text{dissolved solids}}$ in supernatant = The weight of dry solids from the supernatant

$W_{\text{dry solid}}$ = The weight of dry sample (Kaur and Singh, 2006)

Color: The color of ground extrudate powders was determined by using Hunter's color lab. The sample color is denoted by the three dimensions L, a* and b* where L accounts for lightness of the productranging from 100 (white) to 0 (black), a* value denotes redness/greenness and b* denotes yellowness/blueness (Hunter Associates Lab., Ind., USA).

Texture: The textural characteristics of expanded snacks in terms of hardness and crispness were measured in triplicate using a Texture Analyser (TAXT Plus, Stable Microsystems, Surrey, UK). Cut test: a Warner-Bratzler shear blade with guillotine probe was used with test speed of 1 mm sec^{-1}. The cut was performed perpendicularly to the main axis of the snack until completely breaking it. The peak force obtained in newtons was taken to be the result from the test.

Sensory analysis: Semi-trained panels consist of staff and post graduate students of IARI were selected for sensory analysis on a basis of 9-point hedonic scale. The panelists were naive to project objectives. Samples were coded using random three-digit numbers and served with the order of presentation counter-balanced. Panelists were provided with a glass of water and instructed to rinse and swallow water between samples. They were given written instructions and asked to evaluate the products for acceptability based on its appearance, texture, taste, color and overall acceptability using nine-point hedonic scale (1 = dislike extremely to 9 = like extremely) (Meilgaard *et al*., 1999).

Experimental design: The experimental design was a full factorial with three replication comprising of variable extrusion variables (HTHM, HTLM, LTHM, LTLM) and three composite blend formulations (SB_1, SB_2, SB_3 prepared with different proportions of sorghum, barley on a dry weight basis and constant ratio of horse-gram (10%) in each blend as discussed in previous study. Different parameters viz. WAI, WSI, total phenolic content, antioxidant activity, calcium, zinc, iron, protein, bulk density and expansion ratio were measured. Further for each parameter, Analysis of Variance (ANOVA) was carried out to compare the mean values. All significant differences are reported at p = 0.05 level. All the analysis was done using SAS 9.3 Software (SAS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Bulk density and expansion ratio: Expansion Ratio (ER) and Bulk Density (BD) of the extrudates developed from three different blend formulations of sorghum-barley and horse-gram viz. (SB_1, SB_2, SB_3) varied significantly ($p<0.05$), under the influence of the selected extrusion parameters (HTLM, HTHM, LTLM and LTHM). The results are presented in Table 2. ER is a manifestation of puffing and high values of ER is a desirable characteristic of snack extrudates. In general, ER ranged from 2.88-4.29 and all composite blends processed at HTLM (120°C, 14%) registered highest values for ER.Blend SB_2 registered in ER, probably due to an optimum blend comprising of sorghum and barley in equal ratios or in other words an optimal content of starchand fibre. Barley is known to be high in soluble dietary fibre and low in starch content whereas sorghumflour is high in starch (Al-Rabadi *et al*., 2011). Optimum blend composition when subjected to favourable moisture and temperature might have contributed to higher ER. Similar results have been obtained by Chakraborty *et al*. (2009) in millet and legume extruded snacks. Expansion is a function of viscosity and elasticity of dough governed by ratio of starch, protein and fiber. Water acts as a plasticizer and binds with starch and fibre to undergo a glass transition during extrusion, thus facilitating the deformation of the mixture and influence expansion. The lower ER in SB_3 extrudates could be attributed to high fibre content that rupture cell walls and prevent air bubbles to expand to the maximum level (Anton *et al*., 2009). There was significant difference ($p<0.05$) between blend combinations, treatments and their interaction (Table 3).

BD is inversely proportional to ER and is indicative of compact and nonporous structure of extruded snacks which profoundly affects textural properties. Extrusion conditions significantly ($p<0.05$) affected BD of different blends. BD in blends ranged from 51.00-130.77, depicting, 2.54 variation, processed under variable extrusion conditions. Low BD is a desirable character and minimum values were observed for SB_2 blend (51.29-121.75) followed by SB_3 (64.68-125.87) and SB_1 (66.53-130.77). High starch in SB_1 (sorghum: barley: 60:30) and high fibre in SB_3 (sorghum: barley: 30:60) originating from native flour may be the obvious reason for high BD of the respective extrudates. It is well known that the degree and ease of puffing is affected by many factors such as: type of starch contained, feed moisture and the extrusion variables. Type of starch and its gelatinization influences the puffing characteristic during extrusion processing. Sorghum has higher ratio of amylopectin whereas barley starch is primarily amylose. Protein-starch and fiber-starch interactions tend to

Table 2: Effect of extrusion parameters and flour blends on Bulk Density (BD) and Expansion Ratio (ER) of sorghum and barley snacks

Composite flour blends	HTHM (120°C, 20%)		HTLM (120°C, 14%)		LTHM (100°C, 20%)		LTLM (100°C, 14%)	
	BD	ER	BD	ER	BD	ER	BD	ER
SB_1	125.16±2.52	3.21±0.27	66.53±0.56	3.35±0.26	130.77±0.81	3.26±0.29	65.40±1.53	3.51±0.35
SB_2	121.75±1.23	3.29±0.29	51.29±1.86	4.29±0.13	101.85±1.35	2.88±0.32	67.74±1.58	3.46±0.34
SB_3	120.13±1.21	3.51±0.27	64.68±0.75	3.40±0.24	125.87±1.53	3.23±0.16	74.90±0.90	3.52±0.44

SB_1 = Sorghum:Barley::60:30; SB_2 = Sorghum:Barley::45:45; SB_3 = Sorghum:Barley::30:60; H = Stands for high; L = Stands for low; T = Stands for Temperature; M = Stands for moisture; BD = Bulk Density; ER = Expansion Ratio

Table 3: Effect of extrusion parameters and flour blends on water absorption Index and water solubility Index of sorghum and barley snacks

Composite flour blends	Unextruded (Control)		HTHM (120°C, 20%)		HTLM (120°C, 14%)		LTHM (100°C, 20%)		LTLM (100°C, 14%)	
	WAI (g/g)	WSI (g/g)	WAI (g/g)	WSI (g/g)	WAI (g/g)	WSI (g/g)	WAI (g/g)	WSI (g/g)	WAI (g/g)	WSI (g/g)
SB_1	2.14±0.10	2.69±0.16	6.32±0.09	11.46±0.06	4.71±0.16	23.28±0.38	6.16±0.08	12.04±0.14	5.26±0.08	20.53±0.41
SB_2	2.28±0.32	3.60±0.02	6.50±0.09	13.37±0.05	4.85±0.05	20.52±0.47	5.34±0.09	15.69±0.49	4.58±0.21	23.52±0.31
SB_3	2.19±0.20	3.56±0.05	6.30±0.10	14.83±0.05	4.75±0.09	20.68±0.46	6.61±0.08	12.66±0.07	4.36±0.10	22.15±0.29

SB_1 = Sorghum:Barley::60:30; SB_2 = Sorghum:Barley::45:45; SB_3 = Sorghum:Barley::30:60; WAI = Water Absorption Index; WSI = Water Solubility Index; H = Stands for high; L = Stands for low; T = Stands for Temperature; M = Stands for moisture

decrease the free expansion of amylopectin chains and inhibit the release of water vapour, thus limiting expansion and increasing density (Seker, 2005). Fibres may also bind water more strongly than starch, inhibiting water loss at the die and reducing its ability for expansion. Similar results have been reported by Camire *et al*. (1990) in barley and rice extrudates. With respect to processing variables, there was significant ($p<0.05$) difference between HTHM, LTHM and HTLM, LTLM. Low BD was observed in blend formulations with 14% feed moisture, irrespective of the barrel temperature (100 and 120°C). At optimum moisture levels, a significant structural breakdown of starch granules takes place, causing significant alteration in molecular structure of starch, promoting desirable BD. Generally, BD of extruded products is a function of moisture content; excess of moisture has a lubricating effect, causes inadequate cooking, impedes gelatinization and decreases the radial and volumetric expansion, leading to a dense product. Also, high moisture content may hinder the sudden vapour pressure release due to lack of pressure barrier. The results are in agreement with previous research on barley extrudates (Chakraborty *et al*., 2009; Stojceska *et al*., 2009).

Water Absorption Index (WAI) and Water Solubility Index (WSI): Hydration properties of snack extrudates expressed in terms of WAI and WSI in blend formulations are presented in Table 4. WAI is an index of swelling behaviour of starch component and is indicative of hydrolytic breakdown of starch as well as protein denaturation and complex macro molecular formulation (Dogan and Karwe, 2003). There was significant increase ($p<0.05$) in WAI and WSI in all blend formulations, irrespective of variable extrusion conditions. WAI in native and processed extrudates ranged from 2.14-2.28 and 4.366.61%, respectively, thus depicting 2-3 fold increase over control (native flour). The response values in decreasing manner were HTHM (6.50- 6.30)<LTHM (6.61-5.34)<LTLM (5.26-4.36)<HTLM (4.85-4.71). Increased WAI as a result of high damaged starch is a common phenomenon observed during extrusion processing and our results are in agreement with previous research (Martinez *et al*., 2014; Sarawong *et al*., 2014). Damaged starch has higher water retention or swelling capacity due to gelatinization and extrusion-induced fragmentation of amylose and amylopectin molecules (Rodriguez-Miranda *et al*., 2011).The interaction effect of all treatments was also significant at 5% level of significance (Table 3).

Similar trend was observed in WSI, an indicative of starch breakdown or dextrinization. Extrusion processing caused significant ($p<0.05$) increase in WSI of all formulated blends, processed under the influence of extrusion parameters. The values in native flour and extrudates ranged from 2.69-3.56 and 11.46-23.28, respectively depicting, 5-10 fold increase. The values in decreasing order was HTLM (23.28-20.52)>LTLM (23.52-20.53)>LTHM (15.69-12.04)>HTHM (14.83-11.46). Amongst the selected extrusion variables, extrudates processed at HTLM (120°C, 14%), recorded the best response in terms of high WSI, irrespective of blend formulations. Interestingly, this also corresponded to low WAI values (4.71-4.85) observed for the same treatment. This could be probably due to optimum moisture content which favoured high dextrinization or starch melting that prevailed over the gelatinization phenomenon (Rodríguez-Miranda *et al*., 2011). There was significant difference ($p<0.05$) between SB_1, SB_2 and SB_3blend and SB_2blend (containing equal content of sorghum and barley) registered high values of WSI. Optimum blend composition and a combination of

Table 4: Effect of extrusion parameters and flour blends on mineral content of sorghum and barley snacks

Composite flour blends	Unextruded (Control)			HTHM (120°C, 20%)			HTLM (120°C, 14%)			LTHM (100°C, 20%)			LTLM (100°C, 14%)		
	Ca (ppm)	Zn (ppm)	Fe (ppm)	Ca (ppm)	Zn (ppm)	Fe (ppm)	Ca(ppm)	Zn(ppm)	Fe(ppm)	Ca (ppm)	Zn (ppm)	Fe(ppm)	Ca (ppm)	Zn (ppm)	Fe (ppm)
SB_1	102.67± 2.08	15.00± 2.00	244.67± 5.51	73.33± 3.06	9.33± 1.53	84.67± 3.06	55.67± 2.52	11.33± 3.51	212.33± 4.16	98.67± 3.06	13.67± 2.08	134.00± 3.00	61.00± 2.00	12.00± 1.00	89.67± 3.06
SB_2	98.67± 3.06	18.67± 2.08	134.00± 4.00	64.00± 2.00	12.67± 2.52	104.67± 4.04	24.67± 4.51	17.00± 3.00	105.33± 4.04	89.00± 4.00	15.33± 1.53	116.33± 4.04	80.67± 2.08	11.00± 1.00	120.67± 4.04
SB_3	102.00± 3.00	16.00± 2.65	153.67± 5.51	67.00± 2.65	8.33± 1.53	80.33± 4.16	35.00± 2.65	13.00± 2.00	123.00± 2.00	99.00± 2.00	13.00± 2.00	99.67± 2.08	76.33± 2.52	11.00± 1.00	145.33± 2.52

SB_1 = Sorghum:Barley::60:30; SB_2 = Sorghum:Barley::45:45; SB_3 = Sorghum:Barley::30:60; H = Stands for high; L = Stands for low; T = Stands for Temperature; M = Stands for moisture

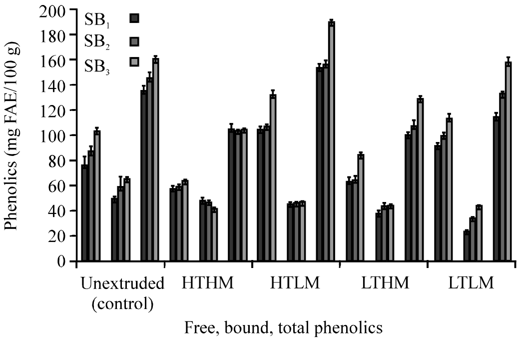

Fig. 1: Effect of extrusion parameters and flour blends on phenolic content of sorghum and barley snacks

temperature, screw speed and optimum moisture content caused an increase in the amount of degraded starch granules resulting in an increased formation of water-soluble products.

Total phenolic content (free and bound): The results of free, bound and total phenolics are represented in Fig.1. The FPC in native sorghum, barley and horse-gram flour was 51.35±5.79, 125.85±19.03 and 339.79±35.18, respectively. The FPC in SB_1, SB_2 and SB_3 in native (un-extruded) blend formulations ranged from 76.02-102.94 mg FAE/100 g. Incremental increase in barley led to significant ($p<0.05$) increase in FPC of SB_2 and SB_3 blends respectively. This is basically because of inherent high FPC found in native barley (125.85 mg FAE/100 g) than in sorghum (51.35 mg FAE/100 g). Extrusion processing under variable parameters (temperature and moisture) significantly ($p<0.05$) affected the FPC of the extrudates. There was a significant ($p<0.05$) decrease in FPC under all treatments except for extrudates processed at HTLM (120°C, 14%) which showed an appreciable increase. FPC content ranged from 104.87-131.78 mg FAE/100, depicting an increase of 25-40%, over un-extruded native flour. Phenolic compounds are heat-liable and can break upon exposure to high temperatures. Therefore, loss in the FPC content of formulations under extrusion are expected to occur, due to break down or oxidative degradation of phenolics acids and formation of complex acids (Fleuriet and Macheix, 2003). Feed moisture also can affect total phenolic content; optimum moisture protects phenolics from degradation, thus maintaining their stability, however high moisture may further favour loss. In view of above, loss of FPC under extrusion conditions of HTLM, LTHM and LTLM could be attributed to the combined effect of moisture and temperature conditions. Similar reduction in phenolic content has also been reported for extruded bean (19-21%), oat cereals (24-46%), oat extrudates (50%) and barley extrudates (8-30%) (Korus *et al*., 2007; Sharma *et al*., 2012). Total phenolics in barley extrudate samples was reduced by 60-68% and 46-60%, respectively compared with that of the unprocessed barley flour (Altan *et al*., 1990).

A similar trend was observed with respect to Bound Phenolic (BP) and results are presented in Fig. 1. The content in native flour and extrudates ranged from 48.74-64.97 and 23.37-47.75 mg FAE/100 g. Extrusion cooking resulted in significant decrease in the bound phenolics. Breakdown of conjugated BP to release free phenolic acidsoccurs as a result of breakdown of cell walls at high temperature during extrusion processing. Phenolic compounds are linked with cellulose lignin and proteins through ester bonds. More specifically, phenolics such as ferulic acid and p-coumaric acid are easily released during extrusion conditions of high temperature and moisture (Nayak, 2011). Similar results have been reported by Gui and Ryu (2014) in white and red ginseng powder.

Total Phenolic Content (TPC) represents a sum of free and bound phenolics and results are presented in Fig. 1. TPC in native blend formulation ranged from 134.36-160.45 mg FAE/100 g. As expected, extrudates processed at HTLM showed a significant increase by 23-34% in comparison to native blends. This is in line with the previous trend of enhanced free phenolic content as explained above. Han and Baik (2008) reported a similar increase in TPC in cooked and soaked chickpeas, yellow peas, green peas, soybeans following extrusion. Leaching of soluble fibres, proteins and other non-phenolic soluble components such as mono-, di- and oligosaccharides as a result of breaking of conjugated phenolics may also account for increase of TPC (Nayak, 2011). Statistical analysis of data (free, bound and total phenolic content) revealed significant interaction between blends and treatments (Table 3).

Table 5: Effect of extrusion parameters and flour blends on protein content of sorghum and barley snacks

Composite flour blends	Unextruded (Control)	HTHM (120°C, 20%)	HTLM (120°C, 14%)	LTHM (100°C, 20%)	LTLM (100°C, 14%)
SB_1	11.47±1.37	8.59±1.22	9.55±0.34	9.98±0.51	8.99±0.56
SB_2	12.63±0.60	9.86±0.87	10.88±0.70	10.41±0.43	9.79±0.50
SB_3	11.18±1.03	9.40±0.63	10.59±0.53	10.22±0.35	9.31±0.36

SB_1 = Sorghum:Barley::60:30; SB_2 = Sorghum:Barley::45:45; SB_3 = Sorghum:Barley::30:60; H = Stands for high; L = Stands for low; T = Stands for Temperature; M = Stands for moisture

Table 6: Effect of extrusion parameters and flour blends on color of sorghum and barley snacks

	Unextruded (Control)			HTHM (120°C, 20%)			HTLM (120°C, 14%)			LTHM (100°C, 20%)			LTLM (100°C, 14%)		
Blends	L	a*	b*	L	a*	b*	L	a*	b*	L	a*	b*	L	a*	b*
SB_1	101.35	-19.85	67.42	77.72	3.36	15.75	84.89	2.81	18.53	78.16	3.02	14.88	84.43	3.06	17.04
SB_2	101.87	-21.59	66.26	78.38	3.18	15.45	82.12	2.94	18.34	76.94	3.47	16.26	80.01	3.18	18.10
SB_3	103.11	-23.56	65.57	77.29	3.49	17.19	80.45	3.14	18.62	75.81	3.15	15.93	80.52	2.99	17.71

SB_1 = Sorghum:Barley::60:30; SB_2 = Sorghum:Barley::45:45; SB_3 = Sorghum:Barley::30:60; H = Stands for High; L = Stands for Low; T = Stands for Temperature; M = Stands for Moisture

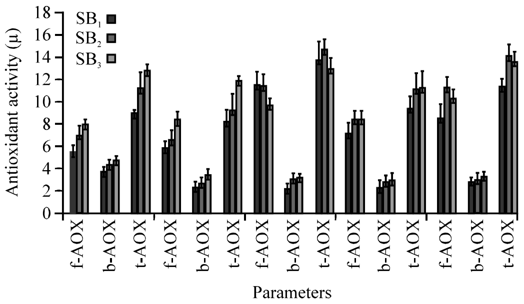

Fig. 2: Effect of extrusion parameters and flour blends on antioxidant activity of sorghum and barley snacks

Antioxidant Activity (AOX): The free bound and total Cupric Antioxidant activity (CUPRAC) has been designated as f-AOX, b-AOX and t-AOX, respectively. The results are presented in Fig. 2. The f-AOX in native blend formulations ranged from 5.49-8.11 μmol TE g^{-1} and incremental increase in barley in proportion to sorghum, lead to significant increase in f-AOX in SB_2 and SB_3blends. High f-AOX of these blends was attributed to the high TPC of native barley flour. It is a documented fact that phenolic compounds are major determinant for enhanced AOX in foods. Extrusion processing significantly ($p<0.05$) increased the f-AOX (5.80- 11.45 μmol TE g^{-1}) in extrudate blends, however maximum content (9.69-11.45 μmol TE g^{-1}) was registered in extrudates processed at HTLM (120°C and 14%). Overall there was 1.2-2 fold increase in f-AOX. The t-AOX in native blends ranged from 9.09-12.80 μmol TE g^{-1}. Small differences was observed in extrudates processed at HTHM, LTHM and LTLM. However, extrudates processed at HTLM showed significant increase; values ranged from 13.72-14.60 μmol TE g^{-1}. This was in line with the previous trend of enhanced free and total phenolics as explained in section 3.3. Release of free phenolics from bound phenolics as a result of high thermal conditions during extrusion processing, results in significant increase in total antioxidant activity. Zielinski and Troszynska (2000) reported that extracts from extruded cereal grain or cereal bran, exhibited a greater AOX than those from unprocessed grain or bran due to the formation of Maillard browning pigments which enhanced the AOX (Ruffian-Henares and Delgado-Andrade, 2009; Gumul *et al*., 2010; Nayak *et al*., 2011). Although, difference between blend SB_2 and SB_3 was statistically insignificant, overall extrusion conditions at HTLM proved to more promising than the rest (Table 3).

Mineral and protein content: The mineral and protein content of the different extrudates produced from sorghum-barley blends are presented in Table 5-6, respectively. Respective calcium, zinc and iron content of the native flour blends ranged from 98.67-102.67, 15.00-18.67 and 134.0244.67 ppm and there was significant decrease in mineral content following extrusion processing. Calcium suffered maximum loss to the tune of 75.69% followed by iron (67.16%) and zinc (55.38%). Amongst the different extruder variables, maximum retention of calcium was observed in extrudates processed by LTHM (100°C, 20%) in blend SB_1. Scanty information is available with respect to effects of extrusion on, mineral content however, few researchers have reported the substantial increase in iron content following extrusion which has been attributed to increased level of iron arising from metallic pieces, mainly screws or use of tap water for conditioning (Razzaq *et al*., 2012).

The results of protein content of native flour and different extrudates are presented in Table 6. Content in native flour and the extrudates ranged from 11.18-12.63

Table 7: Statistical comparison among different blends and treatment means under study

	Blend			Treatment				
Parameters	SB$_1$	SB$_2$	SB$_3$	Control	HTHM	HTLM	LTHM	LTLM
WAI	4.92^{a}	4.71^{b}	4.84^{a}	2.21^{d}	6.37^{a}	4.77^{c}	6.04^{b}	4.73^{c}
WSI	14.12^{c}	15.34^{a}	14.77^{b}	3.46^{e}	13.22^{d}	21.50^{b}	13.46^{c}	22.07^{a}
Free phenolic	78.44^{c}	82.75^{b}	99.03^{a}	88.28^{c}	59.49^{e}	114.70^{a}	69.95^{d}	101.29^{b}
Bound pehnolics	42.41^{b}	46.15^{a}	47.42^{a}	58.21^{a}	45.07^{c}	47.60^{b}	41.82^{d}	33.94^{e}
Total phenolics	120.86^{c}	128.90^{b}	146.45^{a}	146.48^{b}	104.56^{e}	162.30^{a}	111.77^{d}	135.23^{c}
Free Antioxidant activity (f-AOX)	7.68^{b}	8.94^{a}	8.96^{a}	6.85^{d}	6.93^{d}	10.85^{a}	8.01^{c}	10.02^{b}
Bound Antioxidant Activity (b-AOX)	2.66^{c}	3.14^{b}	3.52^{a}	4.20^{a}	2.87^{b}	2.89^{b}	2.64^{b}	2.94^{b}
Total Antioxidant Activity (t-AOX)	10.35^{b}	12.08^{a}	12.48^{a}	11.05^{b}	9.80^{c}	13.74^{a}	10.64^{b,c}	12.95^{a}
Calcium	78.27^{a}	71.27^{c}	75.87^{b}	101.11^{a}	67.89^{d}	38.44^{e}	95.56^{b}	72.67^{c}
Zinc	12.27^{b}	14.93^{a}	12.27^{b}	16.56^{a}	10.11^{c}	13.78^{b}	14.0^{b}	11.33^{c}
Iron	153.07^{a}	116.20^{c}	120.40^{b}	177.44^{a}	89.89^{e}	146.89^{b}	116.67^{d}	118.56^{c}
Protein	9.72^{b}	10.72^{a}	10.14^{b}	11.76^{a}	9.28^{c}	10.34^{b}	10.20^{b}	9.36^{c}
Bulk Density	96.97^{c}	85.66^{a}	99.89^{b}	-	122.35^{b}	60.83^{d}	124.16^{a}	69.35^{c}
Expansion ratio	3.33^{b}	3.63^{a}	3.41^{a,b}	-	3.34^{b}	3.68^{a}	3.33^{b}	3.50^{a,b}

Means with the same letter indicate that they are not significantly different; WAI = Water Absorption Index; WSI = Water Solubility Index; AOX = Antioxidant;SB$_1$ = Sorghum:Barley::60:30; SB$_2$ = Sorghum:Barley::45:45; SB$_3$ = Sorghum:Barley::30:60; H = Stands for High; L = Stands for Low; T = Stands for Temperature; M = Stands for Moisture

and 8.59-10.88%, respectively. It was apparent from the data that extrusion causes significant reduction in protein content, irrespective of blend formulation or extrusion variables. There was significant difference ($p<0.05$) between blend formulations and extrusion variables, however, interaction effect was insignificant (Table 3). Decrease in protein content is due to heat induced changes leading to transamination and deamination reactions and hence, reduction in amino acids (Yaqoub *et al.*, 2008). Gelatinization during extrusion processing may also cause reduction in protein content (Anuonye *et al.*, 2010, 2012).

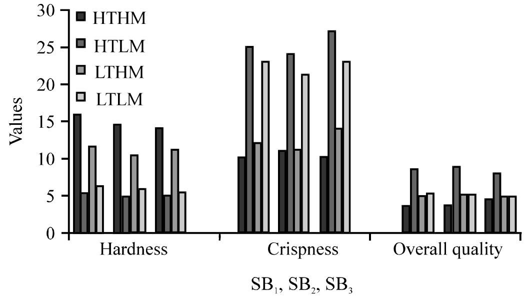

Fig. 3: Effect of extrusion parameters and flour blends on hardness, crispness and overall acceptability of sorghum and barley snacks

Color: Color is an important quality factor that typically relates to the acceptability, marketability and wholesomeness of foods. Color attributes L, a* ,b* of variably processed blends (SB$_1$, SB$_2$, SB$_3$) are presented in Table 7. Extrusion treatment significantly ($p<0.05$) affected L-values obtained at variable extrusion conditions. L-value in native flour ranged from 101.355-103.11 and there was significant reduction after extrusion processing. The values dropped to 75.81-84.89. There was a significant decrease in L-values of formulated blends processed by various extrusion conditions, however, extrudates processed at HTLM and LTLM showed had higher values then those extruded at HTHM and LTHM. Lower L-value are an indicative of heat employed during extrusion that leads to maillard reactions which produce the brown pigments (Sharma *et al.*, 2012) and these compounds markedly contribute to the aroma, taste and color of foods and are influenced by many factors including temperature, reactant concentration, reaction time and water activity .

The a* value for native flour and extrudates at variable extrusion conditions ranged from -19.855-23.56 and 2.81-3.495, respectively. The b* value in native flours ranged from 65.57-67.42 and significant ($p<0.05$) decrease was observed following extrusion processing. The values in different extrudates ranged from 14.88-18.62. The difference in the color characteristics of extruded puffs was quite dependent on their flours used in three different composite blends and also relates to composition of feed, feed moisture and screw speed (Liu *et al.*, 2000).

Hardness, crispness and overall quality: Hardness and crispness of expanded extrudates are results of the human being perception associated with the expansion and cell structure of the product (Ding *et al.*, 2005). Crispness relates to fragility and "ease of breakdown" whereas hardness is the maximum force required for a probe to penetrate a given commodity. Results of texture (hardness and crispness) of extrudatesfrom respective formulations and selected extrusion parameters are presented in Fig. 3. Hardness and crispness ranged from 4.98-15.79 kg and 10-27, respectively. There was no significant ($p<0.5$) difference between extrudates developed from different blends (SB$_1$, SB$_2$, SB$_3$). The extrudates produced under HTLM (120°C, 14%) showed the best response for texture minimum and maximum values for hardness and crispnessm, respectively. Processing at HTHM (120°C, 20%) and LTHM (100°C, 20%) resulted in harder and less crispy extrudates than their corresponding counterparts. Most probably, a combined effect of selected extrusion parameters (moisture, temperature and screw-speed) had a pronounced effect on extrudate hardness; increased moisture, reduced crispness. Our results are in agreement with previous research in rice based extruded snacks (Ding *et al*., 2005). Previous studies also reported that the hardness of extrudate increased as the feed moisture content increased. It might due to the reduced expansion caused by the increase in moisture content. The fact has been verified by several workers (Saeleaw *et al*., 2012), increased moisture content might cause reduction of expansion by consequent reduced formation of air bubbles and number of internal cells in the extrudates. The reduced starch conversion and compressed bubble growth would result in a dense product and reduced crispness of extrudate. This commensurate with our previous findings on high bulk density, reduced expansion ratio coupled with low WSI index in extrudates processed at HTHM and LTHM (Table 2 and 4).

To examine the overall quality, extrudates having the maximum expansion ratio were selected for sensory evaluation. Nine-point hedonic scales were adopted and the categories were rated from 1(absent/extremely dislike) to 9 (very high/ extremely high) in order to evaluate the extrudates in terms of flavour, colour, texture and overall acceptability. As expected, extrudates processed at HTLM were rated as best. The percentages of “liked” responses were: 25% “liked slightly” (assigned value 6), 33% “liked regularly” (assigned value 7), 23% “like” (assigned values 8).

CONCLUSION

The study revealed that blend composition and extrusion conditions significantly alter the functional and antioxidant properties of sorghum, barley and horse-gram based snacks. Sorghum, barley, horse-gram in ratio of 45:45:10 processed at optimised extrusion variable of temperature 120°C, moisture 14% and screw speed 300 rpm produced the most acceptable snack with best textural properties and overall acceptability. Optimized processing at HTLM, recorded the best response in terms of high expansion ratio, enhanced free phenolic content and total antioxidant activity. Furthermore, extrusion processing significantly altered the functionality of extrudates as manifested by increased hydration properties.

REFERENCES

Altan, A., K.L. Mccarthy and M. Maskan, 2009. Effect of AOAC., 1990. Official Methods of Association of Official Analytical Chemists. 15th Edn., Association of Official Analytical Chemist, Washington DC., USA., pp: 807-928.

Al-Rabadi, G.J., P.J. Torley, B.A. Williams, W.L. Bryden and M.J. Gidley, 2011. Particle size of milled barley and sorghum and physico-chemical properties of grain following extrusion. J. Food Eng., 103: 464-472. extrusion cooking on functional properties and in vitro starch digestibility of barley-based extrudates from fruit and vegetable by-products. J. Food Sci., 74: 77-86.

Anton, A.A., R.G. Fulcher and S.D. Arntfield, 2009. Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (*Phaseolus vulgaris* L.) flour: Effects of bean addition and extrusion cooking. Food Chem., 113: 989-996.

Anuonye, J.C., A.A. Jigam and G.M. Ndaceko, 2012. Effects of extrusion-cooking on the nutrient and anti-nutrient composition of pigeon pea and unripe plantain blends. J. Applied Pharmaceut. Sci., 2: 158-162.

Anuonye, J.C., J.O. Onuh, E. Egwim and O.S. Adeyemo, 2010. Nutrient and antinutrient composition of extruded acha/soybean blends. J. Food Process., 34: 680-691.

Apak, R., K. Guclu, M. Ozyurek and S.E. Karademir, 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine:? CUPRAC method. J. Agricultural Food Chem., 52: 7970-7981.

Asare, E.K., S. Sefa-Dedeh, E. Sakyi-Dawson and E.O. Afoakwa, 2004. Application of response surface methodology for studying the product characteristics of extruded rice-cowpea-groundnut blends. Int. J. Food Sci. Nutr., 55: 431-439.

Behall, K.M., D.J. Schol?eld and J. Hallfrisch, 2004. Diets containing barley signi?cantly reduce lipids in mildly hypercholesterolemic men and women. Am. J. Clin. Nutr., 80: 1185-1193.

Camire, M.E., A. Camire and K. Krumhar, 1990. Chemical and nutritional changes in foods during extrusion. Critical Rev. Food Sci. Nutr., 29: 35-57.

Chakraborty, S.K., D.S. Singh, B.K. Kumbhar and D. Singh, 2009. Process parameter optimization for textural properties of ready-to-eat extruded snack food from millet and pulse-brokens blends. J. Texture Stud., 40: 710-726.

Chakraborty, S.K., D.S. Singh, B.K. Kumbhar and S. Chakraborty, 2011. Millet-legume blended extrudates characteristics and process optimization using RSM. Food Bioprod. Process., 89: 492-499.

Ding, Q.B., P. Ainsworth, G. Tucker and H. Marson, 2005. The effect of extrusion conditions on the physicochemical properties and sensory characteristics of rice-based expanded snacks. J. Food Eng., 66: 283-289.

Dogan, H. and M.V. Karwe, 2003. Physicochemical properties of quinoa extrudates. Food Sci. Technol. Int., 9: 101-114.

Dykes, L. and L.W. Rooney, 2006. Sorghum and millet phenols and antioxidants. J. Cereal Sci., 44: 236-251.

Fleuriet, A. and J.J. Macheix, 2003. Phenolics in Fruits and Vegetables. In: Flavonoids in Health and Disease, Rice-Evans, C. and L. Packer (Eds.). CRC Press, Boca Raton, FL., pp: 1-42.

Gui, Y. and G.H. Ryu, 2014. Effects of extrusion cooking on physicochemical properties of white and red ginseng (powder). J. Ginseng Res., 38: 146-153.

Gumul, D., J. Korus, K. Czechowska, H. Barton and M. Folta, 2010. The impact of extrusion on the content of polyphenols and antioxidant activity of rye grains (*Secale cereal* L.). Acta Scientiarum Polonorum Technologia Alimentaria, 9: 319-330.

Han, H. and B.K. Baik, 2008. Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*) and their quantitative changes during processing. Int. J. Food Sci. Technol., 43: 1971-1978.

Kaur, L., N. Singh, N.S. Sodhi and H.S. Gujral, 2002. Some properties of potatoes and their starches I. Cooking, textural and rheological properties of potatoes. Food Chem., 79: 177-181.

Kaur, M. and N. Singh, 2006. Relationships between selected properties of seeds, flours and starches from different chickpea cultivars. Int. J. Food Propert., 9: 597-608.

Koksel, H., G.H. Ryu, A. Basman, H. Demiralp and P.K. Ng, 2004. Effects of extrusion variables on the properties of waxy hulless barley extrudates. Nahrung, 48: 19-24.

Korus, J., D. Gumul and K. Czechowska, 2007. Effect of extrusion on the phenolic composition and antioxidant activity of dry beans of *Phaseolus vulgaris* L. Food Technol. Biotechnol., 45: 139-146.

Le Leu, R.K., I.L. Brown, Y. Hu, A.R. Bird, M. Jackson, A. Esterman and G.P. Young, 2005. A synbiotic combination of resistant starch and Bifidobacterium lactis facilitates apoptotic deletion of carcinogen-damaged cells in rat colon. J. Nutr., 135: 996-1001.

Martinez, M.M., C.M. Rosell and M. Gomez, 2014. Modification of wheat flour functionality and digestibility through different extrusion conditions. J. Food Eng., 143: 74-79.

Meilgaard, M., G.V. Civille and B.T. Carr, 1999. Sensory Evaluation Techniques. 3rd Edn., CRC Press, Boca Raton, FL., Pages: 387.

Nayak, B., J.D.J. Berrios, J.R. Powers and J. Tang, 2011. Effect of extrusion on the antioxidant capacity and color attributes of expanded extrudates prepared from purple potato and yellow pea flour mixes. J. Food Sci., 76: 874-883.

Razzaq, R.M., M.F. Anjum and M.I. Khan, 2012. Effect of extruder variables on chemical characteristics of Maize (*Zea mays* L.) extrudates. Pak. J. Food Sci., 22: 108-116.

Rodriguez-Miranda, J., I.I. Ruiz-Lopez, E. Herman-Lara, C.E. Martinez-Sanchez, E. Delgado-Licon and M.A. Vivar-Vera, 2011. Development of extruded snacks using taro (*Colocasia esculenta*) and nixtamalized maize (*Zea mays*) flour blends. Food Sci. Technol., 44: 673-680.

Rufian-Henares, J. and C. Delgado-Andrade, 2009. Effect of digestive process on maillard reaction indexes and antioxidant properties of breakfast cereals. Food Res. Int., 42: 394-400.

Saeleaw, M., K. Durrschmid and G. Schleining, 2012. The effect of extrusion conditions on mechanical-sound and sensory evaluation of rye expanded snack. J. Food Eng., 110: 532-540.

Sarawong, C., R. Schoenlechner, K. Sekiguchi, E. Berghofer and P.K. Ng, 2014. Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour. Food Chem., 143: 33-39.

Sawant, A.A., N.J. Thakor, S.B. Swami and A.D. Divate, 2013. Physical and sensory characteristics of ready-to-eat food prepared from finger millet based composite mixer by extrusion. CIGR Int. J. Agric. Eng., 15: 100-105.

Seker, M., 2005. Selected properties of native or modified maize starch/soy protein mixtures extruded at varying screw speed. J. Sci. Food Agric., 85: 1161-1165.

Sharma, P., H.S. Gujral and B. Singh, 2012. Antioxidant activity of barley as affected by extrusion cooking. Food Chem., 131: 1406-1413.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol., 299: 152-178.

Stojceska, V., P. Ainsworth, A. Plunkett and S. Ibanoglu, 2009. The effect of extrusion cooking using different water feed rates on the quality of ready-to-eat snacks made from food by-products. Food Chem. J., 144: 229-232.

Thirukkumar, S. and G. Sindumathi, 2014. Studies on preparation of processed horse gram (*Macrotyloma uniflorum*) flour incorporated chappati. Int. J. Sci. Res., 3: 275-277.

Wolf, B., 2010. Polysaccharide functionality through extrusion processing. Curr. Opinion Colloid Interface Sci., 15: 50-54.

Yagoub, A.E.G.A., M.A. Muhammed and A.A.A. Baker, 2008. Effect of soaking, sprouting and cooking on chemical composition of bioavailability of minerals and *in vitro* protein digestibility of roselle *Hibiscus sabdaritta* (L.) seed. Pak. J. Nutr., 7: 50-56.

Young, S.L., M.A. Simon, M.A. Baird, G.W. Tannock and R. Bibiloni *et al*., 2004. Bifidobacterial species differentially affect expression of cell surface markers and cytokines of dendritic cells harvested from cord blood. Clin. Diagnosis Laboratory Immunol., 11: 686-690.

Zhang, X., H. Chen, N. Zhang, S. Chen, J. Tian, Y. Zhang and Z. Wang, 2013. Extrusion treatment for improved physicochemical and antioxidant properties of high-molecular weight polysaccharides isolated from oarse tea. Food Res. Int., 53: 726-731.

Zhu, L.J., R. Shukri, N.J. de Mesa-Stonestreet, S. Alavi, H. Dogan and Y.C. Shi, 2010. Mechanical and microstructural properties of soy protein-high amylose corn starch extrudates in relation to physiochemical changes of starch during extrusion. J. Food Eng., 100: 232-238.

Zielinski, H. and A. Troszynska, 2000. Antioxidant capacity of raw and hydrothermal processed cereal grains. Polish J. Food Nutr. Sci., 9: 79-83.