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Shelf life studies of the optimized extruded product developed by severe extrusion processing

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Abstract
This study was conducted to investigate effect of two different packaging materials on the quality attributes of extruded snacks prepared form rice and chickpea flour through severe extrusion. For storage studies of the extrudates high-density-polyethylene and laminated pouches were used. The snacks were stored for a period of six months and were evaluated at an interval of one month, for different parameters - peroxide value, free fatty acids (%), water activity, moisture content (%), total plate count (CFU/g) and overall acceptability. Results showed that the packaging material as well as storage period had significant effect ($p<0.05$) on extruded snacks. However, more quality changes were observed in extrudates packed in high-density-polyethylene than in laminated pouches. So, it is suggested that laminate pouches can be used to safely store the extruded snacks for a period of 6 months with least changes in the quality of snacks.

Keywords: High amylose rice, chickpea, HDPE, laminated pouches

Introduction
Snack consumption has been on the increase as an outcome of urbanization. Food based industries can exploit this development by fabricating nutritious snack foods. Extrusion has been extensively utilized during the past two decades due to its versatility, efficiency, low cost, product quality and eco-friendliness (Guy, 2001) [10]. This technology is used worldwide to produce snack foods, ready-to-eat cereals such as corn, rice and wheat (Choi and Phillips, 2007) [6], baby foods, pasta and pet foods (Deshpande and Poshadri, 2011) [7]. The important parameters affecting the keeping properties of food product are a function of its micro-environment like gas composition (oxygen, carbon dioxide, inert gases, and ethylene), pressure or mechanical stresses, relativehumidity (RH), light and temperature. This in turn depends on two other critical factors such as packaging and storage conditions (Singh, Cadwallader, 2003) [19]. Shelf life testing is gaining momentum owing to consumers' perception about the quality of the packaged products; good storage quality of processed food is an essential attribute to extend their utilization.

Legumes are high in nutrient especially in protein (18-24%) than cereal grain. Thus it can be used to provide amino acids such as lysine, tryptophan, or methionine (Potter, 1986). Unless certain raw or cooled cooked foods are considered, legumes are the only foods that contain substantial amounts of resistant starch (RS) (Marlett and Longacre, 1996) [17]. Resistant starch increases amount of indigestible substances in the colon and demonstrates the physiological benefits of dietary fibre. Annelisse *et al*. (2011) [2] reported that incorporation of RS into a cereal matrix may increase the intake of dietary fibre and hence help against chronic disease such as cardiovascular disease and type 2 diabetes. The purpose of this study is to determine the physicochemical and sensory attributes of chickpea flour substituted extruded snacks with rice flours.

Material and methods
Raw material
The raw materials used in this study were high amylose rice (cv. Pusa Sugandh-3) and chickpea (var SKUAST-233). The paddy was milled in a modern rice mill. The broken rice was collected and was finely ground in a lab mill alongside chickpea. Both the rice and chickpea flour samples were stored in high density polyethylene bags at 4 °C for further use.

Extrusion
Extrusion experiments were performed on a co-rotating twin screw extruder (Basic Technology Pvt. Ltd., Kolkata, India). The length to diameter ratio (l/d) was 8:1. The extruder has three barrel zones. Temperature of first and second zone was maintained at 60 and 80 °C throughout the experiment, whereas in third zone (die section) it was varied according to experimental design. The circular die of 4.0 mm was used. Samples were prepared as per the obtained optimized conditions. Required moisture content of the blends was adjusted by conditioning through moisture addition. After following the obtained optimized conditions extrusion cooking was carried out and the product was packed in two different packaging materials i.e. HDPE and laminated pouches (LP) and stored for six months under ambient conditions (25±2 °C, 60-62% RH). Evaluation tests were carried at 0, 30, 60, 90, 120 and 180 days for moisture content, water activity, peroxide value (PV), free fatty acid (FFA) content, total plate count (TPC) and overall acceptability.

Moisture Content (%)
Standard AACC (AACC, 2000) procedure was followed for determination of moisture content. Two grams of samples in triplicates were dried in a clean, dry and pre-weighed moisture dish at 130±1 ºC for 1 hour were dried to constant weight at 70 °C, in an oven, cooled in the desiccators and weighed. The moisture loss was calculated and expressed in percentage.

Water Activity (a_w)
Water activity was measured using water activity meter (AQUA, LAB, SN: PRE000197)

Peroxide Value
Five grams of weighed sample was placed in a 250 ml conical flask. 30 ml reagent I (Acetic acid-chloroform solution) was added and after shaking 0.5 ml potassium iodide was added. The solution was allowed to stand with some shaking and 30 ml distilled water was added. The solution was titrated with 0.1N sodium thiosulphate with constant shaking until yellow colour disappeared. The 0.5 ml starch solution was added and titration was continued till all blue colour disappeared. Blank test was also conducted in the same pattern. The peroxide value as peroxide per kg sample was calculated as:

$$\text{Peroxide Value} = \frac{(\text{Sample reading} - \text{Blank reading}) \times \text{Normality of NaS203 solution} \times 1000}{\text{Weight of sample (g)}}$$

Free Fatty Acids (%)
Standard AOAC procedure (AOAC, 2000) [3] was followed for free fatty acids determination in extruded products. Product sample (5 g) was taken in flask and 50ml benzene was added and kept for 30 min for extraction of free fatty acids. After extraction, 5 ml extract, 5 ml benzene, 10 ml alcohol and phenolphthalein as indicator were taken in flask and titrated against 0.02 N KOH till light pink colour disappeared. The fatty acid value was calculated by using the following formula:

$$\text{Fatty acid value as \% oleic acid} = \frac{282 \times 0.02 \text{ N KOH} \times \text{ml of alkali} \times \text{Dilution Factor}}{1000 \times \text{wt of the samples (g)}} \times 100$$

Total Plate Count (cfu/g)
Microbial analysis was carried out to determine the microbial stability of the extruded samples over time. 10 g of grated sample was aseptically taken from each package and homogenized in 90 mL of the sterilized physiological solution. Three-fold serial dilution was done and each dilution was plated out in triplicates on nutrient agar plate for aerobic mesophilic bacteria using spread plate method. Plates were allowed to cool and incubated at 37 °C for 48 h (Adegoke, 2004) [1]. The number of visible colonies produced on each plate of different dilutions and colonies were counted using the digital colony counter and expressed as CFU/g as given below:

CFU⁄g = No. of colonies × Dilution factor/Volume of sample used (ml)

Overall acceptability
Samples were evaluated for sensory attributes like appearance, color, texture, flavor, mouthfeel and overall acceptability through a panel of semi-trained judges using 5-point scale ranging from five (excellent) to one (poor).

Results and Discussion
The results obtained during the storage studies are presented in Table 1. It was observed that not just the storage time but the packaging material as well had significant ($p<0.05$) effects on all the parameters.

Moisture content
Irrespective of the packaging material, the moisture content was found to increase significantly ($p<0.05$) from 2.13% to 4.35% in HDPE and to 3.88% in laminated pouches after storage of six months. This may be due to the reason that the extruded products have a hygroscopic nature which resulted in the increase in moisture content during storage (Kocherla *et al*., 2012) [16]. In addition to this, it was observed that HDPE resulted in higher moisture increase as compared to that of LP. This is probably due to low barrier properties of HDPE (Khalifa, 2016) [14]. Similar results have been obtained by Butt *et al*. (2004) [5].

Water activity
The water activity of extrudates on day one was found to be 0.167 which augmented to 0.379 and 0.283 in snacks packed HDPE bags and laminated pouches respectively on the 180th day of storage. The increase in water activity of the extrudates may possibly be due to the change in humidity of the surrounding environment (Syed *et al.*, 2014) [20]. The range (0.4–0.6) of water activity values denotes the potential for a shelf stability of the products (Vadukapuram *et al*. 2014) [22]. Lipids are most stable to oxidation when water activity is within the range of 0.3–0.6 and all water activities under 0.75 values are considered acceptable in preventing microbial growth (Jensen and Risbo, 2007) [12]. Water activity for both extrudates stored in both HDPE and LP at ambient at ambient conditions were in safe limit for microbial safety.

Free fatty acid (FFA)
During the storage period of six months, the FFA content of the snacks was found to significantly increase from 0.04 to 0.41% in snacks packed in HDPE and to 0.28% in those packed in laminated pouches. However, the increase in FFA was within the permissible limits of 0.5% specified by FSSAI for foods. This increase in FFA content is due to oxidation of lipids caused during storage which leads to breakdown of long chain fatty acids to smaller ones (Khan *et al*., 2011). In addition to this, it was observed that formation of free fatty acids was higher in snacks packed in HDPE as compared to that of laminated pouches. This is probably due to its low oxygen and water vapour barrier properties of HDPE.

Peroxide Value (PV)

PV value is an important indicator of amount of peroxides formed due to oxidation during storage (Ozkan *et al*., 2007). With incresase in storage time the PV of snacks increased significantly ($p<0.05$) regardless of the packaging material from 0.25 to 0.67 meq/100 g and 0.58 meq/100 g in extrudates stored in HDPE bags and LP respectively after six months of storage. Increase in peroxide value during storage was comparatively higher in snacks stored in HDPE bags, due to poor moisture and oxygen barrier properties of HDPE compared to that of LP (Khalifa, 2016) [14].

Total Plate Count (TPC)

Owing to low water activity of the snacks and high temperature thermal processing the total plate count of snacks on day one of storage was negligible in snacks. Frazier and Westhoff (1988) [8] stated similar the reasons for low total plate count of extruded snacks, but after six months of storage, the total plate count was found to be 2.8 x 10^3 and 1.9 x 10^2 cfu/g in case of HDPE bags and laminated pouches respectively (Table 1). However, the TPC at the end of storage period was within the permissible limits ensuring microbiologically safe extrudates (ICMFS, 2010) [11].

Overall acceptability (OAA)

During storage period of six months, the overall acceptability score of snacks showed a significant decrease from 3.43 to 2.70 in extrudates stored in HDPE and to 2.90 in those stored in laminated pouches. The most likely cause for the lowering of sensory scores during storage is loss of crispness of snacks due to moisture gain, especially in the snacks stored in HDPE. Not only this, but, hike in FFA and peroxide value during storage period might as well have caused the loss of organoleptic properties of the snacks.

Conclusion

It can be concluded from this study, that quality attributes of rice and chickpea flour extrudates were significantly affected by packaging material and storage. Moisture content, water activity, free fatty acids and peroxide value of stored products increased in both packaging materials, while as sensory scores reduced. However, higher increase in moisture, free fatty acids, peroxide value and microbial level were noticed in HDPE, in comparison to LP. Above all, the results of storage and sensory studies showed the feasibility of LP for rice-chickpea flour extrudates for better storage upto six months than HDPE where in the products showed more susceptibility towards moisture gain, loss of crispness and oxidation.

Table 1: Effect of storage time and packaging material on chemical properties of extrudates during storage

Storage Period	Moisture (%)		Water activity		Peroxide Value (meq/Kg)		Free fatty acids (%)		Total plate Count (cfu/g) × 10^3		Overall acceptability	
	HDPE	LP	HDPE	LP	HDPE	LP	HDPE	LP	HDPE	LP	HDPE	LP
Ist Day of storage	2.13	2.13	0.167	0.167	0.25	0.25	0.04	0.04	N. D	N. D	3.43	3.43
30-DAS	2.45	2.24	0.182	0.181	0.34	0.31	0.08	0.05	0.2	N. D	3.37	3.4
60-DAS	2.75	2.45	0.196	0.188	0.41	0.37	0.11	0.09	0.4	0.3	3.3	3.35
90-DAS	3.29	2.68	0.236	0.206	0.49	0.45	0.18	0.11	0.7	0.5	3.25	3.3
120-DAS	3.53	2.93	0.273	0.236	0.52	0.49	0.27	0.16	1.1	0.9	3.14	3.21
150-DAS	4.17	3.32	0.295	0.251	0.58	0.53	0.35	0.21	1.6	1.2	2.96	3.05
180-DAS	4.35	3.88	0.379	0.283	0.67	0.58	0.41	0.28	2.3	1.8	2.7	2.9
Mean	3.23	2.8	0.24	0.21	0.423	0.425	0.2	0.13	1.05	0.94	3.16	3.23
C.D(p<0.05)	Days = 0.369		Days =0.028		Days = 0.055		Days = 0.024		Days = 0.125		Days = 0.40	
	PM = 0.197		PM= 0.015		PM= 0.029		PM = 0.013		PM= 0.067		PM=N. S	
	Days × PM = N.S		Days × PM = 0.040		Days × PM = N.S		Days × PM = 0.034		Days × PM =0.177		Days × PM = N.S	

C.D. Critical difference; ND: Not Detected; DAS: Days after storage; PM: Packaging material; LP: Laminated Pouches

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