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Research Article

Study on Evaluation of Functional Properties of Blends of Soy and Jackfruit Seed Floor Based Extruded Products

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Abstract: The present study aims in preparing novel products with better functional properties (expansion ratio, bulk density and antioxidant properties) from jackfruit seed flour and soy flour, whose availability is increasing in India. Extrusion of soy flour was standardized on the basis of product response which gave the highest expansion ratio, lowest bulk density, and maximum *in-vitro* antioxidant properties. The optimized parameters were further applied for the preparation of extrudates from composite blends of Jackfruit seed flour incorporated at different levels from 20-80% in soy flour. A portion of equilibrated composite blends of soy and jackfruit seed flour was subjected to analysis prior to extrusion in terms of functional and antioxidant properties. Total flavonoid content was found to be slightly higher in post-extruded samples. *In-vitro* antioxidant activities showed significantly higher to a marginal increase in antioxidant properties post-extrusion. HPLC analysis of the products showed the presence of phenolic entities like ascorbic acid, gallic acid, vanillin; and flavones like catechin and daidzein. From texture analysis, hardness of the extrudates was found to be acceptable. Post extrusion analysis of the newly developed product showed increased functional values compared to control mix containing only soy flour. Hence, it was concluded from the study that addition of jackfruit seed flour improved the overall functional property of extruded products.

***Keywords*:** optimization, extrusion, Jackfruit seed flour, soy flour, functional property, antioxidant activity

1. Introduction

Food industries are facing the challenge of developing food products with novel or special health-enhancing characteristics. To meet this challenge, it must identify new source of nutraceuticals and other natural materials having the desirable functional and nutritional characteristics [1]. In recent years, there has been a marked increase in the search for natural antioxidants and other bioactive compounds from less known sources, especially of plant origin. Not only the fruits, but the seeds also contain a large number of bioactive components which can impart remedial uses. In view of this, various plants and plant products are under investigation for the detection of antioxidant and bioactive rich compounds.

New kinds of plant-based raw materials are in use in recent scientific works. Food industries are in search of such raw materials with functional and novel properties. Although not common in food formulations, jackfruit seed has the ability to convey functional benefits and promote good consumer health as it imparts added physiological benefits along

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with nutrition [2-3]. In a recent study jackfruit seeds are claimed to be the accompaniment to functional foods. These seeds are underutilized and non-conventional, but have immense nutritional benefits and can be considered as a possible functional food ingredient [4]. The seeds contain a rich amount of carbohydrates and proteins and are a good source of fiber and vitamins. Jacalin, is a major protein present in jackfruit seeds that possess immunological properties; however, it is also a lectin that bear antinutritional properties. Jackfruit seeds are explored in terms of nutritional and antioxidant properties [2]. It is found to contribute more than 70% to the total antioxidant activity, an appreciable amount of phenolic compounds and carotenoid content [3, 5].

Soybean, on the other hand, is an indispensable part of functional foods, and at the same time could be used for the enrichment of product quality [6]. Soybean seed is an important commodity, rich in essential macro nutrients, phenolic compounds and flavonoids; predominantly isoflavones. These provide immense benefits by preventing a number of chronic diseases like diabetes, cancer, coronary heart disease and osteoporosis [7]. A combination of two seed flours namely jackfruit seed and soy flour will eventually result in better functional benefits

Extrusion cooking technology has become a cornerstone of the food industry, primarily in cereal, dairy, bakery, confectionery and pet food industries and most recently was used in the development of expanded, novel, value-added foods [8-9]. Research work shows that antioxidant activity changes during various types of food processing technologies, especially involving heat treatment. It is observed that extrusion cooking does not affect the phytate content of oat products but the amount of dietary fiber is altered [10].

Thus, the present study is based on the determination of the physical and functional properties of extruded blends of jackfruit and soy seed flour and the determination of the antioxidant activity of the composite flour blends before and after extrusion. The study also aims to investigate possibilities of utilization through the production of antioxidant-rich jackfruit seed-based snack food using a twin-screw extrusion process after successful optimization of process parameters.

2. Materials and methods

2.1 *Plant seed material, chemicals and reagents*

Jackfruit seed is an important edible seed popularly available and consumed at house level and in culinary uses. Local variety of Jackfruit seeds were purchased from the market of Howrah (West Bengal), India, sold by greengrocers. Soy flour was generously gifted by Progressive Exim Limited, Raipur, India. Sugar and salt were procured from local groceries; powdered sugar was used in this study. Distilled water was used for blend conditioning. All the chemical reagents and solvents used were of MERCK (Emerck India Ltd. Mumbai, India). HPLC-grade methanol, acetic acid and Milli-Q water were used for HPLC solvent system. Authentic antioxidant standards (Gallic Acid, Vanillin, Ascorbic acid, Tannic acid, Ferrulic acid, Catechin, Diadzein and Biochanin) used for quantitative antioxidant analysis were of Sigma-Aldrich Pvt. Ltd.

2.2 *Preparation of seed flours*

The jackfruit seeds flour was prepared following the method of [11] with minor alteration. Seeds were manually cleaned and white outer skin was removed followed by lye-peeling, to remove the thin brown layer. The peeled seeds were then washed under running water, sliced into thin chips and dried at 50 °C in an air oven (LASIN Itherm AI-7981, Mumbai, India) for 12 h. The dried chips were ground and pulverized to get jackfruit seed flour. Sieving was done with 100 mesh size screen to obtain flour of 150 micron particle size, using a laboratory vibrating sieve shaker machine (Model-911MPELMS-200, Canada). Soy flour was also sieved to obtain uniform particle size. Flour was stored in refrigerator for further analysis and composite blend preparation. The flours used in the study had the following composition which were determined using approved methods of [12], jackfruit seed flour has a good amount of carbohydrate 68.0 ± 0.49 $g.100g^{-1}$ and fiber 10.2 ± 0.04 $g.100g^{-1}$. Protein content was recorded to be 12.4 ± 0.34 $g.100g^{-1}$, fat content was around 5.0 ± 0.05 $g.100g^{-1}$; fair amount of ash of 2.8 ± 0.16 $g.100g^{-1}$ and moisture of 10.1 ± 0.0 $g.100g^{-1}$ was recorded on dry basis (db), whereas soy flour had high content of protein, 43.8 ± 0.28 $g.100g^{-1}$. Carbohydrate, fiber and ash percentage were 31.64 ± $g.100g^{-1}$, 5.3 ± 0.21 $g.100g^{-1}$, 7.5 ± 0.17 $g.100g^{-1}$ respectively (db). Moisture content of soy flour was found to be 11.0 ± 0.10 $g.100g^{-1}$ (db).

2.3 *Optimization of process parameter and preparation of composite flour blend and extruded products*

Table 1. Optimization of extrusion process parameters for functional properties and antioxidant activity of extrudates from different blends

SET No	RUN No.	Extrusion Process Parameters					Functional Properties and Antioxidant activity			
		Blend Proportion (%)		Moist %	Temp °C	Screw speed (RPM)	Expansion Ratio	Bulk Density (kg.cm^{-3})	Radical Scavenging Activity (%)	Total Antioxidant Activity (mg TE/g db)
		SF	JSF							
S-I	Run-1	100	0	10	100	300	2.61 ± 0.01	0.12 ± 0.01	50.11 ± 0.23	186.36 ± 0.42
	Run-2	100	0	12	100	300	2.70 ± 0.01	0.13 ± 0.00	51.01 ± 0.31	186.17 ± 0.25
	Run-3	100	0	14	100	300	2.76 ± 0.03	0.16 ± 0.00	49.97 ± 0.58	186.84 ± 0.51
	Run-4	100	0	16	100	300	2.84 ± 0.03	0.17 ± 0.01	51.09 ± 0.22	186.22 ± 0.54
	Run-5	100	0	18	100	300	2.81 ± 0.01	0.18 ± 0.00	49.00 ± 0.61	186.47 ± 0.51
S-II	Run-1	100	0	12	100	300	2.83 ± 0.02	0.16 ± 0.00	51.06 ± 0.49	189.16 ± 0.19
	Run-2	100	0	12	110	300	2.90 ± 0.02	0.14 ± 0.00	50.96 ± 0.64	188.36 ± 0.48
	Run-3	100	0	12	120	300	2.84 ± 0.03	0.13 ± 0.01	50.03 ± 0.53	186.44 ± 0.51
	Run-4	100	0	12	130	300	2.84 ± 0.02	0.12 ± 0.01	49.91 ± 0.47	186.13 ± 0.17
	Run-5	100	0	12	140	300	2.71 ± 0.01	0.11 ± 0.01	49.07 ± 0.35	182.18 ± 0.51
S-III	Run-1	100	0	12	130	250	2.84 ± 0.01	0.13 ± 0.01	50.39 ± 0.49	175.52 ± 0.51
	Run-2	100	0	12	130	275	2.86 ± 0.00	0.12 ± 0.00	49.81 ± 0.54	177.10 ± 0.21
	Run-3	100	0	12	130	300	2.90 ± 0.02	0.12 ± 0.00	50.79 ± 0.49	183.98 ± 0.65
	Run-4	100	0	12	130	325	2.97 ± 0.01	0.12 ± 0.00	50.96 ± 0.56	187.88 ± 0.48
	Run-5	100	0	12	130	350	2.96 ± 0.00	0.11 ± 0.00	50.14 ± 0.54	187.71 ± 0.47
S-IV	Run-1	80	20	12	130	325	2.97 ± 0.00	0.12 ± 0.00	52.15 ± 0.63	189.04 ± 0.58
	Run-2	60	40	12	130	325	3.06 ± 0.02	0.13 ± 0.00	57.80 ± 0.50	191.96 ± 0.15
	Run-3	50	50	12	130	325	3.08 ± 0.07	0.11 ± 0.00	63.79 ± 0.54	194.44 ± 0.51
	Run-4	40	60	12	130	325	3.11 ± 0.01	0.10 ± 0.00	72.04 ± 0.63	198.41 ± 0.49
	Run-5	20	80	12	130	325	3.06 ± 0.05	0.10 ± 0.00	72.02 ± 0.54	198.27 ± 0.51

Results are triplicate mean ± SD, SF- Soy flour; JSF- Jackfruit seed flour, RPM- Revolution per minute, TE- Trolox equivalent

For optimization of the process parameters for preparation of extruded products by twin-screw extrusion cooking with maximum antioxidant properties and expansion ratio and other desirable functional properties, soy flour equilibrated to different moisture levels was extruded at varying barrel temperatures and screw speed. A set of flour blends was hydrated (a part of precondition process, which may be defined as a prerequisite processing step of blending steam and water with dry ingredients to achieve temperature and moisture equilibrium, which in turn results in more uniform cooking of ingredients) [13] and mixed using an electric blender (Model-NOVA NM-79WB, made in China) for 10 min in order to achieve uniformity before extrusion. The hydrated blends were kept in sealed food-grade plastic bags and allowed to equilibrate at refrigeration temperature for 24 h before extruding. The equilibrated blends were extruded using a co-rotating lab scale twin-screw extruder (LAB Model EB-10, Basic Technology Pvt. Ltd, Kolkata, India). The length-to-diameter (L/D) ratio for the extruder was approximately 20:1. A circular cross-section die with a diameter of 3 mm and die length of 27 mm was used. Optimization has been tabulated in Table 1. In SET-I, 2 kg of soy flour was separately equilibrated to feed moisture of 10-18% (db) with 2% increments at each level of run and conditioned for 24 ± 1 h was extruded at 120 ± 5 °C temperature and 300 ± 10 rpm barrel screw speed. Followed by the observations based on the properties of the extrudates, the second set (SET-II) of the experiment containing 2 kg soy flour equilibrated with the moisture level of 12% (optimized), conditioned for 24 ± 1 h. Four sets of the experimental study were carried out for extruded food preparation. Ingredient components of the composite flour blend were extruded at a temperature ranging from 100-150 °C with 10 °C increments and 300 ± 10 rpm barrel screw speed. Further, in the next set (SET-III) optimized moisture equilibrated flour was extruded at 130 °C temperature (as found optimum from the observations of the previous sets) at various screw speeds ranging 250-350 ± 5 rpm with increments of 25 rpm. Finally, in the last set of run (SET-IV) blends of soya flour and jackfruit seed flour were prepared at different feed ratios (80:20, 60:40, 50:50, 40:60 and 20:80) and extruded at the optimized parameters of moisture level (12%), temperature 130 °C and screw speed of 325 rpm. The proportion of salt (2%) and powdered sugar (20%) addition was kept invariable in each set throughout the experimental run.

Samples from each trial were dried by exposing to 45% relative humidity (RH) and 40 °C temperature and the functional properties including Expansion Ratio and antioxidant activity of the extrudates were recorded. Followed by the experiment, the composite blend of 40:60 (soy flour: jackfruit seed flour) with 12% blend moisture content, 130 °C barrel temp and screw speed of 325 rpm was considered optimum for product development, with better antioxidant and functional properties in this study. The jackfruit seed flour-based extrudates were collected in trays and dried in a convection oven at 105 °C till 4-5% moisture content is reached (moisture determined by [12]). The product was cooled and tempered at 4 °C before being stored into food-grade polyethylene bags for further analysis. Each of the blends of feed mixes of the sets prior to extrusion was simultaneously studied for physical and antioxidant properties along with the extruded products for comparison.

2.4 *Physical and functional properties*

Moisture content was determined following the official method of the Association of Official Analytical Chemists [12] and was expressed in terms of percentage. Physical properties like expansion ratio (ER) were measured using [14]. Functional properties like water holding capacity (WHC), oil holding capacity (OHC), water absorption index (WAI), water solubility index (WSI), and bulk density (BD) were evaluated for both the raw blends and extrudates using [15].

2.5 *Color parameters*

Color parameters were evaluated using a handheld Konica Minolta Color reader (CR-10), calibrated with standard black and white color before use and reported as L^*, a^* and b^* values. L^* is a measure of lightness, a^* represents the color range from green to red and b^* represents the color range from blue to yellow [16]. A powdered test sample was taken in a 50 × 12 mm glass petri dish and color was measured in triplicate values per sample.

2.6 *Hardness*

Hardness is technically defined as the peak force of the first compression cycle. Hardness of the extruded samples was carried out using a Texture Analyzer (ModelStable Micro Systems TA.XT Express Enhanced, UK) equipped with P/5 aluminium cylinder probe of 5 mm diameter. Randomly selected extruded sample was placed below the probe and compressed twice and the first highest peak value was recorded as the measurement of hardness and analyzed by Texture Exponent LiteExpress software program (version 6.0) [17]. The hardness was measured in triplicate for each sample.

2.7 *Antioxidant assay*

2.7.1 *Preparation of sample extract for antioxidant assay*

100 ml of methanol was added to 10 g of ground sample. Then it was extracted for 24 h at room temperature with constant stirring. The supernatant was collected by filtration and the solvent was evaporated to make crude extract. The crude extracts were used for various analyses by volume adjustment using methanol at 1% extract concentration.

2.7.2 *Qualitative tests for preliminary phytochemical screening*

Preliminary phytochemical screening for minor constituents like tannins, flavonoids, phlobatannins, saponins, glycosides and steroid of methanolic extracts of the flour blends, before and after extrusion was carried out using [18]. Iso-flavone was detected using [19].

2.7.3 *Determination of total phenolic compounds*

Total phenolic content of sample extracts was carried out following the Folin-Ciocalteu method. Here, 0.1 ml of the sample extract was taken in test tubes and made up to the volume of 1ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of 20% sodium carbonate solution were added in each tube in a triplicate manner. After vortexing the reaction mixture, the test tubes were incubated in dark for 40 minutes and the absorbance was recorded at 725nm against a reagent blank. All tests were carried out in triplicate. The concentrations of phenolic compounds in the extracts were obtained through a linear calibration curve ($y = 0.371x + 0.044$), where 'x' represent the concentration and 'y' represent the absorbance, obtained with gallic acid at concentrations between 0 and 0.5 g/L. The results were expressed in mg of gallic acid equivalents (GAE) per g of sample (db) [20].

2.7.4 *Determination of flavonoids*

Total flavonoid content was determined using [21]. The total flavonoid concentration in each sample extract was determined by UV-VIS spectrophotometer (Jasco V-630, Jasco International Co.Ltd, Japan). Quercetin was used as Standard. A linear calibration curve of $y = 0.145x + 0.055$ was obtained with quercetin at concentrations range of 0.02 and 0.20 g/L. Concentration of sample extract was obtained based on the standard curve of quercetin. The results were expressed in mg of quercetin equivalents (QE) per g of sample.

2.7.5 *In vitro antioxidant assays*

Radical Scavenging Activity (RSA)- The radical scavenging efficacy of sample extracts was analyzed using 1, 1-Diphenyl Picryl Hydrazyl (DPPH) [22]. 1 ml of sample aliquots (1 mg/ml) was mixed with 60 mM 1, 1-Diphenyl Picryl Hydrazyl (DPPH) solution in methanol and (DPPH) % scavenging = 100 × [As/Ac]; where *As* is the sample absorbance and *Ac* is the control absorbance at 517 nm. Triplicate measurements were conducted, and mean results were tabulated. IC_{50} values of DPPH scavenging assay was also carried out with sample aliquots at different concentration 0.2-1 mg/ml.

Ferric Reducing Antioxidant Power (FRAP)- FRAP assay was carried out using a modified experimental protocol [23]. The FRAP reagent containing sodium acetate pH 3.6, 2,4,6-Tripyridyl-S-triazine (TPTZ) from Sigma Aldrich, and ferric chloride was prepared freshly. The FRAP reagents were mixed with 10 µL aliquots of sample extract. Then the mixture was incubated and absorbance was read at 593 nm. The FRAP value was determined by plotting in a standard curve produced by the addition of ferrous sulfate to the FRAP reagent. Results were expressed as µmol Fe+2 per g (db).

Reducing Power Assay- The reducing power of the sample extracts was determined by a slight modification of the method followed by [24]. 1 ml aliquots of sample extract mixed with 2.5 ml phosphate buffer and 2.5 ml potassium ferricyanide (1%) were incubated at 50 °C for 20 min followed by addition of 2.5 ml of 10% trichloroacetic acid and centrifuged for 10 min at 3000 rpm. 2.5 ml supernatant was mixed with the same volume of distilled water and 0.5 ml $FeCl_3$ (0.1%) solution, and the absorbance was measured at 700 nm spectrophotometrically using Jasco V-630, UV-VIS Spectrophotometer.

Total antioxidant capacity- The total antioxidant capacity of methanol extracts of samples was evaluated by the phosphomolybdenum method of [25]. An aliquot of 0.5 ml of sample solution (0.2, 0.4, 0.6, 0.8, 1 mg/ml) was combined with 5 ml reagent solution (0.6 m sulfuric acid, 28 mm sodium phosphate and 4 mm ammonium molybdate). The tubes were capped and incubated at 95 °C for 1h and 30 min. On cooling, the absorbance of the aqueous solution of each sample was measured at 695 nm against a reference cell on Spectrophotometer. Reference solution was prepared and incubated under the same conditions. Antioxidant capacity is expressed as equivalents of ascorbic acid.

2.8 *Evaluation of phenolic compounds and flavones by HPLC*

2.8.1 *Phenolics*

Analysis was performed with minor modifications to the method of [26] temperature and time on the extraction of total phenolic content, total flavonoids and antioxidant capacity (FRAP and DPPH. The sample extract was prepared using 1000 mg of powdered sample in 10 ml of methanol with constant stirring at 35-40 °C for 24 h. The extract was filtered using Whatman filter paper Grade No-1 (size-110 mm) twice. The total extract was condensed and evaporated using a Rotary vacuum evaporator (Heidolph Laborota-4000, Sigma Aldrich, Germany); the extract was reduced under a high vacuum to obtain the dry weight of the sample extract. The extract was dissolved in a known amount of non-polar solvent and it was subjected to water wash at least thrice to remove any water-soluble carbohydrates. Then the extract was vacuum dried and a known amount was dissolved in methanol. This extract was checked for the presence of any triglycerides by Thin Layer Chromatography which might have got extracted in methanol during extract preparation. These steps were adopted to obtain pure antioxidant extracts. The HPLC method has been standardized in the present study for the purpose of analysis of different components in the solvent extracts from both the blends before and after extrusion. The 20 µL of Standard antioxidants (ascorbic acid, tannic acid, ferulic acid, gallic acid and vanillin) and sample extracts were separately injected into Agilent Technologies 1200 series model HPLC system, India. Separation was done in a 5 µm reversed phase column RPC-18. The mobile phases were A) Methanol and B) 1% acetic acid in water. Its solvent system was run in isocratic mode and the flow rate was 1 ml/min. for 20 min run time. The eluents were continuously monitored in a DAD-UV detector at 254 nm. Peak identification was based on the retention time in comparison with standard phenolics (Sigma-Aldrich).

2.8.2 *Flavones*

Flavones were characterized by slight modification of method [27]. 500 mg sample blend was extracted with 5 mL of dimethyl sulfoxide (DMSO): methanol (1:4 v/v) and heated at 50 °C for 12 h in sealed containers. Sample extracts were centrifuged and supernatants filtered using 0.2 µm TF filters. For isoflavones quantification 20 µL aliquots of the extracts were injected into HPLC system (Agilent Technologies 1200 series model) equipped with DAD-UV detector at 260 nm. Isoflavones separation was done in a 5 µm reversed phase column RPC-18. Isoflavones were detected and quantified by comparison with standard curves of genistin and daidzein. Peak identification was based on the retention time in comparison with standard isoflavones (Diadzein, Catechin and Biochanin) (Sigma-Aldrich).

2.9 *Statistical analyses*

Statistical analysis was performed using analysis of variance (ANOVA) and the comparison of the means across the group was done by Tukey test. All the analysis was carried out in triplicates and repeated at least twice with the Origin pro 8 statistical software. The significant levels were determined at $p \leq 0.05$.

3. Results and discussion

3.1 *Process parameter optimization*

Feed moisture content, barrel temperature and screw speed of the extruder were found to influence the expansion and distribution of antioxidant activities of the products as can be seen in Table 1. Optimization of process parameters revealed that 12% feed moisture, 130 °C barrel temperature and 325 rpm screw speed were most suitable for the production of soy-flour based extrudates with a maximum expansion ratio of 2.97 and higher antioxidant properties. Higher feed moisture resulted in poor expansion; however, moisture below 12% resulted in the formation of semi-cooked products with lower expansion, which might be due to a lack of proper gelatinization of starch material of the feed due to lack of sufficient moisture. Likewise, temperature below 120 °C resulted in undercooked products and temperature beyond 140 °C resulted in slight browning of the products, which also affected the antioxidant activities. Initially at a constant barrel temperature of 100 °C, the bulk density of the soy-based extrudates was found to increase with increase in moisture content. The increase in bulk density with increasing moisture content during extrusion could probably be due to a reduction in the elasticity of the dough mass as a result of plasticization of the melt, causing a reduction in gelatinization and an increase in the bulk density of the extrudates as also reported by [28]. However, as temperature increased in the next set of run the bulk density was found to decrease gradually. The probable cause could be that high temperature could have increased the degree of heating of water in the extruder which boosts the bubble formation and a decrease in melting viscosity leading to reduced density in all the extrudates. A similar kind of observation was reported by [28]. Screw speed also showed to affect the bulk density and an inverse relation was observed between them; with increased screw speed BD was found to decrease. This might have occurred due to the structural breakdown of protein and complex carbohydrates under influence of high screw speed which might have created a high shear environment resulting in lower BD. This inverse relationship was also reported by [29]. Increased expansion leads to lower-density products. Also the revolution of the barrel screw at 325 rpm gave the highest scavenging activity in products. This indicated that higher residence time results in the reduction of antioxidant activity. Based on the optimized parameters, blends of jackfruit seed flour and soy seed flour at different ratios were extruded, which showed that 60% replacement of soy flour by jackfruit seed flour produced the most acceptable results in terms of antioxidants and functional properties. The expansion ratio was found to increase slightly by up to 4.71% when compared to the control soy-based product at similar optimized process conditions. An increase in the expansion ratio could be due to the incorporation of co-ingredient namely jackfruit seed flour which has a comparatively much lower protein content than soy flour. As high protein results in hardness and inhibits the expansion of extrudates as thus reduction in protein content might have increased the expansion in the products, this is already reported by many researchers including [30]. This composite blend also resulted in the lowest bulk density of 0.10 g cm^{-3}. Lower bulk densities in extruded foods resulted due to significantly low moisture and high temperature. A similar observation was reported by [13]. The highest RSA of 72.04% and total antioxidant activity of 198.41 mg TE/g db was obtained as product response, at 60% replacement of soy flour by jackfruit seed flour.

3.2 *Functional properties*

The optimized composite blend gave the following results before and after extrusion in terms of functional properties (Table 2). Maximum moisture reduction (63.80%) was noted post extrusion in product containing composite flour blend. There is a significant difference ($p \geq 0.05$) in ER between the control product and the jackfruit seed flour incorporated product under similar extrusion process conditions. Jackfruit seed flour being mostly non-conventional flour rich in carbohydrate moiety contains starch that induces gelatinization during extrusion cooking. Gelatinization of starch moieties is known to induce expansion in extruded snacks [31].

Post extrusion study revealed a decrease in BD in the case of both the control and the jackfruit seed flour incorporated product. No significant difference was found in WAI content of the control and composite product; values were 5.29% and 5.26% respectively. WAI as can be seen increased by 59.39% post-extrusion. Increase in WAI in the extruded product might be due to loss of moisture from the pre-extruded hydrated blend that generally results during extrusion cooking. Increase in water content led to higher values of WAI [32]. WSI increased post-extrusion in the case of both the control product and the composite product. Increasing solubility index might have resulted due to the formation of some simplified and water-soluble products during extrusion. A similar explanation was reported that WSI is associated with the number of soluble solids, which indicates degradation and dextrinization of starch [33]. It also measures the degree of starch conversion which is related to the amount of soluble polysaccharide released from the starch particles during extrusion. Percentage increase in WSI of around 52.88% was found in the composite products and around 27.76% increase in control. There was a huge increase in WHC post-extrusion. However, a very minor difference was noted between the control (335) and composite product (337) post-extrusion. Decrease in OHC was observed post-extrusion. The possible reason behind the decrease in OHC could be due to the simplification of complex carbohydrates and the fiber present in the sample post extrusion leading to the formation of simpler carbohydrates that are hydrophilic in nature which in turn decreases their affinity towards non-polar substances like oil. The composite products showed 40.42% decrease in OHC, which can be considered beneficial in terms of low oil-absorbing food products.

Table 2. Functional and Color Properties of composite blend mix and control at optimized condition before and after extrusion cooking

Parameters	Pre-extrusion		Post-extrusion	
	Control mix	Composite blend mix	Control product	Composite Product
Moisture (% db)	12.21 ± 0.02	12.06 ± 0.05^{a}	5.29 ± 0.01	4.46 ± 0.04^{c}
Expansion Ratio	NA	NA	2.97 ± 0.01	3.11 ± 0.01^{b}
Bulk Density (kg.cm^{-3})	0.39 ± 0.01	0.31 ± 0.00^{c}	0.12 ± 0.00	0.10 ± 0.00^{a}
WAI (%)	3.28 ± 0.01	3.51 ± 0.01^{d}	5.29 ± 0.01	5.26 ± 0.01^{a}
WSI (%)	13.36 ± 0.35	11.1 ± 0.17^{c}	17.07 ± 0.53	16.97 ± 0.53^{d}
WHC (%)	108.93 ± 1.00	221.83 ± 0.76^{b}	335.00 ± 1.03	337.00 ± 2.06^{e}
OHC (%)	12.43 ± 0.09	11.85 ± 0.11^{b}	9.04 ± 0.00	7.06 ± 0.01^{b}
L^*	64.10 ± 0.56	59.00 ± 0.28^{c}	56.15 ± 0.07	54.30 ± 0.21^{c}
a^*	9.40 ± 0.28	10.10 ± 0.00^{b}	11.50 ± 0.14	10.90 ± 0.07^{b}
b^*	22.80 ± 0.07	22.10 ± 0.07^{a}	20.00 ± 0.00	19.20 ± 0.07^{b}
Hardness (N)	NA	NA	425.66 ± 0.16	202.00 ± 2.64^{d}

Values in rows with different superscript are significantly different at $p < 0.05$ Data are means ± SD; n = 3.
NA-Not applicable.

3.3 *Color parameter*

Color is an important attribute of extruded products. Shear forces generated during extrusion cooking speed up the reactions between amino acids and reducing sugars present in the feed material [34]. The color value of extruded food products is tabulated in Table 2. Pre-extruded flour showed the highest L^* value whereas post-extruded products have comparatively low L^* values. Composite flour blend was found to have lower L^* value both pre- and post-extrusion when compared to the control values. However, a remarkable observation is made where it was seen that the L^* value of the control mix post extrusion was reduced by 12.4% whereas L^* value of the composite mix was reduced by only 7.9%. A similar observation was reported where L^* value of extruded product decreases with an increasing amount of finger millet flour [14]. Likewise, the parameter a^* indicative of green to red color increased from 9.4 ± 0.28 to 11.5 ± 0.14 in the control product and 10.1 ± 0.00 to 10.9 ± 0.07 in the composite product after extrusion. The dark color formed during the caramelization of sugar from the Maillard reaction, which might have increased redness (a^*) value post extrusion [14]. The values indicate that jackfruit seed flour incorporation in soy can effectively minimize browning during extrusion, which results in Maillard reaction product formation.

3.4 *Hardness*

Texture profile is an important parameter in evaluating the physical nature of extrudates. Table 2 showed the hardness (N) of the extrudates. The optimized composite blend-based extrudates have a lesser and acceptable hardness, when compared to the control extrudates. From the present study it was found that Jackfruit seed flour incorporation by up to 60% resulted in 52.62% decrease in the hardness of extrudates, or in other words, increased soy flour level in extrudates resulted in increased hardness. The possible reason behind the increased hardness could be due to the incorporation of protein-rich material namely soy flour, which increased the hardness of products. A similar finding reported that increase in finger millet flour level in feed composite mix resulted in increased hardness of extrudates [14]. The hardness of extrudates is affected by moisture and barrel temperature, while screw speed has no effect on hardness. Application of the optimal conditions of 14% feed moisture content, 148 °C temperature and 549 rpm screw speed, produced extrudates with optimum hardness (peak force) [13].

3.5 *Preliminary phytochemical screening*

Values of phytochemical screening of the optimized composite blend pre and post extrusion are detailed in Table 3. Glycosides and flavonoids were present in composite flour before and after extrusion. Tannin and steroids were present in composite blends before extrusion but absent in extruded products, the reason for this could be high temperature and the presence of oxygen during extrusion cooking that brings about changes in these groups. Saponins and phlobatannins were absent in the composite flour mix before and after extrusion. The presence of isoflavones has been confirmed UV Spectrophotometrically and the highest peaks were obtained at 268 nm and 348 nm at a wavelength range of 250-270 nm and 330-350 nm respectively [35].

Table 3. Phytochemical screening of optimized composite flour blend before and after extrusion

Phytochemical test	Pre extrusion	Post extrusion
Test for Tannin (Ferric chloride test)	+	-
Test for Phlobatannins	-	-
Test for Glycosides (Keller-Killainitest)	+	+
Test for Flavonoids (Shinoda test)	+	+
Isoflavones	+	+
Test for Saponins	-	-
Steroid test	+	-

Present: (+), Absent: (-)

3.6 *Antioxidant properties*

Polyphenol content and antioxidant activity of extruded food are greatly dependent on the feed moisture content and extrusion barrel temperature [36]. This is evident from the present study as well. Figure 1 represents the antioxidant properties of the samples. Total phenolic content (TPC) showed a slight reduction in post extruded composite product, similar change was noted in pre and post-extruded control samples (Figure 1a). The result showed a positive co-relation with that obtained from HPLC data, where phenolic acid like-ascorbic acid was found to reduce post extrusion. Total flavonoid was found to increase slightly in post-extruded samples as seen in Figure 1b. Among the in-vitro antioxidant studies, DPPH Radical Scavenging Activity (RSA), FRAP assay and reducing power assay showed higher antioxidant activity than control. Also post extrusion study revealed a slight increase, about 4.78% to 3.23% in RSA and FRAP values in composite samples respectively (Figure 1c-e). DPPH assay is one of the widely used in vitro analyses to determine the radical scavenging efficacy of the antioxidants, in sample extracts. DPPH scavenging activity of the sample extracts was found to be directly proportional to the amount of jackfruit seed flour added to soy flour. Radical scavenging activity of extracts with 60% jackfruit seed flour incorporation was found to be highest; 75% in pre-extruded blend and 78% in post extruded blend.

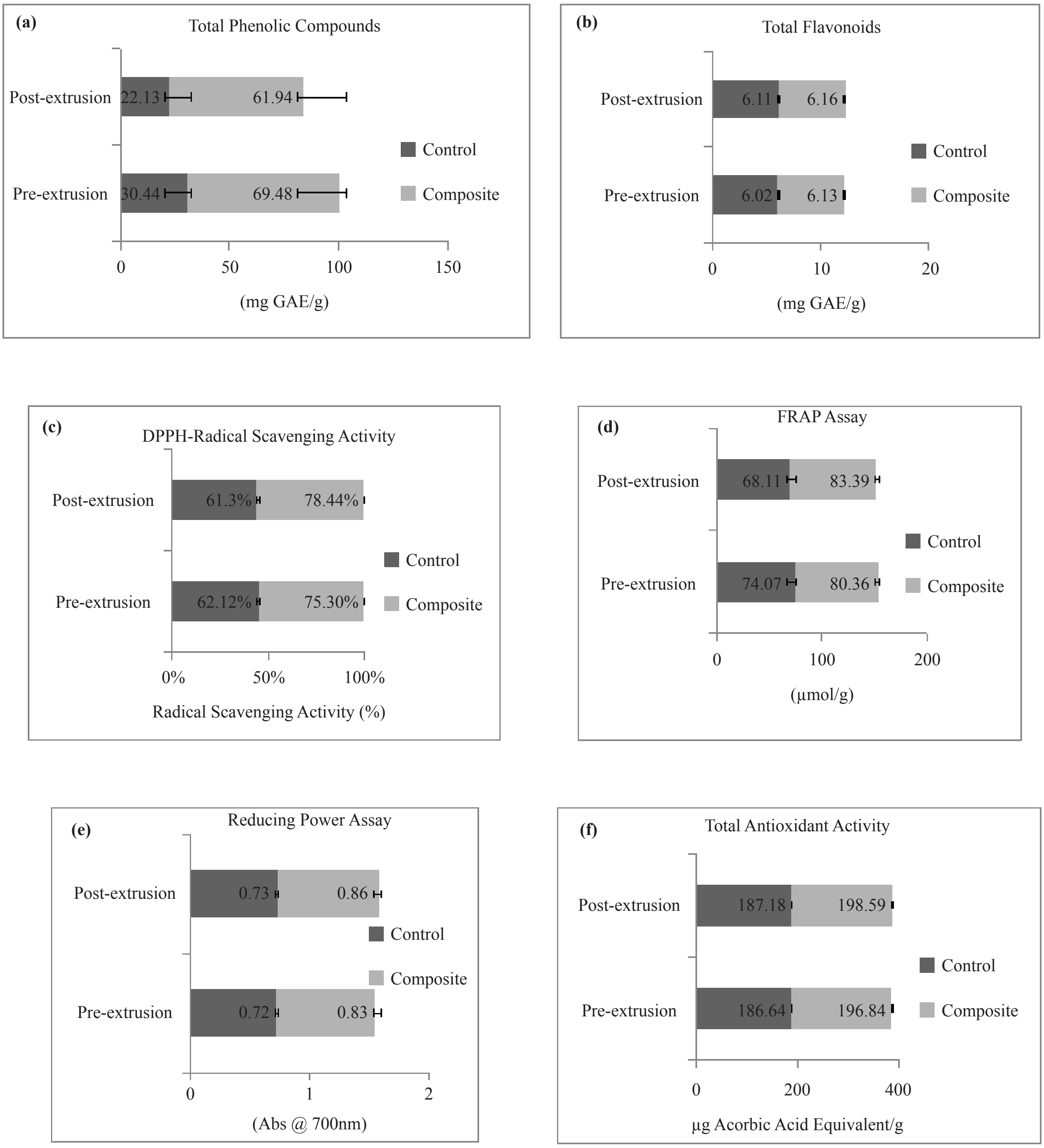

Figure 1. Antioxidant properties of optimized product mix before and after extrusion (a) represents total phenolics, (b) represents total flavonoids (c) represents radical scavenging activity (d) represents FRAP values (e) represents reducing power assay (f) represents total antioxidant activity. Values are represented as mean ± SD (n = 3).

IC_{50} of a sample is inversely related to antioxidant efficacy. The lower the IC_{50} value higher is the antioxidant activity. IC_{50} values were found to be lowest (0.63 mg/ml) in extruded sample containing composite blend whereas other samples fall in increasing order of pre-extruded composite blend (0.66 mg/ml) < control post extruded soy (0.80 mg/ml) < pre-extruded soy samples (0.81 mg/ml). Total antioxidant activity of the extrusion-treated samples was also found to be slightly higher as compared to the pre-extruded control and blend as represented in Figure 1f.

3.7 *Determination of antioxidant by HPLC*

Antioxidants like isoflavones, flavonoids, flavonol, phenolic acids, hydroxybenzoic acid and phenolic aldehyde were studied for, in the flour and composite blend mix pre and post-extrusion. Out of the eight compounds considered in this study, only five compounds were detected and quantified in the samples. The retention time of the antioxidant moieties matched with their standards. The concentration of the antioxidant-rich compounds was quantified and presented in mg per 100mg sample (Table 4). Catechin was found to be a major compound present in jackfruit seed flour at a high concentration (8.44 ± $mg.100^{-mg}$) which was not detected in soy flour. Jackfruit seed was also found to contain isoflavones moiety (daidzein), although at a lower concentration (0.19 ± $mg.100^{-mg}$) than soy flour (0.37 ± $mg.100^{-mg}$). Among others ascorbic acid, gallic acid and vanillin were found to be present at significantly higher concentrations compared to soy flour. Post-extruded composite blends showed a decrease in the amount of ascorbic acid and vanillin by around 22.35% and 20.37% respectively, whereas other compounds did not show a significant reduction in antioxidant-rich compounds post-extrusion. The reduction in the concentration of ascorbic acid can be justified by the fact that ascorbic acid being a heat-labile moiety might have lost from the sample during thermal processing by twin screw extrusion cooking. Also, vanillin being a highly volatile compound was affected after extrusion cooking.

Table 4. Quantification of antioxidants by HPLC of flour samples and composite blends pre and post-extrusion

Compounds (Categorically)	RT (min)	Concentration in $mg.100^{-mg}$ Sample ± SD			
		Soy flour	Jackfruit seed flour	Composite blend (pre-extrusion)	Composite blend (post extrusion)
Isoflavones					
Diadzein	12.744	0.366 ± 0.00	0.19 ± 0.00	0.25 ± 0.01	0.24 ± 0.00
Flavonoids					
Biochanin	13.619	ND	ND	ND	ND
Flavonol					
Catechin	9.612	ND	8.41 ± 0.02	6.91 ± 0.02	6.92 ± 0.01
Phenolic Acids					
Ascorbic Acid	16.212	1.42 ± 0.01	1.51 ± 0.01	2.55 ± 0.01	1.98 ± 0.03
Tannic Acid	9.918	ND	ND	ND	ND
Ferrulic acid	11.406	ND	ND	ND	ND
Hydroxybenzoic Acid					
Gallic Acid	8.892	0.08 ± 0.00	0.24 ± 0.01	0.13 ± 0.01	0.17 ± 0.01
Phenolic aldehyde					
Vanillin	11.114	1.75 ± 0.01	3.25 ± 0.01	2.65 ± 0.01	2.11 ± 0.00

All data are expressed as mean ± SD. (n = 3); RT- retention time, ND- not detected;

4. Conclusion

The present study emerges with the observation that jackfruit seed flour has distinct antioxidant activity as evident from the results obtained from the antioxidative determinations and in vitro antioxidant assays. The composite blend of jackfruit seed flour and soy flour also has good antioxidant activity. The preponderance of the compounds recognized in *A. heterophyllus* seed powder incorporated sample extract is beneficial for health, as they show strong antioxidant activity and are also found to be a potent source of catechin. This composite blend even after extrusion cooking retains the antioxidant activity in the extruded food. From the functional point of view the products are well optimized to exert a high expansion ratio and low bulk density which are the two most desirable characteristics of extrudates, among others. The products also showed good color properties and a way better texture profile as compared to the control soy-based product.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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