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# Influence of different mixtures of ingredients on the nutritional properties of a high protein extruded snack

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#### Abstract

An effort was made to find out the suitability of inclusion of powdered chicken meat and jackfruit bulb and seed powder for the development of extruded snack product and to study the effect of inclusion of different levels of ingredients on the nutritional characteristics as well as contents of total phenolics and dietary fibre. One control ( with rice flour and Jackfruit bulb and seed powder in the ratio-80:20) and three treatment formulations (T1 – 62.69% rice flour, 15.62% jackfruit bulb and seed powder and 21.69% powdered chicken meat; T2- 62.23% rice flour, 15.5% jackfruit bulb and seed powder and 22.21% powdered chicken meat; T3- 57.32% rice flour, 14.33% jackfruit bulb and seed powder and 28.35% powdered chicken meat; Were extruded employing screw speeds ranging from 230-262 rpm and barrel temperatures ranging from 120 °C to 140 °C. It was observed that the protein content of the extradites was found to improve with the inclusion of powdered chicken meat in the feed mix. The content of fat was also higher for T3 resulting in highest calorific contents. Control samples had significantly higher amounts of ash and moisture in them. Dietary fibre and total phenolics were found to be higher in extrudates with non-meat ingredients with acceptable levels maintained in treatments too. Inclusion of meat had been found to improve the nutritional characteristics and jackfruit bulb and seed powders had been found to confer functional properties to the extruded snacks.

Keywords: Chicken meat incorporated extruded snack, jackfruit bulb and seed powder, total phenolics, dietary fibre

# Introduction

Extrusion cooking is one of the areas of health and nutrition that is now being explored the most, and it is expected to continue for a long time and a lot of research has been done on the retention and improvement of nutritional value of food during extrusion cooking. Moreover, this high temperature short time process has now been counted as one of the most interesting techniques of functional food production (Kasprzak *et al.*, 2018)<sup>[12]</sup>.

While starch-based snacks provide all the features for production of highly acceptable extruded snack foods, its nutritional value is far from satisfying the needs of health-conscious consumers. Inclusion of meat in such snacks can increase the protein, mineral and vitamin levels and can improve their palatability.

Jackfruit (*Artocarpus heterophyllus*) is one of the tropical fruits, which grows abundantly in India, especially in Kerala. Many functional, medicinal and physiological properties of this fruit have been reported. Inclusion of this cheaply available fruit into the snacks can benefit by improving the nutritional properties, functionality as well as their palatability.

Many researchers who had studied the nutritional aspects of food extrusion had stated that extrusion cooking might change dietary fibre, protein and amino acid profiles, vitamins, and other nutrient with both positive and negative effects on the food, with the functional quality being improved with moderate conditions (short duration, high moisture, low temperature), whereas a negative effect was found to occur at a high temperature (at least 200 °C), low moisture (less than 15%), or improper components in the mix. (Areas, 1992; Singh *et al.*, 2006) <sup>[5, 25]</sup>.

Therefore, the objective of the study reported in presented paper was production of extruded rice-based snacks supplemented with powdered chicken meat and jackfruit bulb and seed powder and to evaluate the effect of both the ingredients and the process parameters of extrusion cooking on the nutritional composition as well as contents of total phenolics and dietary fibre.

# **Materials and Methods**

# Materials

The study was conducted at the department of Livestock Products Technology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala. The processing facilities and technical expertise of Fish Processing Division, ICAR- Central Institute Fisheries Technology, Kochi, Kerala were utilised.

Broiler chicken meat (skinless) procured from the local market in Vythiri, Wayanad district was washed and cut into pieces of size 20 mm X 20mm X 10mm. The pieces of meat were subjected to 12 h of drying at 60° C in a cabinet dryer. The dried meat was ground into a fine powder using a mixer grinder (Panasonic Corp., Japan). The powdered meat was packed in LDPE pouches and stored frozen until it was used. Jack fruit bulb and seed powders from fresh and fully matured un-ripened jackfruits were procured locally (M/S Nutritivo Food products, KINFRA Industrial Park, Chundale, Wayanad, Kerala). Rice flour (M/S Manjilas Food Tech Pvt. Ltd., Thrissur) was procured from the local market.

#### **Preparation of extruded snacks**

Three treatment formulations (incorporated with meat) and one control formulation (without meat) were prepared at the Division of Fish Processing, ICAR-Central Institute of Fisheries Technology, Kochi. The composition of the feed mix and process variables employed for extrusion cooking are given in table. 1.

 
 Table 1: The composition of the feed mixes and process variables employed for extrusion cooking

Extrusion cooking Variables	Control	<b>T1</b>	T2	<b>T3</b>
Rice flour (% of feed mix)	80	62.69	62.23	57.32
Jackfruit bulb and seed powder in the ratio 1:1 (% of feed mix)	20	15.62	15.5	14.33
Powdered chicken meat (% of feed mix)	0	21.69	22.21	28.35
Barrel temperature (°C)	120	130	123	140
Screw speed (rpm)	250	262	254	230

Extrusion cooking was carried out using a laboratory model co-rotating twin screw extruder (Basic Technology Pvt. Ltd., Kolkata, India). The extruder had a length to diameter (L/D) ratio of 14.4:1 with a circular die having a diameter of 3.0 mm. The extruder had three barrel temperature zones. Temperatures of the first two zones were maintained at 70 and 90 °C, respectively. The temperature of the last zone was adjusted to match the feed mix extruded and accordingly it varied between 120 °C for control, 130 °C for T1, 123°C for T2 and 140 °C for T3.

The three treatment blends and control blend were prepared by mixing rice flour, jackfruit seed and bulb powder and powdered chicken meat in required proportions by adding 2.5% of salt. Water was added by sprinkling to adjust the required moisture content (15%) and mixed thoroughly by hand mixing. The feed mixes after proper mixing were sieved through a 1 mm mesh to get uniform particle size. The sieved mixes were then kept for conditioning at low temperature (4 °C) for 30 min. Once the steady state was attained (30 min) in the last zone of the extruder, the mixes were fed one by one into the feed hopper and the feeding rate was adjusted for easy and non-choking operation. Barrel received feed material from the co-rotating feeder and it got cooked inside the barrel. The cooked material was driven to the die by the rotation of the screws (screw speed set at 250 rpm for control, at 262 rpm for T1, at 254 rpm for T2 and at 230 rpm for T3) and were extruded. The extruded products were immediately cut into pieces by an automatic cutting knife. The extrudates were collected in a tray and were dried at 100  $^{\circ}$  C for 2 minutes to remove the surface moisture.

#### Determination of nutritional composition Moisture

Moisture contents of samples were estimated as per AOAC (2016) <sup>[4]</sup>. About 20 g of accurately weighed and ground sample was taken in an evaporating dish and was kept in a hot air oven (Rotek, Mumbai) set at  $100\pm2^{\circ}$ C for 18 h. The weight of the dried sample was taken after cooling in a desiccator. The difference in the weight was the moisture content of the sample and it was expressed as the percentage weight of the sample.

Moisture (%) = 
$$\left(\frac{W2 - W3}{W2 - W1}\right) \times 100$$

W1= Weight of empty dish; W2= Weight of dish + sample;

W3 = Weight of dish + banpie, W3 = Weight of dish + dried sample

#### Protein

Protein content was estimated as per (AOAC, 2016)<sup>[4]</sup>. Accurately weighed moisture free sample (approximately 2 g) was digested using Micro-Kjeldahl digester (Kelplus-KES06LE, Pelican Equipment, Chennai) in the presence of catalyst (90 parts sodium sulphate + 10 parts copper sulphate) and 25 ml concentrated (36N) sulphuric acid. Tubes were heated gently until frothing ceased, then boiled rapidly until the solution became clear. The samples were cooled and transferred to a 200 ml volumetric flask and made up the volume using distilled water. Ten millilitres of diluted sample was distilled with 20 ml of 40 percent sodium hydroxide using Micro Kjeldahl distillation unit (KelplusDistyl-EMBA, Pelican Equipment, Chennai). Steam was distilled over 5 ml of 2 percent boric acid containing mixed indicator (1 part 0.2% methyl red + 2 parts 0.2% bromocresol green dye in ethanol) for collecting 35 ml of distillate. The ammonia trapped in boric acid was determined by titrating with 0.1N sulphuric acid. The nitrogen percentage was calculated using the following formula.

Nitrogen(%) = 
$$\left(\frac{(A - B) \times 0.0014 \times V1}{W \times V2}\right) \times 100$$

A = Titrated value for sample; B = Titrated value for blank; V1 = Total volume made; V2 = Volume distilled; W= Weight of sample taken

Protein percentage was determined by conversion of nitrogen percentage to protein by using conversion factor (6.25), assuming that all the nitrogen in meat was present as protein i.e.

Protein (%) =  $N\% \times 6.25$ 

#### Fat

Fat contents of samples were estimated as per AOAC (2016) <sup>[4]</sup>. Fat content of about three grams of accurately weighed moisture-free sample was extracted with petroleum ether (boiling range 60–80 °C) using Soxhlet solvent extraction

system (SOCS Plus SCS 06E, Pelican Equipment, Chennai) for a period of 2.5 h. Ether extract obtained was dried to a constant weight at 100 °C, cooled, and weighed. Fat content on a dry matter basis was converted to a wet matter basis and expressed as percentage weight of the sample.

$$Fat(\%) = \left(\frac{W2 - W3}{W2 - W1}\right) \times 100$$

W1= Weight of empty oil flask; W2= Weight of oil flask + fat; W3= Weight of sample taken.

#### Ash

Ash percentage was determined by the gravimetric method as described by (AOAC 2016)<sup>[4]</sup> using a muffle furnace (Rotek, Mumbai).Accurately weighed ground sample (10 g) was transferred to pre-weighed crucible, charred on a hot plate at 100°C for 30 min and transferred to muffle furnace at 550°C for 5 h. Afterward, the sample was transferred to a desiccator having fused calcium chloride as desiccant. After 1 h the crucible was weighed. The ash content was calculated by the formula,

Ash(%) = (W2/W1) X100

W1= Weight of sample taken; W2= Weight of Ash

#### **Total Phenolics**

The concentration of total phenolics in acetone extract was determined by the Folin-Ciocalteus (F-C) assay (Escarpa and Gonzalez, 2001)<sup>[8]</sup> with slight modifications.

#### Standard tannic acid graph for phenolics

Accurately weighed 0.002g of tannic acid was dissolved in 20 ml distilled water and was used as standard tannic acid solution (0.1mg/ml). Folin-Ciocalteu reagent (1 N) and 20 percent sodium carbonate were prepared. Standards of different concentrations of tannic acid were prepared as shown in Table 2 and absorbances were measured at 725 nm. The standard graph (fig. 1) was prepared by plotting optical density against micrograms of tannic acid.

<b>T</b> 11 <b>A</b>	D /*	c (		1	c		
Table 2:	Preparation	ot	standard	solution	ot	tannic	acid
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Tube	Tannic acid solution (0.1 mg/ml), (ml)	Fannic acid solution (0.1 ng/ml), (ml)Distilled water (ml)Sodium carbonate solution (ml)		Folin Ciocalteu reagent (ml)	
Blank	00	0.50	0.25	1.25	
T1	0.02	0.48	0.25	1.25	
T2	0.04	0.46	0.25	1.25	
T3	0.06	0.44	0.25	1.25	
T4	0.08	0.42	0.25	1.25	
T5	0.10	0.40	0.25	1.25	



Fig 1: Standard graph for tannic acid

#### Estimation of total phenolics in product

Total phenolics in product were determined by extracting the phenolics by 70 percent acetone. Accurately weighed 5 g ground sample was blended with 25 ml 70% v/v aqueous acetone. The blended mixture was kept in refrigerator for overnight chilling and 0.1 ml supernatant was transferred to test tubes and made up to 0.5 ml using distilled water. To the testtubes 0.25 ml 20 percent sodium carbonate solution and 1.25 ml Folin-Ciocalteu reagent (1 N) were added. Reaction mixture was mixed thoroughly by vortexing. The absorbances were measured at a wavelength of 725nm in a spectrophotometer (UV/VIS Biomate 3S, Thermo fisher scientific, Bangalore) using a blank containing 0.5 ml distilled water, 0.25 ml 20% sodium carbonate and 1.25 ml 1N Folin-

Ciocalteu reagent. By referring to the standard graph of tannic acid, total phenolics of samples were calculated and expressed as mg of tannic acid /100g of sample.

#### **Determination of dietary fibre**

The extruded samples were analysed for dietary fibre at Interfield Laboratories, Kochi Kerala (India) as per McCleary *et al.* (2013) <sup>[15]</sup>.

# Statistical analysis

Data recorded were analysed statistically using SPSS Software Version 21.0. One-way ANOVA followed by Duncan's multiple range test, Pearson's correlationwere used.

# Results and Discussion

Nutritional composition of the enriched snack

Proximate composition of the extrudates with respect to moisture, fat, protein and ash contents were analysed on day of preparation. The carbohydrate content was arrived at by calculation. The energy content of the extrudates was also calculated. The results are shown in table 3.

		F-value			
Proximate principles	Control	T1	T2	T3	(P-value)
Moisture (%)	$8.26\pm0.09^{a}$	$8.32\pm0.19^{\rm a}$	$8.54\pm0.09^{a}$	$7.68\pm0.15^{b}$	7.452** (0.004)
Fat (%)	$0.88\pm0.03^{\rm b}$	$1.08\pm0.05^{\rm b}$	$1.3\pm0.14^{\text{b}}$	$1.62\pm0.15^{a}$	9.042** (0.002)
Protein (%)	$5.67 \pm 0.11^{\circ}$	$12.58\pm0.23^{b}$	$13.07 \pm 0.06$ <sup>b</sup>	$16.077 \pm 0.08^{a}$	1038.92** (<0.001)
Ash (%)	$3.80\pm0.14^{a}$	$2.53\pm0.09^{b}$	$2.56\pm0.17^{b}$	$2.06\pm0.03^{b}$	37.74** (<0.001)
Carbohydrate (%)	$81.4 \pm 0.08^{a}$	$75.49\pm0.3~^{\rm b}$	$74.54 \pm 0.19^{\circ}$	$72.58\pm0.16^d$	369.64** (<0.001)
Energy (Kcal)	$356.15 \pm 0.7$ <sup>c</sup>	$361.98\pm0.9^{b}$	$362.09 \pm 1.22^{b}$	$369.17\pm1.3^a$	25.57** (<0.001)
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Table 3: Proximate composition of the control and treatment extradites

\*\* Significant at 0.01 level

Means having different small letters as superscript differ significantly within a row;

The protein content of the extrudates were found to improve with the inclusion of powdered chicken meat in the feed mix, with T3 samples recording the highest value. The content of fat was also higher for T3 samples resulting in highest calorific content, even though the carbohydrate level was less. Control samples had significantly higher amounts of ash in them. The samples T3 had a significantly lower moisture content than all other samples.

Fortification of cereal-based snacks with protein sources like legumes, fish and meat had been reported to have a positive effect on chemical properties with considerable negative effects on the physical and consequently sensory characteristics (Obradović, 2014) <sup>[16]</sup>. Adding whey protein concentrates or isolates or legume-based protein into starch-based foods had improved the nutritional profile of snacks (Onwulata and Konstance, 2006 <sup>[17]</sup>; Pastor-Cavada *et al.*, 2011) <sup>[20]</sup>. Similar to the observations in the study, also observed an enhanced protein value of 18.2 g/100g in extruded tripe snack from buffalo rumen meat and corn flour. Comparable values were also cited by Pathania *et al.* (2013) <sup>[21]</sup> in ground nut based extruded instant mixes, which had a protein content ranging from 16.53-17.22%.

Kuna *et al.*  $(2013)^{[14]}$  reported that the carbohydrate content of fish powder incorporated samples (included at 10% level) was lower, but protein and fat contents were higher than the control extrudates. The energy value of the fish incorporated products were however comparable which ranged from 392 to 437 Kcal. Pandi *et al.*  $(2019)^{[19]}$  who incorporated 7% (w/w) oil sardine powder to extruded snacks reported that the carbohydrate contents of sardine incorporated snacks were 90.15 percent as against 83.56 percent in control snacks without sardine powder. The fat content in treatment snacks varied from 1.08 to 1.62 percents as against 0.88 percent in control samples. Ganesan *et al.* (2017)<sup>[9]</sup> extruded fish snack with 30% blanched dried sardine fish powder and recorded a fat content of 11.32 percent against 3.52 percent in control extruded snack. Sardine being a fatty fish contributed to the increased fat content in contrast to chicken meat which is comparatively low in fat content.

Gbenyi *et al.* (2016) <sup>[10]</sup> opined that minerals are generally found to be stable in extruded snacks, since extrusion temperature and feed moisture were not expected to cause a significant change in their composition. The control samples in the present study were found to contain more ash level than the treatment samples. Kuna et al. (2013) [14] however observed higher ash contents in fish powder incorporated extruded snacks. Abedin et al. (2012)<sup>[1]</sup> observed that the ash content of jackfruit was found to vary from 2.13- 4.07% among different varieties of the fruit. Similar to the observation in the present study, Kaushal et al. (2019) <sup>[13]</sup> reported that incorporation of jackfruit bulb flour in rice flour and pigeon pea flour extrudates resulted in ash contents ranging from 1.86 to 2.93%. With increased levels of chicken meat, T3 samples were shown to have decreased moisture content.

# **Total phenolics**

The total phenolic content of the extrudates are shown in table 4. Control samples were found to contain the highest amount of total phenolics which varied significantly (P<0.001) from the treatments.

Table 4: Total phenolics of the control and treatment extrudates

Extrudates	Control	T1	T2	Т3	F-value (P-value)
Day 0	$18.14\pm0.68^a$	$8.43\pm0.65^{b}$	$10.39\pm0.21^{b}$	$8.63\pm0.77^{b}$	55.133** (<0.001)
** Significant at	0.01 level				

\*\* Significant at 0.01 level

Means having different small letters as superscript differ significantly within a row

Control samples with non-meat ingredients contained the highest amount of phenolics, which could be attributed to the enhanced content of jackfruit bulb and seed flours in the control. The total phenolic content had been reported to be 23.3 to 33. 9  $\mu$ g/g in digested and undigested extracts of jackfruit by Pavan *et al.* (2014) <sup>[22]</sup> and Altan *et al.* (2009) <sup>[2]</sup> stated that high extrusion temperatures could cause reduction in the chemical reactivity of phenolic compounds, or could reduce the extraction of phenolics brought in by

polymerisation which was further supported by the findings of Sharma *et al.* (2012) and Wang *et al.* (2000) <sup>[21, 29]</sup>. However, in the present study, conditions of extrusion cooking did not seem to have any particular effect on the total phenolic contents as it did not differ significantly among the treatments. The observations are in accordance with those of Ozer *et al.* (2006) <sup>[18]</sup> and Shi *et al.* (2009) <sup>[24]</sup> in extruded snacks.

#### **Dietary Fibre**

The dietary fibre contents of the extruded samples are shown in table 5. Control samples prepared with 80% rice flour and 20% of jackfruit bulb and seed flour in them had the highest content of dietary fibre, 12.8%. In T1, T2 and T3, rice flour and jackfruit bulb and seed flours were in the ratio, 80:20. T1 had 62.69% rice flour, 15.62% jackfruit seed and bulb flour and 21.69% powdered chicken meat and had a dietary fibre content of 7.4%. In T2, rice flour contributed to 62.23% with, jackfruit seed and bulb flour contributing to 15.5% and powdered chicken meat contributing to 22.21 percent and its dietary fibre content was 6.52%. T3 with57.32% rice flour, 14.33% jackfruit seed and bulb flour and 28.35% powdered chicken meat recorded the lowest dietary fibre, 3.57%.

The dietary fibre content of the extrudates in the present study were seen influenced by the amounts of non- meat ingredients in them, which was evident with the control samples recording the highest while, T3 with the highest meat content recording the lowest.

**Table 5:** Dietary fibre content of the extruded samples

Treatments	Dietary fibre content (%) on day 0
С	12.8
T1	7.4
T2	6.52
T3	3.57

Along with the non- starch polysaccharides like cellulose, hemi-celluloses, lignin, glucans, pectin, gums, and mucilages, resistant starch also is considered in the analysis of dietary fibre (Elleuch et al., 2011)<sup>[7]</sup>. Also, extrusion is known to promote formation of resistant starch, by gelatinisation and retrogradation of the starch (Huth et al., 2000) [11]. Moreover, the Maillard reaction that takes place during extrusion can lead to the formation of a protein-polysaccharide complex, which also is resistant to enzymatic degradation (Robin et al., 2012). Rice flour had a dietary fibre content of 2-5% (Obradović et al., 2014) [16], and the crude fibre content of jackfruit bulb and seed were reported as 4.06% and 3.13%, respectively (Veena and Suma, 2016). Not only this, but also the resistant starch and resistant protein -polysaccharide complexes along with the non-starch polysaccharides contributed to the relatively higher dietary fibre contents in the extrudates studied. However, for enhancing the dietary fibre content, fruits and vegetables were added to extruded snacks similar to the present study (Bisharat et al., 2003, Tyl et al., 2021) [6, 26].

# Conclusion

With the objective of nutritional enhancement of extruded snacks, different proportions of rice flour, jackfruit bulb and seed powder and powdered chicken meat were extruded under different levels of screw speed and barrel temperature. The inclusion of chicken meat powder had been found to improve the nutritional characteristics while jackfruit bulb and seed powders in the feed mix had contributed functional properties to the extrudates. Inclusion of meat and other functional ingredients in extrusion cooking could be adapted by the food industry to develop healthier products and satisfy market trends.

# **Conflict of interest**

Authors hereby declare that they have no conflicts of interest.

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