Food Processing & Technology

Navam et al., J Food Process Technol 2014, 5:8
http://dx.doi.org/10.4172/2157-7110.1000359

Research Article Open Access

Physio-Chemical and Sensory Properties of Protein-Fortified Extruded Breakfast Cereal/Snack Formulated to Combat Protein Malnutrition in Developing Countries

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Abstract

Protein malnutrition is prevalent in the developing parts of the world and children are the most affected. Millet, beans, and rice mixed with soymeal flour were processed to produce an extruded product using a single screw extruder. The Central Composite Design (CCD) of the Response Surface Methodology at varying temperatures (190-275°C) and screw speeds (60-110 rpm) was used to obtain best extrusion conditions to develop a protein-enriched extruded product with desirable physio-chemical and sensory properties. Sensory properties were evaluated in terms of color and overall visual acceptability of the extruded products using a nine-point hedonic scale. The results suggested that the two extrusion variables, barrel temperature and screw speed, influenced the extrudate physio-chemical and sensory properties. The fat absorption capacity and water solubility index were unaffected by the extrusion variables. The best extrusion condition was obtained at a screw speed of 60 rpm and a barrel temperature of 190°C based on expansion ratio, bulk density, water holding capacity, texture, color and overall visual acceptance of the extruded products. This study demonstrated that acceptable extruded products with higher protein content can be prepared from varying blends of millet, beans and rice flour under optimized extrusion conditions by incorporating up to 30% of soymeal flour. This is the first attempt to produce a soymeal-based protein enriched extruded product with millet and beans that can be included in a child's meal as a breakfast cereal or a snack to fight protein malnutrition.

Keywords: Extrusion; Extrudates; Physio-chemical properties; Sensory properties

Practical Application: This product could be supplied to the developing parts of the world which are prone to protein energy malnutrition, especially in Asia and Africa.

Introduction

Protein malnutrition is responsible for half of the deaths of children under the age of five each year in developing countries. More than 4, 26, and 70% of children are protein deficient in Latin America/ Caribbean, Africa, and Asia respectively [1]. Snack foods prepared using cereals grains are most preferred by consumers due to good expansion characteristics, although they tend to be lower in certain nutrients including protein [2]. As a result, a demand for a snack with enhanced nutrition exists [3].

Extrusion technology has been widely used in the preparation of cereal-based snack products [4]. Extrusion is believed to yield safe foods that have a long shelf life; hence extrusion is a useful, economical processing technology to prepare food products and to manage food scarcity during drought conditions [5]. Due to its versatility, low cost, efficiency, product quality and eco-friendliness, extrusion has been in wide use during the past two decades [6]. Extrusion-cooking parameters, such as barrel temperature (BT), screw speed, moisture content, die diameter, and feed rate, have been reported to influence the physio-chemical characteristics of extrudates [7,8].

Many authors have supported the idea of developing products using cereal/ legume blend to fight problems of hunger and malnutrition in developing countries [9-12]. Several reports have demonstrated that mixing legumes and cereals, which are staple foods in the tropics, can complement each other by supplementing the essential amino acids and assist the nutrition profile [13,14]. Researchers developed an extruded cereal product using wheat flour and enhanced its protein content with wet okara, the residue left after soymilk or tofu production [15]. Extrusion of millet and soybean blends in the production of "fura", a traditional Nigerian food, has been reported [16] while other researchers developed a chickpea-flour-based protein rich snack [2]. An extruded puffed product has been developed with corn flour supplemented with soybean and safflower pastes to improve the protein content of the extrudates [17]. This study also showed that other protein sources, such as defatted wheat germ, casein, and soy paste, when used, can improve the quantity and quality of the protein present in corn-based snack.

A sensory analysis is imperative for the success of a newly developed food product in the market. Consumers are concerned about food flavor and overall acceptability of the product [18]. Consumer acceptability evaluation is crucial for indicating the degree of liking or preference of a product [4]. It is essential to test the consumer acceptability of protein rich food products developed for the undernourished in order to successfully market them.

Millet, rice, and bean are staple foods in the developing parts of the world especially in Africa. These are inexpensive and affordable,

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Received April 18, 2014; **Accepted** May 21, 2014; **Published** June 19, 2014

Citation: Navam SH, Tajudini AL, Srinivas JR, Sivarooban T, Kristofor RB, et al. (2014) Physio-Chemical and Sensory Properties of Protein-Fortified Extruded Breakfast Cereal/Snack Formulated to Combat Protein Malnutrition in Developing Countries. J Food Process Technol 5: 359. doi:10.4172/2157-7110.1000359

and have been used in the preparation of our extruded product. In this research, the low millet starch content was fortified using rice, while bean and soymeal flour were selected to enhance the protein content of the breakfast cereal/snack. There are limited studies in the literature on how these four flours can be combined to prepare a snack using an extrusion technology and how their physico-chemical and sensory properties are affected by the extruder conditions such as barrel temperature and screw speed. Hence, the overall objective of this work was to develop a breakfast cereal/snack with a single-screw extruder using millet (*Panicum miliaceum*), beans *(Vigna unguiculata)* and rice *(Oryza sativa*) along with soybean *(Glycine max)* meal flour blend for protein enrichment, and to determine the physio-chemical characteristics and sensory analysis of the developed breakfast cereal/snack product. Sensory properties were evaluated in terms of overall visual color acceptability of the extruded products.

Materials and Methods

Materials

Millet and Bean flour were supplied by the Institut National pour la Recherche Agronomique du Niger (INRAN), Niamey, Niger. Rice flour and sea salt were purchased from a local store. Soybean meal was supplied by GROVAC Systems (McKinney, TX, USA). All chemicals used were reagent grade.

Proximate analysis

Official American Association of Cereal Chemists (AACC) methods were used to analyze moisture (44:19), protein (Nx6.25) (46.10), lipid (30.25), fiber (32.05), total starch (76.13) and ash (08.01) contents in rice, millet, bean, and soymeal flour [19].

Experimental design and extrusion conditions

Central Composite Design (CCD) was used to investigate the effects of extrusion conditions on the process and product responses of the millet, bean, rice, and soy-flour-based breakfast cereal/snack. Results from preliminary reported literatures were used to select a suitable extruder operating window. The independent variables considered for this study were screw speed and barrel temperature with two different levels, 60 and 110 rpm, and 190 and 275˚C, respectively. Experiments were randomized in order to minimize the systematic bias in the observed responses due to extraneous factors.

Rice (10%), beans (16%), soy (16%), and millet (20%) flours were passed through a 60 mesh sieve (250 microns) to obtain uniform particle size. Flours were mixed with salt (0.5%), glyceryl monostearate (6%), and water (31.5%) in a mixer (KitchenAID Inc., St. Joseph, Michigan, USA). The homogeneously mixed product was extruded at varying barrel temperatures (190-275˚C) and screw speeds (60-110 rpm). The extrudates were dried in a dehydrator (Harvest Saver, Model R-4, Commercial Dehydrator System Inc., Eugene, Oregon, USA) at 40˚C.

A single-screw extruder (Killion Extruders Davis-Standard Corporation, Serial # 30337, Cedar Grove, New Jersey, USA) with 939.8 mm length, 1168.4 mm height, 660.4 mm width, three heating zones (feed zone, central zone, and die zone) and a screw and die diameter of 19.1 and 31.8 mm, respectively were employed in this study. The barrel temperature in the die zone was varied from 190 and 275˚C along with the screw speed from 60 to 110 rpm using CCD whereas the temperatures of feed and central zones were set at 35˚C and 15˚C lower than that in the die zone. An in-built thermostat and a temperature control unit were set to maintain the desired barrel temperature by circulating tap water.

The raw material was fed into the extruder, which passed through the three temperature control zones. During extrusion, the temperature zones were adjusted at varying levels. The screw speed was varied between 60 and110 rpm. The extrusion conditions are shown in Table 1. The extrudates were collected, dried, and physio-chemical and sensory properties were evaluated.

Physio-chemical and sensory properties

Expansion Ratio (ER): The method developed by Fan and colleagues was used to determine the expansion ratio [20]. The mean of 10 random measurements of the extrudate diameter was determined using a vernier caliper. Expansion ratio was calculated using the equation:

$$ER=D/d$$

Where D=mean diameter of the extrudate in mm and d=diameter of the die in mm

Bulk Density (BD): The method developed by Deshpande and Poshadri was followed to determine the bulk density [21]. The average diameter and average length of 25 readings of extrudates were measured and the volume of the extrudates computed as:

$$\text{Vol. } (cm^3)=\pi d^2 L/4.$$

Where d=average diameter of extrudates in cm, L=average length of extrudates in cm, the bulk density was calculated using the formula:

$$BD\ (Kg/cm^3)=\text{Mass of extrudate (Kg)}/\ \text{Volume of extrudates } (cm^3)$$

Moisture Retention (MR): The feed and extrudate moisture contents were determined by AACC official method [19].

$$MR\ (\%)=(\text{Product moisture}/\ \text{Feed moisture})\times 100$$

Water Holding Capacity (WHC): A modified method of Deshpande and Poshadri was followed to determine the WHC of the extruded product [21]. Approximately 5 g of fine ground and sieved (60 mesh or 250 microns particle size) of extrudate were weighed. The sample was allowed to absorb water over night after adding 35 mL of deionized water. The suspension was centrifuged at 750xg for 15 min and the weight of the wet extradate flour was recorded.

Treatments no.	Independent variables in coded form[a]		Experimental variables in their natural units	
	SS	BT	SS	BT
1	0	0	85	232.5
2	a	0	60	232.5
3	+	+	110	275
4	0	A	85	275
5	-	−	60	190
6	A	0	110	232.5
7	0	a	85	190
8	+	−	110	190
9	-	+	60	275

Treatments No: 1=85 rpm SS & 232.5°C BT; 2=60 rpm SS & 232.5°C BT; 3=110 rpm SS & 275°C BT; 4=85 rpm SS & 275°C BT; 5=60 rpm SS & 19 °C BT; 6=110 rpm SS & 232.5°C BT; 7=85 rpm SS & 190°C BT; 8=110 rpm SS & 190°C BT; and 9=60 rpm SS & 275°C BT. SS=Screw Speed (revolutions per minute) and BT=Barrel Temperature.

Table 1: Central composite design (CCD) design of the Extrusion Experiments in their coded form and natural units

WHC (%)=(Weight of wet sample-weight of dry extrudate/Weight of dry extrudate)x100.

Tap Density (TD) and True Density (TeD): The tap and true density were determined following the method of Deshpande and Poshadri (21). The extrudate flour was filled in a 50 mL cylinder up to 20 mL and tapped 10 times. The weight of the 20 mL of extudates was measured and TD was calcualted

TD (g/mL)=(Weight of 20 mL (g)/Volume of the sample (20 mL)) Approximately 1 g of ground extrudate was added to a 10 mL cylinder containing toluene and the rise in toluene level was measured. True density was calculated using the formula:

TeD (g/mL)=(Weight of ground sample of extrudates (g)/ Rise in toluene level (mL)

Mass Flow Rate (MFR): The mass flow rate was determined according to the method developed by Singh and colleagues [22]. The extrudate was collected in plastic plates for two minutes as soon as it came out of the die and its weight was taken instantly after cooling to ambient temperature.

MFR (g/s)=Weight of sample collected (g)/Time taken to collect sample (s)

Fat Absorption Capacity (FAC): A 0.5 g sample of the extrudate powder was mixed with 10 mL canola oil and centrifuged at 750 g for 15 min. The supernatant was drained off for 30 min using cheese cloth and the weight gained reported as oil absorption capacity [23].

FAC (g)=Weight gained in oil-initial weight extrudate powder 7

Milk Absorption Capacity (MAC): A 4 g sample of extrudate was placed in 30 mL of milk (1% fat) at 8°C for 3 mins, and the milk was drained from the extrudated using a stainless steel mesh screen (2.8 microns) for 10 seconds [24]. The milk absorption capacity was calculated using the following formula:

MAC (%)=((Drained extrudate weight (g)-initial extrudate weight (g))/Initial extrudate weight (g))x100….i

Protein Solubility (PS): A 10 mL of the filtrate obtained from the water holding capacity experiment was analyzed for nitrogen using a Kjeldahl method. Nitrogen to protein conversion factor of 6.25 was used to derive the percentage soluble protein [25].

Water Absorption Index (WAI) and Water Solubility Index (WSI)

The technique developed for cereals by Anderson and colleagues was used to measure the WAI and WSI [26]. A 2.5 g sample of extrudate powder was suspended in 30 mL of distilled water at 30°C in a 50 mL tube, stirred continuously for 30 min, and centrifuged at 3,000xg for 10 min. The supernatant was poured carefully into a tared evaporating aluminium dish. The remaining gel was weighed and the WAI calculated from its weight. The amount of dried solids recovered by evaporating the supernatant from the water absorption test was expressed as percentage of dry solids.

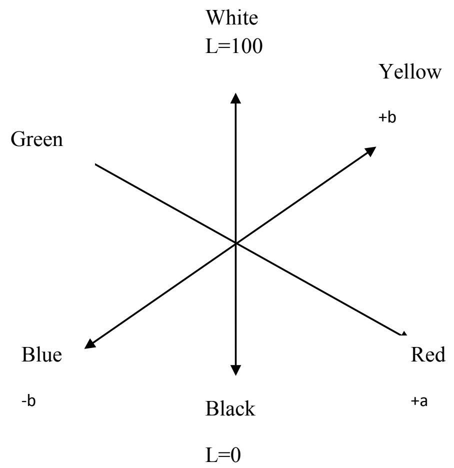
WAI=Weight of sediment/Weight of dry solids

WSI (%)=(Weight of dissolved solids in supernatant/ Weight of dry solids)x100 7.**11**

In Vitro Starch Digestibility (IVSD)

A solution of defatted extrudate flour and amylase solution (50 mg/mL) in 0.2 M phosphate buffer of pH 6.9 and 0.4 mg of pancreatic amylase (1300 u/mg) in one milliliter of 0.2 M phosphate buffer of pH 6.9 were prepared, respectively. A 0.5 mL of the amylase solution was added to the extrudate flour suspension at 20°C for 2 h. At the end of incubation period, 2 mL of 3,5-di-nitrosalicyclic acid reagent prepared was added to the mixture and boiled for 5 min [27]. After cooling, the absorbance of the filtrate was measured at 550 nm with glucose as a standard and the values were expressed as µg of glucose released per gram of sample [28]. A standard curve with glucose solutions (0, 20, 40, 60, 80, and 100 µg/mL) was prepared.

Color: The color of the extruded products was determined using a Minolta colorimeter CR-300 (Minolta Camera Co., Ltd., Chou-Ku, Osaka, Japan) and recorded using L a b color system.



Texture: Samples of 5 g of the extrudates were placed in the load cell of an Alliance RT/1 texture analyzer (Systems, Eden Prairie, MN, USA) MTS to determine the shear force of the extruded products. A clean 10-set Kramer shear blade at a pre-load speed of 5.0 mm/s, strain endpoint of 120.0 mm/mm, a post-test speed of 5 mm/s, and a test speed of 10 mm/s was used and the test was conducted using the MTS compression (2.0) method. The force used by the blade to fracture the extruded products, known as the peak load (N) value, was noted for triplicate samples.

Sensory evaluation: Hedonic sensory tests were conducted by 38 untrained panelists. The samples were placed on white plates coded with alphabetic letters under normal lighting conditions at room temperature. Sensory attributes, including color (liking and either the color is light or dark) and overall visual acceptability of the extruded products appearance were evaluated using a nine-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). The nine extruded products were placed at the same time on the table for ranking by the panelists. No identity of the panelists was recorded. Extrudates were considered acceptable if their mean score for overall acceptance was above 6 like slightly [4].

Statistical analysis: The data reported are means of triplicate observations except for ER and BD which data are means of 10 and 20 observations respectively. A central composite design with two factors was used to select the best extrusion conditions. Data were subjected to a two-way analysis of variance (ANOVA) with extruder screw speed and barrel temperature as main effects using JMP Pro 9.0 software (SAS

Inst. Inc., Cary, N.C., USA) and Student's t significance test difference was used to separate means at P<0.05.

Results and Discussion

Model description

Studies were conducted using the Central Composite Design (CCD) in modeling the extrusion conditions to prepare a breakfast cereal/snack as affected by the process variables Screw Speed (SS) and Barrel Temperature (BT) as shown in Table 1.

Proximate analysis of raw materials

The results of the proximate analysis means of three observations in percentage Dry-Basis (db) of rice, soy, bean, and millet are shown in Table 2. Soy and beans flours had the highest protein (N×6.25) with 52.2 and 25.8%, respectively. Soy and bean flours were also rich in total dietary fiber 21.4 and 17.9% and high in ash content with 6.6 and 4.6% for soy and bean, respectively. Iwe and Ngoddy reported the protein and ash contents of the soy used in their research to be 50.9% and 7.0%, respectively [29]. However, the fat content obtained was 0.5% compared with 13.8% in this study. This disparity was due to the difference of the type of soymeal flour used. The moisture, protein, fat, and ash contents of beans were similar to the findings of Sammán and colleagues [30]. Rice and millet had the highest total starch with 90.1 and 75.4%, respectively. The results of moisture, protein, fat, and ash contents of rice were also similar to that reported previously [31]. The millet used in this study had 9.4% protein, 6.1% lipid and 2.2% ash, which were comparable to that reported by researchers who worked with similar cereal foods [32].

Moisture and protein contents of the extruded products

Mean moisture and protein contents of the extruded products are shown in Table 3. No interaction between the BT and SS was observed for protein content (P=0.76). The mean observed values of protein for the extruded products ranged from 19.5-20.0% indicating an increase in protein content with fortification of the blend with soymeal flour. Protein content was measured as nitrogen (Nx6.25) and the present protein content was not affected by extrusion temperature since nitrogen is not known to be affected by heat treatment [33]. However, there is a significant difference in the moisture content (P<0.0001) among the extruded products ranging from 6.8-12.7% when the extruder was operated at a SS of 85 rpm and BT of 275°C and 110 rpm and 190°C, respectively. The three lowest moisture contents (6.8, 6.9, and 7.9%) were observed at the greatest BT (275°C). This is explained by the fact that at high temperature (275°C), the initial moisture content of the flour mixture fed into the extruder was significantly decreased as it got cooked when compared to other products extruded at lower BTs (190 and 233°C).

Physico-chemical and sensory properties

The determination of physio-chemical and sensory properties is crucial to the overall quality of the extruded breakfast cereal/snack. Expansion ratio, bulk density, moisture retention, water holding capacity, tap density, true density, mass flow rate, fat absorption capacity, milk absorption capacity, protein solubility, water absorption index, water solubility index, in vitro starch digestibility, and color data are listed in Table 4.

Bulk Density (BD) and Expansion Ratio (ER) are important characteristics of extruded products and play a role in the consumer acceptability of the product. BD is linked with the expansion ratio in explaining the degree of puffing in extruded product. It is expected that most extruded products will have a puffed structure to a certain degree [34]. Barrel temperature and SS individually affected the BD (P<0.0001 and P=0.01 respectively). An interaction between BT and SS was observed on ER (P<0.0001). Although the interaction was significant, only the BT significantly affected ER (P<0.0001). The mean values of ER and BD ranged from 0.2-0.3 and 0.016-0.036 kg/cm^3 respectively when the extruder was operated at a SS of 110 rpm and a BT of 232.5°C and at a SS of 110 rpm and a BT of 190°C, respectively for ER and at a SS of 60 rpm and a BT of 275°C and at a SS of 85 rpm and a BT of 232.5°C,

Treatments no.	Independent variables in coded form[a]		Experimental variables in their natural units	
	SS	BT	SS	BT
1	0	0	85	232.5
2	a	0	60	232.5
3	+	+	110	275
4	0	A	85	275
5	-	−	60	190
6	A	0	110	232.5
7	0	a	85	190
8	+	−	110	190
9	-	+	60	275

Treatments No: 1=85 rpm SS & 232.5°C BT; 2=60 rpm SS & 232.5°C BT; 3=110 rpm SS & 275°C BT; 4=85 rpm SS & 275°C BT; 5=60 rpm SS & 19 °C BT; 6=110 rpm SS & 232.5°C BT; 7=85 rpm SS & 190°C BT; 8=110 rpm SS & 190°C BT; and 9=60 rpm SS & 275°C BT. SS=Screw Speed (revolutions per minute) and BT=Barrel Temperature.

Table 1: Central composite design (CCD) design of the Extrusion Experiments in their coded form and natural units.

	Flour			
Composition (%)	Rice	Millet	Beans	Soy
Moisture	15.0 ± 0.1	9.1 ± 0.1	8.8 ± 0.1	5.7 ± 0.1
Protein	8.8 ± 0.5	9.4 ± 0.1	25.8 ± 0.3	52.2 ± 0.8
Lipid	2.4 ± 0.3	6.1 ± 0.2	1.9 ± 0.2	13.8 ± 1.2
Total starch	90.1 ± 0.1	75.4 ± 0.2	47.3 ± 0.1	10.2 ± 0.1
Total dietary fiber/ gram	4.2 ± 0.1	10.7 ± 0.1	17.9 ± 0.1	21.4 ± 0.1
Ash	0.5 ± 0.1	2.2 ± 0.1	4.6 ± 0.1	6.6 ± 0.1

Composition was determined using AACC official methods on dry-weight basis. Values are presented as means ± standard error of triplicates.

Table 2: Moisture, Protein, Lipid, Total Starch, Total Dietary Fiber, Ash Content in Rice, Millet, Beans, and Soy Flours

	Flour			
Composition (%)	Rice	Millet	Beans	Soy
Moisture	15.0 ± 0.1	9.1 ± 0.1	8.8 ± 0.1	5.7 ± 0.1
Protein	8.8 ± 0.5	9.4 ± 0.1	25.8 ± 0.3	52.2 ± 0.8
Lipid	2.4 ± 0.3	6.1 ± 0.2	1.9 ± 0.2	13.8 ± 1.2
Total starch	90.1 ± 0.1	75.4 ± 0.2	47.3 ± 0.1	10.2 ± 0.1
Total dietary fiber/ gram	4.2 ± 0.1	10.7 ± 0.1	17.9 ± 0.1	21.4 ± 0.1
Ash	0.5 ± 0.1	2.2 ± 0.1	4.6 ± 0.1	6.7 ± 0.1

Composition was determined using AACC official methods on dry-weight basis. Values are presented as means ± standard error of triplicates.

Table 3: Moisture, Protein, Lipid, Total Starch, Total Dietary Fiber, Ash Content in Rice, Millet, Beans, and Soy Flours

Physico-chemical Properties	Treatment no								
	1	2	3	4	5	6	7	8	9
MR	28.3 ± 0.3^{d}	23.3 ± 0.3^{e}	18.2 ± 0.2^{g}	17.9 ± 0.1^{g}	30.5 ± 0.2^{b}	24.0 ± 0.1^{e}	29.7 ± 0.2^{c}	33.3 ± 0.4^{a}	20.8 ± 0.2^{f}
MFR	0.36 ± 0.0^{ab}	0.38 ± 0.0^{ab}	0.17 ± 0.0^{d}	0.15 ± 0.0de	0.27 ± 0.0^{c}	0.33 ± 0.0^{bc}	0.38 ± 0.0^{ab}	0.43 ± 0.0^{a}	0.07 ± 0.0^{e}
FAC	0.45 ± 0.0^{ns}	0.42 ± 0.0^{ns}	0.42 ± 0.0^{ns}	0.45 ± 0.0^{ns}	0.43 ± 0.0^{ns}	0.44 ± 0.0^{ns}	0.42 ± 0.0^{ns}	0.40 ± 0.0^{ns}	0.42 ± 0.0^{ns}
PS	0.27 ± 0.0^{g}	0.32 ± 0.0^{ab}	0.28 ± 0.0^{f}	0.25 ± 0.0^{h}	0.33 ± 0.0^{a}	0.3 ± 0.0^{de}	0.31 ± 0.0^{bc}	0.3 ± 0.0^{cd}	0.29 ± 0.0^{ef}
MAC	0.3 ± 0.0^{c}	0.4 ± 0.0^{b}	0.6 ± 0.0a	0.6 ± 0.02^{a}	0.2 ± 0.0^{d}	0.2 ± 0.0^{d}	0.2 ± 0.0^{d}	0.2 ± 0.0^{d}	0.4 ± 0.0^{b}
TD	0.57 ± 0.0^{bc}	0.61 ± 0.0^{a}	0.59 ± 0.0^{ab}	0.56 ± 0.0^{cd}	0.53 ± 0.0^{d}	0.58 ± 0.0^{abc}	0.57 ± 0.0^{bc}	0.58 ± 0.0^{abc}	0.56 ± 0.0^{cd}
TrD	1.38 ± 0.1^{bcd}	1.31 ± 0.0^{bcd}	1.48 ± 0.0^{abc}	1.29 ± 0.0^{cd}	1.60 ± 0.1^{a}	1.39 ± 0.0^{bcd}	1.22 ± 0.0^{d}	1.30 ± 0.0^{bcd}	1.49 ± 0.0^{ab}
WHC	149.7 ± 11.0^{b}	153.1 ± 8.0^{ab}	165.1 ± 4.7^{ab}	181.7 ± 13.2^{a}	85.4 ± 7.3^{c}	156.9 ± 15.8^{ab}	80.9 ± 6.8^{c}	77.0 ± 5.0^{c}	175.9 ± 7.8^{ab}
WAI	16.1 ± 0.1^{bc}	15.3 ± 0.2^{c}	16.7 ± 0.2^{ab}	16.3 ± 0.9^{abc}	13.9 ± 0.0^{d}	15.8 ± 0.1^{bc}	13.9 ± 0.2^{d}	13.8 ± 0.3^{d}	17.3 ± 0.3^{a}
WSI	11.3 ± 0.2^{ns}	11.5 ± 0.3^{ns}	10.6 ± 0.0^{ns}	10.9 ± 1.8^{ns}	12.3 ± 0.1^{ns}	10.7 ± 0.2^{ns}	11.9 ± 0.3^{ns}	12.6 ± 0.1^{ns}	11.7 ± 0.3^{ns}
BD	0.036 ± 0.0^{a}	0.026 ± 0.0^{b}	0.019 ± 0.0^{cde}	0.016 ± 0.0^{de}	0.022 ± 0.0^{bcd}	0.036 ± 0.0^{a}	0.023 ± 0.0^{bc}	0.025 ± 0.0^{b}	0.016 ± 0.0^{e}
ER	0.18 ± 0.0^{c}	0.24 ± 0.0^{d}	0.29 ± 0.0^{b}	0.29 ± 0.0^{b}	0.32 ± 0.0^{ab}	0.17 ± 0.0^{d}	0.32 ± 0.0^{ab}	0.34 ± 0.0^{a}	0.24 ± 0.0^{c}
IVSD	153.4 ± 2.8^{b}	157.2 ± 1.1^{ab}	160.7 ± 0.9^{a}	137.3 ± 0.9^{c}	121.5 ± 0.6^{d}	158.9 ± 1.1^{a}	83.9 ± 1.0^{f}	112.7 ± 1.0^{e}	111.7 ± 1.0^{e}
Color (L*)	51.8 ± 0.0^{c}	50.6 ± 0.0^{e}	50.6 ± 0.0^{d}	50.6 ± 0.0^{d}	50.6 ± 0.0^{d}	53.9 ± 0.0^{b}	54.2 ± 0.0^{a}	45.7 ± 0.0^{g}	48.1 ± 0.0^{f}
Color (a*)	2.1 ± 0.0^{e}	1.7 ± 0.0^{g}	2.1 ± 0.0^{d}	2.8 ± 0.0^{a}	1.9 ± 0.0^{f}	2.3 ± 0.0^{c}	2.5 ± 0.0^{b}	1.6 ± 0.0^{h}	1.9 ± 0.0^{f}
Color (b*)	6.4 ± 0.0^{e}	6.1 ± 0.0^{g}	6.4 ± 0.0^{d}	7.4 ± 0.0^{c}	6.3 ± 0.0^{f}	7.8 ± 0.0^{b}	8.4 ± 0.0^{a}	3.1 ± 0.0^{i}	1.9 ± 0.0^{h}

Values are presented as means ± standard error of triplicates except for ER and BD which are means of 10 and 20 readings respectively. Values connected by the same letter in each row are not significantly different (P<0.05).

Treatments No: 1=85 rpm SS & 232.5°CBT; 2=60 rpm SS & 232.5°CBT; 3=110 rpm SS & 275°CBT; 4=85 rpm SS & 275°CBT; 5=60 rpm SS & 190°CBT; 6=110 rpm SS & 232.5°CBT; 7=85 rpm SS & 190°CBT; 8=110 rpm SS & 190°CBT; and 9=60 rpm SS & 275°C BT. SS= Screw speed, BT= barrel temperature, rpm= revolutions per minutes, and ns=not significant.

Physico-chemical properties: MR=Moisture Retention (%), MFR=Mass Flow Rate (g/sec), FAC=Fat Absorption Capacity (%), PS=Protein Solubility (%), MAC=Milk Absorption Capacity (%), TD=Tap Density (g/cm^3), TrD=True Density (g/mL), WHC=Water Holding Capacity (%),
WAI=Water Absorption Index, WSI=Water Solubility Index (%), BD=Bulk Density (kg/cm^3) and ER=Expansion Ratio, IVSD=*In Vitro* Starch Digestibility (µg of glucose per gram of sample). Color (L)=brightness with L=100 is white and L=0 is black, Color (a+)=red, Color (a-)=green, Color (b+)=yellow, and Color (b-)=blue.

Table 4: Physico-chemical Properties of the Nine extruded products

Treatment no.	Color Liking	Color light or Dark	Appearance
1	3.0^{d}	7.5^{a}	3^{e}
2	3.6^{cd}	6.4^{bc}	4.6^{cd}
3	4.8^{b}	4.4^{d}	3.7^{de}
4	4.9^{b}	4.8^{c}	4.8^{c}
5	6.7^{a}	3.1^{e}	6.6^{a}
6	2.9^{d}	7.1^{ab}	3.8^{de}
7	6.0^{a}	3.3^{e}	6.2^{a}
8	6.8^{a}	2.8^{e}	5.9^{ab}
9	4.5^{bc}	6.3^{c}	5.1^{bc}

Values are presented as means ± standard error of triplicates and those connected by the same letter in the each column are not significantly different (P<0.05). Treatments No: 1=85 rpm SS & 232.5°C BT; 2=60 rpm SS & 232.5°C BT; 3=110 rpm SS & 275°C BT; 4=85 rpm SS & 275°C BT; 5=60 rpm SS & 190°CBT; 6=110 rpm SS & 232.5°C BT; 7=85 rpm SS & 190°C BT; 8=110 rpm SS & 190°C BT; and 9=60 rpm SS & 275°C BT. SS=Screw speed, BT= barrel temperature, rpm=revolutions per minutes, and ns=not significant. Color liking and appearance scale: 9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, 1=dislike extremely. For color light or dark scale: 9=extremely dark, 8=very much dark, 7=moderately dark, 6=slightly dark, 5=neither dark nor light, 4=slightly light, 3=moderately light, 2=very much light, 1=extremely light.

Table 5: Sensory Evaluation of the Nine extruded Products

Amount of Soy (%)	Moisture (%)	Protein (%)		Color		Texture (N)
			L*	a*	b*	
22	14.5 ± 0.3^{a}	22.7 ± 0.2^{b}	53.1 ± 0.01^{b}	2.8 ± 0.01^{b}	7.9 ± 0.01^{b}	419.3 ± 4.4^{b}
30	14.5 ± 0.2^{a}	25.7 ± 0.4^{a}	55.5 ± 0.01^{a}	3.1 ± 0.00^{a}	9.9 ± 0.01^{a}	549.7 ± 16.9^{a}

Values are presented as means ± standard error of triplicates. Values connected by the same letter in each column are not significantly different (P<0.05).

Table 6: Enhancing protein in the optimized extruded product (Treatment No: 5) with 22% and 30% soymeal flour

respectively for BD. The lowest BD (0.016 kg/cm^3) was obtained at the highest BT which was in agreement with a previous study who reported that increasing the BT reduced BD [35]. However contrary to this study who reported an increase in ER with BT, the current study observed a decrease in ER was with an increase in BT. This can be explained by the fact that the ER is highly dependent on the chemical composition of the raw materials used, and the effects that the processing will have on the transformations of these materials will depend on their composition [36].

An interaction between BT and SS was observed for Moisture Retention (MR) which was significant (P<0.0001). The SS did not have any effect on MR (P=0.07) whereas BT had a strong correlation (R^2 of 0.994) to MR. The highest MR (33.3%) was obtained at a SS of 110 rpm and at BT of 190°C and the lowest MR (17.7%) when the extruder was operated at a SS of 85 rpm and a BT of 275°C. A 46.3% decrease of MR was achieved as the BT increased from 190°C to 275°C. It was expected that the MR would decrease as the BT increased. At higher BT (275°C), the heat is intense enough to reduce more water from the initial feed moisture content of the blended flour as compared to other temperatures (190 and 232.5°C).

No interaction between BT and SS was observed on Water Holding Capacity (WHC) (P=0.78). Although the interaction was not significant, BT significantly affected and strongly correlated with WHC (P<0.0001 and R^2 Adj=0.899). The largest WHC (181.7%) was obtained at a SS of 85 rpm and a BT of 275°C, and the lowest (77.0%) when the extruder was operated at a SS of 110 rpm and a BT of 190°C. It was expected that products extruded at highest BT (275°C) would have the highest WHC. This is because at high BT, extruded product lost highest amount of water in comparison to feed moisture content as a result of excess heat. Therefore, they were expected to have a better WHC. A 57.6% increase in WHC was achieved as the extrusion temperature increased from 275°C to 190°C.

Both BT (P=0.03) and SS (P=0.02) significantly affected and strongly correlated (R^2 Adj=0.709) with Tap Density (TD). Moreover, an interaction was observed between the two factors on TD (P=0.01). The TD values ranged from 0.5-0.6 g/cm^3 when the extruder was operated at a SS of 60 rpm and a BT of 190°C and at a SS of 60 rpm and a BT of 232.5°C respectively.

True Density (TeD) was affected by the variation in the combination of BT and SS (P=0.03). Although the interaction was significant, BT alone did not have any effect on TeD (0.49) while only SS affected TeD (P=0.02). The largest TeD (1.6 g/mL) was obtained at a SS of 60 rpm and a BT of 190°C and the lowest (1.2 g/mL) when the extruder was operated at a SS of 85 rpm and a BT of 190°C BT.

The interaction between BT and SS affected Mass Flow Rate (MFR) (P value=0.03) which ranged from 0.1-0.4 g/sec at a SS of 60 rpm with a 275°C BT and 110 rpm SS with a 190°C BT. Lowest MFR values were obtained when the extruded was operated at the highest BT (275°C). It was observed that the greatest and lowest MFR value were obtained at the highest and lowest SS (110 and 60 rpm), respectively. This was expected as the speed at which the screw was operated, determined how fast the extruded products would come out from the extruder die; therefore higher the extruder SS, higher the MFR [37].

Fat Absorption Capacity (FAC) denotes the amount of oil which can be bound to matrices in a particular food system and used as the index of hydrophobicity of the food and it is expressed as the grams of oil bound per gram of the extrudates [21]. Both BT and SS did not affect FAC (P value=0.56 and P value=0.46 respectively) and also, no interaction between the two factors was observed with respect to FAC (P=0.75). The values of FAC ranged from 0.4-0.5 g at a SS of 110 rpm and a BT of 190°C and at a SS of 85 rpm and a BT of 232.5°C, respectively. This was in agreement with previous studies [38] who observed that extrusion conditions had little effect on FAC when rice flour and faba bean protein concentrate blend was used and other researchers [39] who reported that the FAC of the extruded flours were similar to that of the un-extruded rice flours.

An interaction between BT and SS (P<0.0001) was observed with respect to Milk Absorption Capacity (MAC). The two factors strongly correlated to MAC (R^2 Adj=0.979). The MAC values ranged from 0.2-0.6% at a SS of 60 rpm at a BT of 190°C and at a SS of 85 rpm at a BT of 275°C. It was expected that products extruded at highest BT (275°C) would have the highest MAC observation. This was because at high BT, extruded products were the most dehydrated as a result of excess heat; and therefore expected to have a better MAC.

There was an interaction between BT and SS (P=0.0004) with respect to Protein Solubility (PS). The two factors were both strongly correlated to PS (R^2 Adj=0.962). The PS values ranged from 0.26-0.33% at a SS of 85 rpm at a BT of 275°C and at a SS of 60 rpm at a BT of 190°C respectively. The lowest PS (0.26%) obtained when the extruder was operated at a SS of 85 rpm and a BT of 275°C might be due to the complexes formed by tannins and polyphenols with proteins in the flour blend which consequently increases the degree of cross-linking and decreases the solubility of proteins [40,41].

Water Absorption Index (WAI) is the weight of gel obtained per g of dry product and Water Solubility Index (WSI) is the amount of dried solid recovered by evaporating the supernatant from the WAI test [42]. No interaction between the BT and SS was observed on WAI (P=0.29). Although the interaction was not significant, BT significantly affected and strongly correlated with WAI (P<0.001 and R^2 Adj=0.855), respectively. The greatest WAI (17.3) was obtained at a SS of 60 rpm with a BT of 275°C and the lowest (13.8) when the extruder was operated at a SS of 110 rpm with a BT of 190°C. The highest WAI was obtained at the greatest BT which was consistent with the results of previous studies [36] who reported a complementary relation between WAI and the BT.

WSI was not affected by BT and SS (P=0.08 and P=0.54 respectively) and no interaction between the two factors was observed with respect to WSI (P=0.7844). This result was in disagreement with previous reports [26,35,42,43]. This might be due to the difference in food ingredients used in other studies since the impact of the extruder process depended on the chemical composition of the raw materials [36]. The values of WSI ranged from 10.6-12.6% at a SS of 110 rpm and a BT of 275°C and at a SS of 110 rpm at a BT of 190°C respectively.

Both BT and SS significantly affected and strongly correlated to *in vitro* Starch Digestibility (IVSD) (P<0.0001 and R^2 Adj=0.995). Moreover, an interaction was observed between the two factors on IVSD (P<0.0001). The IVSD values ranged from 83.9-160.7 µg of glucose/g when the extruder was operated at a SS of 85 rpm at a BT of 190°C and at a SS of 110 rpm at a BT of 275°C respectively. It was observed that greatest IVSD value (160.7 µg of glucose/g) was achieved at the highest SS of 110 rpm at a BT of 275°C respectively. This could be related to the reduction of trypsin and chymotrypsin inhibitory activities at higher extruder BT which eventually improves the IVSD [44].

Color: Any product for commercialization needs to be consumer acceptable. The two major attributes are texture and color. Color

Citation: Navam SH, Tajudini AL, Srinivas JR, Sivarooban T, Kristofor RB, et al. (2014) Physio-Chemical and Sensory Properties of Protein-Fortified Extruded Breakfast Cereal/Snack Formulated to Combat Protein Malnutrition in Developing Countries. J Food Process Technol 5: 359. doi:10.4172/2157-7110.1000359

characteristics of extruded products can be affected by various treatments. The data on color is shown in Table 4 where an interaction between barrel temperature and screw speed was observed on color 'L*', a*, and b*' values (P<0.0001). The highest (54.2) and the lowest (45.7) L* values were obtained when extruded at a 60 rpm SS and 110 rpm SS respectively with the same BT of 232.5°C. The significant difference in color between the two products was due to the difference in the screw speed indicating that at a low screw speed, the color of the extruded products was lighter or brighter ('L*' value) than at a faster screw speed, which was darker colored than others. The highest values for a* (2.8 and 8.4), which represents the color range between red to green, were obtained when extruded at 85 rpm SS with a BT of 190°C and 110 rpm SS at a BT of 232.5°C respectively. The color range between yellow and blue which is represented by the b* value was highest (1.6 and 3.1) at 60 rpm SS with a BT of 232.5°C and at 110 rpm SS with a BT of 232.5°C, respectively. The positive values for a* and b* establish a yellowish red color to the extruded product at the optimal BT and SS which is agreeable for a breakfast cereal or snack product.

Texture: Texture is also an essential characteristic of food acceptance by consumers [45] and therefore, an important step in quality assessment [46]. Figure 1 shows the sheer force of the nine extruded products. It was observed that both BT and SS significantly affected the sheer force of the extruded products (P<0.0001). The treatments numbers: 3, 4, and 9 extruded at the highest BT (275°C) had the lowest shear force observations with 318.5, 362.2, and 507.3 (N), respectively when compared to other treatment combinations. This was because at the highest BT, the extruded products became very dried, and bristle that they are easily broken by the texture analyzer. It was observed that the shear force of the extruded products decreased with increase of extruder BT. Similar findings were reported where increase in BT decreased the shear force of a breakfast cereal/snack made from purple rice (Hom Nil) and soybean flour blend [47].

Sensory evaluations: A sensory analysis is imperative for the success of a newly developed food product in the market. There was consistency in the overall panel performance when evaluating the extruded products as demonstrated by the ANOVA result. It is accepted in many descriptive profiling procedures that sensory panelists are a significant source of variation for all the attributes [48]. The ANOVA result indicated that BT and SS of the extruder affected the appearance and color of the extruded products as shown in Table 5. Products numbers: 5, 7 and 8 extruded at BT of 190°C scored 6.8, 6.7, and 6.0 points for 'color liking' and 6.6, 6.2, and 5.9 for 'appearance' respectively. These products were the most appealing to the panelists in comparison to the other extruded samples. Products 5, 7, and 8 received best scores since extrudates were considered acceptable if their mean score for overall acceptance was above 6 or 'Like slightly' [4]. It has been reported that, depending on the textural characteristics, a cereal product will show a variety of hues [48]. Figure 2 shows the pictures of the nine extruded products. It was observed that products 1, 2, and 6 extruded at 232.5°C were darker in color (with average of 7.5, 6.4, and 7.1 respectively) than the other products and were the least desirable in both appearance and color liking (average of 3.0, 4.6, 3.8 for appearance and 3.0, 3.6, and 2.9 for color liking). Chen and others reported that color changes in extruded products were due to decomposition of pigments and product expansion, which caused color to fade and to react with chemicals and other components in processes such as caramelization of carbohydrates [49]. Based on the sensory analysis, treatment no. 5, which had the best results for both color liking and appearance, was selected among the others for further analysis to increase the protein content by increasing the soymeal amount in the flour blend.

Protein enhancement: As the demand for a breakfast cereal/snack with enhanced nutritional value increases, the protein contents of soy and bean flours in the preparation of the breakfast cereal/snack will help meet this demand. The incorporation of legumes, such as soy and bean, in the breakfast cereal/snack will contribute to meeting the protein needs of poor in addition to solving some of the world's

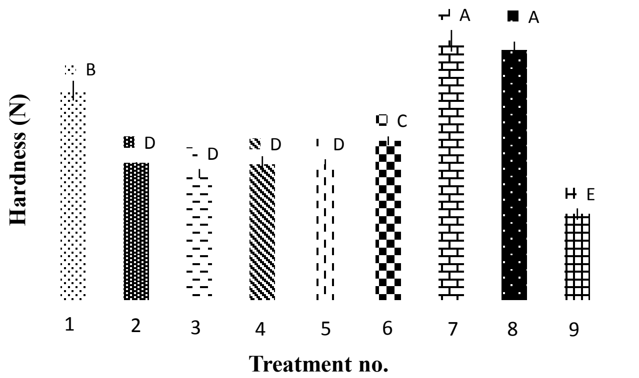


Figure 1: Shear force (N) of the Nine Extruded Products as a measure of texture Values are presented as means ± standard error of triplicates. Values connected by the same letter between the bars are not significantly different (P<0.05). Treatments no.: 1=85 rpm SS & 232.5°CBT; 2=60 rpm SS & 232.5°CBT; 3=110 rpm SS & 275°CBT; 4=85 rpm SS & 275°C BT; 5=60 rpm SS & 190°CBT; 6=110 rpm SS & 232.5°CBT; 7=85 rpm SS & 190°CBT; 8=110 rpm SS & 190°CBT; and 9=60 rpm SS & 275°CBT. SS=Screw Speed, BT=Barrel Temperature and RPM=Revolutions Per Minute.

Figure 2: Pictures of the Nine[a] Extruded Products[b] [a]Treatments (SS-BT) : 1=85 rpm-232.5°C, 2=60 rpm-232.5°C, 3=110 rpm-275°C, 4=85 rpm-275°C, 5=60 rpm-190°C, 6=110 rpm-32.5°C, 7=85 rpm-190°C, 8=110 rpm-190°C, and 9=60 rpm-275°C. [b]SS=Screw Speed, BT=Barrel Temperature, and RPM=Revolutions Per Minute.

health-related issues [50]. Choi and colleagues used defatted peanut as a nutritional ingredient to improve the protein content in the preparation of a snack food [4]. Previous study has shown that legume proteins such as soy, pea, bean levels of up to 30-35% may be added and still maintain high quality final products [51]. In order to increase the protein content of the extruded product, the soy content of the mixture was increased from 16% to 22% and 30% using the extrusion conditions of treatment no: 5. Table 5 shows the results of moisture and protein contents, color, and texture of the improved protein-extruded product (22% and 30% soy) based on conditions of treatment no. 5 at 60 rpm SS at a BT of 232.5°C. The protein content, color and the texture analysis of the extruded product were significantly different between the extruded products with 22% and 30% soy in the flour blend. It was observed that the protein content of the snack increased from 19.5% to 22.7%. Previous research has shown that increasing soybean in a flour mixture increased the nutrient or protein content of the snack [50,52]. Due to the high temperature-short time process, extrusion technology retains considerable amounts of nutrients and eliminates anti-nutritional factors. Moreover, there was an improvement in the textural properties of the snack. This is because as the amount of soy tends to increase in the mixture, the textural properties of the snack shift to consistent hardness. This is in agreement with Veronica and colleagues who reported that the addition of protein to starch-rich flours produced harder extrudates [50]. It was observed that, except for moisture content, there was a significant difference in protein content, texture, and color of the extruded product with 22 and 30% soy, which increased in protein from 22.7-25.7% and texture from 419.3 to 549.7 N respectively. It is noted that as the soy content increased in the flour mixture a^* and b^* values of the color increased, which could be the result of a browning reaction. The breakfast cereal/snack extruded with 30% soy had greater protein content, a harder texture, and a lighter color compared to that extruded with 22% soy (Table 6).

Conclusion

Central Composite Design was used to select the best extrusion conditions to develop a breakfast cereal/snack with millet, bean, rice, and soymeal flour blend using a single screw extruder. The extruder BT and the SS were observed to influence the extrudate physio-chemical and sensory properties both independently and interactively except for fat absorption capacity and water solubility index. The extruder BT was shown to be the most significant factor that affected the properties of the extruded products. This study demonstrated that extruded products, acceptable to consumers, could be prepared from blends of millet, beans, soy and rice flour under a range of extrusion conditions by supplementing with up to 30% of soymeal flour. Therefore, this product could be supplied to the developing parts of the world, including Asia and Africa, which children are suffering from protein malnutrition.

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Citation: Navam SH, Tajudini AL, Srinivas JR, Sivarooban T, Kristofor RB, et al. (2014) Physio-Chemical and Sensory Properties of Protein-Fortified Extruded Breakfast Cereal/Snack Formulated to Combat Protein Malnutrition in Developing Countries. J Food Process Technol 5: 359. doi:10.4172/2157-7110.1000359