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Research Article

Influence of basil seed flour supplementation on micro and macro nutritional profile of RTE corn based functional snacks

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Abstract

The study was conducted with an aim to explore basil seeds for development of functional snacks. Central composite rotatable design (CCRD) was used to investigate the effect of independent variables such as feed proportion (Basil seed flour: Corn flour), feed moisture (FM), screw speed (SS), and extruder barrel temperature (BT) on system and product responses. Significant effect ($p < 0.05$) of independent variables on system and product responses was observed. High coefficient of determination (R2 ≤ 0.99) was observed for all regression models. The optimum conditions for development of basil supplemented functional snack was basil: corn snack was: 6.50:93.40% Basil: Corn, feed moisture of 11%, barrel temperature of 90oC and screw speed 200 rpm. The developed snack (BCS) recorded significantly higher protein (8.75g/100g), fat (8.93g/100g) and fibre (14.07g/100g) content than control (100% corn flour) (CS). Vitamin and mineral content of BCS also increased as compared to CS. Amino acid profiling revealed presence of all the essential and non-essential amino acids. BCS (12.47g/100g) recorded predominantly higher percentage of α-linolenic acid as compared to CS (0.03g/100g). Phytochemical screening showed that BCS contained higher percentage of epicatechin (19.02mg/100g), followed by ferulic acid (8.02mg/100g) and p-coumaric acid (2.24mg/100g) when compared to CS.

Keywords: Basil Seeds; Extrusion; Amino Acid; ALA; caffeic Acid

Introduction

Lifestyle-based diseases such as cardiovascular sicknesses (CVDs), hypertension, obesity, diabetes, etc among common masses have been on rise due to excessive intake of low fibre and less nutritious foods [1]. Attention towards medicinal plants and their parts has begun to grow due to their high nutritive values and associated health benefits. Different medicinal plants have been explored by food scientists and technologists for development of wholesome foods with suitable sensory qualities. Consumption of certain plant materials such as seeds, leaves, fruits and roots have been associated with reduced risk of chronic diseases [2].

Basil seed, also known as king of herbs, has gained tremendous importance now-a-days due to its immense nutritive and therapeutic value. Ellipsoidal black seeds, Basil are good source of fiber and α-linolenic acid (ALA). Basil seeds produce gelatinous mass (mucilage) when placed in aqueous medium due to the presence of poly saccharide layer on outer epidermis wall of seed [3]. Two major fractions of poly saccharides have been reported from basil seeds, glucomannan (43%) and (1-4)-linked xylan (24.29%) and a minor fraction of glucan (2.31%) [4]. Besides dietary fibre, basil seeds are rich in proteins, omega 3 fatty acids, minerals, flavonoids, and polyphenols, all of which are attractive characteristics for the food industry and consumers looking for foods with healthy properties. In addition, they have remarkable phenolic composition which is beneficial in relation to human health and disease prevention. Presently, basil seeds are consumed raw or used to prepare traditional beverages (sharbat) and many ice desserts like falooda [2]. However, basil seeds have not been explored for extrusion cooking. Several studies have shown that consuming whole of seeds (fenugreek, chia, basil) could not increase the plasma α-linolenic levels [5]. Thus, extrusion becomes necessary for seed coat to breakdown and release seed oil in order to exert health benefits after consumption.

Extrusion cooking is a baro-thermal process which simultaneously applies high pressure and temperature in order to develop quality snacks. Extruded foods are known for their precise nutrient and quality component blending. Owing to versatile nature, affordability, and high productivity of extrusion process, extrusion cooking process is extensively used by most of the food processing industries [6]. Since extruded snacks are convenient to eat, have good nutritive value, appearance and texture, thus has huge demand among customers [7]. Thus, the present study was planned to develop a functional food from basil using extrusion cooking.

Materials and Methods

Raw material

Basil seeds (*Ocimum basilicum* var. *thyrsiflora*) were procured from farms of Ganderbal District, Jammu and Kashmir. Corn flour (var C-7) was acquired from Mountain Research Center for Field Crops, Khudwani, Sher-e-Kashmir University of Agricultural Science and Technology of Kashmir (SKUAST-K). Basil seeds were ground to a fine powder by means of Cemotec™ laboratory mill. Both the samples were packed as separate entities in laminated aluminum pouches till further requirement.

Extrusion cooking

Extrusion cooking was carried out using a twin-screw extruder (Basic Technology Pvt. Ltd. in Kolkata, India) having barrel diameter of 2.5 mm, die of 3.mm dia and an 8:1 length-to-diameter ratio. During the experiment, Ist, second-, and third-barrel zones' temperature was kept constant at 40°, 60°, and 80° C, respectively, while the temperature of the fourth zone was adjusted in accordance with the experimental design (Table 1). Prior to extrusion, moisture content of feed blend to be extruded was adjusted as per experimental design (Table 1). The developed snacks were collected in trays and packed for further analysis.

Experimental design and optimization conditions for development of extruded basil-corn snacks

The effect of independent variables viz. Feed proportion (A) (Corn flour-basil seed flour: 95:5::75:25), Feed moisture (B) (11-15%), Barrel temperature (C) 150-190ºC and Screw speed (D) (160-280rpm) for developed extruded basil-corn puffs was investigated by means of 4 factor 5-level central composite rotatable design (CCRD). RSM provided by Design expert-10 (Stat-ease Inc-Minneapolis, MN, USA) statistical package was employed to investigate the effect of independent variables on dependent variables viz. specific mechanical energy (SME), expansion ratio (ER), bulk density (BD), Breaking strength (BS), water solubility index (WSI), water absorption index (WAI), L*, a*, b* values and overall acceptability (OA). Mathematical models in the form of second order polynomial equations were used to study the effect of independent variables on each individual response. CCRD́s desirability function approach was used to determine the optimum condition. Numerical optimization was carried out by following optimization criteria to maximize ER, SME, WAI, WSI, L* value and OA and to minimize BD, BS, a* and b* values.

Effect of extrusion on system and product responses of basil-corn extrudates

System responses

Specific mechanical energy (SME)

SME which measures the mechanical energy required to move the extrudate across the extruder is calculated by the following formula [8]

$$SME = \frac{Actual\ screw\ speed(rpm)/Rated\ Screw\ speed \times motor\ torque(\%)}{100 \times motor\ power\ rating/mass\ flowrate(\frac{kg}{h})} \times 1000 \quad \text{---------(1)}$$

Product response

ER was determined by means of digital Vernier caliper with the accuracy of 0.001mm as per the formula given below

$$ER = \frac{thickness\ of\ extrudate}{diameter\ of\ die} \quad \text{----------(2)}$$

Bulk density of extrudates was calculated as the ratio of weight of the extrudates to that of the volume occupied [8]. Water absorption index (WAI) and Water Solubility index (WSI) were measured as per the formulae given by Wani and Kumar [9].

$$WAI(g/g) = \frac{weight\ gain\ by\ gel}{weight\ of\ dry\ extrudates} \quad \text{----------(3)}$$

$$WSI\ (\%) = \frac{weight\ of\ slids\ dissolved\ in\ supernatent}{weight\ of\ dry\ solids} \times 100 \quad \text{---------(4)}$$

Breaking strength (BS) of extruded basil-corn snacks (BCS) was determined using P50 compression probe of the Texture analyzer (TA-XT2i) as per Pardhi., *et al.* [10]. Color values viz. (L*, a*, b*) were measured by Hunter Lab Colorimeter (Model No. A60-1010-617). Sensory evaluation of the developed extrudates were carried out on different attributes i.e., colour, taste, appearance, texture and overall acceptability by following the procedure laid down by Altaf., *et al.* [11]. A panel of 10 semi-trained judges from the scientific staff of Division of Food Science and Technology, SKUAST-K, Shalimar carried out the sensory evaluation of developed extrudates in separate booth. Overall acceptability was calculated by taking the average score received against each attribute by different extrudates.

Physico-chemical analysis

Standard AOAC [12] procedures were followed for the determination of basic nutritive parameters viz moisture, crude protein,

S. No.	A: Feed proportion (BSF:CF)	B: Feed moisture	C: Barrel temp	D: Screw speed	SME	ER	Bulk density	WSI	WAI	BS	OA	L*	a*	b*
	%	%	ºC	rpm	(Wh/kg)		(kg/m³)	(%)	(g/g)	(N)				
1	10:90	12	110	200	92	4.99	101	53	2.69	15.45	7.63	72.47	1.74	23.36
2	20:80	12	110	200	70	3.67	152	20	5.23	21.66	5.69	63.22	3.14	16.33
3	10:90	14	110	200	85	4.64	102	51	2.28	16	7.23	71.44	1.76	24.65
4	20:80	14	110	200	62	3.57	156	13	4.89	22.69	5.24	63.18	3.17	17.5
5	10:90	12	150	200	89	5.19	93	57	2.48	15.37	7.78	72.54	1.79	23.68
6	20:80	12	150	200	66	3.58	140	23	5.03	21.52	5.82	63.16	3.25	16.63
7	10:90	14	150	200	86	4.84	101	47	2.11	15.54	7.41	71.03	1.83	24.81
8	20:80	14	150	200	64	3.61	155	15	4.73	21.75	5.52	61.84	3.36	17.75
9	10:90	12	110	250	94	4.82	89	55	2.57	15.41	7.69	72.36	1.85	23.43
10	20:80	12	110	250	72	3.75	144	22	5.11	21.59	5.76	62.03	3.42	16.48
11	10:90	14	110	250	88	4.55	103	53	2.19	15.57	7.35	70.71	1.86	24.73
12	20:80	14	110	250	64	3.87	162	11	4.81	22	5.33	61.81	3.49	17.52
13	10:90	12	150	250	90	5.12	86	59	2.37	15.32	7.86	71.48	1.89	23.84
14	20:80	12	150	250	71	3.89	133	26	4.96	21	5.87	61.78	3.54	16.74
15	10:90	14	150	250	83	4.99	96	49	2.04	15.48	7.52	70.1	1.91	24.89
16	20:80	14	150	250	61	4.02	142	19	4.65	22.12	6.57	60.93	3.58	17.89
17	5:95	13	130	225	98	5.16	76	62	1.59	15	8.53	75.32	1.42	27.49
18	25:75	13	130	225	57	2.46	170	16	6.24	29	4.65	58.37	4.49	13.65
19	15:85	11	130	225	84	4.42	108	42	3.78	17.61	6.9	68.32	2.13	19.62
20	15:85	15	130	225	73	4.16	130	40	3.27	18.12	6.14	67	2.28	21.53
21	15:85	13	90	225	81	4.22	129	31	3.84	18	6.31	68.26	2.17	20.44
22	15:85	13	170	225	76	4.57	117	39	3.29	17.77	6.56	66.72	2.38	20.98
23	15:85	13	130	175	72	4.2	122	30	3.69	17.83	6.34	68.54	2.25	20.62
24	15:85	13	130	275	76	4.42	112	31	3.41	17.73	6.6	66.2	2.7	20.74
25	15:85	13	130	225	77	4.44	118	32	3.44	17.51	6.61	66.24	2.29	20.76
26	15:85	13	130	225	78	4.45	113	33	3.48	17.52	5.87	65.28	2.52	20.79
27	15:85	13	130	225	79	4.55	118	34	3.51	17.53	6.61	66.26	2.42	20.81
28	15:85	13	130	225	80	4.56	112	28	3.54	16.98	6.25	66.27	2.52	20.84
29	15:85	13	130	225	81	4.57	106	36	3.57	17.55	5.87	66.22	2.38	20.88
30	15:85	13	130	225	82	4.58	107	37	3.6	17.21	6.45	65.98	2.42	20.93

Table 1: Effect of independent responses on quality and sensorial attributes of developed snacks.

BSF: Basil Seed Flour; CF: Corn Flour; SME: Specific Mechanical Energy; ER: Expansion Ration; WSI: Water Solubility Index; WAI: Water Absorption Index; BS: Breaking Strength; OA: Overall Acceptability, L*: Luminosity; a*: Redness; b* Yellowness

crude fiber, ash and crude fat of basil-corn snacks (BCS) and control. Carbohydrate content was calculated by difference method and energy value was determined using formula

$$\text{Carbohydrate (\%)} = 100 - (\%moisture + \%fat + \%protein + \%ash + \%fiber) \quad \text{--- (5)}$$

$$Energy\ (Kcal/100g) = (4 \times \%carbohydrate) + (9 \times \%fat) + (4 \times \%protein) \quad \text{---- (6)}$$

Mineral analysis

The Atomic Absorption Spectrometer (Perkin-Elmer, USA) procedure of AOAC [12] were followed for estimation of mineral content in both the CS and BCS.

Vitamin analysis

Vitamin content of both samples (BCS and CS) was determined after following the standard AOAC [12] procedure. Vitamin A, C and

E were measured spectrophotometrically. Samples were treated with acetic anhydride reagent and absorbance was measured at 620nm after 15and 30 seconds intervals for estimation of vitamin A. 2,6-dichlorophenol indophenol method was followed for ascorbic acid estimation and absorbance of the samples was spectrophotometrically recorded at 520nm. Samples treated with dipyridyl were spectrophotometrically analysed at 520nm and tocopherol content was recorded. HPLC (YounglinR 930D) method provided by Marzougui., *et al*. [13] was used to determine thiamine and niacin content of both samples (BCS and CS). A reverse phase HPLC equipped with two pumps, control panel, injector port, column (Eurospher 100C-18.5) (250 ×4.6mm; 5µm id) and ultraviolet absorbance detector (254mm) was used to carried out vitamin estimation. A mixture of methanol (9%), crystalline acetic acid (10ml) and distilled water was used as mobile phase. Prior to injection, solvent was added to sulfonic acid pentane and sulfonic acid octane and deaerated through agitation followed by filtration through 45µm Teflon filter. Deareated solvent is pumped across the column with a constant flow rate between 0.5 and 2.1ml/min. The obtained peaks were calculated using electronic integrator and developed into a chromatogram. By comparing the retention times of standards, quantification of vitamin was done and the results were expressed as µg/100g.

Amino acid profiling

Amino acid quantification of control (100% Corn flour snacks -CS) and basil-corn snacks (BCS) was carried out by method provided by Dhillon., *et al.* (2014). A reversed-phase HPLC method was used after hydrolysis of samples with 6N HCl for 24 h at 110°C under nitrogen atmosphere. Prior to HPLC, samples undergo derivatization by treatment with 60 µl of AccQ-Fluor borate buffer (WAT052880-Waters Corporation, USA) added at a rate of 20µl/30sec to samples followed by through mixing and heating at 55°C in order to facilitate complete derivatization of amino acids. Reversed phase AccQ Tag Silica-bonded Amino Acid Column C-18 maintained at 37°C was employed to carry out the HPLC separation of amino-acids. The mobile phase (AccQ Tag Eluent A) diluted to 10% in Milli-Q water and 60% in acetonitrile was made to flow over the solvents in a separation gradient at a flow rate of 1.0 mL/min. Photo diode array (PDA) detector at 254nm was used to detect the separated amino-acids. Identification of the amino acids in the samples was carried out by comparison with the retention times of the standards.

Fatty acid profiling

Fatty acid profiling of BCS and CS was carried out using gas chromatography-mass spectrographic (Shimadzu GCMS-QP2010 Plus) method (GC-MS) method laid down by AOAC (2005). Prior to GC-MS, acid hydrolysis method was used for lipid extraction from samples and conversion of extracted oil to fatty acid methyl esters (FAME). FAME was resolved into individual fatty acids by means of GC-MS equipped with flame ionization detector, an autosampler and column (60m × 250µm × 0.25 µm). The temperature of column was set at 105°C which was increased to 250°C at 25°C/min. FAME were injected to injector port at the rate of 3.5°C/min which was maintained at the temperature of 230°C while detector was set at 280°C. Helium as carrier gas was passed through the GC at a linear viscosity range of 1.4mL/min. By comparing the retention times (RT) of fatty acids with those of authentic standards led to the identification of fatty acids while peak to area ratio was calculated for quantification of resolved fatty acids. Results for each individual fatty acids were expressed in relative percentages (g/100g).

Antioxidant activity and characterization of phytochemical compounds

Preparation of extracts

Samples were turned to fine powder in a pestle and mortar followed by dissolution in 80% methanol. The methanolic solution was placed on shaking incubator overnight for proper extraction. Afterward, the extracts were recovered by centrifugation at 6000 rpm for 15 min. The extracts of control (CS) and basil seed enriched snacks (BCS) were used for estimation and characterization of phenolic compound and antioxidant activity.

Determination of antioxidant activity

The procedure of DPPH radical scavenging activity laid down by Kumar., *et al*. [15] was followed to determine the antioxidant activity of CS and BCS. 3.9ml of 105mol/L DPPH solution was added to 100 µL of extract (100 mg/mL). Afterwards, the solution was rested for period of 30 minute followed by spectrophotometrically measurement of its absorbance at 515nm. Antioxidant activity of CS and BCS was measured as percent inhibition of DPPH.

Determination of phyto-chemicals

Chromatography technique proposed by Martinez-Cruz and Paredes-lopez [16] was used to determine phenolic compounds from crude extracts of CS and BCS. For separation, identification, and quantification of phenolic compounds, an Agilent 1290 infinite LC system (Agilent Technologies, Santa Clara, CA) equipped with a binary pump, degasser, and automated purge valve, an auto-sampler, thermostated column compartment, and diode array detector was employed. A binary mobile phase comprising of solvent A (2 percent acetic acid in water) and solvent B (2 percent acetic acid, 30 percent acetonitrile, and 68 percent water) was employed for efficient separation of phenolic components. Prior to separation, the solvents were filtered through a 0.45 membrane. For a total run time of 18 minutes, the rate of solvent flow was held constant at 0.4 mL/min Extracts of CS and BCS were diluted to an injection volume of 1l and fed to the head of column and absorbance was measured at 280, 325 and 260 nm. Standards were prepared in 80% methanol and later diluted using distilled water to give serial concentrations in a range of 0.0004125–0.1650 mg/mL for phenolic acids. All measurements were performed in triplicate, and the results were expressed as mg/100g of samples.

Statistical analysis

For the purpose of statistical analysis, experiments were conducted in triplicate and results were presented as average mean ± SD. Analysis of data was done by Student's t-test in SPSS software. Mean values were compared by Duncan's Multiple Range test at $p < 0.05$ level of significance.

Results and Discussion

Fit summary and regression models

The effect of parameters feed proportion (A), feed moisture (B), Barrel temperature (C), and screw speed (D) on the responses were studied. Table 1 lists the responses for SME, EI, BD, BS, WSI, WAI, OA, color (L*, a*, b*) for each experimental run. The developed polynomial equations were used to study the effect of each individual parameter on SME, EI, BD, BS, WSI, WAI, OA, color (L*, a*, b*). Analysis of variance (ANOVA) for different responses indicating the statistical validity is given in table 1. Least square regression models were used to fit the obtained data. For EI, WAI, SME and BS quadratic models were suggested, while for color, WSI, OA and BD linear models were suggested. R^2, p-value and lack of fit values described how well the models fitted to experimental data. Models with high correlation coefficients (R^2 = 0.9151 -0.9965) were developed which indicated that developed models were highly significant. Predicted R^2 was found to be in close agreement with adjusted R^2. Coefficient of variation (CV), which studies the variability of data and is used for accuracy and reproducibility of models, ranged from 0.95 to 12.77%. A highly desirable adequate precision range (32.04–286.84) was observed in all the models, which suggested the adequacy of model discrimination in all the parameters. Non-significant lack of fit was recorded for all the parameters, which implies that all the second order polynomial models correlated well with the measured data.

System response

Specific Mechanical Energy (SME)

SME determines the rate of starch conversion and is related to snack expansion. Higher SME values are desired as the more energy delivered, the greater the expansion [8]. In the present study, SME values of extrudates ranged from 57-98Wh/Kg.

$SME = 79.50-21.58A-6.08B-2.25C + 1.42D-5.38D^2$-----(7)

The regression model shown above describes the significant ($p < 0.05$) linear effect of all the independent variables and quadratic effect of screw speed (D). SME decreased when basil flour percentage in the feed blend was increased as shown by linear term of feed proportion (A). The fibre content from basil decreased the percentage of starch in the feed mix consequently decreasing SME. Fiber increases the viscosity of feed melt inside extruder by forming hydrophilic bonds with water thereby decreasing the water accessibility of starch which in turn reduced SME [17]. Increase in feed moisture (B) resulted in reduced SME (equation 7) due to lubricative effect of water on feed mix which leads to less energy requirement.

SME decreased with increase in barrel temperature (C) as higher temperatures favors starch gelatinization process which decreases the SME. Similar observations have been presented by Pradhi., *et al*. [10] for brown rice grit extruded snacks. SME showed a positive relation with screw speed (D). As the screw delivers more energy, it increases the screw shear rate which leads to increase in SME [18]. However, excess increase in screw speed reduced the SME of extrudates by causing fragmentation of starch molecules. Dissociation of these fragmented molecules requires less energy and subsequently lesser SME [19].

Product response

Bulk Density (BD) and Expansion ratio (ER)

Bulk density as well as expansion of extruded snacks is important parameter as far as consumer acceptance is considered as it depicts the product quality. BD and ER are inversely related and are used for determining its packaging requirements [8]. BD and ER of the basil: corn snacks ranged from 76-170kg/m^3 and 3.58-5.19 respectively.

$BD = 119.77 + 50.08A + 10.25B-7.25C-5.4D$----------(8)

$EI = 4.53-1.22A-0.1200B + 0.1733C + 0.1133D + 0.3200AB-0.2250AC + 0.3200AD-0.6367A^2-0.011B^2$------(9)

The fitted regression equations (8 and 9) shown above depicted significant ($p < 0.05$) effects of all the linear variables on BD and ER of the developed extrudates. Significant ($p < 0.05$) quadratic effect of feed proportion (A) and feed moisture (B) was also observed on ER. Interactive effects of feed proportion and feed moisture (AB), feed proportion and barrel temperature (AC), feed proportion and screw speed (AD) were also found to be significant ($p < 0.05$).

Increase in basil seed flour in feed mix yielded denser snacks with decreased expansion due to high protein and fibre content of basil as compared to corn. Fibre causes damage to cell walls of molecules in the molten dough and thus impedes air bubble formation leading to denser product formation [20]. Bulk density of the developed extrudates was found to increase as the moisture content of feed mix was increased. High moisture content restricts the dough fluidity by plasticization of melt as a result of which SME and expansion decreases leading to formation of denser extrudates. As the temperature of barrel increased, expansion of the extrudates increased (equation 8 and 9) as high temperature favors expansion leading to decrease in bulk density by reducing dough viscosity and favouring growth of bubbles. Also, the complex formation between amylases and lipids is hampered by higher temperature which results in formation of expanded snacks. The results are in concomitance with Altaf., *et al.* [11] for chickpea incorporated extrudates.

Bulk density of extruded snacks decreased while expansion increased at higher screw speed probably due to change in molecular structure of proteins and starch which resulted in decrease in length of polymeric chains of molecules and denaturation of proteins as well under high shear conditions [10]. High screw speed lowers the residence time of dough in the barrel which results in incomplete gelatinization of starch and thus favors higher expansion and reduced density of snacks.

Water Solubility Index (WSI)

WSI indicates the solvability of biomolecules like starches, sugars, proteins fibers before and after being subjected to processing in abundance of water [21]. WSI of basil-corn snacks varied from 11 to 62% for all the developed extrudates.

$$WSI = 35.47-30.58A-5.08B \text{-----}(10)$$

The fitted regression equation 10 demonstrated the negative linear effect of Feed proportion A and Feed moisture (B) on WSI of the developed extrudates. Increase in basil seed flour in the feed blend as well as its moisture content reduced WSI values of the developed extrudates. Since basil seed is rich in fibre and protein in comparison to corn as a result these components dominate starch which result in decreased WSI. Extrusion when proceeds at low temperature favours formation of water-soluble molecules while as reverse effects have been observed at high feed moisture level which generally favors' gelatinization and impedes the process of protein denaturation with minimum damage to starch molecules [22]. The results are in concomitance for those reported by Beigh., *et al.* [18] for water chestnut incorporated snacks.

Water Absorption index (WAI)

WAI is an indicator of starch digestibility as it provides the rate of conversion of starch to dextrin's and degree of gelatinization [11]. For all the experimental runs, WAI ranged from 1.59 to 6.24g/g.

$$WAI = 3.52 + 2.50A-0.3133B-0.2083C-0.1083D + 0.3833A^2 \text{--------}(11)$$

The fitted regression equation represents significant ($p < 0.05$) linear effect of all the independent variables as well as quadratic effect of feed proportion on water absorption index (WAI) of basil-corn snacks. WAI increased with increase of basil seed flour (A) in the feed blend probably due to high fibre content of basil seeds. In addition, the gel-forming property of basil seeds when soaked in water may also contribute to increased WAI.

Increased in moisture content (B) of feed mix reduced WAI of developed extrudates which may be ascribed to greasing effect of moisture on dough mix that diminishes the degree of cooking and dissolvability of starch at ambient temperature [21]. High barrel temperature (C) resulted in reduced WAI values (eq) of developed extrudates as high temperature enhances process of starch degradation and accelerated rate of starch conversion to dextrins. Altan., *et al*. [23] in his study revealed that if process of dextrinization or starch liquefying process takes precedence over gelatinization process, WAI decreases with increase in temperature. Decrease in WAI of basil-corn snacks with higher screw speed (D) occurs because of high shear on molecules which leads to breakdown of molecules at higher screw speed resulting in shortening of polymeric chains and making them in order to bind water.

Breaking strength (BS)

The hardness/BS of an extruded snack is a human sensory perception that is related to the product's expansion and cell structure. The average force required for a probe to penetrate the extruded product is defined as hardness. In the present study, the developed snacks recorded hardness ranging from 15-29N.

$$BS = 17.38 + 6.25A + 0.4042B + 4.46A^2 \text{-----------}(12)$$

The Eqn.10 shown above describes the significant ($p < 0.05$) positive linear effect of Feed proportion (A) and feed moisture (B) on BS values of developed extrudates. Quadratic effect of feed moisture on BS was also found to be significant. As the level of basil seed flour increased in the feed mix, harder extrudates were obtained due to increase in fibre content. which restrict the expansion process leading to harder snacks. Increase in feed moisture content also increased hardness of extrudates. Water exerts plasticizing effect on the molten material inside the extruder barrel, which reduces starch resistance to deformation, as well as the rate of energy dispersal and vapor pressure, thus hampering bubble formation subsequently resulting in denser snacks with increased hardness [21].

Overall acceptability (OA)

In the present study, all the developed extrudates scored overall acceptability values In the range of 4.65-8.53

OA = 6.53-1.87A-0.2875B + 0.2442C------(13)

The fitted regression equation 13 describes the significant ($p < 0.05$) negative linear effect of Feed proportion (A), Feed moisture (B) and Barrel temperature (C) and positive linear effect of screw speed (D) on over all acceptability (OA) of basil infused corn-based snacks. An increase in percentage of basil seed flour resulted in the decrease in OA of snacks which can be due to higher protein, gums and fiber content of basil seed which hamper the expansion process during extrusion and result in denser and harder products where are not generally preferred by consumers. OA decreased with increase in feed moisture because high moisture favors formation of denser and harder snacks generally snacks light in weight and crisp in texture are preferred. With increase in barrel temperature during extrusion process, developed extrudates recorded high OA values as high temperature favours most of the biochemical processes viz. Millard reaction, gelatinization, modification of protein structures and many more which are necessary for proper color and flavor development of snacks. Our findings are in agreement with those reported by Altaf., *et al.* [11] for chickpea incorporated snacks.

Color

Color is an important characteristic feature for any product and has a direct impact on the acceptability of food products in market. Luminosity (L*) of developed extruded snacks varied from 58.37 - 75.32 while redness (a*) and yellowness (b*) ranged from 1.42 - 4.49 and 13.65 - 27.49 respectively (Table 1).

L* = 66.84-9.01A-0.8867B-0.6200C-1.03D-----(14)

a* = 2.43 + 1.54A + 0.0950C + 0.2000D + $0.6108A^2$ -----------(15)

b* = 20.68-7.02A + 1.09B + 0.2758C ----------(16)

Fitted regression equations (14), (15) and (16) depicted that all the independent factors had a ($p < 0.05$) significant effect on l* and a* values of basil-corn snacks while feed proportion (A), feed moisture (B) and barrel temperature (C) only affected yellowness of the developed extrudates. In addition, a significant ($p < 0.05$) positive quadratic effect of A was also found on redness of developed extrudates. Increase in basil seed flour percentage in the blend decreased the brightness and yellowness while increased redness of basil-corn snacks which may be ascribed to dark black color of basil seeds. Furthermore, proteins and sugars from basil and corn led to formation of melanoidins which give darker hue to extrudates. Similar observations have been recorded by Nithya., *et al.* [24] when rice flour was replaced with Bengal gram. Decrease in L* values with the increase in screw speed was recorded. High screw speed leads to expansion of extrudates with enhanced air bubbles which absorb light thereby reducing the luminosity of extrudates. Redness and yellowness values were found to increase while as brightness decreased with increase in feed moisture. High moisture of feed leads to compact air cells which absorb more-light consequently resulting in darker color of extrudates [20]. High barrel temperature increases the redness and yellowness but decreased lightness of developed extrudates as higher temperatures also favor Millard reactions and sugar caramelization leading to darker extrudates [18].

Optimization

By applying the desirability function method and covering our criteria as discussed in material and method section, the best solution was selected for development of snacks on the basis of highest desirability value (0.843) (Figure 1). The optimum predicted conditions obtained for development of functional snack were as feed proportion of 6.50:93.50% (Basil seed flour: Corn flour), Barrel Temperature of 90°C; feed moisture content of feed of 11% and Screw speed of 200 rpm). Predicted values for SME, BD, ER, BS, WAI, WSI, OA, L*, a*, b* were recorded 100.5Wh/kg, 76.96kg/m^3, 5.19, 15.850N, 2.422g/g, 63.037%, 8.07, 76.498, 1.307 and 25.223. After following the optimized condition, the snack with SME of 103.19Wh/kg, BD of 78.89kg/m^3, ER of 5.308, BS of 16.01 N, WAI of 2.46g/g, WSI of 64.19%, OA of 8.283, L* value of 78.478, a* value of 1.325 and b* value of 26.152. were acquired which corresponded to the predicted values with difference of less than 3.68% thus showed excellent predicted patterns of the proposed models. The results therefore, confirmed the validity and adequacy of the models for predicting dependent variables.

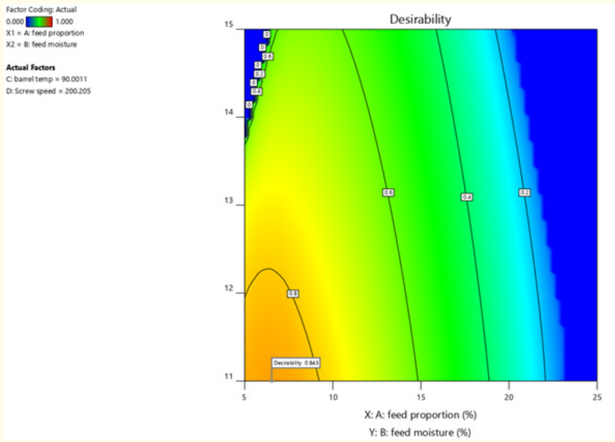

Figure 1: Desirability function response surface plot.

Proximate composition of developed basil-corn snacks

Proximate composition of basil-corn snacks (BCS) and control (100% corn flour-based snacks) (CS) is shown in table 2. A significant ($p \leq 0.05$) decrease in moisture and carbohydrate content and increase in protein, fiber, ash and fat content was recorded in BCS as compared to CS. Incorporation of 6.54% basil seed flour could be the possible reason for enhancement in protein, fat, fibre and

Parameters	Control (CS)	Basil -corn snack (BCS)
Moisture (%)	4.54[a] ± 0.04	2.34[b] ± 0.02
Protein (%)	5.45[b] ± 0.04	8.75[a] ± 0.04
Fat (%)	2.10[b] ± 0.02	8.93[a] ± 0.2
Ash (%)	1.09[b] ± 0.3	3.45[a] ± 0.1
Crude fiber (%)	3.40[b] ± 0.3	14.07[a] ± 0.03
Carbohydrate (%)	83.42[a] ± 0.3	62.46[b] ± 0.5
Energy (kcal/100g)	374.38[a] ± 2.05	365.21[b] ± 3.12
Minerals (mg/100g)		
Calcium	10.56[b] ± 0.22	94.05[a] ± 1.23
Iron	2.78[b] ± 0.02	7.83[a] ± 0.54
Magnesium	64[b] ± 0.02	104[a] ± 0.28
Phosphorus	13.12[b] ± 1.21	23.83[a] ± 0.54
Potassium	5.54[b] ± 0.23	77.07[a] ± 0.07
Sodium	17[b] ± 0.02	21.82[a] ± 1.25
Zinc	0.12[b] ± 0.01	0.32[a] ± 0.57
Copper	0.03[b] ± 0.24	0.21[a] ± 0.03
Manganese	0.06[b] ± 0.19	0.22[a] ± 0.09
Vitamin analysis (ug/100g)		
Vitamin A	11[b] ± 0.02	36.04[a] ± 0.43
Vitamin E	4.9[b] ± 0.23	13.52[a] ± 0.23
Vitamin C	70[b] ± 0.04	119.23[a] ± 0.05
Thiamin	3.4[b] ± 0.54	54.53[a] ± 0.02
Niacin	9[b] ± 0.02	15.75[a] ± 1.06
Folate	0.08[b] ± 0.02	5.46[a] ± 0.28

Table 2: Proximate composition, minerals, vitamin analysis of basil seed flour corn-based snacks and control snacks.

Values are presented as mean ± SD. Values of different parameters with different letters in a single row are statistically different.

ash content of BCS. In addition, extrusion processing enhances the digestibility of protein and consequently protein content of snacks by causing inactivation of enzyme inhibitors and denaturation of proteins in the raw material which exposes them to enzyme attack [11]. Incorporation of basil seed flour also led to an increase in fibre content of the snacks due to its high fibre content of basil seeds. Further, during extrusion cooking a shift from insoluble dietary fibre to soluble dietary fibre takes place, besides formation of resistant starch and enzyme-resistant indigestible glucans by transglycosidation [25]. The increase in ash content of snacks over rice flour can be attributed to 6.5% incorporation of basil seed flour as well as the enhanced bioavailability of minerals during extrusion by inactivation of anti-nutritional factors. Decrease in moisture content of BCS as compared to CS may be ascribed to proteins and fiber content of basil seed flour which binds with the moisture and thus limits its availability [11]. Reduction in carbohydrate content of BCS in comparison to CS is due to significant ($p < 0.05$) increase in its fat, fibre and protein content. BCS had significantly ($p < 0.05$) higher calorific value as compared to CS due to predominant increase in fat and fibre content. Similar observations were reported by Altaf., *et al*. [11] for chick pea incorporated rice-based snacks

Mineral analysis

Table 2 lists the different minerals identified and quantified in BCS And CS. A considerable increase in mineral profile of BCS was observed during extrusion cooking when compared to CS. Calcium, iron, magnesium, zinc, phosphorus copper, sodium, manganese and potassium showed a significant ($p < 0.05$) increase during extrusion cooking in basil enriched snacks. Minerals are relatively heat stable due to their crystalline nature, and extrusion cooking alters the distribution of fibers, which form complexes with other organic molecules, increasing mineral concentration [26]. Besides, addition of 6.5% basil in functional snacks would enrich the of the developed snack while enhancement in iron content of extrudates may also be attributed to iron migration from the extruder. Similar results for enhancement in mineral content of extruded snacks was inferred by Wani and Kumar [9].

Vitamin analysis

The vitamin content of basil corn snacks is depicted in table 2. An increase in Vitamin A content from 11 to 36.04µg/100g, vitamin E from 4.9 to 13.52µg/100g, vitamin C from 70 to 119.23µg/100g, thiamin from 3.4 to 54.53, niacin from 9 to 15.75 µg/100g and folate 0.08 to 5.46 µg/100g was observed. This can be attributed to incorporation of 6.5% basil seed flour as basil is an excellent source of vitamins [27]. Similar observations for enhancement in vitamins were recorded by Giacomino., *et al*. [28] when flaxseed meal was incorporated in cereal based extruded snacks.

Amino acid profile

The amino acid profile of extruded snacks enriched with basil seed flour (BCS) and control (100% Corn flour-based snacks-CS) is depicted in figure 2. From graph, it was evident that amino acid content of BCS was higher than CS. Overall, amino acid composition of extruded snacks enhanced by incorporation of basil seed. Lysine and methionine, limiting amino acids in cereal foods, were recorded in BCS despite being heat sensitive. Phenylalanine, leucine, isoleucine, valine, tyrosine, alanine, glutamic acid and glycine being heat stable were least affected by extrusion conditions. Adeleye., *et al*. [29] reported that most of the amino acids are loss at temperatures of 120°C and above but the temperature used in developing the extruded snacks was 90°C which may have favored the least degradation and retention of most of the amino acids in the developed snacks. Similar observations were recorded by Khattab [30] for cookies enriched with defatted flaxseed and seasame seeds.

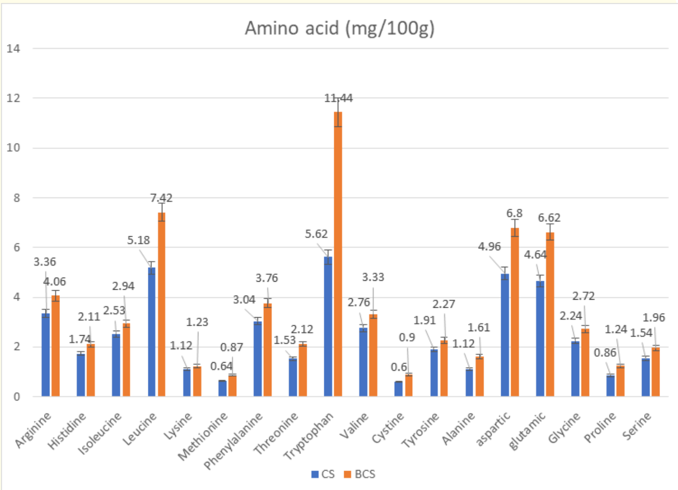

Figure 2: Comparative amino acid profile of control (100% corn flour snacks -CS) and basil-corn snacks (BCS).

Fatty acid profile

Fatty acid profile of control (CS) and basil enriched snacks (BCS) is shown in figure 3. Incorporation of basil seeds enhanced the overall fatty acid profile of extruded snacks, which can be attributed to rich fatty acid profile of basil seeds [31]. Among all the fatty acids observed in the samples, α-linolenic content of BCS was predominantly high as compared to CS. The α-linolenic acid content of BCS and CS was recorded as 12.74% and 0.03% respectively while slight increase was recorded for other fatty acids of BCS in comparison to CS. Lipid enters into reaction with amylose to form amylose-lipid complexes [11] which might be the probable reason for slight increase in fatty acids content of the developed functional snacks (BCS) as compared to control (CS).

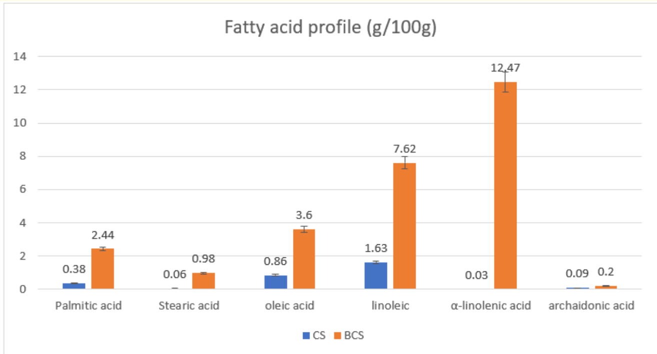

Figure 3: Comparison of fatty acid profile of control (100% corn flour snacks -CS) and basil-corn snacks (BCS).

Antioxidant activity

The comparative anti-oxidant activity of basil-corn snacks (BCS) and corn-based snacks (CS) are represented in table 3. The total antioxidant activity of BCS was recorded as 5.24% which was higher that than reported for CS (0.76). Incorporation of 6.5% basil seeds might have resulted in increased antioxidant activity of BCS over CS as total phenolic content of basil is higher than other Lamiaceae plants [32]. The retention of polyphenols during extrusion process and their ability to scavenge radicals is primarily responsible for their antioxidant activity. The positive correlation of total phenols with antioxidant activity has been observed by Aryal., *et al*. [33]. In addition, synthesis of melanodins and maillard reaction products during extrusion cooking may also contribute to increased antioxidant activity of BCS over CS as these compounds are known to possess antioxidant activity [34]. Furthermore, higher contrent of vitamin E and C in BCS as compared to CS may have added to its increased antioxidant activity. Similar results for enhancement in antioxidant activity was reported by Sharma., *et al*. [34] for extrudates made from grits of barley.

Antioxidant activity (% DPPH activity	CS	BCS
Phytochemicals (mg/100g)	0.76^{b}±0.04	5.24^{a}±0.09
Gallic acid	0.07^{b}±0.01	0.53^{a}± 0.02
caffeic acid	0.002^{b}±0.02	0.96^{a}±0.05
chlorogenic acid	0.04^{b}±0.07	0.61^{a}±0.34
ferulic acid	0.0001^{b}±0.12	8.02^{a}±0.28
Epicatechin	0.024^{b}±0.24	19.02^{a}±1.21
p-coumaric acid	0.0013^{b}±0.06	2.24^{a}±0.54

Table 3: Comparison of anti-oxidant activity and characterization of phytochemicals in control (100% corn flour snacks-CS) and basil-corn snacks (BCS).

Values are presented as mean ± SD. Values of different parameters with different letters in a single row are statistically different.

Phytochemical profile

The different phytochemical components identified in basil corn snacks (BCS) and corn snacks (CS) have been shown in table 3. The results inferred from the bar graph indicate that BCS recorded predominantly higher content of all the phenolic compounds as compared to CS which may be attributed to higher percentage of phytochemical compounds present in the basil seeds. Incorporation of 6.5% basil seed flour enhanced Gallic acid from 0.07 in BCS to 0.53mg/100gm in CS. Similarly, Caffeic acid, chlorogenic acid, ferulic acid, epicatechin and p-coumaric acid was recorded as 0.002mg/100g, 0.04mg/100g, 0.001mg/100g, 0.024mg/100g and 0.002mg/100g respectively in CS while incorporation of 6.5% basil seed flour increased caffeic acid, chlorogenic acid, ferulic acid, epicatechin and p-coumaric acid to 0.96mg/100g, 0.61mg/100g, 8.02mg/100g, 19.02mg/100g and 2.24mg/100g respectively. During extrusion cooking breakdown of cell wall structure occurs thereby releasing phenolic compounds which are covalently bonded to the cell wall as a result of which bound phenolic acids are released and are easily extractable consequently increasing their concentration. The results are in concomitance with those reported by Kasprzak., *et al*. [35] for extruded corn snacks supplemented with Kale.

Coefficient	ER	L*	a*	b*	OA	BS	WSI	WAI	BD	SME
Intercept	4.53	66.84	1.54	20.68	6.53	17.38	35.47	3.52	119.77	79.50
A-feed proportion	-1.22*8	-9.01**	0.0533*	-7.02*	-1.87**	6.52**	-30.58**	2.50**	50.08**	-21.58**
B-feed moisture	-0.1200*	-0.8867**	0.0950^{NS}	1.09**	-0.2875**	0.4042*	-5.08**	0.3133**	10.25*	-6.08*
C-barrel temperature	0.1733*	-0.6200*	0.2000^{NS}	0.2758**	0.2442**	-0.2275**	2.75^{NS}	-0.2083**	-7.25**	-2.25**
D-screw speed	0.1133**	-1.03	1.54^{NS}	0.0875^{NS}	0.1792^{NS}	-0.1408**	1.42^{NS}	-0.1083*	-5.42**	1.42**
AB	0.3200**	-	-	-	-	0.4375*	-	0.0600^{NS}	-	-1.25^{NS}
AC	-0.2250**	-	-	-	-	-0.2075**	-	0.0150^{NS}	-	1.25^{NS}
AD	0.3200*	-	-	-	-	-0.0825**	-	0.0100^{NS}	-	0.7500^{NS}
BC	0.0700^{NS}	-	-	-	-	-0.1175**	-	0.0300^{NS}	-	1.75^{NS}
BD	0.1550^{NS}	-	-	-	-	-0.0325**	-	0.0250^{NS}	-	-2.75^{NS}
CD	0.1700^{NS}	-	-	-	-	0.2425**	-	0.0200^{NS}	-	-2.25^{NS}
A^2	-0.6367^{*}	-	-	-	-	4.46**	-	0.3883^{NS}	-	-1.88^{NS}
B^2	-0.1567^{NS}	-	-	-	-	0.3296*	-	-0.0017^{NS}	-	-0.8750^{NS}
C^2	-0.0517^{NS}	-	-	-	-	0.3496*	-	0.0383^{NS}	-	-0.8750^{NS}
D^2	-0.1367^{NS}	-	-	-	-	0.2446*	-	0.0233^{NS}	-	-5.38
Model (p value)	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001
R^2	0.9853	0.9800	0.9921	0.9988	0.9151	0.9941	0.9191	0.9965	0.9503	0.9881
Adjusted R^2	0.9716	0.9768	0.9847	0.9986	0.9015	0.9886	0.9061	0.9933	0.9424	0.9770
Predicted R^2	0.9235	0.9732	0.9652	0.9984	0.8873	0.9701	0.8796	0.9819	0.9358	0.9552
Adequate precision	35.02	69.12	48.21	286.84	32.04	54.40	33.07	74.53	42.18	38.48
F-value	72	307	134	527	72	180	71	307	120	89
CV (%)	2.33	1	3.56	1.57	4.32	1.86	13.56	2.62	4.86	2.14
Lack of Fit	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table S1: Analysis of variance for the fit of experimental data to response surface models.

Conclusion

The effect of independent variables such as feed proportion (Basil seed flour: Corn flour), feed moisture, barrel temperature and screw speed on the dependent variables viz. SME, ER, BD, WAI, WSI, BS, colour attributes (L*, a*, b*) and OA was investigated. All the dependent variables were effect by all the four independent variables. The study indicated that 6.54% of basil seed flour can be incorporated to corn flour to obtain nutritionally superior and healthy snacks at a barrel temperature of 90°C, screw speed of 200 rpm, moisture content of feed 11%. The developed functional snacks (BCS) were found superior to corn-based snacks in terms of nutritional constituents, mineral profile and vitamin content. Amino acid revealed that the resultant snacks comprised of higher amount of all the essential as well as non-essential amino acids. α-linolenic acid was also found predominantly higher in developed snacks while its phenolic content was also found to be excellent which led to its enhanced antioxidant activity. The results of the study inferred that basil seed flour at 6.5% can be put forth for enrichment of extruded snacks and can pe explored for development of several functional food items. Large production of such food items could be adopted for improvement of nutritional status

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Conflict of Interest

There is no conflict of interest for the work

Author Contribution

Tabeen Jan: Formal analysis, writing original draft; Syed Zameer Hussain: Conceptualization; Asima Rafiq: Data curation, supervision; resources availability; Tahiya Qadri: Reviewing original draft; Shahnaz Mufti: Validation.

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