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Optimization of Machine and Process Parameters for Development of Protein and Antioxidant Rich Composite Food using Twin Screw Extruder

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ABSTRACT

Background: Nutritional quality of extruded products can be enhanced through protein, minerals and antioxidants rich foods *viz.* legumes, green leafy vegetables, dietary fibres, by-products, *etc*., leading to improvement in functional properties of products and health of consumers.

Methods: Extrudates were prepared from maize, defatted soy meal, sesame and spinach using a co-rotating twin screw extruder and dried in a tray dryer at 50°C up to about 5.5% moisture content (w.b.) and analyzed for important product quality parameters.

Result: Quality characteristics of developed food grains-spinach based composite food were affected by variability in independent studied parameters. Optimized extrusion conditions for desired composite food from food grains-spinach were as 14% moisture content, 117°C die head temperature (DHT) and 335 rpm screw speed. These conditions resulted in the products with 18.26% protein, 73.88% *in vitro* protein digestibility, 2.46 protein efficiency ratio, 2.84% total minerals, 4.67 mg/100 g iron, 190 mg/100 g calcium, 24.05% antioxidant activity and 92.52% consumer acceptability of ≥7.

Key words: Antioxidants, Extrusion, Optimization, Protein efficiency ratio, Spinach.

INTRODUCTION

Extrusion is a promising high-temperature-short-time process, provides versatility to develop food grains based low-cost nutritious and convenience foods. High throughput during extrusion with continuous processing lowers the cost, higher retention of heat labile nutrients and environment friendly processing are some of the key features of food extrusion. Food extrusion denatures proteins, gelatinizes starch, inactivate enzymes, microbes and many anti-nutrients, thereby enhanced the digestibility of starch and proteins (Alam *et al*., 2019). Many extruded snack foods made from starchy material are commercially available in Indian market but with poor nutritional fact (Giri and Mridula, 2016). Consumers' demand for such extruded snacks will raise, if overall nutritional quality and biological value of these starchy snacks will be enhanced for health purposes and better marketability. Therefore, the use of protein, minerals and antioxidants rich raw materials like maize, sesame, defatted soy meal and spinach, would be helpful in increasing nutritional qualities.

Maize is one of the raw material component for extruded breakfast products and ready-to-eat expanded foods due to its high starch content. Edible grade defatted soy meal or flour is a very valuable food material for enrichment of protein and overall nutritional quality of cereal foods (Ghoshal and Kaushik, 2020). Sesame is exceptionally high in calcium and can be considered for calcium enrichment in extruded products (Mridula *et al*., 2017; 2021) and bioactive compounds (Pathak *et al*., 2019). Spinach a green leafy vegetable is considered a 'protective food' due to being rich in vitamin and minerals, carotenoids and phytochemicals such as lutein, zeaxanthin, neoxanthin and chlorophylls (Nemzer *et al*., 2021). Food processing parameters/ techniques affect the antioxidant activity of bioactive compounds, present in raw food materials (Toydemir *et al*., 2022). Extrusion processing requires shorter exposure time that favours the better retention of nutrients and bioactive components. In view of this, present study was carried out with objective to optimize the important extrusion parameters for development of acceptable quality nutritious antioxidants rich composite food utilizing maize, sesame, defatted soy meal and spinach.

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MATERIALS AND METHODS

Raw materials

Maize, defatted soy meal (DSM) and sesame, procured from local market, Ludhiana, India were pulverized to reduce the particle size (0.954 mm). Fresh spinach was steam blanched, dried in a tray dryer at 55±2°C to 14% moisture content and coarsely ground. Feed formulation for extrusion was prepared using maize (74%), sesame (5%), DSM (15%) and dried spinach (6%).

Experimental design

Design expert 8.0.5 software was considered for experimental planning using Box-Behnken design with three independent process variables *viz.* die head temperature (DHT; 100-120°C), feed moisture (14-18%) and screw speed (SS) 330-350 rpm (Table 1). Dependent variables (responses) for this study were bulk density, expansion ratio, colour quality, water absorption index, water solubility, antioxidant activity, total phenols, flavonoids, protein, total minerals, calcium, iron content and overall acceptability.

Extrusion processing

Rod shaped extrudates samples were prepared using a co-rotating twin screw extruder and dried in a tray dryer at 50°C for 2 h (Mridula *et al*., 2017) up to about 5.5% moisture content (w.b.). Thereafter, extruded samples were packaged in polyethylene bags (thickness 70 micron) and stored at 25±2°C and 60% RH for further quality analysis.

Physicochemical properties

Bulk density (BD) and expansion ratio (ER) of extrudate samples was determined by measuring the actual dimensions with digital vernier calliper with a least count of 0.01 mm while mass of extrudates was noted using an electronic balance.

$$\text{Expansion ratio} = \frac{\text{Diameter of extrudate}}{\text{Die diameter}}$$

$$\text{Bulk density (Pb)} = \frac{4w}{\pi d^2 l}$$

Where,
l = Length of extrudate (cm).
w = Mass (g).
d = Diameter (cm).

Water absorption index (WAI) and water solubility index (WSI) of developed extrudates were determined using the method, suggested by Mridula *et al.* (2021).

Colour (L, a and b values) of developed and control (L0 78.29, a0 2.53 and b0 22.26) extrudates were determined using Hunter Colorimeter. Whiteness index (WI) and total colour difference (ΔE) were calculated using following formulas:

$$WI = 100 - \{(100 - L)^2 + a^2 + b^2\}^{0.5}$$

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

Where,
$\Delta L = L - L0$.
$\Delta a = a - a0$.
$\Delta b = b - b0$.
L, a and b = Measured values.
L0, a0 and b0 = Values of control extrudates without spinach.

Nutritional quality

Moisture, protein, fat, carbohydrate, total minerals, iron, calcium content and total calories of developed extrudates were determined using AOAC methods while protein efficiency ratio (PER) was determined (AOAC, 960.48) using disease free albino rats with IAEC approval no. GADVASU /2016/ IAEC/ 38/03 at Small Animal Colony, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab (AOAC, 2000). *In-vitro* protein digestibility of optimized extrudates was determined using pepsin and pancreatin enzymes (Akeson and Stachman, 1964). Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (DPPH inhibition) in extrudates was quantified using the methods suggested by Mridula *et al*. (2020) and Arora *et al*. (2019).

Sensory characteristics

Sensory characteristics of extrudate samples were evaluated by following a method mentioned by Deshpande and Poshadri (2011). Samples for sensory evaluation were prepared using 5 mL refined oil, 2.5 g salt and 0.5 g mixer of green chilli powder, cumin seed per 100 g sample for flavoring purposes.

Statistical analysis

Results of this study were analyzed using the Design-Expert (Stat-Ease Inc, Minneapolis, MN, USA). The optimized design was validated using a triplicate analysis of the independent variable parameters.

RESULTS AND DISCUSSION

Physicochemical properties

Consumer acceptability of extruded product is primarily affected by physical qualities such as expansion ratio and bulk density. ER of extrudates varied between 2.53 to 3.37 (R^2=0.959) while BD ranged from 166.31 to 384.34 kg/m^3 (R^2=0.976). Feed Moisture negatively affected the ER while no significant (p>0.05) impact of SS and DHT was observed on ER of studied samples (Table 2a). ER and BD exhibited inverse relation with a negative and positive correlation, respectively with increasing feed moisture (Table 2a). Seth *et al.* (2015) studied the effect of moisture content on expansion ratio of yam-corn-rice based extrudates and concluded that decreased moisture content increases the drag force and therefore exerts more pressure at the die resulting in greater expansion of extrudate at the exit. This study indicated significant (p<0.05) effect of feed moisture and DHT on BD of extrudates with spinach (Table 2a). At a given DHT *i.e.*, 120°C, BD increased from 263.60 kg/m^3 to 337.94 kg/m^3 in sample with 18% feed moisture. Minimum BD (166.306 kg/m^3) was observed at 14% moisture while maximum (384.335 kg/m^3) at 18% feed moisture at 110°C and 100°C DHT, respectively. The increase in bulk density at higher moisture content while extrusion could happened due to a reduction in dough elasticity through plasticization of raw materials melt in barrel, thereby causing reduced gelatinization and increase in the density of the final product as also observed by Ding *et al.* (2005). Effect of higher extrusion temperature are in line with Fletcher *et al*. (1985) where the higher extrusion temperature could have increased the degree of superheating of water and thereby a decrease in melting viscosity leading to lower bulk density than at low extrusion temperature.

$$ER = + 2.95 + 0.037A - 0.017B - 0.31C + 0.036AB + 0.027AC - 0.023BC - 0.15A^2 - 0.097B^2 + 0.18C^2$$

$$BD\ (kg/m^3) = + 249.67 - 29.48A - 1.06B + 55.11C - 13.88AB - 7.15AC + 2.62BC + 52.02A^2 + 28.26B^2 - 56.01C^2$$

WAI of extrudates ranged from 4.55 to 4.78 g/g while WSI was found between 21.11 to 27.92%, which was affected ($p<0.05$) with DHT, having no impact of SS and feed moisture (Table 2a). Maximum WAI (4.78 g/g) was found at 100°C DHT, 325 rpm SS with 14% feed moisture whereas minimum *i.e.*, 4.55 g/g was observed at 120°C DHT, 300 rpm SS with 16% feed moisture. WSI in developed extrudate samples were increased with increasing DHT ($p<0.001$), while SS and feed moisture did not show any significant effect on WSI of spinach incorporated extrudates. The results indicated maximum WSI (27.92%) at 120°C DHT, 325 rpm SS and 14% feed moisture. Conversely, minimum WSI (21.11%) was found at 100°C DHT along with 325 rpm and 18% of SS and feed moisture, respectively. This could be due to higher starch degradation at higher extrusion temperature and greater shearing action of the blend (Seth *et al*., 2015). Die head temperature was observed to have the greatest effect on gelatinization. The maximum gelatinization occurred at high die head temperature as also reported by Ding *et al*. (2005). Decrease in WAI at higher DHT in this study could probably be due to higher dextrinization, which also could have led to increase in WSI (Peluola-Adeyemi and Idowu, 2014). Regression model showed a significant impact of studied variables on WAI (R^2=0.887) and WSI (R^2=0.994) of extrudates with non-significant lack of fit (Table 2a).

$$WAI\ (g/g) = + 4.66 - 0.063A + 4.342E - 004B - 2.049E - 003C + 0.022AB + 0.041AC + 2.505E - 003BC + 1.961E - 004A^2 - 0.012B^2 + 0.014C^2$$

$$WSI\ (\%) = + 24.49 + 2.63A - 0.25B - 0.54C + 0.19AB + 0.089AC + 0.022BC + 0.21A^2 - 0.27B^2 - 0.094C^2$$

Colour index *i.e*., 'L', 'a' and 'b' value of extrudates was observed between 60.02 to 62.87, -3.04 to -3.46 and 22.80 to 24.14, respectively while the whiteness index and colour difference ranged from 17.24 to 22.959 and 52.85 to 57.92, respectively. Though variation was observed in 'L', 'a', 'b' value of extrudates but no statistical difference ($p>0.05$) was observed in colour quality except for b values of samples ($p\leq0.05$) due to variability in independent parameters (Table 2a). Minus 'a' value of samples indicated the greenness of the sample, which is due to presence of spinach in feed formulation. Colour difference values of extrudate samples at 14% feed moisture, decreased from 19.91, 19.25 and 18.75, respectively at 300, 325, 350 rpm SS with no statistical similarity ($p>0.05$). There was a slight reduction in whiteness index from 55.54, 54.45 and 53.74, respectively at 14%, 16% and 18% feed moisture at constant temperature 100°C but it was statistically insignificant ($p>0.05$).

Table 1: Experimental design of independent variables for composite food with food grains-spinach.

Experimental	Temperature (°C)		Screw speed (rpm)		Feed moisture(%)	
run	Coded	Actual	Coded	Actual	Coded	Actual
1	-1	100	1	350	0	16
2	-1	100	0	325	1	18
3	1	120	1	350	0	16
4	0	110	0	325	0	16
5	-1	100	0	325	-1	14
6	0	110	0	325	0	16
7	1	120	-1	300	0	16
8	1	120	0	325	1	18
9	0	110	-1	300	-1	14
10	0	110	1	350	1	18
11	0	110	-1	300	1	18
12	-1	100	-1	300	0	16
13	1	120	0	325	-1	14
14	0	110	1	350	-1	14
15	0	110	0	325	0	16
16	0	110	0	325	0	16
17	0	110	0	325	0	16

$$L\ value = +61.05 + 0.20A - 0.087B - 0.62C + 0.021AB + 0.44AC - 0.57BC + 0.13A^2 + 0.22B^2 + 0.7C^2$$

$$a\ value = -3.34 + 8.750E-003A + 0.028B + 3.437E-003C + 0.060AB - 3.750E-003AC - 0.078BC + 0.075A^2 + 0.052B^2 + 0.16C^2$$

$$b\ value = +23.68 + 0.22A + 0.069B - 0.29C + 0.016AB + 0.090AC + 0.21BC + 0.14A^2 - 0.083B^2 - 0.086C^2$$

$$Colour\ difference\ (\Delta E) = +20.06 - 0.31A + 0.21B + 0.59C - 0.037AB - 0.64AC + 0.80BC + 0.67A^2 + 0.080B^2 - 0.22C^2$$

$$Whiteness\ index\ (WI) = +55.29 + 0.17A - 0.22B - 0.38C + 0.013AB + 0.54AC - 0.81BC - 0.68A^2 - 0.043B^2 + 0.19C^2$$

Nutritional quality

Nutrients *i.e.* protein, iron, calcium and total minerals in developed extrudate samples varied between 18.26 to 18.29%, 4.60 to 4.70 mg/100 g, 180.4 to 190 mg/100 g and 2.84 to 2.96%, respectively, which was higher than the maize based extrudates. The proximate composition of maize based extrudates showed 9.44% protein, 1.28% total minerals, 26 mg/100 calcium and 1.13 mg/100 g iron at 4.62% moisture content, indicating the significant improvement in the nutritional quality with the studied formulation having defatted soy meal, sesame and spinach along with maize as a major portion for the developed protein and antioxidant rich composite food in this study. Though there was some variability in nutritional quality, but ANOVA results indicated no significant (p>0.05) impact of studied variability of DHT, SS and feed moisture on extrudates (Table 2b). This might be due to similar feed formulation for all extrudate samples and shorter thermal exposure to affect the proximate composition of developed extrudates. Coefficient of regression for protein, total

Table 2a: Analysis of variance of independent variables on quality responses of composite food with food grains-spinach.

Particulates	Expansion ratio	Bulk density	L value	a value	b value	Colour difference	Whiteness index	WAI	WSI
Model	18.01**	31.66**	1.23	2.08	2.18	0.33	0.27	6.08**	128.07**
Die head temperature, °C (A)	1.64	33.59**	0.43	0.057	5.05*	0.22	0.074	41.86**	1083.54**
Screw speed, rpm (B)	0.34	0.043	0.081	0.60	0.53	0.10	0.12	1.973E-003	9.50**
Feed moisture, % (C)	119.79**	117.40**	4.12	8.771E-003	9.30**	0.80	0.37	0.044	46.39**
A*B	0.77	3.72*	2.276E-003	1.34	0.013	1.590E-003	2.338E-004	2.45	2.82
A*C	0.43	0.99	1.03	5.219E-003	0.44	0.47	0.38	8.66**	0.62
B*C	0.31	0.13	1.75	2.27	2.39	0.75	0.85	0.033	0.039
A*A	13.85**	55.07**	0.089	2.19	1.19	0.55	0.63	2.119E-004	3.60**
B*B	6.01**	16.25**	0.28	1.05	0.40	7.699E-003	2.493E-003	0.73	5.82**
C*C	20.85**	63.83**	3.12	10.07	0.43	0.060	0.049	1.01	0.72
Lack of Fit	4.08	3.56	1.46	0.24	1.66	0.37	0.40	2.41	2.71
C.V. %	2.79	5.51	1.40	3.24	1.14	9.17	3.19	0.59	0.93
R^2	0.959	0.976	0.613	0.728	0.737	0.296	0.261	0.887	0.994

Significance: *p≤0.05, **p≤0.01.

Table 2b: Analysis of variance of independent variables on quality responses of composite food with food grains-spinach.

Particulates	Protein content	Total minerals	Iron content	Calcium content	Antioxidant activity	Total phenolic content	Flavonoids	Overall acceptability
Model	0.94	0.42	0.88	1.03	220.32**	149.16**	87.44**	3.93**
Temperature, °C (A)	0.63	0.31	2.47	1.76	1893.96**	1293.16**	765.61**	5.28*
Screw speed, rpm (B)	0.11	0.45	9.357E-003	2.68	0.20	7.96*	0.82	4.73
Feed moisture, % (C)	0.67	1.131E-004	0.088	0.19	0.042	0.44	0.60	9.72*
A*B	0.26	4.114E-003	0.95	2.77	3.95*	1.15	0.40	0.87
A*C	0.29	0.76	1.23	0.28	0.80	0.41	0.045	0.58
B*C	0.56	0.71	2.11	0.38	0.40	13.37**	2.70	3.582E-003
A*A	1.27	0.064	0.035	0.038	76.88**	24.25**	15.00**	0.038
B*B	4.29	1.49	0.81	0.065	3.03	0.80	1.58	0.83
C*C	0.26	0.064	0.22	1.13	0.54	0.89	0.91	13.68**
Lack of Fit	8.99	1.66	0.18	3.65	5.91	0.29	0.90	0.51
C.V. %	0.064	1.47	0.66	1.51	0.15	0.53	0.39	4.19
R^2	0.547	0.352	0.532	0.570	0.997	0.996	0.991	0.835

Significance: *p≤0.05, **p≤0.01.

minerals, iron and calcium was 0.55, 0.35, 0.53 and 0.57, respectively with non-significant lack of fit for these nutritional parameters.

Protien content (%) = + 18.27 + 3.271E - 003A + 1.391E + 003B + 3.369E - 003C + 2.971E - 003AB + 3.119E - 003AC + 4.370E - 003BC + 6.397E - 003A^2 + 0.012B^2 - 2.915E -003C^2

Total minerals (%) = +2.90 + 8.447E - 003A - 0.010B - 1.604E - 004C - 1.368E - 003AB + 0.019AC + 0.018BC - 5.277E - 003A^2 + 0.025B^2 - 5.278E -003C^2

Iron content (%) = + 4.65 + 0.017A - 1.057E - 003B - 3.251E - 003C - 0.015AB + 0.017AC + 0.022BC - 2.833E - 003A^2 + 0.014B^2 + 7.139E - 003C^2

Calcium content (%) = + 188.04 - 1.34A + 1.65B - 0.44C + 2.38AB - 0.75AC + 0.87BC - 0.27A^2 + 0.36B^2 + 1.48C^2

TPC of extrudates ranged from 331.74 to 386.53 mg GAE/100 g. SS and DHT brought a significant impact on TPC in developed extrudates with no impact of feed moisture (R^2=0.996). TPC in extrudates decreased with increasing DHT while an inverse relation was observed with the SS (Table 2b).

Loss in the TPC of feed formulation under extrusion by increasing die head temperature is expected to occur due to heat-liable nature of phenolic compounds. However, the increased screw speed showed the comparatively higher retention of TPC, regardless of feed moisture and extrusion temperature, thus explaining the positive effect of screw speed on TPC retention in the final product, might be due to the dual effects of high shear and shorter residence time in the screw barrel. Higher shearing and friction effect might have caused degradation of cell walls, breakdown of conjugated or bound phenols, thus releasing free phenolic compounds. Further, reduced residence time in screw barrel ensued lower thermal degradation and thus indicated higher retention of phenolic compounds (Patil *et al*., 2016). At a given value of SS *viz.* 300 rpm, increasing DHT from 100°C to 120°C showed a substantial decrease in TPC from 380.35 at 100°C to 352.90 mg GAE/100 g at 110°C with further decreased to 334.59 mg GAE/100 g at 120°C. On the other hand, increasing SS from 300 to 350 rpm resulted in increased TPC from 380.50 to 386.34 mg GAE/100 g, respectively at a given DHT of 100°C. Maximum TPC (386.53 mg GAE/100 g) was observed at 100°C DHT, 350 rpm SS and 16% moisture while minimum TPC (331.74 mg GAE/100 g) was found at 120°C DHT with 325 rpm SS and 14% feed moisture.

TPC (mgGAE/100 g) = + 354.18 - 24.16A + 1.90B + 0.45C - 1.02AB + 0.61AC - 3.47BC + 4.56A^2 + 0.83B^2 - 0.87C^2

TFC in developed extrudates ranged from 7.70 to 8.39 mg QE/100 g. DHT was found to be the most influencing variables (R^2=0.991). At a constant 300 rpm SS, TFC decreased from 8.36 mg QE/100g at 100°C DHT to 8.01 mg QE/100g at 110°C DHT which further decreased to 7.77 mg QE/100g at 120°C DHT; with no significant impact due to variable SS and feed moisture. The higher loss of TFC at higher DHT could be due to heat sensitive properties of flavonoids and showed the thermal destruction. Thermal effect *i.e.* retention or degradation of flavonoids, during heat dependent processing is a collective effect of different parameters such as nature of matrix, type of processing or processing conditions particularly temperatures used during thermal processing (Moussa-Ayoub *et. al*., 2015; Patil *et. al*., 2016).

TFC (mg QE/100 g) = +8.01 -0.31A -0.010B + 8.662E - 003C - 9.954E - 003AB - 3.370E - 003AC - 0.026BC + 0.060A^2 - 0.019B^2 - 0.015C^2

Antioxidant activity (DPPH inhibition) in extrudates samples decreased from 24.96% at 100°C to 24.51% at 110°C DHT, with further decreased to 23.76% at 120°C DHT and constant 300 rpm SS (R^2=0.9965). This antioxidant activity of developed extrudates was due to presence of DSF, sesame and spinach in extrudates, which are rich in bioactive compounds. Reduction in antioxidant activity at higher DHT in this study might be attributed to the reduction in flavonoid and phenolic contents as also been observed in other studies (Patil *et al*., 2016). Maximum antioxidant activity (24.97%) was found at 100°C DHT along with 325 rpm SS and 18% feed moisture, while minimum antioxidant activity (23.75%) was indicated at 120°C DHT with 300 rpm SS and 16% feed moisture (Fig 1a-b).

Antioxidant activity (% DPPH inhibition) = 4.55 - 0.57A + 5.892E - 003B + 2.695E - 003C + 0.037AB + 0.017AC - 0.012BC - 0.16A^2 - 0.031B^2 - 0.013C^2

Overall acceptability

Sensory evaluation is an important factor for consumer acceptability and marketability of newly developed products (Sharif *et al*., 2017). Overall sensory acceptability of extruded samples varied from 6.57 to 8.31, which was found affected ($p \leq 0.05$, R^2=0.835) due to variable extrusion conditions (Table 2b). Maximum overall acceptability score (8.31) was observed for extrudates prepared at 120°C, 325 rpm SS with 14% feed moisture while minimum at 100°C DHT with 325 rpm SS and 16% feed moisture. At constant feed moisture (14%), overall acceptability of extrudates was found as 7.42, 7.78 and 8.13 at 100, 110 and 120°C DHT, respectively. Contrary to this, increasing feed moisture brought a significant reduction in the sensory acceptability from 7.42 at 14% to 6.99 at 18% feed moisture. The inverse relation of overall acceptability with feed moisture was attributed to basically higher expansion with lower bulk density at lower feed moisture.

Overall acceptability = +6.94 + 0.24A + 0.23B - 33C - 0.14AB - 0.11AC - 8.929E - 003BC -0.028A^2 - 0.13B^2 + 0.54C^2

Optimization and validation of independent variables

Numerical optimum extrusion conditions were determined following maximum ER, iron, calcium, antioxidant activity, WSI and overall acceptability with minimum BD keeping the studied extrusion conditions within the range. Optimized conditions with maximum desirability (0.845) using RSM were as 14% feed moisture, 117.07°C DHT and 335.01 rpm SS (Table 3), which were validated by evaluating the quality responses of developed product. Quality responses of validation experiment were found statistically similar ($p>0.05$), which were as 18.26% protein, 73.88% *in vitro* protein digestibility, 2.84% minerals, 4.67 mg/100 g iron and 190 mg/100 g calcium with 8.16 overall acceptability score, thereby indicating the validation of numerical optimization of extrusion variables for development of food-grains and spinach based expanded food.

Protein efficiency ratio (PER) of composite food grains-spinach based extrudates, prepared using validated extrusion conditions was 2.462. It was significantly higher

Table 3: Optimized conditions for development of composite food with food grains-spinach using twin screw extruder.

Particulars	Target	Experimental range		Importance	Optimum value	Desirability
		Minimum	Maximum			
Feed moisture (%)	In range	14	18	3	14.00	
Screw Speed, rpm	In range	300	350	3	335.01	
Die head temperature (°C)	In range	100	120	3	117.07	0.845
Responses					Predicted values	Observed/experimental values
Expansion ratio	maximize	2.53462	3.37222	5	3.37	3.39
Bulk density, kg/m^3	minimize	166.306	384.335	5	147.89	146.09
Antioxidant activity, %	maximize	23.7515	24.9719	1	24.05	24.11
WSI, %	maximize	21.1075	27.9169	5	26.74	26.98
Calcium content, mg/100 g	maximize	180.4	192.0	3	190.45	190.0
Iron content, mg/100 g	maximize	4.60117	4.6973	1	4.64	4.67
Overall sensory acceptability	maximize	6.57	8.31	5	8.08	8.16

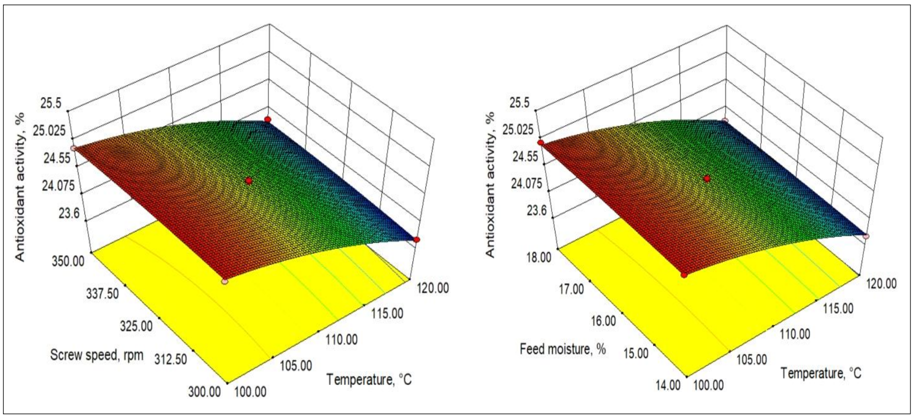
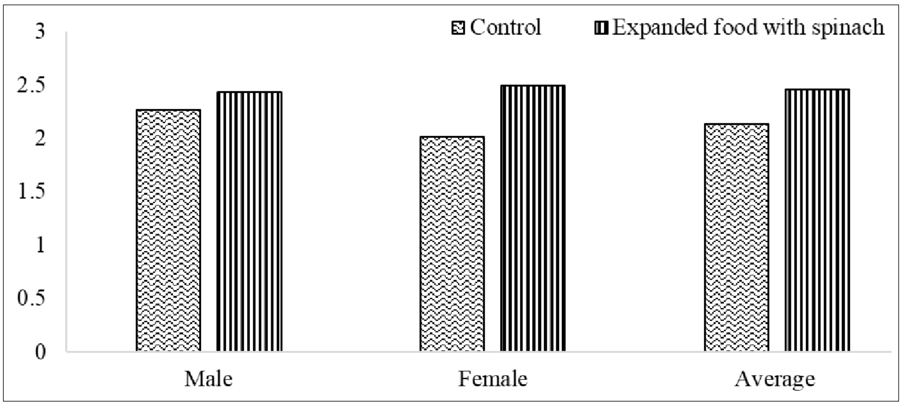


Fig 1: Effect of die head temperature, feed moisture and screw speed on antioxidant activity of composite food with food grains-spinach.



than of control group (Fig 2) ($p<0.001$). PER of male and female rats for this extrudate sample was 2.429 and 2.494, respectively. The better protein quality of the developed extrudates in terms of higher PER than the control sample explicated that product is good enough to consider for improving the nutritional status.

Consumer acceptability of extrudate developed using standardized conditions showed a very good overall sensory acceptability with only two people (*i.e.*, 1.87%) scored as <6 at nine point hedonic scale. Out of 107 people, who had evaluated the sample, 92.52% (*i.e.*, 99 people) scored the developed product with ≥7 while 5.61% (*i.e.*, 6 people) between 6-6.99 overall mean sensory score at nine point hedonic scale. This showed that the developed composite food grains-spinach based expanded food has enormous market potential and can be adopted for commercial production.

CONCLUSION

Quality characteristics of developed composite food using twin screw extruder were affected by variability in independent studied parameters. Increasing feed moisture reduced expansion ratio with higher bulk density at lower DHT. WAI was affected only with DHT. However, showing no impact of SS and feed moisture while WSI was influenced by all three studied extrusion variables. Though proximate composition was not much affected but TPC, TFC and antioxidant activity reduced with increasing DHT. The extrudate can be developed using 117°C DHT, 335 rpm SS and 14% moisture with maize (74%), DSM (15%), sesame (5%) and dried spinach (6%) based formulation. Total carbohydrates, crude fibre, fat, minerals, iron, calcium and antioxidant activity in this expanded food were 67.02%, 2.60%, 4.59%, 2.84%, 4.67 mg/100 g, 190 mg/100 g and 24.05%, respectively indicating consumer acceptability ≥7 by 92.52%. Calorific value was 382 kcal/100 g with 18.26% protein, 73.88% *in-vitro* protein digestibility and PER as 2.46 thereby fulfilling >75% protein, >20% calorie, >25% calcium and >30% iron requirement of 7-9 years aged Indian children. This expanded food, with good nutritional characteristics and organoleptic acceptability, has remarkable potential for marketability and improving undernutrition.

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