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Development of Corn-Rice Flour Blend based Extruded Product Enriched with Gamma Oryzanol Concentrate isolated from Rice Bran Oil-An RSM approach

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Abstract—*Gamma oryzanol, one of the principal nutraceutical components of rice bran oil was isolated and oryzanol fortified extruded product of corn and rice flour blend was prepared by substituting this flour blend with oryzanol concentrate at low (1.5g/100g) and high (3.0g/100g) level. A five-level, three factor central composite rotatable design was employed to investigate the effect of three independent variables i.e. barrel temperature(90-110^{0}C), screw speed (270-290 rpm) and feed moisture(17-21% wb) on dependent variables like lateral expansion, bulk density, water absorption index, hardness and colorimetric values(L*, a* and b*). Increased barrel temperature significantly increased the lateral expansion, water absorption index, a* and b* value of the extrudates($p<0.05$). Screw speed was positively related to the bulk density, a* and b* value ($p<0.05$). Feed moisture content had significant positive effect on bulk density, hardness and a* value ($p<0.05$). A numerical optimization technique was used to obtain optimum processing conditions. For low level fortification, the optimized conditions were barrel temperature (110^{0}C), screw speed (289.763rpm), feed moisture (17.00%) and the responses were lateral expansion (115.44%), bulk density (0.28g/cm3), water absorption index (5.21g/g), hardness (15.46N), L*(81.14), a*(-1.042) and b*(22.899). For high level, the optimised processing conditions were barrel temperature (99.52^{0}C), screw speed (270rpm), feed moisture (17%) and responses were lateral expansion (113.85%), bulk density (0.32g/cm3), water absorption index (5.27g/g), hardness (23.00N), L*(82.27), a*(-1.092) and b* (21.48). The retained amount of oryzanol in the optimised samples after extrusion from the two designs was detected by HPLC analysis and it came out to be 1.07 g/100g and 1.55g/100g of oryzanol for low and high level of fortification respectively. The components detected were cycloartenyl feulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate and β-sitosteryl ferulate.*

Keywords*: Oryzanol, fortification, retention.*

1. INTRODUCTION

The term functional foods are considered as innovative and promising products which can provide additional health benefits beyond basic nutrition. There is a growing interest in the development of novel food products supplemented with natural antioxidants which are derived from fruits, vegetables, whole grains, oilseeds as well as their by products. These antioxidants are thought to protect animal tissues from free radical-mediated degenerative diseases and ageing. Out of several natural sources, rice bran contains high levels of several phytochemicals which possess antioxidant activities as well as other reported health beneficial properties (4). Gamma oryzanol is a natural antioxidant extracted from rice bran oil, it contains a mixture of at least ten phytosterol ferulates among which cycloartenyl ferulate, 24-methylene cycloartanyl ferulate and campesteryl ferulate are the prominent ones[17]. Rice bran oil is nutritionally superior oil as compared to other common vegetable oils[8].

Rice bran is one of the richest sources of oryzanol. For physically refined rice bran oil, the oryzanol content ranges from 1.1% to1.74% and for chemically refined oil, it varies from 0.19% to 0.20% [7]. Many extraction solvents have been used for the extraction of gamma oryzanol. In this work, gamma oryzanol concentrate with purity level (20-25%) was isolated from rice bran oil by following the process adopted by Ricela, manufacturers of rice bran oil from A. P. Solvex Pvt. Ltd., Dhuri, Punjab, India with slight modifications. Oryzanol fortified extruded product of corn and rice flour blend was prepared by substituting this flour blend with oryzanol concentrate at low (1.5g/100g) and high (3.0g/100g) level. A five-level, three factor central composite rotatable design was employed to investigate the effect of three independent variables i.e. barrel temperature(90-110^{0}C), screw speed (270-290 rpm) and feed moisture(17-21% wb) on dependent variables like lateral expansion, bulk density, water absorption index, hardness and colorimetric values(L*, a* and b*). A numerical optimization technique was used to obtain optimum processing conditions. The retained amount of oryzanol in the optimised samples after extrusion from the two designs was detected by HPLC analysis and it came out to be 1.07 g/100g and 1.55g/100g of oryzanol for low and high level of fortification respectively. The components detected were cycloartenyl feulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate and β-sitosteryl ferulate.

2. MATERIALS AND METHODS

2.1 Procurement of raw materials and chemicals

Ricela brand rice bran oil from A. P. Solvex Pvt. Ltd., Dhuri, Punjab, India was procured from local market of District Sangrur, Punjab (India). The oryzanol standards were purchased from TCI (Japan). All other reagents were of used were of analytical grade. Corn flour and rice flour were procured from local market of Longowal, Punjab.

2.2. Preparation of flour blend for extrusion

Corn flour and rice flour were selected as base materials for extrusion. Equal proportions of corn and rice flour (50:50) was taken as control. This blend was substituted with oryzanol concentrate at two levels i. e. low (1.5 gms) and high (3.0gms) level. All the blended samples were prepared in 100 gm batch. The moisture content was adjusted by sprinkling calculated amount of distilled water in all the dry flour blends fortified with oryzanol. All the samples after mixing in a food processor with mixer attachment were stored in polyethylene bags at room temperature for 24 hrs. The moisture content of all the samples were further estimated by using hot air oven method [11]. Extrusion was done with a twin screw extruder (Basic Technology Pvt. Ltd. Kolkata, India).

2.3 Experimental Design

Response surface methodology was employed in order to generate the experimental designs, statistical analysis as well as regression model with the help of design Expert Software (version 9.0 by STAT-EASE inc. USA). RSM is commonly used as a major tool in analyzing experimental data resulting in the optimization of processes. The central Composite Rotatable design with a quadratic model [3] was employed. The major advantage of RSM is the reduced number of experimental runs. It was employed to derive the optimum levels of the independent variables by using a three-factor five level Central Composite rotatable design.The independent variables choosen include barrel temperature(90-110^{0}C), screw speed(270-290rpm) feed moisture(17-21%, wb). The five responses selected were lateral expansion, bulk density, water absorption index, hardness, colorimetric values (L*, a*and b*). A total of 20 different combinations including six replicates of center point each signed the coded value (0). The α-values in the design outside the ranges were also selected for the rotatability of the design [15]. Results were the means of three triplicates. A second order polynomial equation was used to express as a function of three independent variables which is cited below:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2$$

where x_1, $x_2$and x_3 are the coded values of barrel temperature, screw speed and feed moisture respectively. The coefficient of the polynomial were represented by b_0 (constant), b_1,b_2 and b_3 are the linear effects, b_{12}, b_{13} and b_{23} are the interactive effects and b_{11}, b_{22} and b_{33} are the quadratic effects. The anova tables were generated and the effect and regression coefficients of individual linear, quadratic and interactive terms were determined.

2.4 Physical Properties of the exrudates

2.4.1. Lateral expansion: It was calculated as the ratio of the diameter of extrudates and the diameter of die[2]. Six lengths of extrudates were selected randomly. The diameter of the extrudates was then measured at ten different positions along the length of each of the six samples using a vernier caliper. Lateral expansion was then calculated using the mean of the measured diameter:

LE (%) = diameter of product-diameter of die/diameter of die×100

2.4.2. Bulk Density: The bulk density of the extrudates was calculated by measuring the actual dimensions of the extrudates[16]. A digital vernier caliper (model CD-12" C, Mitutoyo corp. Japan) with least count of 0.1mm was used to measure the diameter and length of the extrudates. Ten extrudates were randomly selected and their average was taken

BD (g/cm³) = $4m/\pi d^2L$;

where 'm' is the mass(g) of the extrudate, 'd' is the diameter of the extrudate(cm) and 'L' is the length of the extrudate(cm).

2.4.3. Water Absorpion Index: Water absorption index (WAI) for the extruded samples was determined by using the method of [2] with some modiications. The extrudates were grounded and then passed through a sieve. 2.5 gm of ground extrudate was dispersed in 25 g of distilled water followed by stirring with a glass rod for 30 minutes at room temperature. It was then rinsed into centrifuge tubes and was made upto 32.5 g with distilled water. Centrifugation was done at 4000 rpm for 5 minutes. The supernatant was decanted into an evaporating dish with an evaporating dish with a known weight. Water absorption index was then computed by using the following formulae:

WAI (g/g) = Weight of sediment/Weight of sample

2.4.4. Hardness: Hardness of the extruded sample was determined by using a TA-XT2 Texture Analyser(Serial No. 4650, Stable Micro system, USA). Each extrudate(10 cm long) was subjected to compression with a probe three point bend ring with a target mode distance of 3 mm and load cell of 50 kg. A three point bend ring with a pre-test speed of 1.00

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mm/sec, test speed of 2 mm/sec and post test speed of 10mm/sec was used. Mode distance was kept 3mm. 5 Kg load cell was used. Analysis of each sample was performed in triplicates. The compression generates a curve with the force over distance.

2.4.5. Colorimetric values (L*, a* and b*): Colour properties of the extrudates was analyzed by hand held calorimeter (CR-400, Konica Minolta, Chroma meter, Japan) on the basis of L^*, a^* and b^* values where the L^* value indicates the lightness, its value range from 0 to 100, a^* value gives the degree of the red and green colour, with a higher positive a* value indicating more red. The b^* value indicates the degree of the yellow and blue colour, with a higher positive b^* value indicating more yellow in color.

3. RESULTS AND DISCUSSION

3.1. Effect of process variables on the lateral expansion of extruded products

Expansion is the most important physical property of a snack food. The lateral expansion measured for all experimental samples ranged from 107.64% to 117.13% for oryzanol fortification at low level (Table 1) where lateral expansion for all experimental samples for fortification at high level ranged from 106.55% to 114.65 %(Table 2). The quadratic model obtained from regression analysis for lateral expansion in terms of coded level of variables is as under:

$$LE=111.59+1.99x_1-1.42x_2-1.74x_3-0.08x_1x_2-0.95x_1x_3-0.49x_2x_3+0.42x_1^2-0.15x_2^2-0.11x_3^2$$

$$LE=110.73+1.48x_1-0.81x_2-1.85x_3+0.40x_1x_2-0.0.051x_1x_3+0.82x_2x_3-0.043x_1^2-0.25x_2^2-0.065x_3^2$$

The above quadratic equations shows that lateral expansion increased significantly ($p<0.05$) with barrel temperature which is well in line with the finding of [12]. Expansion decreased with the screw speed which is similar to the findings of [12]. Feed moisture also had negative effect which is similar to the result reported by [1, 6].

3.2. Bulk Density

It is a major physical property of the extruded product. A high bulk density is associated with a low expansion index [14]. The bulk density ranged from 0.27 to 0.37 (g/cm^3) and 0.32 to 0.41(g/cm^3) for low and high level oryzanol fortification. The quadratic model obtained from regression analysis for bulk density of two designs in terms of coded levels of the variables is as under:

$$BD=0.33-0.019x_1+0.018x_2+0.012x_3-0.0037x_1x_2-+0.0062x_1x_3+0.0062x_2x_3-0.008x_1^2-0.0045x_2^2-0.0045x_3^2$$

$$BD=0.34-0.014x_1+0.014x_2+0.016x_3-0.005x_1x_2-0.0025x_1x_3+0.0009x_2x_3+0.0049x_1^2+0.0031x_2^2-0.00036x_3^2$$

Bulk density was negatively affected by the linear effects of barrel temperature ($p<0.05$) which is similar to the findings of [1]. Screw speed had positive significant effect ($p<0.05$) of bulk densiy which is well in lining with [12]. Similar effect of feed moisture was reported by [5].

3.3. Water Absorption Index

Water absorption index measures the amount of index absorbed by starch that can be used as an index of gelatinization [2]. WAI measured for all the experimental designs ranged from 5.05 to 5.77 for fortification at low level while for high level fortification it ranged from 5.05 to 5.76. The quadratic model obtained from regression analysis for water absorption index in terms of coded levels of the variables is as under:

$$WAI=5.36+0.087x_1-0.093x_2-0.084x_3-0.16x_1x_2-0.0062x_1x_3+0.026x_2x_3-0.039x_1^2-0.0016x_2^2-0.010x_3^2$$

$$WAI=5.16+0.15x_1-0.092x_2-0.107x_3+0.028x_1x_2+0.02375x_1x_3+0.066x_2x_3+0.106x_1^2+0.027x_2^2+0.037x_3^2$$

From the above equations, WAI increased with the increase in temperature (x_1).At higher temperature, starch granule is disrupted and more water is bound to the starch molecule resulting in increase in WAI. This is well in line with findings of Shan et al., 2015. Screw speed and feed moisture had significant negative effect ($p<0.05$).

3.4. Hardness

It is the average force required for a probe to penetrate the extrudate. The hardness measured for all experimental samples ranged from 15.69N to 22.35N(Table 1) and 18.74N to 24.99N for two designs respectively. The quadratic model obtained from regression analysis for lateral expansion in terms of coded level of variables is as under:

$$Hardness=18.75-1.23x_1-0.80x_2+0.93x_3-0.60x_1x_2-0.40x_1x_3+0.77x_2x_3+0.35x_1^2-0.17x_2^2+0.40x_3^2$$

$$Hardness=22.98-1.41x_1-1.14x_2+0.78x_3-0.56x_1x_2+0.019x_1x_3-0.15x_2x_3-0.48x_1^2-0.17x_2^2-0.069x_3^2$$

Hardness decreased significantly with the increase in barreltemperature, well in line with [13] and screw speed. It increased with increasing feed moisture ($p<0.05$) similar to the findings of [10].

3.5. L*= The higher L* value indicated greater lightness of extrudates. The L* value measured for all experimental samples ranged from 79.64 to 84.11 and 77.42 to 84.21 for two designs respectively. The quadratic model obtained from regression analysis for L*value for two designs in terms of coded level of variables is as under:

$$L^*=81.74-0.92x_1-0.88x_2-0.75x_3-0.20x_1x_2+0.041x_1x_3+0.24x_2x_3+0.16x_1^2-0.071x_2^2-0.028x_3^2$$

$$L^*=80.10-1.22x_1-1.28x_2-1.19x_3+0.36x_1x_2-0.41x_1x_3+0.085x_2x_3+0.36x_1^2+0.35x_2^2-0.15x_3^2$$

Table 1 The effect of independent variables on the response functions for developing gamma oryzanol (at low level) fortified extruded product

Run	Type	BT (°C) X1(x1)	Moisture Content (%, wb) X2(×2)	Screw Speed (rpm) X3(x3)	LE (%)	BD (g/cm³)	WAI	Hardness (N)	L*	a*	b*
1	Factorial	90(-1)	21(+1)	270(-1)	110.65	0.31	5.05	21.03	82.65	-0.98	20.71
2	Center	100(0)	19(0)	280(0)	111.76	0.34	5.37	18.76	81.69	-1.02	22.26
3	Factorial	110(+1)	17(-1)	290(+1)	115.75	0.29	5.22	15.69	79.98	-0.98	22.74
4	Factorial	110(+1)	21(+1)	290(+1)	109.57	0.34	5.10	17.82	79.64	-0.95	22.16
5	Center	100(0)	19(0)	280(0)	110.95	0.33	5.35	18.52	81.57	-1.05	22.32
6	Factorial	110(+1)	21(+1)	270(-1)	113.52	0.29	5.52	19.34	80.98	-0.98	22.07
7	Center	100(0)	19(0)	280(0)	111.65	0.34	5.35	18.59	81.67	-1.06	22.15
8	Center	100(0)	19(0)	280(0)	112	0.33	5.36	18.8	81.63	-1.05	22.2
9	Center	100(0)	19(0)	280(0)	111.2	0.34	5.39	18.76	81.94	-1.04	22.05
10	Axial	100(0)	22.36(+α)	280(0)	107.65	0.35	5.18	21.76	80.12	-0.95	21.45
11	Axial	116.81(+α)	19(0)	280(0)	115.63	0.28	5.39	17.52	80.65	-0.97	23.38
12	Factorial	90(-1)	17(-1)	290(+1)	109.41	0.35	5.38	18.17	82.63	-1.09	22.26
13	Axial	100(0)	19(0)	263.18(-α)	113.82	0.30	5.53	19.57	83.06	-1.12	21.26
14	Factorial	110(+1)	17(-1)	270(+1)	117.13	0.27	5.77	19.86	82.95	-1.18	22.10
15	Factorial	90(-1)	21(+1)	290(+1)	107.64	0.37	5.26	22.35	81.45	-0.97	21.85
16	Factorial	90(-1)	17(-1)	270(-1)	111.09	0.31	5.25	20.38	84.11	-1.25	20.69
17	Center	100(0)	19(0)	280(0)	112.00	0.33	5.34	19.07	81.95	-1.04	22.05
18	Axial	83.18(-α)	19(0)	280(0)	109.65	0.35	5.08	21.99	83.79	-1.07	21.07
19	Axial	100(0)	15.63(-α)	280(0)	114.61	0.30	5.45	18.02	83.23	-1.24	21.76
20	Axial	100(0)	19(0)	296.81(+α)	108.23	0.35	5.15	16.98	80.05	-0.95	22.98

Table 2 The effect of independent variables on the response functions for developing gamma oryzanol (at high level) fortified extruded product

Run	Type	BT (°C) X1(x1)	Moisture Content (%, wb) X2(×2)	Screw Speed (rpm) X3(x3)	LE (%)	BD (g/cm³)	WAI	Hardnes s (N)	L*	a*	b*
1	Center	100(0)	19(0)	280(0)	110.49	0.35	5.18	22.79	79.89	-1.11	20.98
2	Factorial	90(-1)	17(-1)	270(-1)	112.88	0.33	5.56	22.96	84.21	-1.35	21.45
3	Axial	100(0)	15.63(-α)	280(0)	113.63	0.32	5.43	21.34	81.70	-1.21	23.98
4	Axial	83.18(-α)	19(0)	280(0)	108.15	0.39	5.15	24.51	82.92	-1.15	21.12
5	Center	100(0)	19(0)	280(0)	110.61	0.34	5.12	23.11	79.87	-1.05	20.87
6	Factorial	110(+1)	17(-1)	290(+1)	112.41	0.34	5.47	18.74	79.91	-0.99	24.45
7	Factorial	110(+1)	21(+1)	290(+1)	110.13	0.37	5.37	19.96	77.42	-0.96	20.34
8	Factorial	90(-1)	21(+1)	290(+1)	106.55	0.41	5.05	23.38	79.65	-0.97	20.21
9	Center	100(0)	19(0)	280(0)	110.65	0.35	5.17	23.04	80.24	-1.09	20.95
10	Axial	100(0)	19(0)	296.81(+α)	108.78	0.38	5.07	20.36	78.62	-0.95	23.45
11	Factorial	90(-1)	21(+1)	270(-1)	107.10	0.37	5.10	24.69	83.03	-1.15	20.23
12	Axial	100(0)	19(0)	263.18(-α)	111.71	0.33	5.39	24.99	83.14	-1.19	20.67
13	Center	100(0)	19(0)	280(0)	110.98	0.35	5.16	23.40	80.56	-1.08	20.13
14	Center	100(0)	19(0)	280(0)	110.62	0.35	5.17	22.72	80.21	-1.07	21.00
15	Axial	116.81(+ α)	19(0)	280(0)	113.49	0.33	5.76	19.04	78.89	-0.95	22.03
16	Factorial	110(+1)	21(+1)	270(-1)	109.35	0.35	5.42	23.15	78.65	-0.97	21.12
17	Axial	100(0)	22.36(+ α)	280(0)	107.89	0.37	5.09	24.56	77.27	-1.05	20.34
18	Factorial	90(-1)	17(-1)	290(+1)	108.76	0.37	5.13	22.59	81.22	-1.02	23.46
19	Factorial	110(+1)	17(-1)	270(-1)	114.65	0.32	5.67	21.70	82.21	-1.15	22.12
20	Center	100(0)	19(0)	280(0)	110.97	0.35	5.20	22.78	79.87	-1.1	20.89

Barrel temperature, screw speed and feed moisture had significant negative effect on L* value ($p<0.05$).

3.6. a*= The a* value measured for all experimental samples ranged from -0.95 to -1.25 and -0.95 to -1.35 for two designs. The quadratic model obtained from regression analysis for L*value for two designs in terms of coded level of variables is as under

a*=-1.04+0.026x_1+0.05x_2+0.081x_3+0.0075x_1x_2-0.02x_1x_3-0.04x_2x_3-0.008x_1^2-0.0035x_2^2-0.017x_3^2

a*=-1.08+0.005x_1+0.079x_2+0.053x_3-0.042x_1x_2-0.005x_1x_3-0.0375x_2x_3+0.014x_1^2+0.0075x_2^2-0.0136x_3^2

Barrel temperature increased a*value significantly ($p<0.05$) similar to study of. Screw speed also enhanced a* value. Feed moisture also had positive significant effect ($p<0.05$).

3.7. b*= The b* value measured for all experimental samples ranged from 20.69 to 22.98 and 20.13 to 23.98 for two designs. The quadratic model obtained from regression analysis for L*value for two designs in terms of coded level of variables is as under

b*=22.18+0.55x_1+0.46x_2-0.11x_3-0.25x_1x_2-0.027x_1x_3-0.12x_2x_3-0.013x_1^2-0.050x_2^2-0.23x_3^2

b*=20.81+0.31x_1+0.60x_2-0.1.15x_3-0.055x_1x_2-0.080x_1x_3-0.64x_2x_3+0.20x_1^2+0.38x_2^2+0.41x_3^2

Screw speed increased significantly b* value ($p<0.05$).

4. OPTIMIZATION

A numerical optimization technique was used to obtain optimum processing conditions. For low level fortification, the optimized conditions were barrel temperature (110^{0}C), screw speed (289.763rpm), feed moisture (17.00%) and the responses were lateral expansion (115.44%), bulk density (0.28g/cm3), water absorption index (5.21g/g), hardness (15.46N), L*(81.14), a*(-1.042) and b*(22.899). For high level, the optimised processing conditions were barrel temperature (99.52^{0}C), screw speed (270rpm), feed moisture (17%) and responses were lateral expansion (113.85%), bulk density (0.32g/cm3), water absorption index (5.27g/g), hardness (23.00N), L*(82.27), a*(-1.092) and b* (21.48). The retained amount of oryzanol in the optimised samples after extrusion from the two designs was detected by HPLC analysis and it came out to be 1.07 g/100g and 1.55g/100g of oryzanol for low and high level of fortification respectively. The components detected were cycloartenyl feulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate and β-sitosteryl ferulate.

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