Development of designer chicken shred with response surface methodology and evaluation of its quality characteristics

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Abstract Meat is considered to be an excellent source of protein, essential minerals, trace elements and vitamins but negative concerns regarding meat consumption and its impact on human health have promoted research into development of novel functional meat products. In the present study Rice bran oil (RBO), and Flaxseed oil (FSO) were used for attaining an ideal lipid profile in the product. The experiment was designed to optimise the RBO and FSO concentration for development of product with ideal lipid profile and maximum acceptability by the application of central composite rotatable design of Response surface methodology (RSM). Levels of RBO and FSO were taken as independent variables and overall acceptability (OAA), n-6 and n-3 fatty acids as responses. Quadratic fit model was found to be suitable for optimising the product. Sample with RBO (20.51 ml) and FSO (2.57 ml) yielded an OAA score of 8.25, 29.54 % of n-6 and 7.70 % of n-3 having n-6/n-3 ratio as 3.8:1. Optimised product was analysed for physico-chemical, sensory and microbial profile during storage at 4±1 °C for 30 days. Increase in the lipid oxidative parameters was observed during storage but it was not significant ($p < 0.05$). Studies revealed great potential of developing functional poultry products with improved nutritional quality and good shelf stability by incorporating RBO and FSO.

Keywords Rice bran oil · Flaxseed oil · Response surface methodology · n-6/n-3

Introduction

In view of the present health scenario, there is a tendency to replace traditional fats like ghee and coconut oil with other healthier options like RBO and olive oils. The key elements responsible for health benefits of RBO include fatty acids, unsaponifiable matter, antioxidants like Oryzanol, Tocopherols, and Tocotrienols. Fatty acid composition of meat has received increasing attention owing to its implications in human health and product quality. Development and quality improvement of processed meat products with proven functional ingredients is an area of interest for meat processors. These products can be developed using designer meat obtained either through pre-slaughter strategies i.e., through feeding or via post slaughter modifications through incorporation of functional ingredients in meat product formulations. There has been an increasing interest in the use of proven functional ingredients with multi-dimensional health benefits in the product formulation in order to improve the nutritional and functional value of meat products (Yogesh et al. 2015). The ratios of PUFA/SFA and n-6/n-3 are widely used to evaluate the nutritional value of fat (Orellana et al. 2009). Modification of the fatty acid profiles of meat and meat products can be achieved by, feeding animal diets rich in polyunsaturated fatty acids (PUFA's), like fish oil, linseed oil and soya oil (Raes et al. 2004) or alternatively, by processing specific lipid ingredients into meat products (Fernández-Ginés et al. 2005). Several studies have highlighted the addition of non-meat ingredients like oat flour, apple peel flour, green banana pulp flour, green banana peel flour and green banana pulp in beef burgers (Bastos et al. 2014). Oils such as linseed oil, fish oil, olive oil, and soya oil alter product fatty acid profiles producing healthier meat products, such as dry fermented sausages (Ansorena and Astiasaran 2004; Muguerza et al. 2004a, b; Valencia et al. 2006).

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Flaxseed has recently gained attention as a functional food due to its unique nutrient profile. The phenolic compounds of interest that are accumulated in flaxseed include ferulic and vanillic acid (Siger et al. 2008). The high content of n-3 fatty acid present in this oil allows the attribution of functional food, (Vaisey-Genser and Morris 2003). Several studies revealed the positive effects of omega fatty acids on human health (Yalçın and Unal 2010). Pelser et al. (2007) studied the effect of addition of flaxseed oil, canola oil, encapsulated fish oil and encapsulated flaxseed oil in Dutch style fermented sausages and suggested possibility of replacing part of the animal back fat by health promoting oils giving fermented sausages a healthy image. RSM technique gives the effect of an individual parameter as well as interactive effect (Yadav et al. 2007). This statistical tool has been employed for the standardisation and optimizing processing variables (Madamba 2002) and also for product development (Jalarama Reddy et al. 2013a, b).

At present scientists are showing much concern in improving the nutritional quality of meat foods through the manipulation of their fatty acid profiles through nutritional strategies. This present investigation was undertaken to develop poultry product with ideal lipid profile and evaluation of its stability during storage. RBO and FSO in meat and poultry products increase its value and contribute to the development of newer functional products with ideal fatty acid profile.

Materials and methods

Raw materials

Fresh chicken (leg portion) was purchased from local market, Mysore, India within 1–2 h after slaughter and allowed rigor mortis to set in. The portion of meat were cut into uniform size pieces and washed thoroughly with water to remove foreign particles adhered to the surface of pieces and trimmed of all external fat. The Rice bran oil used in present investigation was obtained from M/S. Habib Agro Industries, Mandya, Karnataka, India and Flaxseed oil was obtained from M/S. SSS Industries, Bangalore, India.

Chemicals and reagents

All the reagents and chemicals used for the study were of Analar grade and procured from M/S Sigma Chemicals, Corporation, USA and M/S BDH Company. The standard fatty acid methyl esters used in the estimation of fatty acids by Gas chromatography and the BF₃ - CH₃OH used in the esterification were obtained from M/S. Sigma Chemicals Corporation, USA.

Preparation of designer chicken shreds

RBO and FSO in the ranges of 18–22 & 1–4 ml were added into 100 g of chicken mince along with other ingredients to develop designer chicken shred product. Process flow chart for the preparation of chicken shreds with incorporation of rice bran oil and Flaxseed oil is given below.

Process flow chart for the preparation of designer chicken shreds

Experimental design

The central composite rotatable design (CCRD) of RSM was used for designing the experimental combinations for the development of designer chicken shred with ideal lipid profile (n-6/n-3 as 4:1) and sensory attributes using software State-Ease (Design Expert version 6.0.10). RBO and FSO levels were taken as independent variables and overall acceptability, n-6 and n-3 fatty acids as responses. The number of design points was obtained with the help of statistical software depending on the number of independent variables. Design consisted of four factorial points, five central points and four axial points leading to 13 sets of experiments (Myers and Montgomery 2002). The levels of both the independent variables were taken considering the amount of oil required for

the development of chicken shred by considering the original fatty acid composition in the raw materials. Design of experiments for the development of designer poultry product is shown in Table 1. The results for the central composite designs were used to fit second-order polynomial equation. The regression analysis of the responses namely OAA, n-6 and n-3 were carried out by fitting with suitable models represented by (1) & (2). All variables of the polynomial regression at a significance level of $p < 0.05$ were included in the model, and the coefficient of determination (R^2) was generated in order to assess the accuracy of the model. The response surfaces were generated from the equation of the second order polynomial, using the values of each independent variable to the maximum quadratic response (Sin et al. 2006).

First order Linear Equation (1)

$$Y = \beta_0 + \sum_{i=1}^n \beta_i x_i$$

Second-order polynomial Equation (2)

$$Y = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^n \beta_{ii} x_i^2 + \sum_{i \neq j=1}^n \beta_{ij} x_i x_j$$

where, 0 was the value of the fitted response at the center point of the design, i.e., point (0,0,0) in case of RBO- FSO; i , ii and

ij were the linear, quadratic and cross product (interaction effect) regression terms respectively and n denoted the number of independent variables.

Proximate composition

Proximate composition for the designer chicken shreds was established as per AOAC methods (2000) for moisture, protein, fat, carbohydrate (by difference method) and total ash.

Determination of hunter color values

Color values of RTE chicken shreds samples were determined using Hunter colorimeter (Color Flex, CFLX-45-2, Hunter lab, Reston, VA, USA) in terms of L^* , a^* and b^* values as per the procedure given by Shand (2000). Measurement of the color co-ordinates were determined using a D-65 illuminant with a spectral range of 400–700 nm and a spectral resolution of 10 nm after standardizing the sensor using standard black and white colored tiles. CIE L^* (lightness), a^* (redness), b^* (yellowness) values were recorded. The average of three measurements was taken for each sample.

Estimation of oxidative and hydrolytic rancidity parameters

Thiobarbituric acid reactive substances (TBARS) values were measured for chicken shred samples as an index of lipid oxidation according to the method of Taraldis et al. (1960). The TBARS number is expressed as mg of malondialdehyde (MDA) per kg of sample. Free fatty acids (FFA) for the samples were determined as per the standard procedure AOCS (1993).

Total fatty acid profile of samples by gas chromatographic method

Fatty acid composition of the oil samples were determined by Gas chromatography as fatty acid methyl esters using standard esters of fatty acids.

Esterification of fatty acids

The oil samples were esterified as per the procedure (Metcalf et al. 1966) with slight modifications. About 150 mg of lipid was accurately weighed into a clean and dry stoppered test tube. Four millilitres of 0.5 N alcoholic sodium hydroxide (O) solutions was added and heated for 5 min over a water bath at 90 °C. On cooling, 5 ml of boron tri-fluoride methanol ($\text{BF}_3 \cdot \text{CH}_3\text{OH}$) reagent was added and heated for 5 min at 90 °C over a water bath, followed by addition of 10 ml of saturated sodium chloride (NaCl) solution. The samples were thoroughly cooled to room temperature and 5 ml hexane

Table 1 Design of experiment for the development of designer chicken shreds

Run order ^a	Variables		Responses		
	RBO (ml) ^b	FSO (ml) ^c	OAA ^d	n-6 ^e	n-3 ^f
1	20	2.5	8.32	29.52	7.71
2	18	1	7.2	30.1	4.85
3	20	2.5	8.1	29.41	7.68
4	22	4	7.5	29	9.91
5	22.83	2.5	7.8	29.96	7.18
6	20	4.62	7.1	28.38	11.48
7	20	2.5	8.2	29.43	7.72
8	18	4	7.2	28.25	11.1
9	20	0.38	7.4	30.87	3.25
10	20	2.5	8.2	29.56	7.62
11	22	1	7.4	30.74	4.45
12	20	2.5	8.33	29.55	7.7
13	17.17	2.5	6.8	28.93	8.37

^a Run Order

^b Rice bran oil (ml)

^c Flaxseed oil (ml)

^d 9 point hedonic scale

^e omega 6 fatty acids

^f omega 3 fatty acids

(C₆H₁₄) was added to each tube. It was shaken well and kept undisturbed. The upper hexane layer was drawn out into clean dry conical flask and dried over anhydrous sodium sulphate (Na₂SO₄) to remove the traces of moisture if present. The samples were filtered and transferred to stoppered clean dry tubes for gas chromatographic analysis.

Total fatty acid analysis by gas chromatography

Analysis of total fatty acids was carried out by a Ceres-800, Chemito model gas chromatograph fitted with BPX 70 column (25 m, 0.32 mm ID) and flame ionization detector. Temperature gradient programming was employed from 150 to 220 °C. Split ratio was adjusted to 1:25 and capillary flow of carrier gas to 2.0 ml/min. Injector and detector port temperatures were adjusted as 230 and 240 °C respectively. For FID, hydrogen and oxygen were used and the flow was adjusted as

45 and 450 ml/min respectively. Along with samples, standard esters of fatty acids (Sigma chemical company, St. Louis, USA.) were also injected and the fatty acids were detected by comparing the retention time of the standard esters of fatty acids. The quantification of the fatty acids was carried out by evaluating with the standard fatty acid esters area corresponding to each peak in the chromatogram. Iris-32 software was used to integrate and evaluate the chromatogram in the analysis.

Sensory evaluation

Sensory evaluation of chicken shred samples were carried out by 13 semi-trained panellists on a 9 point hedonic scale (9-like extremely, 1- dislike extremely) as per Murray et al. (2001) to establish the overall acceptability of the product.

Fig. 1 **a** Perturbation graph of two independent variables RBO and FSO for desirability **b** 3D plot depicting effect of independent variables RBO and FSO on Desirability

Design-Expert® Software

Desirability

Actual Factors
A: RBO = 20.00
B: FSO = 2.50

(a)

Design-Expert® Software

Desirability

X1 = A: RBO
X2 = B: FSO

(b)

Microbiological analysis

Ten grams of samples were homogenized with 90 ml of sterile normal saline (0.85 %) for 1 min. The homogenized sample was serially diluted using 9 ml sterile saline (0.85 %) and 0.1 ml aliquot of the appropriate dilutions was inoculated on plate count agar. Plates were incubated for 48 h at 37 °C (APHA 1992). Average counts from triplicates were calculated and expressed as colony forming units per gram (cfu g⁻¹) of the sample.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) and Duncan's multiple range test to evaluate the

statistical significance of the treatments and significance was established at $p < 0.05$. Response surfaces were generated using the Design Expert version 6.0.10 software (Stat Ease Inc., Minneapolis, MN).

Results and discussion

Optimisation of the product

Designer chicken shred was developed using CCRD with minimum possible number of points. Experimental design with variables (RBO & FSO) and responses (OAA, n-6 & n-3) were shown in Table 1. Addition of edible oils like RBO

Fig. 2 **a** Perturbation graph of two independent variables RBO and FSO for sensory scores (OAA) **b** 3D plot depicting effect of independent variables RBO and FSO for sensory scores (OAA)

Design-Expert® Software

OAA
● OAA

Actual Factors
A: RBO = 20.00
B: FSO = 2.50

(a)

Design-Expert® Software

OAA
8.33
6.8

X1 = A: RBO
X2 = B: FSO

(b)

and FSO improves the lipid quality in the meat products in terms of percentages of n-6 and n-3 of fatty acids. Assessment of levels of n-6 and n-3 PUFA is important in meat quality evaluation (Gebauer et al. 2006). Central composite design results were used to fit the second order polynomial equation. However, the regression analysis of all the three responses such as OAA, n-6 and n-3 levels were conducted by fitting the suitable model.

The effect of variations in the levels of independent variables (RBO & FSO) in the present design on three responses (OAA, n-6 & n-3) has been depicted in Perturbation graphs and 3D response plots in Figs. 1, 2, 3 and 4. Figure 1a reveals that the desirability score for the chicken shreds was more affected by the levels of RBO followed by the FSO and 3D response plot for desirability

of the product was shown in Fig. 1b. Perturbation and 3 D response plots for sensory scores in terms of OAA was depicted in Fig. 2a and b respectively, from these Figs it could be observed that levels of RBO has more impact followed by FSO on the OAA of the product. Perturbation and 3 D response plots for both percentages of n-6 and n-3 fatty acids was depicted in Figs. 3a and b and 4a, b respectively and these Figs showed that levels of n-6 and n-3 fatty acids in chicken shred was more affected by the addition of FSO levels than compared to RBO addition. A proportionate increase was observed in percentages of n-3 fatty acids with the addition of higher levels of FSO. Several studies mentioned about role and importance of FSO to increase omega-3 (n-3) fatty acid content in beef (Barton et al. 2007; Juárez et al. 2011).

Fig. 3 a Perturbation graph of two independent variables RBO and FSO for n-6 fatty acids b 3D plot depicting effect of independent variables RBO and FSO for n-6 fatty acids

Design-Expert® Software

n-6
● n-6
Actual Factors
A: RBO = 20.00
B: FSO = 2.50

(a)

Design-Expert® Software

n-6
30.87
28.25
X1 = A: RBO
X2 = B: FSO

(b)

Analysis of variance was calculated and model statistics for all the responses were shown in Table 2. In designer chicken shred product, quadratic model was well suitable and selected for the OAA ($p < 0.0003$), n-6 ($p < 0.0001$) and n-3 ($p < 0.0003$). All the responses showed highly significant and fitted with quadratic model. The p-value showed as $p < F$ which should be always less than 0.05 for model to significant.

Multiple regression equations (in terms of coded factors) generated for all three responses are represented as follows:

Sensory score of designer chicken shreds (OAA)

$$\text{OAA}(Y) = +8.23 + 0.24 * A - 0.041 * B \\ + 0.025 * A * B - 0.45 * A^2 - 0.48 * B^2$$

Omega 6 fatty acids percentages in designer chicken shreds (n-6)

$$\text{n-6}(Y) = +29.49 + 0.36 * A - 0.89 * B \\ + 0.028 * A * B - 0.028 * A^2 + 0.062 * B^2$$

Omega 3 fatty acids percentages in designer chicken shreds (n-3)

$$\text{n-3}(Y) = +7.69 - 0.41 * A + 2.92 * B - 0.20 * A * B \\ + 0.046 * A^2 - 0.16 * B^2$$

Responses were optimized using Design Expert version-6 software. Optimisation of the independent

Fig. 4 a Perturbation graph of two independent variables RBO and FSO for n-3 fatty acids b 3D plot depicting effect of independent variables RBO and FSO for n-3 fatty acids

Table 2 ANOVA and model statistics for the development of designer chicken shred with RBO and FSO

Term model	Responses		
	OAA	n-6	n-3
<i>F</i> value	24.32	496.87	12010.12
<i>P</i> > <i>F</i>	<0.0003	<0.0001	<0.003
Mean	7.66	29.52	7.62
S.D. ^a	0.16	0.054	0.034
C V %	2.10	0.18	0.45
<i>R</i> squared	0.9456	0.9972	0.9999
Adjusted <i>R</i> squared	0.9067	0.9952	0.9988
Predicted <i>R</i> squared	0.6744	0.9945	0.9997
Adequate precision	11.393	70.77	356.28
Model	Quadratic	Quadratic	Quadratic

^a Standard deviation

variable levels (RBO & FSO) was achieved based on the maximisation of the sensory scores (OAA), n-6 fatty acids (lower to higher level), and n-3 fatty acids with targeted values (7.70). From this design best among the suitable desirability was taken as optimised ingredients level. Predicted values resulting from optimised design were as follows with variable factors of RBO (20.51 ml), FSO (2.57 ml), and responses of OAA (8.25), n-6 (29.544), and n-3 (7.709). Designer chicken shred was developed with optimised levels of variables and responses were analysed and verified with the predicted values. Actual values for optimised design as follows of RBO (20.50 ml), FSO (2.50 ml), and responses of OAA (8.20), n-6 (29.00), and n-3 (7.59). It showed that the values of predicted and actual were almost similar consequently, the above levels of ingredients were recommended for the optimization of the product.

Optimised product was subjected for further quality evaluation studies to assess the shelf stability of the product with the incorporation of RBO and FSO.

Proximate composition

Proximate composition of designer chicken shred product reveals that the product is a good source of quality protein and fat with a percentages 16.51 ± 0.61 and 15.02 ± 0.44 respectively. This product is having 66.2 ± 1.02 % moisture, and lesser amounts of total carbohydrates (1.25 ± 0.15 %) providing 211 kcal per 100 g of the product.

Evaluation of lipid oxidative parameters

Lipid peroxidation is one of the primary causes of quality deterioration in meat and meat products, and generates compounds that may be detrimental to human health. TBARS and FFA values can be taken as one of the important chemical parameters for the evaluation of the oxidative profile of meat products. Meat oxidation is an important chemical reaction which influences the quality by affecting flavor, color, texture and nutritive value (Mercier et al. 2004). Designer chicken shred product was analysed for lipid oxidation in terms of oxidative (TBARS) and hydrolytic (FFA) rancidity parameters during refrigerated storage. TBARS and FFA values of the product were depicted in Fig. 5. Results from the study revealed that there was no significant ($p > 0.05$) increase in these oxidative parameters up to 25 days while significant ($p < 0.05$) increase was observed after 25 days of refrigerated storage. Presence of natural antioxidants in RBO controls the lipid oxidation in the product. These results are in agreement with the earlier studies conducted by Jalarama Reddy et al. (2013a,

Fig. 5 Oxidative (TBARS) and hydrolytic (FFA) rancidity parameters of chicken shreds during storage

Table 3 Physico-chemical, sensory and microbial analysis of designer chicken shreds during refrigerated storage

Parameters	Storage period (Days)						
	0	5	10	15	20	25	30
<i>L*</i>	43.13±1.05 ^b	43.45±0.84 ^{ab}	44.22±0.88 ^{ab}	44.98±0.71 ^{ab}	45.10±0.93 ^{ab}	45.37±0.47 ^{ab}	46.12±0.78 ^a
<i>a*</i>	16.66±0.24 ^a	16.52±0.11 ^a	16.03±0.31 ^a	15.64±0.26 ^a	15.21±0.04 ^a	14.67±0.28 ^a	13.85±0.30 ^a
<i>b*</i>	46.31±1.20 ^a	46.21±1.01 ^a	45.88±0.95 ^{ab}	45.60±0.87 ^{ab}	44.89±1.06 ^{ab}	44.52±0.59 ^{ab}	43.42±0.64 ^b
<i>TPC</i>	3.8×10 ¹	5.2×10 ¹	1.2×10 ²	2.4×10 ²	4.8×10 ²	5.5×10 ²	2.3×10 ³
<i>Y & M</i>	1.2×10 ¹	Nil	Nil	Nil	Nil	Nil	Nil
<i>Coliforms</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve
<i>OAA</i>	8.20±0.25 ^a	8.16±0.13 ^a	8.01±0.30 ^a	7.83±0.29 ^a	7.50±0.16 ^a	7.27±0.14 ^a	7.04±0.19 ^b

All values are means ± standard deviation. Different lowercase letters (a-b) in the same column indicate significant difference ($p < 0.05$)

b) on the stability of RBO and FSO blends during 3 months of storage.

Color changes in chicken shred during refrigerated storage

Color is a sensory property with a strong influence on food acceptance as it contributes decisively to the initial perception that one can acquire of the condition, degree of processing, and other characteristics of foods (Alós et al. 2006). Changes in hunter color values with respect to lightness (L^*), redness (a^*) and yellowness (b^*) of the product was determined at 5 days of interval for 30 days at refrigerated storage and results are represented in Table 3. During refrigerated storage L^* and b^* values tend to decrease whereas an increase was observed in a^* values. Dark color of RBO and oxidation of myoglobin pigment leads to the increase in the redness (Mantzioris et al. 1994). No significant changes in color attributes were observed during refrigerated storage. Apart from microbial spoilage, oxidation of meat lipids and myoglobin during storage and display at retail leads to the development of off-flavors and the deterioration of the appealing color (Gray et al. 1996).

The designer product developed was subjected for TPC, Yeast and Mould and Coliforms to establish the microbial safety during storage and the product exhibited excellent microbial profile. From Table 3, it could be seen that the TPC count increased only by 2 log after 30 days and these values are well within the acceptable limit for RTE meat products i.e., 10⁴ cfu/g (<http://www.foodauthority.nsw.gov.au/> (2009)).

The product exhibited good organoleptic attributes as reflected in Table 3. Even though the initial OAA score of 8.20±0.25 did have a reduction after storage, it was not significantly different ($p > 0.05$). The product was found to be acceptable till 30 days of storage in terms of acceptable quality attributes as reflected in Table 3. Incorporation of antioxidant

rich rice bran oil for the development of the product might have contributed for the extension of shelf life up to 30 days (Orthoefer 2005). Other workers have studied the physico-chemical effect of incorporating chicken fat in the development of RTE chicken nuggets and reported to have beneficial effects in the quality attributes of the product (Yogesh et al. 2013).

Conclusion

Designer and restructured meat and poultry products are inviting lot of importance in the present scenario of meat industry due to its wider applicability to meet functional and other healthy attributes. Feasibility of employing RSM in designing the variables w.r.t responses to achieve a functional poultry product was established. Quadratic fit model was found to be suitable for optimising the product. Optimized levels of RBO and FSO yielded a product with a n-6/n-3 ratio as 3.8:1 which is nearer to the ideal ratio of 4:1. Incorporation of RBO not only influenced in the lipid profile but also contributed towards the extension of shelf life of the product due to the presence of natural antioxidants. This type of designer poultry products will have greater applicability amongst civilian and service sector due to its ideal lipid profile and functional attributes.

Conflicts of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing the article.

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