



Development of dietary fibre incorporated tuna sausage employing response surface methodology and quality evaluation during chilled storage using multivariate control charts

C. G. JOSHY, K. S. ASWATHY, A. A. ZYNUDHEEN, S. S. GREESHMA, GEORGE NINAN
AND C. N. RAVISHANKAR

ICAR-Central Institute of Fisheries Technology, Matsyapuri P. O., Willington Island, Kochi - 682 029, Kerala, India
e-mail: cgjoshy@gmail.com

ABSTRACT

In the present study, dietary fibre incorporated functional sausage was prepared by optimising the ingredients viz., tuna mince, wheat and oats dietary fibre using response surface methodology. D-optimal mixture response surface experimental design with 13 runs was formulated for the development of dietary fibre incorporated tuna sausage. The optimum combinations of sausage were found to be 62.5% fish mince, 2.5% wheat and 5% oats fibre and 66.8% fish mince and 1.6% of oats and wheat fibre each. The quality evaluation of combination sausage prepared at optimum conditions along with control sample was carried out based on biochemical, physical and sensory parameters during storage in chilled condition at 2°C. Storage stability studies of the optimised sausage samples indicated a shelf life of 14 days under chilled conditions. Multivariate statistical control charts based on T^2 and PSE statistics were developed to monitor quality variations during storage.

Keywords: Dietary fibre, Multivariate statistical profile, Response surface methodology, T^2 and PSE statistics, Tuna sausage

Introduction

Fish sausage is a highly nutritive food product in human diet as it contains proteins, vitamins, minerals, carbohydrates, fat and healthy omega-3 polyunsaturated fatty acids in good quantity compared to other meat based sausages (Ozpolat and Patir, 2016). Fish sausage prepared from the mixed mince of underutilised and fatty fishes would be a healthy option. The major ingredients in fish sausage include fish mince, starch, fat and spices. The ingredients are properly mixed, stuffed in casings, cooked and cooled to get fish sausage of desirable acceptance (Nithin *et al.*, 2015). Even though, fish sausages have superior health benefits, consumers opt to go for healthier options by way of fortification of the existing product with new ingredients.

Dietary fibre is a plant derivative, which contains non-starch polysaccharides, cellulose, resistant starch, resistant dextrins, inulin, lignins, chitins, pectins, beta-glucans and oligosaccharides (Turner and Lupton, 2011). The presence of these components guarantees dietary fibre to be nutritionally significant ensuring health benefits. Several researchers (Anderson, 2003; Anderson *et al.*, 2009; Lattimer and Haub 2010; Li and Komarek, 2017) have reported in detail the nutrient and health benefits of dietary fibre intake and its applications in food. Fish sausages do not contain any dietary fibre and therefore the incorporation of the same in fishery products improves

the functionality of food products viz., water binding and emulsion capacity, thickening, gelling and other physiological properties (Yang *et al.*, 2017). There have been very limited studies on dietary fibre incorporated fish sausages especially ingredient optimisation studies using statistical methods.

Response surface methodology (RSM) is regarded as a reliable tool for the development of process/product by formulating experimental designs, developing statistical models and optimising the process conditions (Myers and Montgomery, 2007). Daley *et al.* (1978) used mullet mince to develop a sausage type product by response surface methodology. Cardoso *et al.* (2008a, b) developed healthy low fat fish sausage from hake mince containing dietary fibre by replacing pork meat with Swelite (a dietary fibre obtained from inner pea) and Fibruline (a dietary fibre obtained from chicory root) and studied the quality changes of fish sausage containing dietary fibre along with control during storage at 2°C. They found that dietary fibre incorporated fish sausage had good textural advantages compared to the control samples. Marzieh *et al.* (2018) studied the effect of lantern fish (*Benthosema pterotum*) protein isolate incorporated at 4 and 2% levels on the physicochemical and sensory properties of sausages during storage at 4°C. Dincer *et al.* (2017) evaluated the quality changes and stability of surimi sausage prepared from saithe (*Pollachius virens* L., 1758) flesh during cold

storage. Biochemical quality and shelf life of fish sausage prepared from bullseye fish (*Priacanthus hamrur*) was carried out by Maheswara *et al.* (2017) and found that the product had 2 and 25 days of shelf life in ambient and refrigerated conditions, respectively. Similarly, Nithin *et al.* (2015) studied the physico-chemical changes in liquid smoke flavoured yellowfin tuna (*Thunnus albacares*) sausage and control sausage during chilled storage and found that liquid-smoked sausage was better than control.

The present study was undertaken to develop dietary fibre incorporated tuna sausage by optimising different combinations of fish mince, wheat and oats dietary fibres by mixture response surface methodology (RSM). The quality evaluation of optimised dietary fibre enriched tuna sausage was carried out by assessing biochemical, physical and sensory attributes during chilled storage at 2°C. Further, multivariate statistical quality profiling of sausage during storage was done using T² and squared prediction error (SPE) control charts.

Materials and methods

Raw material

Frozen blocks of yellow fin tuna (*Thunnus albacares*) meat purchased locally (Kochi, Kerala) were used for the preparation of sausage. Tuna blocks were brought to the laboratory, thawed, chopped into small pieces, washed in potable water, impurities and bones were removed and then minced using meat bone separator.

Two different types of dietary fibres from VITACEL® brand namely, wheat fibre WF 600R and oats fibre HF 401-30 (M/s Rettenmaier India Pvt. Ltd., Maharashtra, India) were used for the study. Other food grade ingredients like corn starch, fat and spices procured locally were used for the formulation of dietary fibre incorporated tuna sausage. Analytical grade chemicals were used for the study. Commercially available polyamide casings were used for stuffing the ingredient mix.

Proximate composition

Proximate composition of tuna mince and dietary fibres was analysed as per AOAC (2012) method (Table 1). Total carbohydrate was estimated by subtracting weight of the constituents *viz.*, protein, fat, water and ash from the total weight of the sample.

Preparation of sausage

Fish mince was properly mixed with all the ingredients using silent cutter (Garant MTK 661, MADO, France) and stuffed into casings and tied on both ends manually using a twine. Hand operated sausage stuffer was used to stuff the mixed ingredients into polyamide

casings (35 mm dia) to a length of 100 mm. Sausages were cooked at 80°C in an automated water bath for 30 min and immediately cooled in water at 15°C for 15 min. Further they were packed in polyethylene pouches and stored in chilled conditions (2±1°C) for quality analysis. Tuna sausage prepared without incorporation of dietary fibre (control) was used for comparison purpose.

Experimental design

D-optimal mixture response surface design with 13 runs was formulated for the preparation of dietary fibre incorporated tuna sausage for optimising the levels of fish mince as well as dietary fibres. The levels of fish mince used varied from 60 to 70% and that of wheat and oats fibre varied from 0 to 5% (Table 2). The other ingredients were kept as constant for the preparation of sausage namely, salt 2.5%, sugar 1.5%, potassium sorbate 0.1%, polyphosphate 0.2%, guar gum 0.01%, spice mix 0.1%, spice concentrate 1.5%, hydrogenated vegetable fat 5%, corn starch 9% and ice 10% (Pranisa *et al.*, 2001).

Optimisation of ingredients for dietary fibre incorporated tuna sausage

Optimisation of ingredient combination was carried out for the response variables measured from texture profile analysis (TPA), colour measurements, water holding capacity (WHC) and sensory evaluation. Texture parameters like Hardness, Springiness, Cohesiveness, Gumminess, Chewiness, Adhesiveness and Stiffness were measured using a food texture analyser (Lloyd instruments, UK, Model LRX plus). Colour parameters like L* (lightness), a* (redness) and b* (yellowness) values were measured using a Hunter Lab MiniScan® XP Plus spectrophotometer, model No D/8-S (Hunter Associates Laboratory Inc., Reston, VA, USA). Expressible water of sausage was measured as water holding capacity (WHC) using the press method as described by Dincer and Cakli (2010). Sensory evaluation of sausage samples was done on a 9 point hedonic scale (9 being like extremely, 1 denoting dislike extremely and a score of 4 denoting the rejection score) by a ten member expert panel on appearance, colour, odour, flavour, taste and texture of the

Table 1. Proximate composition of raw materials

Proximate composition (%)	Tuna mince	Wheat fibre (WF 600R)	Oats fibre (HF 401-30)
Protein	19.60	1.00	1.71
Carbohydrate	1.70	92.30*	90.01**
Fat	1.60	0.29	0.21
Ash	1.40	1.31	2.82
Moisture	75.70	5.10	5.25

*Carbohydrate contains 74% cellulose and 26% hemicellulose

**Carbohydrate contains 70% cellulose, 25% hemicellulose and 5% lignine

Table 2. Combinations for dietary fibre incorporated tuna sausage

Sample No.	Fish mince (%), x_1	Wheat fibre (%), x_2	Oats fibre (%), x_3
S1	67.50	2.50	0.00
S2	65.00	3.33	1.67
S3	65.00	5.00	0.00
S4	65.00	0.00	5.00
S5	63.33	5.00	1.67
S6	66.67	0.00	3.33
S7	68.33	0.00	1.67
S8	65.00	1.25	3.75
S9	62.50	3.75	3.75
S10	70.00	0.00	0.00
S11	60.00	5.00	5.00
S12	66.67	1.67	1.67
S13	62.50	2.50	5.00

samples. Overall acceptability score (OAS) was obtained by taking the average score of all attributes for each panelist (Fishken, 1990).

Development of statistical models

Linear, quadratic and cubic response surface mixture models were fitted to the experimental data and was used to predict the response variables as a function of fish mince and dietary fibre. The functional forms of linear, quadratic and cubic models are given in Equations (1), (2) and (3), respectively. The models were fitted to the experimental data using Design Expert 7.1.5.

$$\text{Linear model: } Y = \sum_{i=1}^q \beta_i x_i + e \quad \text{.....(1)}$$

$$\text{Quadratic model: } Y = \sum_{i=1}^q \beta_i x_i + \sum_{i < j=2}^q \beta_{ij} x_i x_j + e \quad \text{.....(2)}$$

$$\text{Cubic model: } Y = \sum_{i=1}^q \beta_i x_i + \sum_{i < j=2}^q \beta_{ij} x_i x_j + \sum_{i < j < k}^q \beta_{ijk} x_i x_j x_k + \sum_{i < j=2}^q \delta_{ij} x_i x_j (x_i x_j) + e \quad \text{.....(3)}$$

where Y is the response variable and (components x_1 , x_2 and x_3) are input variables, β_i is linear regression coefficient, β_{ij} is the quadratic regression coefficient, β_{ijk} is the cubic regression coefficient, δ_{ij} is a special cubic regression coefficient. Further 'e' is the error term assumed to be identically and independently distributed with constant variance σ^2 . The regression coefficients were estimated by minimising the error sum of squares using ordinary least squares (OLS) method. Desirability function was formulated and desirability score was computed for optimising multiple responses. The goodness of fit of the model was assessed by coefficient of determination (R^2) and root mean square error (RMSE). The fitted model with highest R^2 and lowest RMSE values was selected for predicting the response variables.

Storage study of sausage samples

Optimised sausages along with control were prepared, packed in polyethylene pouches and stored in chilled room at $2 \pm 1^\circ\text{C}$ for storage stability studies. Sausage samples were drawn on 1, 4, 7, 9, 11 and 14 days of storage and evaluated for biochemical, texture, colour and sensory parameters.

Proximate composition and quality evaluation

Proximate analysis of optimised dietary fibre incorporated tuna sausage and control samples were carried out following AOAC (2012). Total carbohydrate was estimated from the difference in weight of other constituents (protein, fat, water, ash) from the total weight of the sample. Peroxide value (PV) was determined as per the methodology of Jacobs (1938). Total volatile base-nitrogen (TVB-N) and trimethylamine (TMA-N) were measured in mg nitrogen 100 g^{-1} as suggested by Conway (1950). Free fatty acid (FFA) was measured in mg% oleic acid as per AOCS (2017). Thiobarbituric acid (TBA) value was measured in milligram malonaldehyde per kg of sample (Tarladgis *et al.*, 1960). Total plate count (TPC) was determined in tryptic soy agar (TSA) by spread plate method (ICMSF, 1986). Texture profile analysis, instrumental colour parameters and sensory evaluation of sausage samples were also carried out during storage.

Multivariate statistical quality profiling of sausage during storage

Multivariate process monitoring was used to monitor the variability in the quality attributes during storage days to determine the product stability as well as safety and also to identify the attributes responsible for unusual variation (Keunpyo *et al.*, 2003). Multivariate statistics namely, T^2 and squared prediction error (SPE) were computed and the

same was used to construct T^2 and SPE control charts for a given set of quality attributes.

The measured quality attributes viz., $X_1, X_2, \dots, X_p$ of sausage during storage days were assumed to follow multivariate normal distribution with mean vector $\mu = (\mu_1, \mu_2, \dots, \mu_p)'$ and covariance matrix Σ . Principal component analysis (PCA) was performed to transform the original correlated response variables (X_i 's) into a new set of uncorrelated variables (y_j 's), whose variability was maximum (Johnson and Wichern, 2006). The sample variance-covariance matrix S given in Equation (4) was computed and used for principal component analysis.

$$S = \frac{1}{n-1} \sum_{j=1}^n (x_j - \bar{x})(x_j - \bar{x})' = PLP', \quad \dots\dots\dots(4)$$

where P is an orthogonal matrix whose column elements are the eigen vectors and L is a diagonal matrix whose elements are eigen values of sample variance-covariance matrix S . The j^{th} principal component is the linear combination of original variables and it is mathematically represented as:

$$y_j = P'(x_j - \bar{x}), j = 1, 2, \dots, p \quad \dots\dots\dots(5)$$

The first j principal components provide least square solution to the model given in Equation (6)

$$X = YP' + E, \quad \dots\dots\dots(6)$$

where X is the $n \times p$ matrix of centered measured quality attributes, Y is the $n \times j$ matrix of first j principal components, P' is the $j \times p$ matrix of eigen vectors and E is an $n \times p$ matrix of error terms. The T^2 statistic for i^{th} sampling day is computed from j^{th} principal component model and it is defined as:

$$T_{ji}^2 = y_j' L^{-1} y_j = (x_j - \bar{x})' S^{-1} (x_j - \bar{x}) \quad \dots\dots\dots(7)$$

The variability in the remaining $p-j$ principal components is monitored through SPE statistic and it is computed as:

$$SPE_i = \sum_{k=1}^p e_{ik}^2 \quad \dots\dots\dots(8)$$

where e_{ik} is the i^{th} error term of k^{th} quality attribute in the error matrix E .

The T^2 and SPE control charts were obtained by plotting the values of T_{ji}^2 and SPE_i against the storage days. The control limits for T^2 and SPE charts were obtained from the beta distribution. This analysis was carried out in SAS 9.3 (SAS, 2011).

Results and discussion

Statistical model development

Cubic model given in Equation (3) was found to be best fitted model to the response variables as a function of

tuna mince and dietary fibres. The estimated regression coefficients along with R^2 and RMSE values are given in Table 3. The fitted cubic model was then used to predict the response variables.

Texture profile analysis

In general, all the texture characteristics showed an increasing/positive trend with higher levels of fish mince and increasing levels of dietary fibre. The lowest hardness was observed for 68.33% tuna mince and 1.667% oats fibre, but the average hardness increased significantly from 18.21 N with increasing levels of wheat and oats fibre. The linear and cubic coefficients of fitted model of hardness were positive and the quadratic coefficients were negative. The corresponding R^2 and RMSE values were 0.67 and 5.265, respectively. Springiness and chewiness were found to be significantly ($p < 0.05$) increasing with increasing levels of wheat and oats, but gumminess showed increasing trend for increasing levels of wheat and decreasing trend for increasing levels of oats. The values varied from 0.20-7.77 mm, 2.16-5.96 N and 13.85-46.32 N mm, respectively for springiness, gumminess and chewiness. There was no significant change in the cohesiveness values from the average value of 0.232 when the tuna mince was replaced with different levels (0; 1.667; 2.50; 3.333 and 5%) of wheat and oats fibre. The response surface plots of hardness 1 (H1) and chewiness are depicted in Fig. 1a and b, respectively. Stiffness of the sausage developed was linearly related with the levels of wheat and oats fibre. The stiffness value was 4.968 N mm⁻¹ for control which increased to 6.721 N mm⁻¹ for highest levels (5% each) of wheat and oats fibre. Hardness 1, springiness, cohesiveness and chewiness exhibited linear positive regression coefficients with increasing levels of wheat and oats fibre, but gumminess showed a negative trend for oats fibre. The R^2 values ranged from 0.65 to 0.96 and RMSE values ranged from 0.447 to 12.63.

Colour analysis

The effect of addition of wheat and dietary fibre on colour parameters of sausage was also analysed. The R^2 and RMSE values of fitted model for L^* , a^* and b^* were 0.86, 0.89 and 0.72 and 3.90, 1.65 and 0.86, respectively. The colour parameters were found to be increasing with increasing levels of fish mince. The lightness L^* increased with the addition of wheat fibre to the sausage, whereas a^* and b^* values decreased with the addition wheat fibre. Wheat fibre produced a positive linear trend for L^* , whereas a^* and b^* had negative linear trend. The addition of oats fibre increased a^* and b^* values, but decreased the lightness (L^*) values (Fig. 2a, b, c). This might be due to high fibre content of oats. Cardoso *et al.* (2008a, b) reported that a^* showed a decreasing trend with increasing levels of pork meat replacement with fibre in the sausage.

Table 3. Estimated regression coefficients along with R^2 and RMSE values

Response variables	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	$x_1x_2x_3$	$x_1x_2^*(x_1-x_2)$	$x_1x_3^*(x_1-x_3)$	$x_2x_3^*(x_2-x_3)$	R^2	RMSE
Hardness I	0.241	375.750	122.369	-8.596	-2.477	-16.662	0.210	0.047	0.010	0.033	0.670	5.265
Cohesiveness	0.003	4.516	6.108	-0.102	-3.612	-0.133	-0.286	0.003	0.0005	0.0006	0.920	0.447
Springiness	0.073	28.120	300.605	-0.632	-6.770	-6.970	0.068	0.003	0.059	-0.021	0.820	0.678
Gumminess	0.051	16.857	-82.219	-0.411	1.848	-0.798	0.024	0.003	-0.010	-0.030	0.650	1.476
Chewiness	0.328	643.441	496.994	-14.768	-11.032	-39.208	0.506	0.081	0.056	-0.252	0.680	12.630
Stiffness	0.071	264.254	498.379	5.826	11.103	17.061	-0.173	-0.029	-0.057	0.013	0.873	0.754
L^*	0.829	1810.451	-621.748	-39.689	13.227	-29.210	0.328	0.197	-0.061	-0.420	0.860	3.900
a^*	0.024	-1350.484	283.779	29.779	-6.143	29.426	-0.343	-0.150	0.030	0.255	0.890	1.650
b^*	0.256	-454.484	9.195	10.140	-0.195	9.819	-0.103	-0.052	0.001	0.070	0.720	0.860
WHC	0.0001	26.900	14.078	-0.598	-0.309	-0.880	0.009	0.003	0.002	-0.003	0.955	0.001
OAS	0.103	285.124	313.096	-6.386	-6.899	-15.409	0.172	0.033	0.035	-0.027	0.800	0.920

x_1 = Fish mince, x_2 = Wheat fibre, x_3 = Oats fibre

WHC: Water holding capacity, OAS: Overall acceptability score

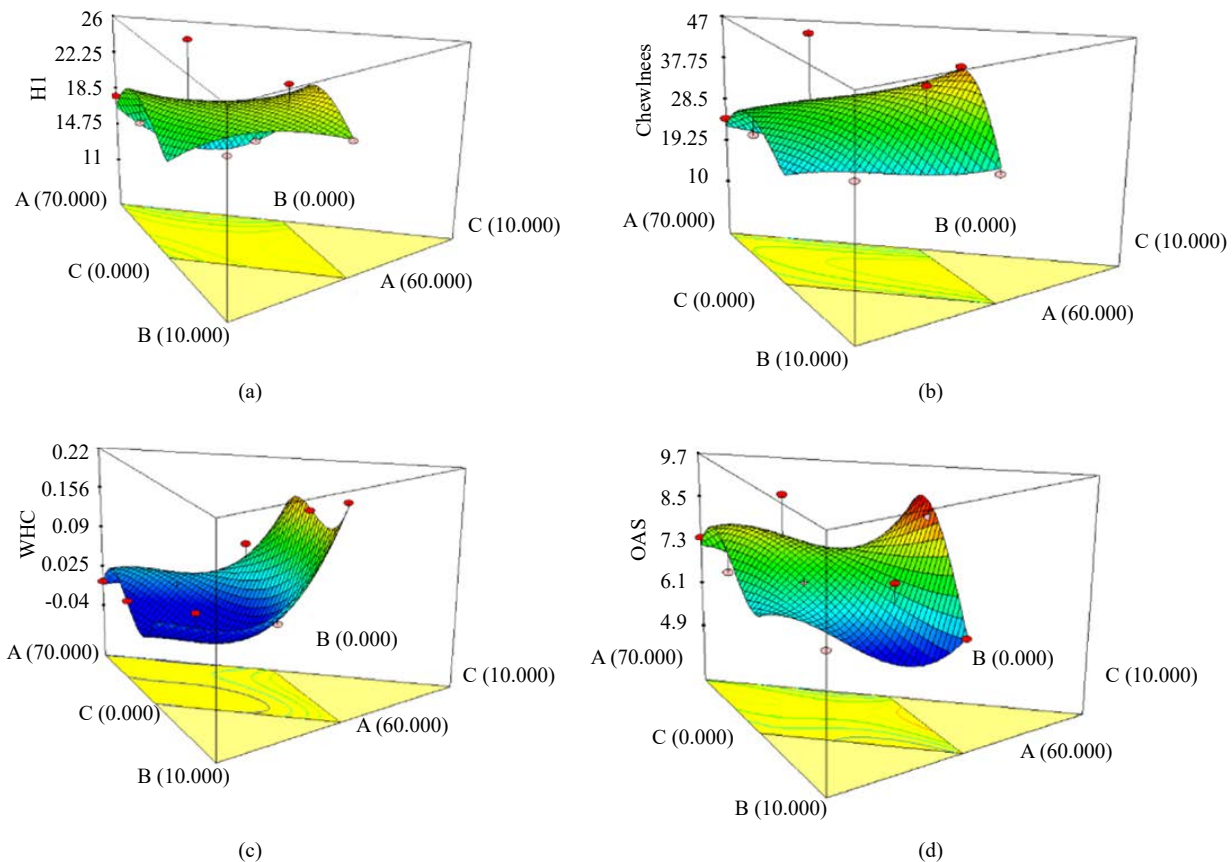


Fig. 1. Response surface plots of (a) Hardness I (H1); (b) Chewiness; (c) Water holding capacity (WHC) and (d) Overall acceptability score (OAS)

Water holding capacity

Water holding capacity (WHC) of the sausages ranged from 0.0009 to 0.203%. Cubic model was fitted to the experimental values with R^2 value of 0.96 and RMSE value of 0.001. The linear regression coefficients of fish mince, wheat and oats fibre produced a positive effect on WHC. The highest value of WHC (0.203%) was obtained for sausages with lowest levels of fish mince and highest

level of wheat and oats fibre. The lowest value (0.0009%) of WHC was recorded for control sausage samples which indicated that the replacement of tuna mince with wheat and oats fibre has significant effect on WHC. This essentially indicated the role of dietary fibre in improving the water retaining capacity of the sausage developed. Similar results were reported by Cardoso *et al.* (2008a). The response surface plot of WHC is given in Fig. 1c.

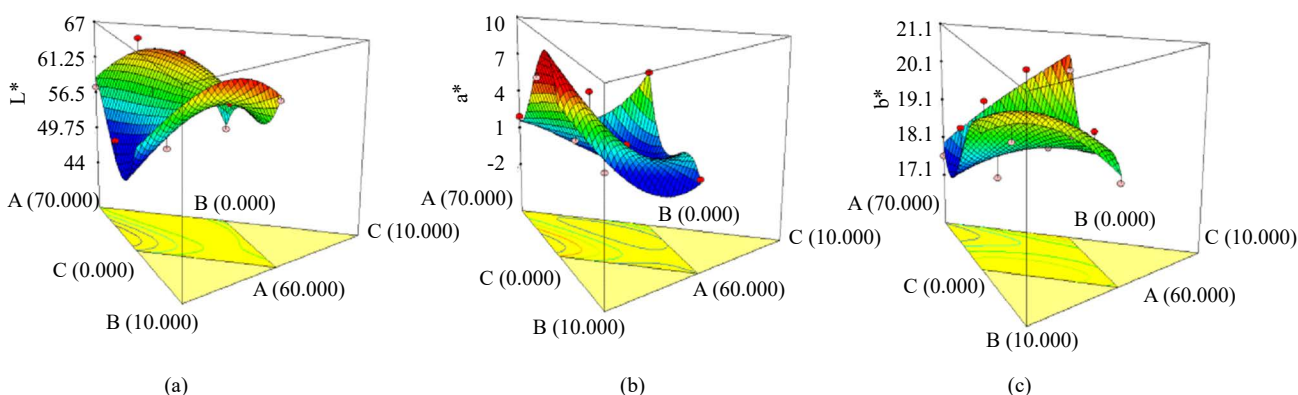


Fig. 2. Response surface plots of colour parameters (a) Lightness (L^*); (b) Redness (a^*) and (c) Yellowness (b^*)

Sensory evaluation

Overall acceptability score (OAS) was computed for different combinations of sausage samples. The lowest OAS of 5.7 was obtained for sausage with 63.33% fish mince, 5% wheat fibre and 1.67% oats fibre followed by 68.33% fish mince, 0% wheat fibre and 1.67% oats fibre. The highest OAS was 8.9 obtained for sausage with 66.67% fish mince, 1.67% wheat and oats fibre followed by sausage containing 62.5% fish mince, 2.5% wheat fibre and 5% oats fibre with a score of 8.7. The R^2 and RMSE values of fitted model for OAS were 0.80 and 0.92, respectively. The linear coefficients of fish mince, wheat and oats fibre were found to have a positive trend, whereas quadratic effects produced negative regression coefficients. Fig. 1d depicts the response surface plot of OAS.

Optimisation of ingredient combinations

The multiple response optimisation was done by formulating the desirability function and best combination was selected for highest desirability score. The best combinations were found to be 62.5% fish mince, 2.5% wheat fibre and 5% oats fibre (OS1) with a desirability score of 0.96; 66.8% fish mince and 1.6% wheat and oats fibres (OS2) with a desirability score of 0.90. Based on the score, these two optimised combinations were selected for further analysis along with control.

Proximate composition

The proximate composition of raw materials used is provided in Table 1. Proximate analyses of control and optimised samples (OS1 and OS2) were also carried out. Proximate composition of sample *viz.*, OS1 indicated a moisture content of 53%, 16% protein, 7% fat, 1% ash and 23% carbohydrate. The control and sample OS2 had no significant difference in proximate composition; both had 18% carbohydrate, 15% protein, 7% fat, 1% ash and

59% moisture. This might be due to less amount of dietary fibre in the sausage. But sample OS1 had higher amount of carbohydrate, protein and less moisture compared to control. The optimised sample had higher amount of fibre content as a healthier functional food.

Quality changes during storage

The pH of control and optimised samples (OS1 and OS2) during storage was found to be static throughout the storage period ranging between 5.60 to 5.70. The PV, FFA, TBA and TVB-N showed an increasing trend for all the samples, but were within the acceptable limits. There was no significant difference ($p>0.05$) in the PV, FFA, TBA and TVB-N values for control, OS1 and OS2 in each sampling day. Hardness 1 showed an increasing trend from the initial value of 13.63 N, 16.83 N and 12.74 N to 20.94 N, 21.0 N and 20.20 N on day 14 of storage for control, OS1 and OS2, respectively. This might be due to the fact that the dietary fibre in the sausage absorbs oil and water in the matrix, leading to increase in hardness of the sausage. Cardoso *et al.* (2008b) also observed that the hardness of dietary fibre incorporated fish sausage increased during storage. The other texture parameters did not show any significant ($p>0.05$) increase in the values. The colour parameters L^* and b^* showed slight increase in values during storage for all the samples, whereas a^* values showed slight decrease. The average values during 14 day storage for L^* were 57.77 ± 1.00 ; 59.44 ± 0.61 and 59.30 ± 1.23 ; a^* values were 0.34 ± 0.87 ; 0.25 ± 1.0 and -0.16 ± 0.54 and b^* values were 16.81 ± 0.26 ; 19.22 ± 0.21 and 17.43 ± 0.29 , respectively for control, OS1 and OS2 samples. These values were not significantly different between samples as well as during storage.

WHC was found to be constant during the storage for all the samples ranging from 0.07 to 0.23%, 0.10 to 0.19% and 0.13 to 0.20%, respectively for control, OS1 and OS2 samples. The initial TPC was 2.20, 3.0 and 2.80 log cfu g^{-1}

respectively for control, OS1 and OS2 samples. However it increased during storage and crossed the rejection limit of 6 log cfu g⁻¹ on 14th day of storage. The TPC values on the previous sampling day (11th day) were 5.49, 4.68 and 5.28 log cfu g⁻¹ for control, OS1 and OS2, respectively. The TPC value on 14th day was 6.25, 6.72 and 6.041 log cfu g⁻¹ for control, OS1 and OS2, respectively. Cardoso *et al.* (2008b) also observed similar growth of microorganisms during storage of dietary fibre incorporated fish sausage.

The overall acceptability score also decreased during storage and on 14th day it reached a value of 5. Hence, though the samples were sensorily acceptable on 14th day of storage, it was not microbiologically acceptable. Cardoso *et al.* (2008b) and Maheshwara *et al.* (2017) noticed decrease in sensory score in different types of fish sausages during storage. Changes in the biochemical indices and OAS are depicted in Fig. 3a, b, c, d, e and f. The control and two optimised samples had shelf life upto 14 days in chill stored condition.

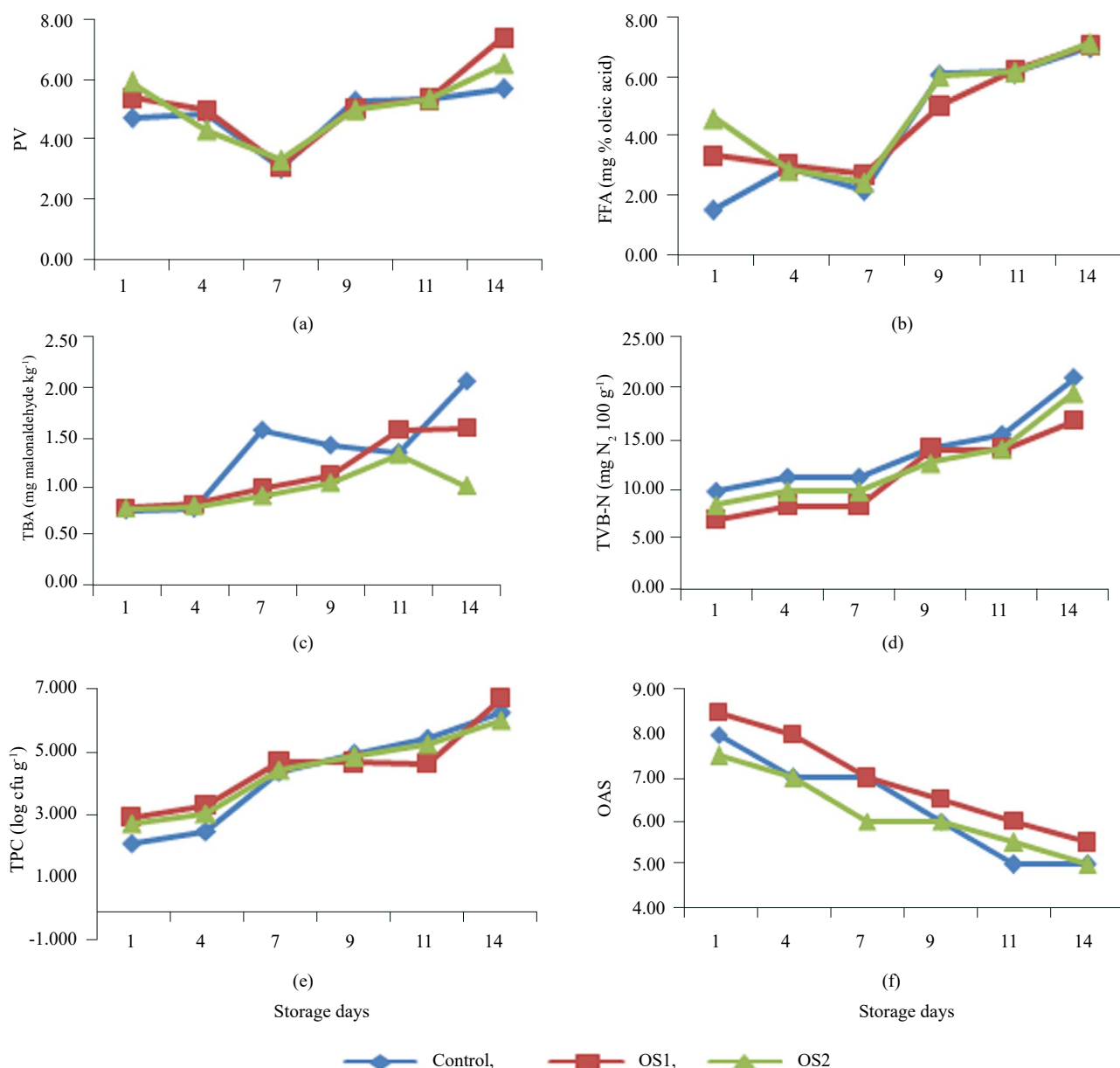


Fig. 3. Changes in the biochemical indices during storage days (a) Peroxide value (PV); (b) Free fatty acids (FFAs); (c) Thiobarbituric acid (TBA) value; (d) Total volatile base-nitrogen (TVB-N); (e) Total plate count (TPC) and (f) Overall acceptability score (OAS)

Multivariate control charts

T^2 and SPE based multivariate control charts were developed for control, OS1 and OS2 samples to evaluate the product stability during storage. Principal component analysis was performed to the raw data obtained from the storage study and principal component based model was developed from the variance-covariance matrix with 4 principal components for control and 3 principal components for OS1 and OS2. The first 4 and 3 principal components explained 92, 90 and 87% of total variability

in the data for control, OS1 and OS2, respectively. These principal components were then used to compute T^2_{ji} and SPE_i given in Equations (7) and (8), respectively. The T^2 and SPE control charts based on the estimated values of T^2_{ji} and SPE_i are given in Fig. 4 for control, OS1 and OS2. It could be inferred from the multivariate control charts that the product was safe/stable till 14th day of storage. These graphs can be used to see if T^2_{ji} and SPE_i statistics computed from a new observation falls in the acceptable limits of T^2_{ji} and SPE_i charts.

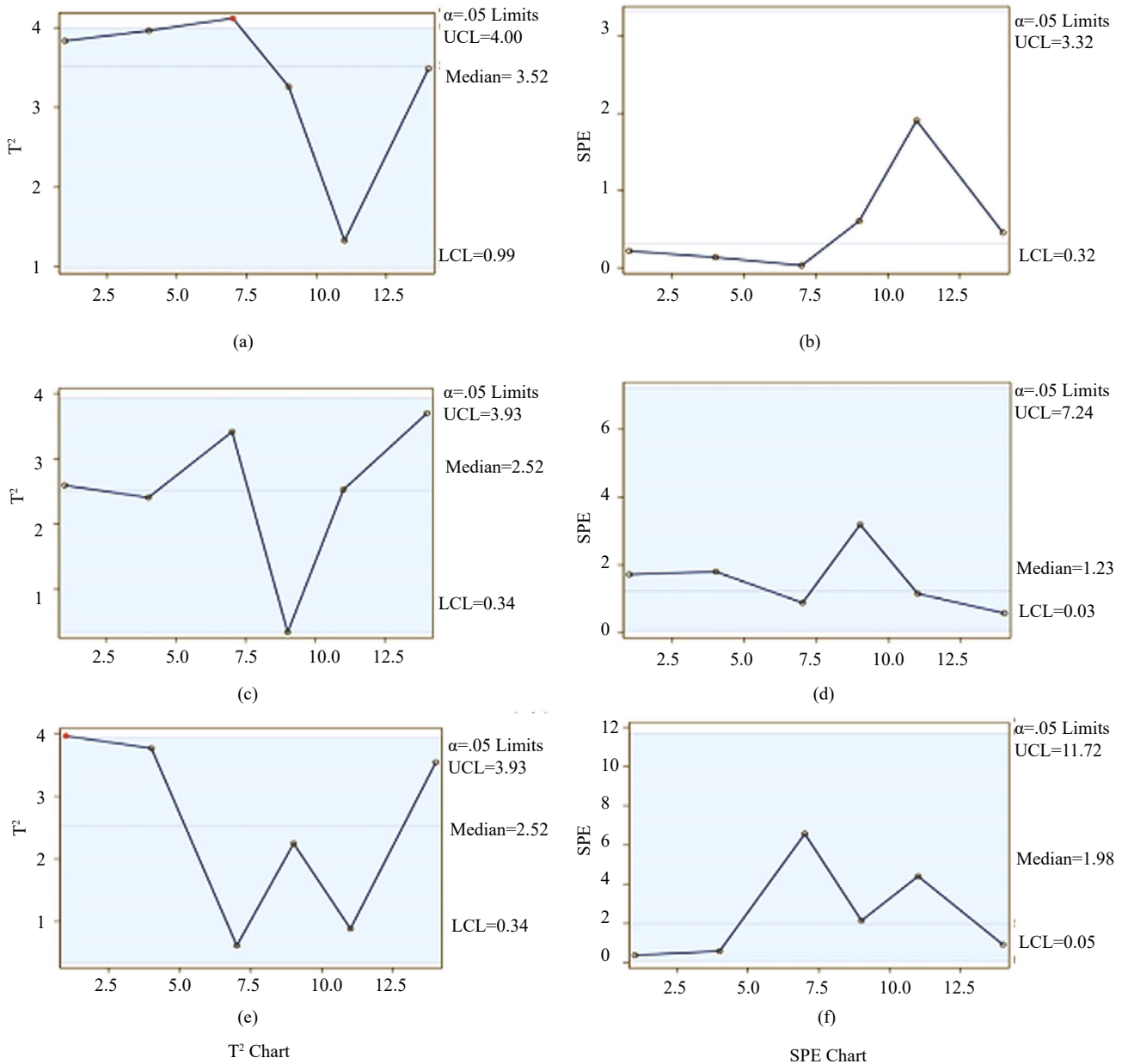


Fig. 4. T^2_{ji} and SPE_i charts of Control, OS1 and OS2 during storage days. a, c and e : T^2 charts for control, OS1 and OS2; b, d and f : SPE charts for control, OS1 and OS2

Acknowledgments

The authors thank the Director, ICAR-CIFT, Kochi for granting permission to carry out this work and Mr. Nobi Varghese and Ms. Susmitha, V., technical assistants and all other staff of Fish Processing Division, ICAR-CIFT, Kochi for the support extended during the study.

References

- Anderson, J. W. 2003. Whole grains protect against atherosclerotic cardiovascular disease. *Proc. Nutr. Soc.*, 62: 135142. doi: 10.1079/PNS2002222.
- Anderson, J. W., Baird, P., Davis Jr., R. H., Ferreri, S., Knudtson, M., Koraym, A., Valerie, W. and Williams, C. L. 2009. Health benefits of dietary fiber. *Nutr. Res.*, 67(4): 188-205. doi: 10.1111/j.1753-4887.2009.00189.x.
- AOAC 2012. *Official methods of analysis*, 19th edn. Association of Official Analytical Chemists, Washington DC, USA.
- AOCS 2017. *The official methods and recommended practices of the AOCS*, 7th edn. American Oil Chemist's Society, Urbana, USA.
- SAS 2011. *Base SAS® 9.3 Procedures guide*. SAS Institute Inc., Cary, North Carolina, USA.
- Cardoso, C., Mendes, R. and Nunes, M. L. 2008a. Development of a healthy low-fat fish sausage containing dietary fibre. *Int. J. Food Sci. Technol.*, 43: 276-283. DOI: 10.1111/j.1365-2621.2006.01430.x.
- Cardoso, C., Mendes, R., Pedro, S. and Nunes, M. L. 2008b. Quality changes during storage of fish sausages containing dietary fiber. *J. Aquat. Food Prod. Technol.*, 17(1): 73-95. DOI: 10.1080/10498850801891249.
- Conway, E. J. 1950. *Micro diffusion analysis of volumetric error*. Crosby Lockwood and Son Ltd., London, UK.
- Daley, L. H., Deng, J. C. and Cornell, J. A. 1978. Development of a sausage-type product from minced mullet using response surface methodology. *J. Food Sci.*, 43(5): 1501-1506. <https://doi.org/10.1111/j.1365-2621.1978.tb02529.x>.
- Dincer, M. T., Yilmaz, E. B. S. and Cakli, S. 2017. Determination of quality changes of fish sausage produced from saithe (*Pollachius virens* L., 1758) during cold storage. *Ege JFAS*, 34(4): 391-399. DOI:10.12714/egejfas.2017.34.4.05.
- Dincer, T. and Cakli, S. 2010. Textural and sensory properties of fish sausage from rainbow trout. *J. Aquat. Food Prod. Technol.*, 19(3): 238-248. <https://doi.org/10.1080/10498850.2010.509539>.
- Fishken, D. 1990. Sensory quality and the consumer: Viewpoints and directions. *J. Sens. Stud.*, 5(2): 203-209. <https://doi.org/10.1111/j.1745-459X.1990.tb00490.x>.
- ICMSF 1986. *Microorganisms in food - 2, Sampling for microbiological analysis: Principles and specific applications*. University of Toronto Press, Toronto, Canada.
- Jacobs, M. B. 1938. *The chemical analysis of food and food products*. D. Van Nostrand, New York, USA, p. 393-394.
- Johnson, R. A. and Wichern, D. W. 2006. *Applied multivariate statistical analysis*. Pearson Education Inc., New Jersey, USA, p. 426-466.
- Keunpyo, K., Mahmoud, A. M. and William, H. W. 2003. On the monitoring of linear profiles. *J. Qual. Technol.*, 35(3): 317-328. <https://doi.org/10.1080/00224065.2003.11980225>.
- Lattimer, J. M. and Haub, M. D. 2010. Effects of dietary fiber and its components on metabolic health. *Nutrients*, 2: 1266-1289. doi: 10.3390/nu2121266.
- Li, Y. O. and Komarek, A. R. 2017. Dietary fibre basics: Health, nutrition, analysis and applications. *Food Qual. Saf.*, 1: 47-59.
- Maheshwara, K. J., Jaya, N., Arun, B. and Jagadish, T. D. 2017. Biochemical and shelf life study of quality of fish sausage in ambient and refrigerated storage. *Biochem. Cell. Arch.*, 17(1): 110-116.
- Marzieh, M. N., Rezvan, M. and Najme, O. 2018. Physicochemical evaluation of sausages prepared by lantern fish (*Benthosema pterotum*) protein isolate. *Food Sci. Nutr.*, 6: 617-626. doi: 10.1002/fsn3.583.
- Myers, R. H. and Montgomery, D. C. 2002. *Response surface methodology - Process and product optimization using designed experiments*. John Wiley and Sons, New York, USA.
- Nithin, C. T., Ananthanarayanan, T. R., Yathavamoorthi, R., Bindu, J., Joshy, C. G. and Srinivasa Gopal, T. K. 2015. Physico-chemical changes in liquid smoke flavoured yellowfin tuna (*Thunnus albacares*) sausage during chilled storage. *Agric. Res.*, 4(4): 420-427. DOI: 10.1007/s40003-015-0189-z.
- Ozpolat, E. and Patir, B. 2016. Determination of shelf life for sausages produced from some freshwater fish using two different smoking methods. *J. Food Saf.*, 36: 69-76. <https://doi.org/10.1111/jfs.12214>.
- Pranisa, C., Nongnuch, R. and Wanchai, W. 2001. Process development of fish sausage. *Int. J. Food Prop.*, 4(3): 523-529. <https://doi.org/10.1081/JFP-100108653>.
- Tarladgis, G. B., Watts, M. B. and Younathan, T. M. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.*, 37: 44-50.

- Turner, N. D. and Lupton, J. R. 2011. Dietary fiber. *Adv. Nutr.*, 2(2): 151-152. <https://doi.org/10.3945/an.110.000281>.
- Yang, Y. Y., Ma, S., X. X. and Zheng, X. L. 2017. Modification and application of dietary fiber in foods. *J. Chem.*, doi. [org/10.1155/2017/9340427](https://doi.org/10.1155/2017/9340427).