JOURNAL OF MEAT SCIENCE AND TECHNOLOGY
Journal homepage: www.jakraya.com/journal/jmst

ORIGINAL ARTICLE

Quality evaluation of fermented sausages as influenced by different fat levels and temperature of fermentation

Saghir Ahmad* and Qasim Nawab

Department of Post Harvest Engineering and Technology, Faculty of Agricultural Science, Aligarh Muslim University, Aligarh, India.

*Corresponding Author:

Saghir Ahmad

Email: alsaghirqadri@gmail.com

Received: 22/07/2014

Revised: 11/08/2014

Accepted: 11/08/2014

Abstract

The investigations were carried out to study the effect of different fat and temperature levels for fermentation on various physico-chemical and microbiological characteristics of developed Semi Dried Fermented Sausage (SDFS). The response surface methodology was applied to study the effect of dependable variables on various physico-chemical and sensory properties. The quality of semi dry fermented sausage was based on physico-chemical characteristics (pH, moisture content, fat content, protein content, ash content, moisture protein ratio, TBA number) and sensory characteristics (colour, flavour, texture, taste, mouth coating and juiciness). The pH of Semi Dried Fermented Sausage was found in the range of 4.8-5.4 for the samples prepared with 33.1 ^{0}C and 20 % fat content and 11.89 ^{0}C. Moisture content and protein content of fresh SDFS were found in the following ranges 39.5-51.8 %, 17.0-18.87 % respectively. The fat incorporation significantly affected the physico-chemical and sensory properties of FDFS. The value of Moisture Protein Ratio ranged between 2.24 and 2.75 for the sample prepared with 15 ^{0}C of temperature and 25% fat and 22.50 ^{0}C temperatures and 12.93 % fat. It was found that increasing fat levels and a medium temperature of 20 ^{0}C improved the sensory characteristics like juiciness, tenderness and texture of SDFS. The score values of these attributes varied from 8.21-8.5 when fat level increased to 20 % and temperature of fermentation remained 15 ^{0}C. Samples were packed in combination film and for shelf life study was conducted under ambient condition. Progress of fermentation (carried out by mixed culture of Lactic Acid Bacteria, LAB) was recorded by drop of pH with respect to time. Increasing temperature was found to be function of drop in pH value. The time for completion of fermentation varied from two to five days. Thus fermentation, smoking and drying reduced the pH and moisture content. The reduced moisture content and low pH was the criteria for extended the shelf life of SDFS.

Keyword: Fermented sausage, response surface methodology, moisture protein ratio, fat levels.

Introduction

Meat and meat products play an important role in human nutrition. However, being highly perishable, their storage and marketing demands considerable amount of energy input, in the form of refrigeration and freezing, which is costly and scantly in India and other developing countries. Development of simple technologies for the manufacture of shelf stable ready-to-eat meat products would not only save energy, bring better returns and provide consumer convenience but would also be a valuable contribution to the growth of meat industry. The role of fermentation as a means of food preservation and food conservation is well established. Stanton (1985) listed the virtues of food fermentation as: it preserves the raw materials at low cost; reduces fuel demand by reducing cooking time and temperature; enhances nutritional quality, by improving digestibility, protein value, vitamin content; destroys toxic or undesirable components and harmful biota, protects against re-infection and adds positive antibacterial components; and improves appearance, aroma, flavour and texture. UN (1679) recommended that future research should include more work on application of fermentation to meat, milk, sea-food etc. Sharma (1987) also indicated about the scope of development of fermented meat products in India. Ahmad and Srivastavas (2007) conducted studies on fermented sausage using culture of Lactic Acid Bacteria (LAB) and the product was found to be shelf stable. A combined culture of LAB further glorified the quality of fermented sausage with respect to colour, texture, juiciness (Ahmad and Amer, 2012).

Materials and Methods

Experimental work was carried out to study the effect of different fat level and temperature for fermentation on quality characteristics of semi dry fermented sausage. Various physico-chemical and sensory properties of fresh samples were evaluated and different levels of fat and temperature (for fermentation) was combined using response surface methodology.

Experimental design

The independent variables (factors) in present study were fat and temperature. The levels of these factors were generated by applying rotatable central composite design (RCCD). The RCCD suits for fitting quadratic surface, which usually works well for optimizing compositions. Lower and higher levels of both the ingredients were selected on the basis of preliminary trials. The CCD for two factors response comprises of following three parts. Its pictorial representation is given in Fig 1.

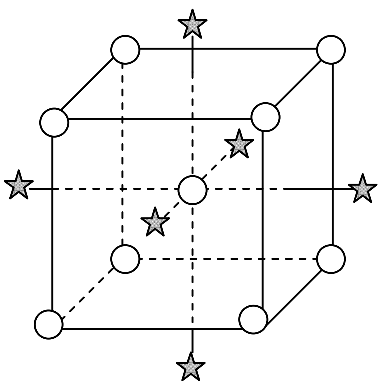

Fig 1: Central composite design for three factors

1. The central points (0, 0) are at centre
2. The factorial points (- 1, - 1); (1, - 1); (- 1, 1) and (1, 1)
3. **The Axial points** $(-\alpha, 0)$; $(\alpha, 0)$; $(0, -\alpha)$ and $(0, \alpha)$

This distance 'α' is measured in terms of coded factor levels and may be chosen from variety of options for alpha; i.e. its coded and actual levels of factors representation is given in Table 1.

- Rotatable $\left(\alpha = 2^{K/4}; K \le 5\right)$, where k is number of factors
- Spherical $\left(\alpha = \sqrt{K}\right)$
- Orthogonal quadratic(α value allows each term to beestimated independently of the block)
- Practical $\left(\alpha = K^{\frac{1}{4}}; \quad K \ge 5\right)$
- Face centred $(\alpha = 1)$

Statistical analysis and optimization

Using the experimental plan as described in the previous section (2.1), 21 different experiments were carried out. Factorial and axial points replicated twice and centre point was replicated five times. Response surface methodology was applied on various response, such as, moisture content, protein content, etc, using design expert® 7.15 software. The response was present by first order, second order or higher order, multiple linear/polynomial regression models. For two independent variables (coded as X_1 *and* X_2), the first order linear regression model is

$$Y = \beta_0 + \beta_1 \chi_1 + \beta_1 \chi_2$$

and second order polemical model is

$$Y = \beta_0 + \beta_1 \chi_1 + \beta_2 \chi_2 + \beta_{11} \chi^2{}_1 + \beta_{22} \chi^2{}_2 + \beta_{12} \chi_1 \chi_2$$

Where, Y is any response variable and $\beta' s$ is regression coefficients. The adequacy of any regression model for the fitting experimental results for any response was cheeked by selecting the available options for models provided by the software giving highest value of coefficient of regression $\left(R^2\right)$. In the response surface plotting the value of dependent variable are represented as a three dimension surface. The value of the independent variables for any two values of independent variable can be determined from the plot.

$$X_1^{opt} = \frac{2\beta_1 \beta_{22} - \beta_2 \beta_{12}}{\beta_{12^2} - 4\beta_{11}\beta_{22}}$$

$$X_2^{opt} = \frac{2\beta_{11}\beta_2 - \beta_1\beta_{12}}{\beta^2{}_{12} - 4\,\beta_{11}\beta_{22}}$$

The value of the response function under this condition is

$$Y^{opt} = \beta_0 + \frac{\beta_1\chi_1^{opt}}{2} + \frac{\beta_2\chi_2^{\ opt}}{2}$$

The overall optimization was done by choosing a desired goal for each factor and response from goals: maximize, target, within range none (for response only) and set to exact value (for factor only). These goals were then combined into an overall desirability function on the desirability objective function D (χ), each response was assigned an importance relative to other response. The importance varies from the least value to 1 to maximize value of 5. For the different important values the desirability function is given as

$$D = \left(d_1^{r_1}\ \mathrm{X}\ d_2^{r_2}\ \mathrm{X}\ \ldots\ldots\ldots \mathrm{X}\ d_n^{r_n}\right)^{\frac{1}{\sum r_i}}$$

Where n is the number of response in the measure and di are value according to which goals for each response was set. Experimental studies were conducted on semi-dry fermented sausages prepared by using different levels of fat and temperature conditions suggested by the RSM, during refrigerated storage (4^0C). The treatment of sodium ascorbate along with sodium nitrite was given to meat mix before preparation (fermentation, filling, smoking and drying). The quality of semi dried fermented sausage was evaluated on the basis of physico-chemical characteristics viz. pH, acidity, moisture content, protein content, fat content, moisture protein ratio (MPR), Thiobarbituric acid (TBA) number, sensory characteristics like colour, aroma, texture, taste, juiciness and mouth feel.

Preparation of meat and non meat ingredient

Meat samples collected from the local meat shop in the study were from buffaloes slaughtered according to traditional *halal* method at slaughterhouse of municipal corporation, Aligarh. The animals were kept in lairage for a period of 18-20 hours. Meat samples from round portion (*Biceps femoris muscle*) of 2.5, 3 and 3.5 years aged female carcasses of good finish were obtained from meat shop within 4 hr. of slaughter. The meet chunks were packed in combination film packaging and brought to the laboratory with in 20 min. Buffalo fat were also packed in combination film packaging and brought to the laboratory. Other non-meat ingredients like spices, salt, condiments and combination film were procured from the local market. This fibrous casing was procured from PRS technologies New Delhi. The meat and fat were kept inside ultra low temperature cabinet at 4^0C.

Preparation of semi dry fermented sausage

Fermented sausages were prepared from comminuted mixture of meat, fat, salt, spices and sugar using bacterial culture there allowed to undergo fermentation under strict conditions of temperature and humidity. Two different lots of semi dry fermented sausages were conducted containing two levels of fat 20% and 25%. The composition of fermented sausages was kept: meat (2 kg), fat (400 and 500 g for 20 and 25% fat samples respectively), mix spices (24 g), chilli powder (12 g), condiments (40 g), salt (50 g), sugar (20 g), dextrose (10 g), sodium ascorbate (1000 mg), mono sodium glutamate (2 g), ice (150 g) and 10 ml of starter culture. The buffalo meat and fat were ground on a -

Table 1: Coded and actual levels of factors

S. No	Point type	Coded value	Temperature Degree C	Fat (%)	No of replications
1	Factorial	$(-1,-1)$	15	15	2
2	Factorial	$(-1,1)$	15	25	2
3	Factorial	$(1,-1)$	30	25	2
4	Factorial	$(1,1)$	30	25	2
5	Centre	$(0,0)$	22.50	20	5
6	Axial	$(-1.141,0)$	11.89	20	2
7	Axial	$(0,-1.414)$	22.50	12.93	2
8	Axial	$(0, 1.414)$	22.50	27.07	2
9	Axial	$(1.414, 0)$	33.11	20	2

grinder (PRS Technologies, India). Then buffalo meat was first chopped and then fat and other non-meat ingredients were added, salt was added at very end of chopping. Bowel cutter (PRS Technologies, India) was used for chopping of meat and other ingredients. Spices, condiment and mono sodium glutamate (MSG) were added to contribute flavour in semi dry sausages. Well-mixed mass was further added with different combined culture and finally placed in a shallow pan and held at 15°C, 85% relative humidity to complete fermentation. The completion of fermentation was indicated by drop in pH of the mixture. After the completion of fermentation the mixture was stuffed into the casing by using the sausage filler machine. Stuffing into fibrous casing (35 mm dia.) was done firmly and carefully to exclude the air inside the casing, which might discolour the meat mix and reduce the shelf life of the sausages. Semi dry fermented sausages then were smoked at temperature 40-60°C for 4 hours to improve flavour and to inhibit bacterial development. The smoked sausages were dried at 20°C and relative humidity 70%. The drying was done at optimum speed, precautions were taken to ensure that sausages neither dried too fast nor retained surface moisture and become sticky. At the end, sausages samples were packed in combination film under atmosphere packaging and stored at refrigerated temperature of 2°C for further study.

Physico-chemical analysis

The pH of the finally minced samples were determined after homogenizing 10g of the sample with 100 ml distilled water using laboratory grinder (Yarco, India). The pH of suspension was recorded using reference and glass electrode portable type Digital pH meter model PH1500 (Eutech, Singapore) (Ranganna, 2002).

Thiobarbituric acid TBA reagent was prepared by dissolving 0.2883 g of Thiobarbituric acid in sufficient quantity of 90% acetic acid and by slight warming, the volume being made up 100 ml with 90% acetic acid. 20 g of meat sample were blended in a blender with 50 ml of cold 20% tricarboxylic acid (TCA) for 2 min. The blended contents were rinsed with 50 ml of distilled water, mixed together and filtered through filter paper and the interstate was collected in a 100 ml capacity-measuring cylinder. The filtrate, termed the TCA extract was used in the estimation of thiobarbituric acid (TBA) number. TBA number was measured by the method described by Strange *et al.* (1977). Five ml of TCA extract was mixed with 5 ml of TBA reagent in test tube. The test tube was kept in a water bath at 100° C for 30 min along with another test tube containing a blank of 10% TCA and 5 ml of TBA reagent. After cooling the tubes in running water about 10 min, the absorbance was measured at 530 nm in a spectrophotometer (Digital spectrophotometer Model 310E, India) and reported as TBA number.

Results and Discussion

The present study was undertaken for development, quality evaluation and shelf life studies of buffalo meat semi dry fermented sausage (SDFS) produced by using different levels of fat and temperature conditions. The quality of fresh sausage was evaluated on the basis of physico-chemical characteristics namely pH, acidity, moisture content, protein content, moisture protein ratio (MPR), fat content and TBA number, microbiological characteristics viz. total plate count, yeast and mold count, and coliform count and sensory characteristics. In the first experiment the composition of the fermented sausage was optimized using Response Surface Methodology. The results of the study have been presented in different tables and graphs and are discussed below:

Effect of factors levels (fat and temperature) on various responses

The effect of fat and temperature on various physico-chemical and sensory evaluation properties of fresh samples of fermented sausage have been summarized in Table 2.

Effect on moisture content

Moisture content of semi dry fermented sausage is an important characteristic, which relates the quality and shelf life. The moisture content is also criteria for deciding the completion of product in case of fermented sausage. The moisture is an important property influencing storage stability and texture of foods. Higher moisture containing foods are more prone to microbial spoilage but they have softer texture. The moisture content in the samples ranged between 39.5% (db) (in the sample prepared by incorporating 27.07% fat and) and 51.8 % (db) (in the sample prepared by incorporating 12.93% fat). The regression model obtained for moisture content.

$$\mathbf{M.C = +45.41 - 0.10^{*}\ A - 4.05^{*}\ B}$$

Table 3 shows that the linear model was suggested by the analysis and was found significant on

Table 2: Physico-chemical and microbiological properties of fresh samples of fermented sausage

Std	Run	Type	Factor 1 Temperature Degree C	Factor B:Fat%	MC % (db)	pH	Acidity %	Protein MPR %	MPR	TBA	Sensory Characte-ristics
5	1	Fact	15	25	41	5.1	0.508	18.3	2.24	0.35	8.62
15	2	Axial	22.5	27.07	40	5	0.557	17	2.35	0.38	8.6
13	3	Axial	22.5	12.93	51.8	5.2	0.475	18.87	2.75	0.23	8.12
12	4	Axial	33.11	20	45.8	4.8	0.607	18.44	2.48	0.3	8.48
11	5	Axial	33.11	20	45.4	4.9	0.585	18.44	2.46	0.31	8.45
20	6	Center	22.5	20	46	5	0.554	18.72	2.46	0.306	8.51
7	7	Fact	30	25	41.2	5.1	0.5	17.56	2.35	0.351	8.5
3	8	Fact	30	15	49	5.2	0.472	18.64	2.63	0.25	8.3
1	9	Fact	15	15	50.09	5.3	0.406	18.76	2.67	0.245	8.25
16	10	Axial	22.5	27.07	39.5	4.9	0.58	17.12	2.31	0.385	8.62
19	11	Center	22.5	20	45.6	5.1	0.504	18.22	2.5	0.295	8.5
8	12	Fact	30	25	41	5	0.552	17.56	2.33	0.355	8.42
14	13	Axial	22.5	12.93	51	5.2	0.46	18.87	2.7	0.235	8.1
18	14	Center	22.5	20	45.5	5.2	0.464	17.2	2.64	0.308	8.4
17	15	Center	22.5	20	45.2	5.2	0.48	17.34	2.61	0.29	8.6
4	16	Fact	30	15	48.5	5	0.55	18.64	2.6	0.26	8.35
2	17	Fact	15	15	49	5.1	0.506	18.76	2.61	0.265	8.23
10	18	Axial	11.89	20	45.62	5.4	0.36	18.13	2.52	0.3	8.46
21	19	Center	22.5	20	45.4	5.2	0.466	17.52	2.59	0.305	8.54
6	20	Fact	15	25	41.5	5	0.556	18.3	2.27	0.345	8.65
9	21	Axial	11.89	20	45.42	5.2	0.476	18.13	2.51	0.303	8.48

Table 3: ANOVA for Moisture Content

SOURCE	SS	Df	MS	F-VALUE	P value Prob>F	
Model	262.95	2	131.47	764.02	<0.0001	Significant
A-Temp.	0.17	1	0.17	1.01	0.3293	
B-Fat	262.77	1	262.77	1527.03	<0.0001	
Residual	3.10	18	0.17			
Lack of fit	1.34	6	0.22	1.52	0.2532	Non-Significant
Pure error	1.76	12	0.15			
R^2	0.9836					

The model showed (R^2 = 0.9836). The coefficient of linear term of factors indicates that the moisture content is negatively correlated with quantity of fat and temperature, whereas the quantity of temperature shows a weak negative correlation, and the F value shows that the fat affected more as compared to temperature. Fig 2 shows the response surface plot, describing the influence of temperature and fat on calculated response variable (moisture content). Starting from the initial point (A=15^{0}C, B=15%) value of moisture content was found 49.56%, the moisture decreased to 45.51% when the fat level was increased to 20%, while it further decreased to 41.46% when the fat level was increased to 25%.

Effect on pH

Table 2 represents the results of pH of semi dry fermented sausage produced by using different levels of fat and temperature conditions. Samples after preparation were packed by atmosphere packaging. pH of sausage samples lowered down after fermentation, smoking and drying. Reduction in pH was due to formation of lactic acid by mixed culture of LAB using carbohydrates (dextrose and sucrose) added in the meat mix. Similar results were obtained by (Ensoy *et al.*, 2010). During refrigerated storage 2^{o}C, pH values were found to significantly (p<0.05) decrease. At the end of 120 days of storage pH values were found to be between 4.39 and 4.43. Increasing the fat level from 20% to 25% increased the pH of all sample and that agreed with the result of studies conducted by (Ahmad, 2005; Liaquati and Srivastava, 2010; Olivares *et al*., 2010). pH plays an important role in preservation of foods. Generally high acidic foods are less prone to bacterial spoilage but have chance of mould spoilage. The value of pH ranged between 4.8 and 5.4 for the sample prepared with 33.11^{0}C of Temperature +20% of Fat and 11.89 ^{0}C of Temperature + 20% Fat, respectively. Following equation is the regression model obtained for pH.

pH = +5.10 - 0.092 * A - 0.069 *

Table 4 shows that the linear model was suggested by the analysis and was found significant on the analysis of variance at (p<0.05), the linear model showed low (R^2 = 0.3113). The coefficient of linear term of factors indicates that the pH is negatively correlated with the amount of temperature whereas the quantity of fat shows a weak negative correlation. The values (F value) of the table also indicate that the temperature has more effect as compared to fat. Fig 3 shows the response surface plot, describing the influence of temperature and fat on calculated response variable (pH). Starting from the initial point (A=15^{0}C, B=15%) value of pH was found 5.26 and decreases to 5.19 when we increase the temperature to 20^{0}C and further decreases to 5.07 when the temperature increases to 30^{0}C.

Effect on acidity

Table 2 represents the results of acidity of semi dry fermented sausage produced by using different levels of fat and temperature conditions according to RSM. Samples after preparation were packed by atmosphere packaging. Acidity of sausage samples increase after fermentation, smoking and drying. The acidity increases due to formation of lactic acid by mixed culture of LAB using carbohydrates (dextrose and sucrose) added in the meat mix. Acidity of semi dry fermented sausage was determined to ascertain the progress of fermentation. During fermentation mixed culture was added in the mix and lactic acid was produced. Acidity plays an important role in preservation of foods. Generally high acidic foods are less prone to bacterial spoilage but have chance of mould spoilage. The value of acidity ranged between 0.36 and 0.61 for the sample prepared with 11.89^{0}C of Temperature +20% of Fat and 33.11^{0}C of Temperature + 20% Fat, respectively. Following equation is the regression model obtained for acidity-

Acidity=+0.51+0.038*A+0.029*B

Table 5 shows that the linear model was suggested by the analysis and was found significant on the analysis of variance at (p<0.05), the linear model showed low (R^2 = 0.2868). The coefficient of linear term of factors indicates that the acidity is positively correlated with the amount of temperature and fat. The value (F value) of the Table 5 also indicates that the temperature has more effect as compared to fat. Fig 4 shows the response surface plot, describing the influence of temperature and fat on calculated response variable (Acidity). Starting from the initial point (A=15^{0}C, B=15%) value of acidity was found 0.439 and increase to 0.497 and 0.514 when fat increase to 25% and temperature to 30^{0}C respectively.

Effect on protein content

Protein content of semi dry fermented sausage is an important characteristic, which relates the quality and shelf life. The protein is the third major class of nutrients. There are thousands of proteins founds in nature which vary in their composition and size. All the proteins contain the elements carbon, hydrogen, oxygen and nitrogen. Proteins are essential for tissue building and maintaining N equilibrium. Protein in the diet should be derived from different sources and provide at least 10 to 12 per cent energy. A good quantity of protein is essential for proper growth. The value of protein content ranged between 17 and 18.87 for the sample prepared with 22.50^{0}C of Temperature + 27.07% of Fat and 22.50^{0}C of Temperature + 12.93% of Fat, respectively (Table 2). Following equation is the regression model obtained for protein content-

Protein content (%) = + 18.12 - 0.053 * A - 0.51 * B

Table 6 shows that the linear model was suggested by the analysis and was found significant on the analysis of variance at (p<0.05), the linear model showed low (R^2 = 0.4324). Fig 4 shows the response surface plot, describing the influence of temperature and fat on calculated response variable (Protein content).

Effect on MPR

Table 2 represents the results of MPR of semi dry fermented sausage produced by using different levels of fat and temperature conditions according to Response Surface Methodology. Semi dry sausages have low moisture content (40-50%). The ratio of moisture content and protein is known as moisture protein ratio (MPR) and it has been considered a standard property. MPR of semi dried fermented sausages was found apparently increased due to increase the percentage of fat. That indicates the decrease in protein content was more than the decrease of moisture content due to increase the level of fat. However, in both levels of fat, the values of MPR-

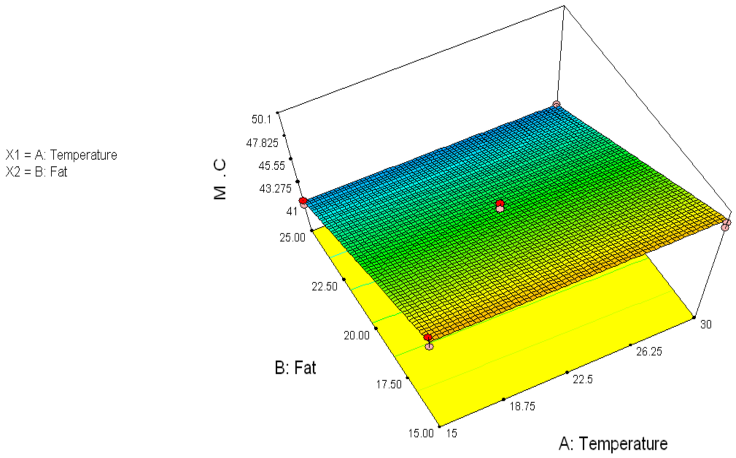

Fig. 2: 3D response surface plot, showing the dependency of FS moisture content (%) on temperature and fat.

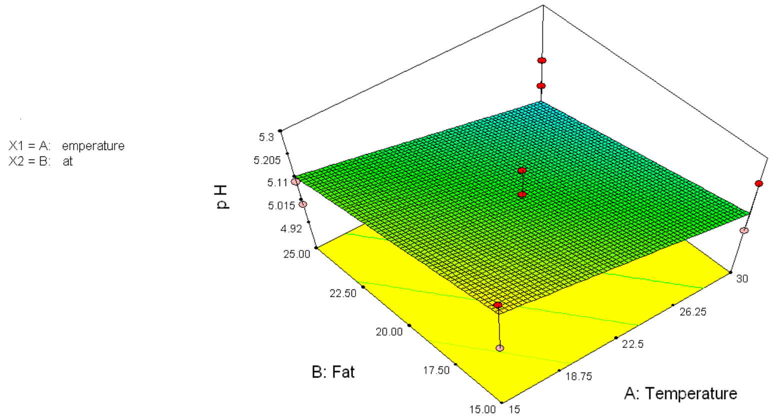

Fig. 3: 3D response surface plot, showing the dependency of pH of FS on temperature and fat

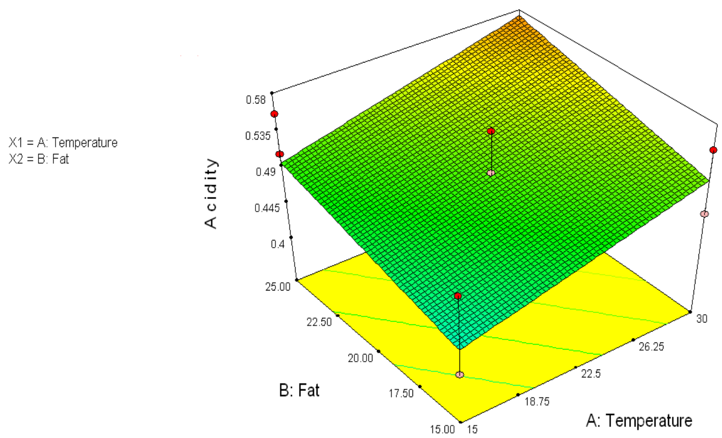

Fig. 4: 3D response surface plot, showing the dependency of FS Acidity on temperature and fat

Table 4 ANOVA for pH

SOURCE	SS	dF	MS	F-VALUE	P value Prob>F	
Model	0.21	2	0.11	9.19	0.0018	significant
A-Temp.	0.14	1	0.14	11.74	0.0030	
B-Fat	0.077	1	0.077	6.63	0.0190	
Residual	0.21	18	0.012			
Lack of fit	0.096	6	0.016	1.71	0.2016	Not significant
Pure error	0.11	12	9.333E-003			
R^2	0.311					

Table 5: ANOVA for Acidity

SOURCE	SS	dF	MS	F-VALUE	P value Prob>F	
Model	0.036	2	0.018	8.68	0.0023	significant
A-Temp.	0.023	1	0.023	10.82	0.0041	
B-Fat	0.014	1	0.014	6.54	0.019	
Residual	0.038	18	2.090E-003			
Lack of fit	0.014	6	2.359E-003	1.21	0.3671	Not significant
Pure error	0.023	12				
R^2	0.2868					

Table 6: ANOVA for Protein Content

SOURCE	SS	dF	MS	F-VALUE	P value Prob>F	
Model	4.25	2	2.12	10.69	0.0009	significant
A-Temp.	0.044	1	0.044	0.22	0.6419	
B-Fat	4.20	1	4.20	21.16	0.0002	
Residual	3.57	18	0.20			
Lack of fit	1.89	6	0.32	2.26	0.1086	Not significant
Pure error	1.68	12	0.14			
R^2	0.4324					

obtained in this study were in the range of 1.751-2.097. MPR should be in the range of 3.7-1.0 (AMI, 1982). That was in accordance with FSIS (1986); Ricke and Keeton (1997); Doyle (2001). Guidance from the Food Safety and Inspection Service/United States Department of Agriculture (FSIS/USDA) requires that shelf stable semi-dry and dry sausage be nitrite cured, fermented, and smoked, and have MPR of ≤3.1:1 and ≤1.9:1, with a final of pH ≤ 5.0 (American Meat Institute Foundation, 1997). The value of MPR ranged between 2.24 and 2.75 for the sample prepared with 15^0C of Temperature + 25% of Fat and 22.50^0C of Temperature + 12.93% of Fat, respectively. Following (Eq.) is the regression model obtained for MPR-

$$\mathbf{MPR = +2.53 - 4.550E\text{-}004 * A - 0.15 * B - 0.036 * A^2}$$

Table 7 shows that the quadratic model was suggested by the analysis and was found significant on the analysis of variance at (p<0.05), the quadratic model showed high (R^2 = 0.8123). The coefficient of linear term of factors indicates that the MPR is negatively correlated with the amount of temperature and fat. The values (F value) show that the fat has more effect as compared to temperature. Fig 6 shows the response surface plots, describing the influence of temperature and fat on calculated response variable MPR. Starting from the initial point (A=15^0C, B=15%) value of MPR was found 2.65 and decreased to 2.49

when fat increased to 20% and further decreased to 2.34 when fat level reached to 25%.

Effect on TBA

Table 2 represents the results of TBA of semi dry fermented sausage produced by using different levels of fat and temperature conditions according to Response Surface Methodology. Thiobarbituric acid (TBA) number is important relevant characteristics of meat product that indicate the oxidation state and later on rancidity of the product. The semi-dry fermented sausages after preparation were packed in combination film under atmospheric packaging systems. The samples contained sufficient fat and therefore samples might be oxidized by atmospheric oxygen and may lead to develop warm over flavour (WOF). WOF is related to sensory quality of the products which is unacceptable by the consumer. TBA measurements have been frequently found to give useful correlation with sensory scores, in looking at the development of WOF in cooked meats (Poste *et al.,* 1986). TBA number was determined as mg of malonaldehyde/kg. Malonaldehyde is produced as a result of fat oxidation and it react with TBA reagent to produce coloured complex with an absorption max/min 530-532 nm. The red pigment produced is the reaction product obtained from condensation of two moles of TBA reagent with one mole of malonaldehyde (Sinnhuber *et al.,* 1958). The value of TBA ranged between 0.23, for the sample prepared with 22.50^0C of Temperature + 12.93% of Fat, and 0.385 prepared with 22.50^0C of Temperature + 27.07% of Fat. Following equation is the regression model obtained for TBA-

$$\textbf{TBA} = +0.30 + 1.306E\text{-}003 * A + 0.050 * B$$

Table 8 shows that the linear model was suggested by the analysis and was found significant on the analysis of variance at (p<0.05). The linear model showed high (R^2 = 0.9729). The coefficients of linear term of factors indicate that the TBA is positively correlated with the amount of temperature and fat. Ahmad *et al.* (2010) and Coşkune *et al.* (2010) reported that TBA number of SDFS increased during refrigerated storage. Ali (2011) reported that TBA values of ground beef increased gradually and significantly (p<0.05) during storage period. The values (F value) show that the fat has more effect as compared to temperature. Fig 7 shows the response surface plot, depicting the influence of temperature and fat on calculated response variable TBA. Starting from the Centre point (A=22.5^0C, B=20%) value of TBA was found 0.302 and decreased to 0.251 when fat decreased to 15% and increased to 0.352 when fat level reached to 25%.

Effect on sensory characteristics

The results of sensory evaluation of semi-dry fermented sausage (SDFS) with different levels of fat temperature condition have been presented in Table 2. Sensory characteristics were measured in terms of colour, aroma, taste, texture, juiciness and overall acceptability on nine point hedonic scale (Ranganna, 2008). Evaluations of sensory characteristics of SDFS were conducted by a group of trained and semi trained panellists on 9-point hedonic scale. '9' expressed liked extremely while '1' expressed dislike extremely. The fermented sausages had bright red colour after smoking and subsequent drying. The complete time taken for fermentation was 1-5 days. At higher temperature and humidity the time decreased and *vice versa.* All the fresh fermented sausages samples which had the score values between '8.1' and '8.65' (It represented condition between liked very much and liked extremely) for the sample prepared with 22.50 ^{0}C of Temperature + 12.93% of Fat and prepared with 15^0C of Temperature + 25% of Fat. Following equation is the regression model obtained for sensory characteristics-

$$\textbf{Sensory Characteristic} = +8.49 - 0.012 * A + 0.15 * B - 0.065 * A * B - 0.067 * B^2$$

ANOVA Table 9 shows that the quadratic model was suggested by the analysis and was found significant on the analysis of variance at (p<0.01). The quadratic model showed high (R^2 = 0.8747). The coefficient of quadratic term of factors indicates that the sensory characteristics are influenced by temperature and fat. The effect of temperature was very weak and fat had a very high effect as indicated by their respective F values. Fig 8 shows the response surface plot, depicting the influence of temperature and fat on sensory characteristics. Starting from the centre point (A=15^0C, B=15%) value for sensory characteristics was found 8.21 and increased to 8.5 when fat increased to 20% and further increased to 8.65 when fat level reached to 25%.

Conclusion

All fermented sausages in fresh condition were good in physico-chemical characteristic like pH (4.8-5.4), TBA No. (0.232-0.385), moisture content (39.5-27.07) and sensory characteristic as described by sensory score values were found 8.1-8.7, sensory attribute were colour, flavor, texture, taste, mouth -

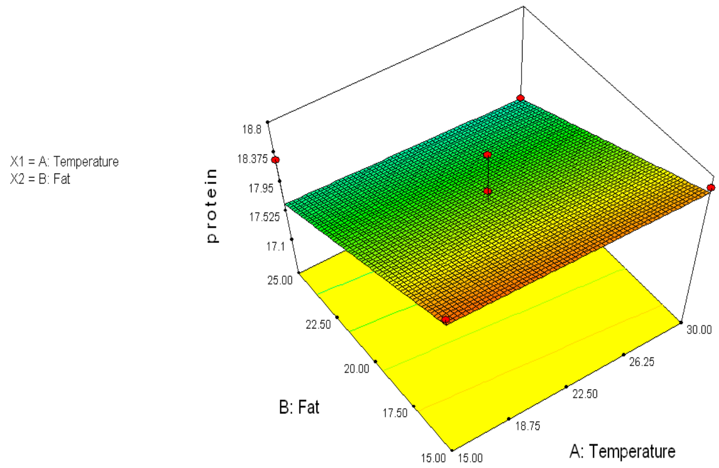

Fig. 5: 3D response surface plot, showing the dependency of FS Protein on temperature and fat

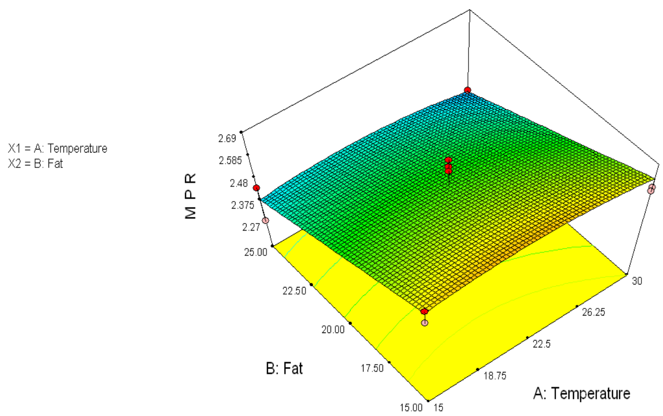

Fig. 6: 3D response surface plot, showing the dependency of Moisture Protein Ratio of FS on temperature and fat

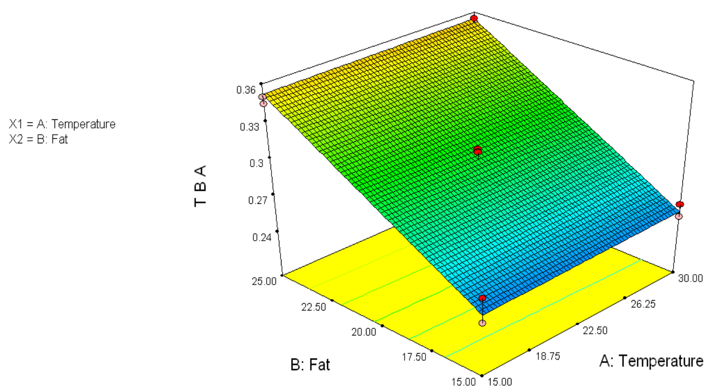

Fig. 7: 3D response surface plot, showing the dependency of TBA No. of FS on temperature and fat.

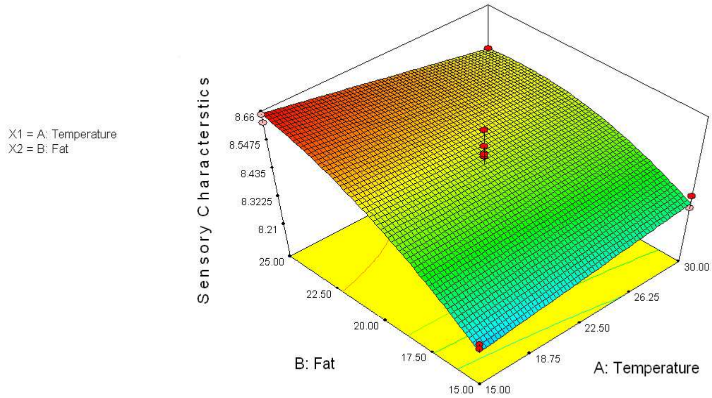

Fig. 8: 3D response surface plot, showing the dependency of sensory characteristics of FS on temperature and fat

Table 7: ANOVA for MPR

SOURCE	SS	dF	MS	F-VALUE	P value Prob>F	
Model	0.39	3	0.13	37.59	<0.0001	significant
A-Temp.	3.312E-006	1	3.412E-006	9.668E-004	0.9756	
B-Fat	0.37	1	0.37	108.38	<0.0001	
A	0.015	1	0.015	4.38	0.0517	
Residual	0.058	17	3.425E-003			
Lack of fit	0.030	5	5.926E-003	2.49	0.0910	Non-significant
Pure error	0.029	12	2.383E-003			
R^2	0.8123					

Table.8: ANOVA for TBA

SOURCE	SS	df	MS	F-VALUE	P value Prob>F	
Model	0.041	2	0.020	437.45	<0.0001	significant
A-Temp.	2.730E-005	1	2.730E-005	0.59	0.4528	
B-Fat	0.041	1	0.041	874.32	<0.0001	
Residual	8.344E-004	18	4.635E-005			
Lack of fit	2.376E-004	6	3.960E-005	0.80	0.5907	Non-significant
Pure error	5.968E-004	13	4.973E-005			
R^2	.9729					

Table 9: ANOVA for Sensory Characteristics

SOURCE	SS	df	MS	F-VALUE	P value Prob>F	
Model	0.47	4	0.12	45.58	<0.0001	significant
A-Temp.	2.356E-003	1	2.356E-003	0.91	0.3544	
B-Fat	0.38	1	0.38	147.72	<0.0001	
AB	0.034	1	0.034	13.05	0.0023	
B	0.053	1	0.053	20.63	0.0003	
Residual	0.041	16	2.590E-003			
Lack of fit	0.014	4	3.523E-003	1.55	0.2512	Non-significant
Pure error	0.027	12	2.279E003			
R^2	0.8747					

coating and juiciness. Progress of fermentation by mix culture of lactic acid bacteria, was recorded by drop of pH with respect to time. In principle fermentation smoking and drying reduced the pH and moisture content. The reduced moisture content and low pH was declaration for extended shelf life of semi dry fermented sausage. The study during the storage stability that semi dry fermented sausage as in combination film had the shelf life of 75 days.

Acknowledgment

Authors gratefully acknowledge Indian Council of Agricultural Research, New Delhi for providing the facility of experiment under Adhac Research Project on fermented sausage.

References

Ahmad S (2005). Studies on quality and shelf life evaluation of buffalo meat sausage incorporated with different levels of heart and fat. *Ph.D. thesis. AMU, Aligarh, India.*

Ahmad S and Amer B (2012). Effect of different cultures of Lactic acid bacteria fermentationon quality and shelflife of semidry fermenrted saudsages of buffalo meat. *Journal of Industrial Research and Technology*, 2(2): 603-609.

Ahmad S and Srivastava PK(2007). Quality and shelf life evaluation of fermented sausages of buffalo meat with different levels of heart and fat. *Meat Science*, 79(2): 603-609.

Ahmad S, Rizawi JA and Srivatava PK (2010). Effect of protein isolate incorporation on quality characteristics and shelf life of buffalo meat emulsion sausages. *Journal of Food Science and Technology*, 47(2): 290-294.

Ali RFM (2011). Antioxidative effects of pomposia extract, on lipid oxidation and quality of ground beef during refrigerated storage. *American Journal of Food Technology*, 6(1): 52-62.

American Meat Institute Foundation (1997). Good manufacturing practices for fermented dry and semi-dry sausage products. www.amif.org/Factsand-Figures/Sausage.pdf

AMI (1982). Good manufacturing practices, fermented dry and semi dry sausage. *American Meat Institute, Washington. D.C.*

APHA (1992). Compendium of methods for microbiological examination of foods. ML Speck (Ed) *American Public Health Association, Washington, DC.*

Coşkuner Ö, Ertas A H and Soyer A (2010). The effect of processing method and storage time on constituents of Turkish sausages (*sucuk*). *Journal of Food Processing and Preservation*, 34: 125-135.

Doyle MP, Beuchat LR and Montville TJ (2001). Fermented meat, poultry and fish products, In food microbiology. *ASM Pub. N.W. Washington*. pg-865.

Ensoy U, Kolsarici N, Candoğan K and Karslioğlu B (2010). Changes in biochemical and microbiological characteristics of turkey *sucuks* as affected by processing and starter culture utilization. *Journal of Muscle Foods*, 21: 142-165.

FSIS (1986). Standards and Labeling Policy Book. Food Safety and Inspection Service. *Standards and Labeling Division. Washington, DC.*

Liaquati S and Srivastava P (2010). Studies on quality and shelf life evaluation of buffalo meat sausages, utilization of byproducts for value addition and improve meat process economy. *LAP Lambert Academic publishing, Germany*. Pp 60-70.

Olivares A, Navarro JL, Salvador A and Flores M (2010). Sensory acceptability of slow fermented sausages based on fat content and ripening time. *Meat Science,* 86: 251-257.

Poste LM, Willemot C, Butler G and Patterson C (1986). Sensory aroma scores and TBA values as indices of warmed-over flavour in pork. *Journal of Food Science*, 51: 886-888.

Ranganna S (2002). Fruit Juices, Concentrate and Beverages In 'Handbook of analysis and quality control for fruit and vegetable products, Second Edition.'*Tata Mc Graw Hill Pub. Co. Ltd. New Delhi.* pp 879-81.

Ranganna S (2002). Sensory Evaluation In 'Handbook of analysis and quality control for fruit and vegetable products Second Edition.' *Tata Mc Graw Hill Pub. Co. Ltd. New Delhi.* pp 623-24.

Ricke SC and Keeton JT (1997). Fermented meat, poultry, and fish products. Ch. 33 in Food Microbiology: Fundamentals and Frontiers, 1st Ed., Doyle, M.P., Beuchat, L.R., and Montville, T.J. (Ed.). *ASM Press, Washington, DC.* pp 610-628.

Sharma N (1987). Processed meat products and their potential in 2000 AD. Advances in meat research. J.B. Khot, A.T. Sherikar, B.M. Jayarao, Pillai (Eds). *Red and Blue Cross Publisher, Bombay.*

Sinnhuber RO, Yu T C and Yu T C (1958). Characterization of red pigment formed in the 2-thiobarbturic acid determination of oxidative rancidity. *Food Research,* 17: 550.

Stanton WR (1985). Food fermentation in the Tropics. In, Microbiology of fermented foods. Vol. 2. J.B. Brian (Ed.), pp 193-211. *Elsevier Applied Sci. Pblishers, London.*

Strange ED, Benedict RC, Smith JL and Swift K (1977). Evaluation of rapid tests for monitoring alteration in meat quality during storage. *Journal of Food Protection*, 40: 843-847.

UN (1979). Workshop on research and development needs in the field of fermented foods. *United Nations International Symposium on microbiological aspects of food storage, processing and fermentation in Tropical Asia, Dec. 14-15, Bogor, Indonesia*.