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Optimisation of buffalo (*Bubalus bubalis*) meat sausage with animal fat and carrot powder using response surface methodology

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Abstract
The prevalence of non-communicable diseases is increasing alarmingly owing to several aetiological factors with dietary regime playing the prime cause. The study was carried out to develop functional sausages of buffalo meat fortified with carrot powder (0-5%) and 20-30% buffalo white fat. Nine variants of sausages were optimized using the response surface methodology and the quality and shelf life of carrot fortified buffalo meat sausages was determined on the basis of physicochemical characteristics *viz*., moisture content (60-62%), ash content (1.8-2.18%), fat content (14-16.20%), protein content (18.20-18.56%), pH value (6.20-6.35), TBA value (0.08-0.089), microbiological quality *viz*., total plate count (2-2.4 log cfu/gm). The sausages were found with improved sensory quality as compared to control. The overall desirability varies between 0.6-0.7 out of unit scale. The sausage sample with fat content 28.54% and carrot powder 4.27% was found with good quality features as compared to control. The sausage samples were found within the acceptable limit of microbial standards.

Keywords: Sausage, buffalo meat, carrot powder, fortification, optimization

1. Introduction
Meat is rich in protein, lipids, minerals, and vitamins which are essential for the structural and functional development of human body. Several plant based foods (e.g. soybean) and algae (e.g. chlorella) have high protein content but are of low biological value. The consumption of meat has drastically increased in recent years. In developing countries the average annual per capita consumption of meat consumption increased from 10 kg in 1960s to 26 kg in 2000 and will reach 37 kg around by 2030 [6]. India produces approximately 56.57% of buffalo meat production world wise [8]. Variety of meat products *viz*., emulsion sausages, cooked sausages, fermented sausages etc, are produced with buffalo meat owing to better emulsion making qualities. The major limitation with its consumption is lack of fibre content.
Several studies have shown the diet rich in dietary fibre is linked with reduction, prevention and treatment of several non-communicable diseases [15, 16]. Apart from the vegetables, rice bran is a good source of dietary fiber and is extensively being incorporated in processed meat products and to fulfill the dietary fiber requirement. The meat balls, incorporated with 10% rice bran were found to have better quality than the other proportion [18]. The rice bran consists of approximately 27% dietary fibre which attributes to laxative property and ability of cholesterol reduction [19]. The fruit waste such as orange peel is being utilized as a source of dietary fiber in bologna sausage [20]. Different levels of pumpkin were incorporated in chicken sausages and the study reported the same sensory quality as well as the acceptability of product as in case of control samples [21].
Meat and meat products needs to be fortified with dietary fibres to make them healthier and functional food. Dietary fibres are most commonly present in plant foods, namely, fruits, vegetables, cereals etc [7]. The The carrot (*Daucus carota L.*) is the important root vegetable of Apiaceae family. The phytochemical analysis of carrot suggested the presence of carbohydrates, essential oil, bioactive components like chlorogenic acid, coumarin, antioxidants like phenolic compounds & carotenoids [1, 2]. Carrot has a number of therapeutic properties *viz*., antidiabetic, antimicrobial, hypotensive, gastro-protective, nephro-protective, hepato-protective, cardio-protective, anti-inflammatory, immune boosting, anticarcinogenic and anticlastogenic [1, 3, 4, 5]. The carrot is generally consumed as salads and in cooked form with other vegetables.

The vegetable incorporation in meat products improves the nutrition value along with the texture, fibres and oxidative stability [9, 10]. A variety of compounds along with dietary fibres are present in the carrots which may prevent different types of cancers by adsorbing or linking with various hydrophobic cancer causing agents [11]. The insoluble fibre-rich fractions prepared from carrot pomace were found to have hypocholesteromic and hypolipidemic effects [12]. The carrot and its different derivatives have been proved beneficial in constipation and to control the cholesterol metabolism due to high amount of dietary fibres. The colour, flavour and overall acceptability were found to be higher in the mutton nuggets incorporated with vegetables (carrot, capsicum and radish) in comparison to control [13]. Different parts of carrot are being used for the preparation of dietary fibres *viz*., carrot peel is utilizing for the production of antioxidant dietary fibre [14] for making fibre-rich meat products. It has been proved that carrot pomace found to have insoluble Fiber-rich fractions (FRFs) composed of pectic polysaccharides, hemicellulose, and cellulose [17].

Although, several studies have been published on the incorporation of carrot powder in pork and poultry meat products. No research has been done on the optimisation of buffalo meat sausage with carrot powder. In this paper, the optimization of animal fat and carrot powder was carried out in buffalo meat sausage by using response surface methodology.

2. Materials and methods

Materials

The buffalo meat and buffalo white fat were collected from the local market in Aligarh Muslim University, India. The buffaloes were slaughtered according to the traditional halal method at slaughter house of municipal corporation Aligarh, India. The animals were kept in lariage for a period of 24 hrs before slaughter. Meat samples from round portion (biceps femoris muscle) of 3.5 yrs aged female carcasses were collected within 2-3 hrs of slaughter. Buffalo fat from brisket and back was collected along with other ingredients. The meat chunks and buffalo fat were packed in low density poly ethylene (LDPE) and were stored at -4 ^{0}C until further use. The other ingredients namely carrots, spices, condiments, were procured from the local market Aligarh, India. The casing material was procured from PRS technologies New Delhi, India. Food grade sodium nitrite and ascorbate was obtained from the meat processing laboratory of Department of Post-Harvest Engineering and Technology, Aligarh Muslim University, Aligarh.

Chemicals

Nutrient agar and potato dextrose agar were procured from Hi-Media, Mumbai, India.

2.1 Preparation of carrot powder

The dark red coloured carrots (*Daucus carota*) were selected and procured from the local market of Aligarh, India. The carrots were subjected to washing, cleaning to remove the adhered dust and dirt at the surface. The carrots were cut, blanched (0.09% saline) to inactivate the enzymes for preservation of colour. Thereafter, blanched carrots were shredded and tray dried (Royal Scientific Instruments 103-B, 2012) at 50-60 ºC for 48 hrs till the moisture content was 5% on wet basis. The dried shredded carrots were grounded to fine powder with mixer grinder (Philips, HL7756/00,750 W). The carrot powder was packed in low density polyethylene packages for further analysis.

2.2 Sausage formulations and processing

The lean meat was chopped and minced. The sausages were prepared by mixing lean meat, buffalo white fat, carrot powder, spices, condiments and other ingredients as depicted in Table 1. The flow chart for the preparation of sausages is shown in Figure1. The ice was added to maintain the temperature between 8-10 °C for stable emulsion. The meat mix was then transferred to stuffer/sausage filler machine (Sirman IS-16/Aries) to mould into a sausage. The sausages were steamed at 70 ˚C for 30 minutes, cooled and thereafter, the casing was removed. The sausages were then packed in low density poly ethylene (LDPE) packages under normal atmospheric condition.

Table 1: Composition of carrot fortified buffalo meat sausage.

Ingredients	Amount (g)
Lean meat	1500
Fat	300
Carrot powder	0-5
Spices and Condiment mix	15
Onion: Ginger: Garlic paste (60:20:20)	40
Sodium nitrate	0.30
Sodium ascorbate	0.75
Salt	As per taste
Ice	100

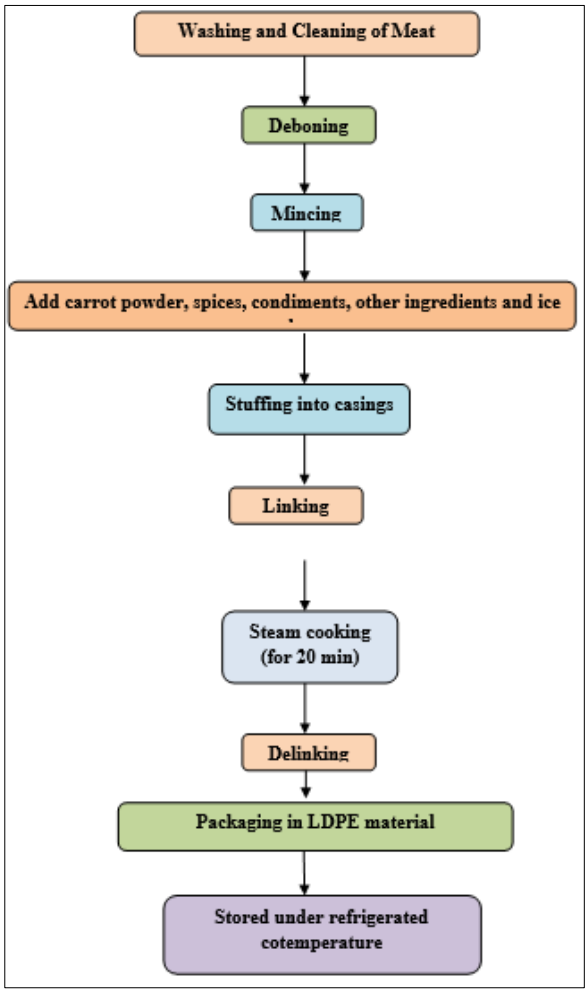

Fig 1: Schematic representation of preparation of carrot fortified buffalo meat sausage

2.3 Analytical methods

2.3.1 pH: The pH of carrot fortified buffalo meat sausages was determined by digital pH meter (Model XT 22, Metzer, New Delhi, India). To 10 g of finely ground sample 50 ml of distilled water was added in a Cyclo Mixer (CM-101, Yorco, New Delhi, India). The extract was filtered through Whatman No. 1 filter paper and the pH of the sample was recorded.

Moisture content: The moisture content of sausages was evaluated as per AOAC method (Association of Official Analytical Chemists) given in Food Industry Manual [18] by using hot air oven (Model ASO, Yorco, New Delhi, India) thermo statistically controlled at 150 ± 5 °C.

2.3.2 TBA number: The TBA value of carrot fortified buffalo meat sausage was determined by the TBARS method [23]. The absorbance was read at 530 nm in a Spectrometer (Model 310E, Electronic Corporation of India, Hyderabad, India).

2.3.3 Microbial analysis: The carrot fortified buffalo meat sausage samples were analyzed for total plate count (TPC, 37 °C, 48 h, nutrient agar) and yeast and mold count (Potato dextrose agar, 3 °C, 48 h) according to the method reported by American Public Health Association [24].

2.3.4 Sensory analysis: The organoleptic evaluation of carrot fortified buffalo meat sausages was carried out using 9-point Hedonic scale by semi-trained panelists from the Department of Post-Harvest Engineering and Technology, Aligarh Muslim University, Aligarh, India, for various attributes namely colour, flavour, texture, taste, mouth feel, juiciness and overall acceptability.

2.4 Experimental Design

RSM was used to determine the best conditions for optimization of the carrot fortified buffalo meat sausages for varied physicochemical parameters. A CCD was performed to evaluate the effect of carrot powder and animal fat on the quality of meat product. The carrot powder and buffalo white fat were independent variables. The levels of these factors were generated by applying rotatable central composite design (RCCD). The RCCD design suits for fitting quadratic surface, which works well for optimizing composition. The minimum and maximum levels of both the ingredients were selected on the basis of preliminary trials.

The CCD for two factors response comprises of following the basis of preliminary trials.

1. The central points (0, 0) were taken at the centre.
2. The factorial points (-1, 0), (1, 0), (0, -1), (0, 1)
3. The axial points (-1.414, -1.414), (-1.414, 1.414), (1.414, -1.414), (1.414, 1.414)

Table 4: The coded and actual levels of factors are represented in Coded and actual levels of factors

S. No.	Point type	Coded value	Fat (%)	Carrot Powder (%)	No. of replicates
1	Factorial	(1, 1)	28.54	4.27	3
2	Axial	(1.414, 0)	30	2.5	3
3	Factorial	(1, -1)	28.54	0.73	3
4	Axial	(0, -1.414)	25	0.00	3
5	Factorial	(-1, 1)	21.46	4.27	3
6	Centre	(0, 0)	25	2.5	3
7	Axial	(-1.414, 0)	20	2.5	3
8	Factorial	(-1, -1)	21.46	0.73	3
9	Axial	(0, 1.414)	25	5	3

2.4.1 Optimization of different levels of fat and carrot powder in sausage samples

The optimization of fortified carrot buffalo meat sausages with varied levels of buffalo white fat and carrot powder was conducted using response surface methodology and the influence of both the variables on different physicochemical characteristics were evaluated.,

3. Results and Discussion

3.1 Moisture content

The moisture content is an important proximate constituent that influences the texture and sensory properties of meat products. The foods with higher moisture content are comparatively more prone to microbial spoilage. The impact of animal fat and carrot powder on the moisture content of sausages has been presented in Fig. 2a. The moisture content of freshly prepared sausages increased with increase in level of incorporation of carrot powder probably due to high water retention and swelling capacity of carrot powder. Improved water binding capacity of meat products had been reported earlier by incorporating 2% of carrot powder in the pork sausages [25]. Similar findings had been reported by incorporating dried carrot powder in the chicken cutlets [26]. While, the other independent variable, the animal fat (21-25%) did not bring any considerable increase in the moisture content of sausages. The response surface plots (Fig. 2a) depicts that the incorporation of carrot powder brought considerable improvement in the moisture content as compared to fat content.

3.2 Ash content

The ash content depicts the mineral content present in the sausages. Buffalo meat is good source of minerals *viz*., iron, zinc, phosphorus, potassium and manganese etc. Fortification with carrot powder further enhanced the mineral content (Fig.2b) Starting from the initial point (A= 21.46%, B= 0.73%) the ash content was found to be 1.958 %. The increase in the ash content attributes to the fact that carrot powder contains 1.1% of total ash content [27]. Similar findings have been reported earlier demonstrating that incorporating dried carrot powder in the chicken cutlets increased the ash content [26]. The animal fat, however, did not considerably affect the ash content of the sausages as animal fat lacks mineral content.

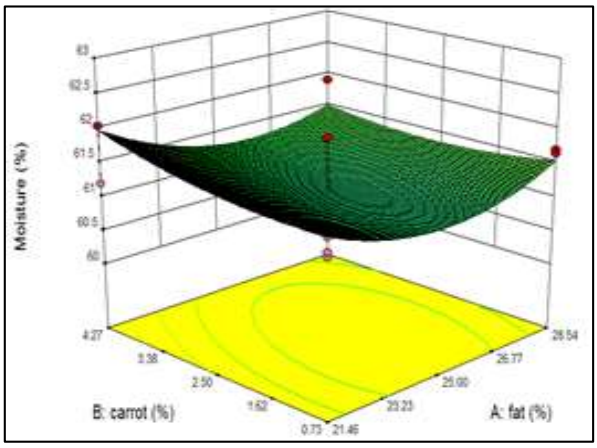

Fig 2a: Moisture

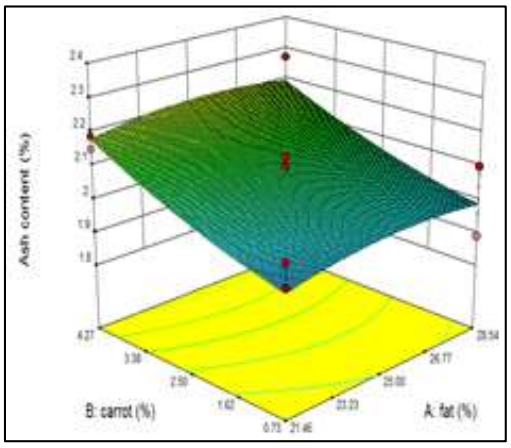

Fig 2b: Ash content

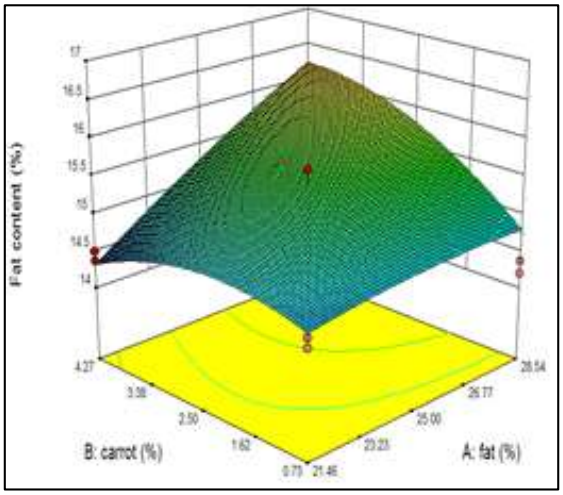

Fig 2c: Fat content

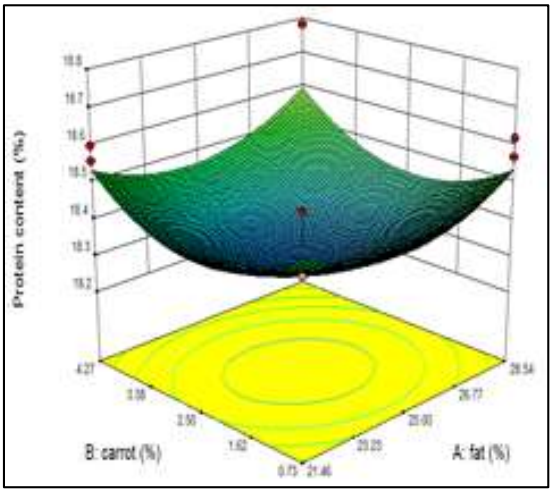

Fig 2d: Protein content

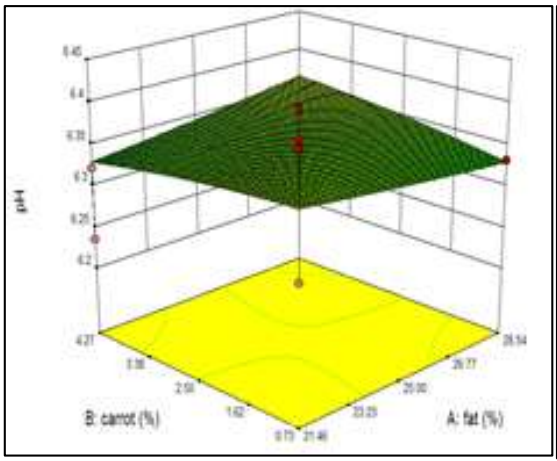

Fig 2e: pH

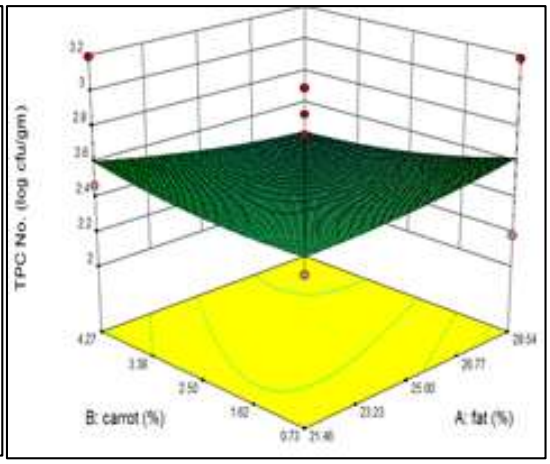

Fig 2f: TPC

Fig 2: Response surface plots showing nutritional and physicochemical parameters dependency on animal fat and carrot powder

3.3 Fat content

Fat content plays a crucial role in emulsification of meat proteins and water. The incorporation of carrot powder did not result in any significant change in fat content. Starting from the initial point (A= 21.46%, B= 0.73%) the fat content was found to be 14.63 %. Fig.2c shows that the total fat content of sausages did not markedly increased by increasing the incorporation of animal fat probably due to fat losses during cooking. The results revealed that some of the fat molecules participated in emulsion formation with muscle proteins while remaining fat molecules were free within the matrix. In contrast to present study, several studies had been performed on the addition and reduction of the animal fat in the sausages and they had reported a linear relationship between the animal fat added and the total fat content of the sausages [28-31].

3.4 Protein content

Protein is the key ingredient in body building and a source of essential amino acids required for various metabolic

processes. In sausage making, protein plays a crucial role in emulsion formation. The protein molecules particularly myofibrillar proteins entrap the fat globules during emulsification. Fig. 3d depicted the slight increase in the protein content with incorporation of both the animal fat and carrot powder. However, this increase in the protein content with animal fat was not significant as the tallow or animal fat does not contain any proteins. This slight increase in the protein content of sausages might be attributes to the 6.16% protein fraction of carrot powder [32]. In contrast to the present study, several researchers performed the investigations by incorporating the carrot powder in chicken cutlets and demonstrated a slight decline in the protein content of meat products [26].

3.5 Effect on pH
The developed sausages were packed in low density polyethylene (LDPE) packages under normal atmospheric condition (T: 25±2 ˚C; P: 1atm). Generally high acidic foods are less prone to bacterial spoilage but have higher likelihood of mould spoilage. The pH of sausages did not changed with the incorporation of animal fat and carrot powder probably due to the stability of meat emulsion.

3.6 Effect on TPC value
The TPC (Total Plate Count) is a microbiological assay that determines the quality and shelf life of the sausages. Meat can be evaluated on the basis of microbial count for different quality *viz*., 10^2 CFU/gm as excellent; 10^4 CFU/gm as good; 10^6 CFU/gm for rejection limit in many commercial meat products, 10^8 CFU/gm gives off flavours and odours while at 10^9 CFU/gm becomes slimy and the texture is lost. Fig.2f. indicates that the TPC value was not affected considerably with the independent variables *viz*., animal fat and carrot powder.

3.7 Sensory Evaluation
The sausages were evaluated by semi trained panel for varied sensory attributes namely colour, texture, taste, juiciness, mouth coating and palatability on 9 point hedonic scale where "9" denoted liked extremely and "1" denoted disliked extremely. The fried sausages were presented to semi-trained panel along with water for rinsing mouth. Fig.3a. exhibits sensory attributes of carrot fortified buffalo meat sausages which showed an upsurge with increase in the level of carrot powder while the increase in fat content showed negative effect. The sensory appraisal results revealed that the incorporation of carrot powder was more acceptable in comparison to the incorporation of animal fat content.

3.8 Desirability of the sausages
Fig 3b. Indicates that the desirability significantly ($p<0.05$) improved with increased levels of carrot powder as well as buffalo white fat. The carrot fortified meat sausages showed desirable effects owing to the high dietary fibre content.

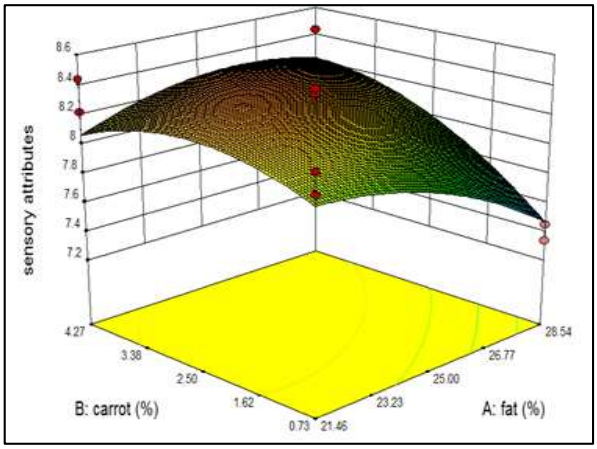

Fig 3a: Sensory Characteristics

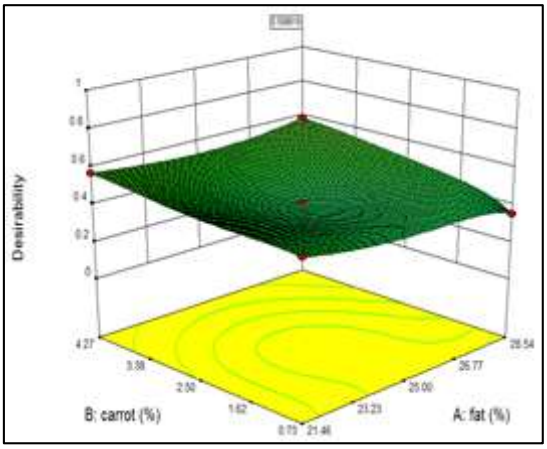

Fig 3b: Desirability

Fig 3: Response surface plots showing sensory attributes dependency on animal fat and carrot powder

4. Conclusion
The results of studies confirmed the optimization of carrot powder and animal fat to produce functional sausages with improved physicochemical, microbial, nutritional and sensory quality. Incorporation of animal fat and carrot powder enhanced the proximate composition besides improving antioxidative potential.

5. Acknowledgements
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6. Conflict of Interest
The authors declare no conflict of interest and the article has not been communicated in any other journal for the publication purposes.

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