

Optimization and quality evaluation of freeze dried mutton manchurian

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<u>Abstract</u>

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Introduction

Development of safe, convenient and nutritionally sound preserved meat variety foods have a unique demand in the consumer market. Meat is known as one of the major source of animal protein and appears in the diet of millions of people around the globe breaking all the codes of region, religion and race. From the nutritional point of view, meat's importance had derived from its high quality protein, containing all essential amino acids and bio-available minerals and vitamins. Major sources of animal proteins are from cattle, sheep, goat, camel, pig and fishes etc. Meat is also specifically valuable as a source of omega 3 fatty acids, bio-available iron, vitamin B12, which is not readily available in vegetarian diet (Bender, 1992; Verma and Banerjee, 2010). Population growth, urbanization, economic growth and flourishing market all leads to the increasing demand of meat and animal products (Delgado, 2003; Costales et al., 2006; Steinfeld et al., 2006). India is the second highly populated country and the requirement of meat protein is mainly met from mutton and chicken. However, in some parts of the country beef and pork also find its place. Mutton has a distinct flavor and texture (Wu et al., 1991) when compared to other meat sources and leads to unfamiliarity and dislike (Griffin et al., 1992; Rhee et al., 2000) among the consumers. In most of the mutton based Indian cuisines, a unique combination of vegetables and different spices are liberally used to overcome or reduce the inferior flavor of mutton, making it popular among the Indian

Studies were conducted on standardization and optimization of Mutton manchurian (MM) using Response Surface Methodology and evaluation of physico-chemical and microbial properties of optimized fresh (FP) and freeze dried (FD) MM products were carried out. The central composite rotatable design for two factors was used to optimize MM with tomato ketchup (80-120 grams) and onion garlic mix (80-120 grams) as independent variables and overall acceptability as a response. Optimized product was FD to extend shelf life and to retain sensory properties. Physico-chemical quality parameters like texture profiles, color values, density, water activity, pH, acidity, moisture, fat, protein, ash and microbial analysis of standard plate count, coli forms, yeast & mold count were determined for the fresh and freeze dried samples. Moisture content and water activity values decreased during FD whereas color, acidity, pH, protein, fat and ash content significantly increased in FD sample as compared to FP sample.

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sub-continent population. According to FAO (2010) India stands sixth in the world in terms of sheep and goat population with a healthy number of 800 million and contributes to the production of milk and meat to meet the country's food concerns. Manchurian is a term related to a unique Chinese method of food preparation using chopped vegetables and different sauces like tomato, green chili and soy in appropriate proportions with some spices. Manchurian is very tasty and has a universal flavor and texture which makes it acceptable to all ages. Different kinds of manchurian's are prepared and served in hotels and restaurants all over India i.e. cauliflower manchurian, baby corn manchurian, soy manchurian etc. Utilizing the same method of preparation in optimizing and developing a high protein manchurian using a design tool has got a wide range of application especially with mutton because of its wide popularity and availability in India. Preserving the same with any dehydration process will help in retaining the nutritional and physico-chemical attributes of the product for the convenient reconstitution.

Dehydration is an important intermediate step in turning a raw food or food product into retail products. Among all the dehydration techniques known, Freeze dehydration (FD) is the most sophisticated and highly efficient method employed in the preservation and storage of biological components. FD is the method of preserving a product by removing the moisture by sublimation process by applying low temperature and vacuum pressure conditions. FD prevents physicochemical deterioration, microbial and enzymatic activities in the product as the whole process is carried out in the absence of liquid water and low temperature and pressure (Ratti, 2001).

Statistical design tools such as response surface methodology (RSM) helps quite effectively in optimizing the independent variable levels in product and process parameters based on statistical and mathematical techniques. The RSM technique gives the effect of an individual parameter as well as interactive effect of the parameters (Yadav et al., 2007). RSM has been successfully employed in optimizing processing variables for the preparation of soy-fortified curd (Yadav et al., 2008), virgin coconut meal incorporated ladoo (Srivastava et al., 2011), carrot dehydration process (Madahar et al., 1989), as well as optimization of pepper based appetizers (Wadikar et al., 2008) and ginger based appetizers (Wadikar et al., 2010). RSM can be used in problems that have ingredients and / processing conditions as variables (Arteaga et al., 1994). RSM has successfully applied to optimize food-processing operations (King and Zall, 1992; Oomab and Mazza, 2001: Madamba, 2002). Therefore attempt was made to develop and establish the nutritional quality parameters of MM to observe consumer acceptance.

Material and Methods

Raw material selection

Mutton leg portion cuts were procured from a local meat vendor in Mysore (India). Large meat cuts were washed thoroughly and cut into medium size pieces. Other ingredients used for the preparation include, Onions, garlic, tomato ketchup, chili sauce, soya sauce, pepper, green chilies, capsicum, monosodium glutamate (MSG), corn flour, salt and refined sunflower oil.

Experimental design

Experiment was designed using response surface methodology (RSM). In this design, two-factor central composite rotatable design (CCRD) was applied to determine the best combination of ingredients to optimize overall acceptability using software State– Ease (Design Expert version 6.0.10). Two factors were onion-garlic mix and tomato ketchup and one response overall acceptability (OAA). It consisted of four factorial points, five central points and four axial points leading to 13 sets of experiments ((Myers and Montgomery, 2002). Quadratic model used to describe the response variables is as follows.

$$Y = b_{o} + b_{1} X_{1} + b_{1} X_{2} + b_{3} X_{1}^{2} + b_{4} X_{2}^{2} + b_{5} X_{1} X_{2}$$

Where Y= Response (Dependent variable): $X_1 =$ Onion Garlic (Independent variable); $X_2 =$ Tomato Ketchup (Independent variable); b_0 , b_1 , b_2 , b_4 , $b_5 =$ Response model variables.

Standardization and processing of MM

To optimize the quantity of onion garlic and tomato ketchup added in MM constraints like upper and lower limits of onion garlic and tomato ketchup values were fixed as 80 and 120, 80 and 120 respectively based on preliminary studies. Other ingredients used in the processing include corn flour, soya sauce, chili sauce, capsicum, green chilies, pepper, monosodium glutamate (MSG), oil and salt.

Mutton was mixed with corn flour and salt then kept aside for 10 min. It was then deep fat fried at 170° C for 2 min. Vegetables were roasted in an open pan and fried mutton was added along with the proportion of other ingredients and cooked thoroughly. Organoleptic evaluation of MM was done on a 9-point hedonic scale by a panel of trained panelists (n = 13) and numerical scores assigned by them to each attribute (Peryam and Pilgrim, 1957). Overall acceptability of each experiment of the product was found out and used for optimization.

Freeze drying

Freeze drying of the optimized MM was carried out in a pilot plant freeze dryer (Martin Christ GmbH & Co KG, Osterode, Germany) equipped with both freezing and drying facilities. Pre-freezing of the samples were done at -40°C for 4 h and contact drying was done at a temperature of 50°C under a chamber pressure of 100–300 Pa for 12 h. After the freeze drying, samples were taken to a low humidity chamber and packed in paper, aluminum foil, low density polyethylene (LDPE) (45 gsm paper/ 20 μ aluminum foil/ 37.5 μ LDPE) packets of size 20 x 12 cm for further studies under ambient temperature (28+5°C, 60-80% RH) conditions.

Rehydration studies of FD MM

Rehydration or reconstitution of FD MM was done as per the procedure and methods depicted by Huang *et al.* (2009). From the rehydration studies, rehydration ratio and rehydration % were calculated as per the following equation.

$$Rehydration ratio (RR) = \frac{W2 - W1}{W1}$$

$$Rehydration (\%) = \frac{Drained weight of rehydrated sample - Dry matter content of sample}{Drained weight of rehydrated sample} x 100$$

Where, W1 and W2 are initial and final weight of the FD MM sample, respectively.

Proximate composition

Proximate composition of the MM before and after freezing was established as per AOAC (1997) for protein, carbohydrate (by difference method), total ether extract as fat, total ash and moisture. All the chemicals used for the study were of AR grade.

Hunter color estimation

Color values of the product before and after freezing were determined using Hunter colorimeter (Color Flex, CFLX-45-2, Hunter lab, Reston, VA,USA) in terms of L, a* and b* values as per the procedure given by Shand, (2000). Measurement of the color co-ordinates were determined using a D-65 illuminant with a spectral range of 400-700 nm and a spectral resolution of 10 nm after standardizing the sensor using standard black and white colored tiles. L value in the Hunter measurements indicates lightness or darkness, a* value indicates the color co-ordinate with respect to redness and greenness, while b* stands for the shift of color between yellow to blue.

Density, water activity (a_w) , acidity, pH and lipid oxidation

Density (ρ) of the product before and after freeze drying was measured based on Archimedes displacement principle using Ultrapycnometer (Quantachrome Instruments, FL, USA). Nearly 20 g of the sample was taken in a medium sized sample cell and readings were recorded against an average of three runs keeping standard deviation to 0.005% at flow mode using Helium gas. Labmaster-aw (Novasina, Switzerland) model water activity meter was used for recording the water activity (a_w) of the manchurian sample before and after freeze drying. Acidity was determined according to Methods of Ranganna (1995) and pH of the fresh and FD (after reconstitution) samples were measured using a digital pH meter (Cyber scan 510, Singapore) at 25°C. In order to record the pH, 10 g of the sample was well homogenized in a mortar with 40 ml of double distilled water and filtered prior to the analysis. Lipid oxidation profile with respect to free fatty acids (FFA) as % oleic acid (AOAC, 1972) was also studied initially before and after freeze drying.

Texture profile analysis (TPA)

TPA of MM was carried out using a 50 Newton (N) load cell connected to TA plus texture analyzer, (Lloyd instruments, Hampshire, UK). Two bite (two cycles) penetration tests at a constant pre and post-test speed of 30 mm/min using a 4 mm ball probe at the geometric center of the MM was performed. Other test conditions were maintained as clearance 3 mm, 93% penetration to the height of sample and trigger force of 8 gram force (gf). Different parameters like hardness (N) during first (hardness 1) and second cycle (hardness 2), springiness (mm), cohesiveness (viscoelasticity), gumminess (hardness x cohesiveness, N), chewiness (gumminess x springiness, Nmm) were calculated from the graph as depicted by Bourne (1978).

Microbiological analysis

Method of analysis depicted in APHA (1992) were used to establish the microbiological quality of the product before and after freeze drying in terms of standard plate count (SPC), coliforms, *E. coli*, yeast and mold (Y&M).

Organoleptic evaluation

Sensory evaluation of FD MM were carried out by 13 trained panelists on a 9 point hedonic scale (9like extremely, 1- dislike extremely) as per Murray *et al.* (2001) for finding overall acceptability of experimental designed MM and to establish the overall acceptability of the rehydrated FD product. Panelists were trained in attributing rating for the sensory characteristics. Hedonic scale (Larmond, 1982) and assigned scores from liked extremely (9) to disliked extremely (1).

Results and Discussion

Optimization of MM

Sensory evaluation was carried out for experimental design using a nine point hedonic scale and the sensory values are depicted in Table 1. Sample with 80 g onion garlic and 120 g of tomato ketchup scored higher with OAA 8.15 \pm 0.05, sample with 71.72 g onion garlic, 100 g of tomato ketchup scored 7.52 ± 0.02 and sample with 120 g onion garlic, 120 g of tomato ketchup scored 7.03 ± 0.04 whereas sample with 80 g onion garlic and 80 g of tomato ketchup scored least OAA as 6.41 ± 0.03 . Values of OAA in different experiments were used for optimizing the onion garlic and tomato ketchup requirements. FD rehydrated product showed sensory score of OAA as 7.60 ± 0.04 . Table 2 showed design of experiments with overall acceptability. Constraints were kept for optimizing the MM namely onion: garlic and tomato sauce in their respective ranges to maximize overall acceptability. Finally, optimized conditions were obtained as 9.977% onion: garlic and 14.967% tomato sauce with a desirability of a 99.2%. The optimized ingredients for MM are shown in Table 3. A three dimensional representation with respect to onion: garlic and tomato sauce corresponding to overall acceptability is shown as Fig 1. It was observed from the figures that overall acceptability increases near

Table 1. Organoleptic characteristics for fresh MM

Sample	Color	Texture	Flavour	Taste	OAA
1	8.34 ± 0.05	7.87 ± 0.07	7.84 ± 0.06	8.54 ± 0.06	8.15 ± 0.05
2	6.24 ± 0.07	6.64 ± 0.08	6.31 ± 0.06	6.45 ± 0.07	6.41 ± 0.03
3	6.95 ± 0.08	6.29 ± 0.04	7.64 ± 0.07	7.24 ± 0.07	7.03 ± 0.03
4	6.67 ± 0.06	6.34 ± 0.05	7.22 ± 0.04	7.27 ± 0.06	6.88 ± 0.02
5	6.05 ± 0.07	6.47 ± 0.07	6.89 ± 0.06	7.31 ± 0.05	6.68 ± 0.05
6	6.67 ± 0.06	6.34 ± 0.05	7.22 ± 0.04	7.27 ± 0.06	6.88 ± 0.02
7	6.67 ± 0.06	6.34 ± 0.05	7.22 ± 0.04	7.27 ± 0.06	6.88 ± 0.02
8	6.67 ± 0.06	6.34 ± 0.05	7.22 ± 0.04	7.27 ± 0.06	6.88 ± 0.02
9	6.44 ± 0.06	6.23 ± 0.05	7.15 ± 0.06	6.92 ± 0.05	6.69 ± 0.03
10	7.60 ± 0.05	6.06 ± 0.04	7.32 ± 0.05	7.15 ± 0.07	7.03 ± 0.04
11	7.79 ± 0.05	6.88 ± 0.06	7.33 ± 0.05	7.85 ± 0.05	7.51 ± 0.03
12	6.67 ± 0.06	6.34 ± 0.05	7.22 ± 0.04	7.27 ± 0.06	6.88 ± 0.02
13	$7.75 \pm .067$	7.84 ± 0.07	6.57 ± 0.05	7.94 ± 0.04	7.52 ± 0.02
Values represents: Mean \pm SD (n =13)					

Table 2. Real designs for response surface methodology (RSM)

(RSM)						
Even anim an ta	Factor 1	Factor 2	Response 1			
Experiments	X1: Onion Garlic (g)	X2: Tomato Ketchup (g)	Overall Acceptance (Y)			
1	80.00	120.00	8.15 ± 0.05			
2	80.00	80.00	6.41 ± 0.03			
3	120.00	80.00	7.03 ± 0.03			
4	100.00	100.00	6.88 ± 0.02			
5	100.00	71.72	6.68 ± 0.05			
6	100.00	100.00	6.88 ± 0.02			
7	100.00	100.00	6.88 ± 0.02			
8	100.00	100.00	6.88 ± 0.02			
9	128.28	100.00	6.69 ± 0.03			
10	120.00	120.00	7.03 ± 0.04			
11	100.00	128.28	7.51 ± 0.03			
12	100.00	100.00	6.88 ± 0.02			
13	71.72	100.00	7.52 ± 0.02			

Table 3. Optimized ingredients for MM



Figure 1. 3-D Graph for over all acceptability (OAA) of MM

the factorial and axial points, from Fig 1. Desirability plot of optimized ingredients is shown in as Fig 2. Analysis of variance indicated that that quadratic model was best fit with probability greater than F value, which was quite less as 0.0031. Lack of fit test also suggests that it is fitting the best as compared to other models. From the model, summary statistics it is clear that R² value was 0.95 and adjusted R² was 0.9142, which was best compared to other model. Standard deviation for the data found as 0.14. From the analysis of variance also it was observed that the model was found significant (p < 0.001) with very





Figure 2. 3-D Graph for Desirability plot of optimized MM

less pure error of 0.030.

The response quadratic model was obtained as:

 $OAA(Y) = 1.4175 + 0.03516X_1 + 0.06633X_2 +$ $0.0003X_1^2 + 0.0003X_2^2 - 0.0011X_1X_2$

Where, Y, X_1 and X_2 are defined earlier.

Then optimized sample was chosen for freeze drying and for further study.

Proximate composition

Proximate compositions for fresh and freeze-dried samples are depicted in the Table 4. For fresh sample, moisture, protein, fat, total carbohydrates and ash content was found to be $61.60 \pm 0.70\%$, 22.53 ± 1.08 %, 2.90 ± 0.45 %, 12.36 ± 0.57 % and 0.61 ± 0.029 % whereas for FD sample it was $3.61 \pm 0.26\%$, 51.01 $\pm 0.80\%$, 17.27 $\pm 0.22\%$, 26.74 $\pm 0.44\%$ and 1.38 \pm 0.36% respectively. Free fatty acid (FFA) values for fresh and FD samples were determined as 1.74 and 1.96 % oleic acid respectively.

Hunter color Estimation

Hunter color values were found as L 38.77, a* (R-G) = 6.091, $b^{*}(Y-B) = 23.706$ and L 51.11, a^{*} (R-G) = 9.93, b^{*} (Y-B) = 27.453 for fresh and FD samples respectively. It shows that FD product showed increase in color values as compared to fresh samples.

Density, water activity (a_{y}) , acidity and pH

Characteristics like water activity, density, acidity and pH of the product were determined initially and after freeze drying process. The a_w for the fresh MM was 0.94 and the lowest value of water activity for normal bacteria, mold and salt tolerant is 0.90, 0.80 and 0.75 respectively (Potter and Hotchkiss, 1996)



Figure 3. Overlap graph for TPA of Fresh MM and FD rehydrated MM

therefore, water activity has to be reduced below 0.75 in order to preserve the food for a long time, hence freeze drying was opted for increasing the shelf life stability to ensure the quality retention in terms of physical, chemical and microbiological parameters. The water activity (a,) of the FD MM was found to be 0.261 and had a positive effect on the microbiological profile of the product after FD process. Density values for the fresh and FD samples were found to be 1.385 ± 0.003 g/cc and $0.547 \pm$ 0.030 g/cc respectively. Density and water activity of the product decreased after FD process. Acidity and pH for both fresh and freeze-dried were found as $0.74 \pm 0.03\%$, 5.15 ± 0.08 at 30.7°C and 1.497%, 5.28 ± 0.069 at 32.2°C respectively, however not much variation in acidity and pH were observed for FD rehydrated MM samples.

Texture profile analysis

TPA of MM was carried out initially and after FD in terms of hardness, cohesiveness, gumminess, chewiness and adhesiveness. Hardness 1 and hardness 2 of both fresh and reconstituted FD samples increased significantly (p < 0.05) as 4.25, 8.46, 3.11 and 6.12 N respectively implicating slight impact of freeze drying and reconstitution on the muscle texture of the product (Fig 3). Bele et al. (1966) reported significant increase in the shear resistance of cooked chicken meat after freeze drying than in the frozen sample. Toughness of freeze dried reconstituted meat and poultry was also reported by Brooks, (1958) and Tappel et al. (1955, 1957). Increase in the hardness of the product after FD process has reflected in the derived parameters like gumminess and chewiness recording 2.77 N and 15.42 N mm against 1.84 N and 18.64 N mm respectively. Adhesiveness property that reflects the stickiness of the product found higher for FD sample with 2.01 N mm whereas 1.13 N mm in fresh sample. Visco-elastic property of the product with respect to cohesiveness after FD has reduced from 0.43 to 0.33 clearly indicating muscle structural change.

Microbiological analysis

Microbiology of the fresh product was carried out in terms of standard plate count, yeast & mold, coliforms and *E. coli*. It was observed that standard plate count for fresh and freeze-dried samples were $2x10^3$ cfu/g and $4.7x10^2$ cfu/g respectively and the yeast & mold count in fresh sample was $2.0x10^1$ cfu/g whereas nil in freeze-dried samples. Coliforms were nil in both fresh and freeze-dried samples.

Rehydration studies

Rehydration/reconstitution study of FD MM indicated the percentage of water uptake as 62.09% and rehydration ratio was found as 0.345.

Conclusion

Freeze-drying (FD) process plays a key role in preservation and retention of its sensory attributes and helps in reduction in volume of the product, easy for packaging, transportation and storage. Response surface methodology helps in standardization of the MM product with various combinations of ingredients. RSM used in the study showed quadratic response surface as the best fit for prediction with minimum standard error and maximum r-square. MM product was freeze dried, which on rehydration retains almost all sensory attributes. Freeze-dried samples showed no significant changes in physic-chemical and, sensory and microbial evaluation compared to fresh one.

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