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RESEARCH ARTICLE

Effect of ground Chicken incorporation on the nutritional, textural and sensory characteristics of shelf stable biscuits

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Abstract

Biscuits are considered as a good vehicle for protein fortification and other nutritional improvement. In this study, attempts have been made to increase the nutritional, sensory and textural characteristics of the biscuits with the incorporation of ground chicken protein to develop a novel functional, non-vegetarian biscuit with good shelf stability. An experimental design was utilized to optimize the levels of meat and baking fat by rotatable central composite design using Response Surface methodology. A ratio of 1:0.93 of baking fat and meat was found to be ideal for obtaining a sensory score of 8.28±0.18 on a 9 point hedonic scale. The developed chicken biscuits were subjected to physico-chemical and sensory analyses. Studies indicated that biscuits with 30% Chicken protein enrichment showed increase in values of protein (25%) and had maximum sensory score. Therefore, the biscuits supplemented with meat proteins may definitely improve the nutritional status of vulnerable groups.

Introduction

Biscuit is the most popular bakery product worldwide. Because of its acceptability in all age group, longer shelf life, better taste and its position as snacks, it is considered as a good product for protein fortification and other nutritional improvement. Some of the reasons for such wide popularity are low cost among other processed foods, varied taste, easy availability and longer shelf life (Gandhi et al., 2001). Biscuits along with bread are considered to be the major bakery product and they account for 82% of all bakery production in India. The demand for the production of biscuits has increased from 650 thousand metric tonnes from 1990-91 and 2043 thousand metric tonnes during 2009-10 (Ministry of Food Processing, 2012).

The development of protein rich biscuits shows promise in improving the nutritive value of the final product. The nature and quantity of ingredients used determine the quality of the biscuit. Several researchers have described the effect of major ingredients in biscuit dough systems and on the final product (Chevallier et al., 2000a; Chevallier et al., 2000b; Maache-Rezzoug et al., 1998). The enrichment of protein has been done through the incorporation of protein rich non-wheat flours (Patel and Rao, 1996; Singh and Chauhan, 1996; Gandhi et al., 2001; Sharma and Chauhan, 2002). Development of biscuits by the incorporation of egg powder has been reported by Roch-Boris et al., (2007) and they have concluded that the ingredients for the preparation of batter has got a direct correlation with the textural profile and overall quality characteristics of the product. But there are no reports found so far on the incorporation of meat in biscuits. As poultry meat is a very popular food commodity around the world due to its low cost of production, low fat content, high nutritional value and distinct flavour (Barbut, 2002; Patsias et al., 2008), it was selected for incorporation in biscuits as a functional ingredient. Therefore, the present study was designed to develop and evaluate the nutritional, sensory and textural characteristics of chicken biscuits and optimize the incorporation level of chicken meat for the preparation of biscuits using response surface methodology. Response surface methodology was used to minimise the number of baking trials while gathering all information relating to ingredient interactions and quality characteristics.

Materials and methods

Raw Materials

Commercially available refined wheat flour, baking fat, spices, salt, and egg were procured from the local market. Fresh chicken meat (from 6-8 weeks old hens, weighing 1.3-1.5kg) was obtained from the local market and was used in the preparation of biscuit making.

Chemicals and Reagents

All the reagents and chemicals used for the study were of Analar® grade and procured from M/s Sigma Chemicals, Corporation, USA and M/s BDH Company. The standard fatty acid methyl esters used in the estimation of fatty acids by Gas chromatography and the BF_3 - CH_3OH used in the esterification were obtained from Sigma Chemicals Corporation, USA.

Preparation of Biscuits

Deskinned, cleaned and deboned chicken were cut into small pieces and subjected for marination with lemon juice, salt and spices. After marination for 3 hours it was subjected for partial cooking and coarsely ground through a 13-mm plate in a meat grinder (Hobart, Model 4812-CE, Offenburg, Germany). The biscuits were prepared using refined wheat flour (40%), baking fat (15%), baking powder (0.1%), eggs (7%) salt (0.6%), chilli powder (0.1%), turmeric powder (0.11%), whole pepper (crushed) (0.5%). A known weight of baking fat, salt and spices were creamed together at medium speed in a dough mixer (Hobart, Legacy® Floor Mixer, 30 quart model) until light and fluffy appearance is formed. Then refined wheat flour, minced chicken meat, egg and baking powder were added to the creamed paste and mixed until uniform smooth dough was obtained. The dough was rolled out to 3mm thickness on a board and cut into round shape of about 5 cm in diameter with a biscuit cutter. The biscuits were placed in a greased baking tray and baked in a laboratory preheated oven at 180°C for 20 min, according to the methods of AOAC (2000). Then cooled to 30±2°C and packed in PFP [paper / foil / polyethylene packages (45 GSM paper / 20μ Al.foil / 37.5μ low density LDPE)] bags (Figure 1) for further analyses.

Experimental design and Analysis

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 baking fat and meat 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 = Baking fat (Independent variable); X_2 = Meat (Independent variable); b_0, b_1, b_2, b_3, b_4, b_5 = Response model variables.

From the data obtained through the experimental design, optimal ingredient levels were identified.

Chemical analysis

Proximate composition of the product was determined by the AOAC (1984) procedure. Lipid extraction of the samples was carried out by the method of Folch et al., (1957). The products initially and during storage were subjected for chemical evaluation by estimating TBARS (Taraldgis et al., 1960) and FFA (AOCS, 1993).

Total fatty acid profile of samples by Gas Chromatographic method

Fatty acid composition of the lipid extracts was determined by Gas chromatography as fatty acid methyl esters using standard esters of fatty acids.

Esterification of fatty acids: The samples were esterified as per the procedure of Metcalf et al., (1966) with slight modifications.

About 150 mg of lipid was accurately weighed into a clean and dry stoppered test tube. Four milliliters of 0.5 N alcoholic sodium hydroxide solutions was added and heated for 5 min over a water bath at 90°C. On cooling, 5 ml of boron tri-fluoride methanol reagent was added and heated for 5 min at 90°C over a water bath, followed by addition of 10 ml of saturated sodium chloride solution. The samples were thoroughly cooled to room temperature and 5 ml hexane was added to each tube. It was shaken well and kept undisturbed. The upper hexane layer was drawn out into clean dry conical flask and dried over anhydrous sodium sulphate to remove the traces of moisture if present. The samples were filtered and transferred to stoppered clean dry tubes for gas chromatographic analysis.

Total fatty acid analysis by Gas Chromatography

Analysis of total fatty acids was carried out by a Ceres-800, Chemito model gas chromatograph fitted with BPX 70 column (25 m, 0.32 mm ID) and flame ionization detector. Temperature gradient programming was employed from 150 to 220 °C. Split ratio was adjusted to 1:25 and capillary flow of carrier gas to 2.0 ml/min. Injector and detector port temperatures were adjusted as 230°C and 240°C respectively. For FID, hydrogen and oxygen were used and the flow was adjusted as 45 ml/min and 450 ml/min respectively. Along with samples, standard esters of fatty acids (Sigma chemical company, St. Louis, USA.) were also injected and the fatty acids were detected by comparing the retention time of the standard esters of fatty acids. The quantification of the fatty acids was carried out by evaluating with the standard fatty acid esters area corresponding to each peak in the chromatogram. Iris-32 software was used to integrate and evaluate the chromatogram in the analysis.

Sensory Analysis

The sensory characteristics of the Chicken Biscuits during storage were evaluated by subjecting these samples to an overall acceptability score on a 9 point hedonic scale by a panel of judges, using the procedure of Murray et al., (2001).

Textural analysis

Hardness as firmness and deformation in freshly prepared and stored samples (2, 4 and 6 months) of the product were determined by three point breaking strength test (snap strength set up) using a pre-calibrated 500 Newton (N) load cell connected to a texture analyzer, (TA Plus, Lloyd instruments, Hampshire, UK) supported with Nexigen 4.0 software for analysis. Textural determinations were made on 6 biscuit samples and the average and standard deviations were calculated. The whole test was carried out at a constant pre and post-test speed of 30mm/min using a three point bending fixture (HDP/3PB). Other test conditions were maintained as clearance 2-3 mm, 10% penetration to the height of sample and a trigger of 8 gram force (gf). The biscuit sample was kept horizontally on to the two point parallel biscuit support fixture attached to the base table of the texture analyzer and the breaking force was applied at the geometric center of the biscuit through another round edged blade fixture which was connected to the moving cross head and sensor. Parameters like firmness and deformation (mm) were calculated from the typical break strength graph as depicted by Bourne (1979).

Microbiological analysis

Microbiological characteristics of the product were evaluated by determining Standard Plate Count, Yeasts and molds, Coliforms and pathogens by standard microbiological procedures (Harrigan, 1998).

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) and Duncan's multiple range test to evaluate the statistical significance of the treatments and significance was established at $p<0.05$.

Results and discussion

Optimization of ingredients by Response Surface Methodology

Designing of the quantity of ingredients employed in Chicken biscuits formulation was standardized by the application of central composite design / RSM as described in the methodology. The ingredients that were thought to influence the sensory and storage properties were considered. Baking fat and meat quantity were taken as variables and the combinations obtained as per the design (Table I). The combinations were subjected for the evaluation of sensory attributes by taking colour, aroma, texture, taste and overall acceptability as parameters. Overall acceptability score on a 9 point hedonic scale was taken as a response against these two variables. From Figure 2, it could be ascertained that variation in the quantity of variables significantly contributes ($p<0.01$) towards the sensory profile as reflected in the overall acceptability score (Table I & Figure 2). Out of the various combinations as per RSM design, a ratio of 1:0.93 of baking fat and meat was found to be ideal showing a score of 8.28±0.18 on a 9 point hedonic scale.

Nutritional composition of Chicken Biscuits

The proximate composition of the Chicken biscuits is presented in Table II. It is a good source of quality meat protein, fat and carbohydrate and provides a calorific value of 456kcal per 100g of the product. The moisture content of the product is 4.8g/100g which is nearer to the BET monolayer value of dried meat products at which the deteriorative changes in quality parameters are minimum (Jayathilakan et al., 2007a).

Evaluation of oxidative and hydrolytic rancidity

Chemical stability of the products was evaluated in terms of lipid oxidative profile. Hydrolytic and oxidative rancidity parameters were estimated in terms of FFA and TBARS respectively. The values pertaining to the storage evaluation has been presented in Figure 3. From the values it could be inferred that the product was stable upto 5 months without any significant ($p>0.05$) variation in TBARS and FFA values. But after 5 months of storage at ambient temperature (30±2°C) significant difference ($p<0.05$) was observed in TBARS and FFA values indicating the extent of oxidative and hydrolytic rancidity that occurred to the products after 5 months.

The increase in FFA could be attributed to the hydrolysis of triglycerides, triggered by the moisture from the food and due to oxidation (Fisch, 1981). The presence of moisture can cause other deteriorative chemical reactions leading to hydrolytic rancidity (Okiy and Oke, 1984; Yoon et al., 1987). The product in discussion is contributed by chicken fat and protein which are prone for oxidative changes during storage. The phospholipids and other unsaturated fractions of the fat component act as a substrate in lipid peroxidation (Angel catala, 2009). Many volatile components are formed during storage which may contribute towards the rancidity development. The product exhibited good stability characteristics in terms of these quality attributes during storage upto 5m at ambient temperature as reflected in Figure 3. Significant difference ($p<0.01$) in TBARS and FFA observed may be due to these reasons and studies have to be conducted for getting enhanced shelf stable products beyond 5m using natural antioxidants. These results are in accordance with the total fatty acid and sensory profile of the product reported in Tables III and V respectively. Earlier studies by Seevaratnam et al., (2012) on the formulation and stability of biscuits incorporated with potato flour revealed a stability of only 60 days at room temperature in terms of rancidity parameters.

Total fatty acid profile by GLC

Total fatty acid profile of chicken biscuits was carried out by GLC after esterifying the samples along with standard fatty acid esters. Eight fatty acids were detected in the product which include saturated fatty acids (Lauric, Myristic, Palmitic, Stearic), mono unsaturated (Oleic) and poly unsaturated (Linoleic, Linolenic and Arachidonic acids). Quantitative estimation of these fatty acids was carried out by comparing with standards using Iris 32 software and the ratio was established as 1:1.13:0.5. Oleic acid (38.99%) was the major fatty acid present followed by palmitic (18.46%), stearic acid (12.56%) and linoleic acid (11.16%). To establish the stability of these fatty acids studies were conducted to monitor the extent of degradation happening in the fatty acid content during storage. The data pertaining to the study has been given in Table III. It is usually observed that the main substrate for lipid oxidation is unsaturated fatty acids (Jayathilakan et al., 2007b). All the unsaturated fatty acids were found to be stable at ambient temperature and a significant decrease ($p<0.01$) in linolenic and arachidonic were noticed after 5 months of storage. This could be clearly attributed to the reasons explained in the earlier part of the discussion with TBARS and FFA and the findings are in correlation ($r^2=0.98$). Gouveia et al., (2008) evaluated the stability of higher unsaturated fatty acids in PUFA enriched functional biscuits and reported that no significant difference in PUFA ($p>0.01$) till 3 months of storage at RT.

Textural Characteristics of biscuits

In texture determination, snap strength test provided a typical firmness peak resulting from the progressive increasing force during breaking and a sudden drop in the force as the biscuit has fractured in to two pieces. As the biscuit has not fractured into many pieces, the test reveals that the biscuit has got a less porous structure (Piga et al., 2005). From Fig 3, it could be seen that the maximum force recorded revealed different evolutions during storage and it increased from 45.92 N in freshly prepared biscuits to 102.82 N in 6 months old sample, whereas deformation was recorded from 0.80 mm to 1.30 mm respectively. The increase in hardness as expressed in terms of Newton (Table IV) may be attributed to the interaction of protein and saccharide during storage (McMahon et al., 2009). The biscuit hardness was positively correlated with the deformation and subjective sensory attributes. From the force peak graph, (Figure 4) it was clear that deformation from trigger force was increasing along with firmness during storage period showing a direct correlation of degree of compression before complete disintegration of the samples. Biscuit was acceptable with respect to hardness up to 4 months of storage subjectively against an objective force of 77.52N and unacceptable after 6 months with a hardness value of 102.82 N.

Sensory evaluation

To evaluate the sensory attributes of the product and also to correlate with the physico-chemical parameters, periodic monitoring of sensory changes was carried out on a 9-point hedonic scale during storage at ambient temperature to establish the shelf stability. Sensory parameters like colour flavour, texture, taste and overall acceptability were evaluated by semi-trained panelists at monthly intervals and the score obtained has been depicted in Table V. From the sensory score, it can be interpreted that upto 5 months the product has been rated very well by the panelists. The physico-chemical data in terms of textural profile and rancidity parameters (TBARS and FFA) as reported in the earlier discussion (Table IV and Figure 3) is in correlation with the sensory profile. It has been reported that due to the oxidation and other chemical changes in fat and protein the acceptability of the product may come down during storage (Sampaio et al., 2012). The product exhibited a very good overall acceptability score of 7.8±0.14 during 5m storage and a significant difference in rating has been noticed after 5 months storage.

Microbiological profile of chicken biscuits

Microbiological status of the product initially and during storage was examined in terms of TPC, Y&M, coliforms and *E.coli*. It was observed that, there was a one log increase in SPC and Y&M during storage for a period of 6 months. All these values were in the acceptable range and the pathogens was totally absent overall the product exhibited good microbiological safety throughout the storage period. The data for the microbiological profile of the chicken biscuits during storage is shown in Table VI. The bacterial count of all biscuits samples was lower than acceptable limit of $1x10^5$ CFU/g of sample (Seevaratnam et al., 2012). It was observed that there was one log reduction in the total plate count during baking compared to the dough (Berghofer et al., 2003). The microbial counts were well within limits of Ready-to-eat foods even after 5months of storage but it was not acceptable due to the changes in physico-chemical and sensory attributes.

Broiler Chicken meat (6-8 weeks old birds)

↓

Deskinning & deboning

↓

Cleaning in running water

↓

Cutting into small pieces

↓

Marinating with salt, chilli powder, turmeric powder

↓

Subjecting to partial cooking for 10mins

↓

Grounding

↓

Mixing with maida, baking fat & eggs until smooth dough obtained

↓

Rolling the dough and cutting into biscuit shapes using biscuit cutter

↓

Baking at 180°C for 20mins

↓

Cooling to 30°C and packing in PFP pouches

Figure 1. Flow diagram for preparation of Chicken Biscuits

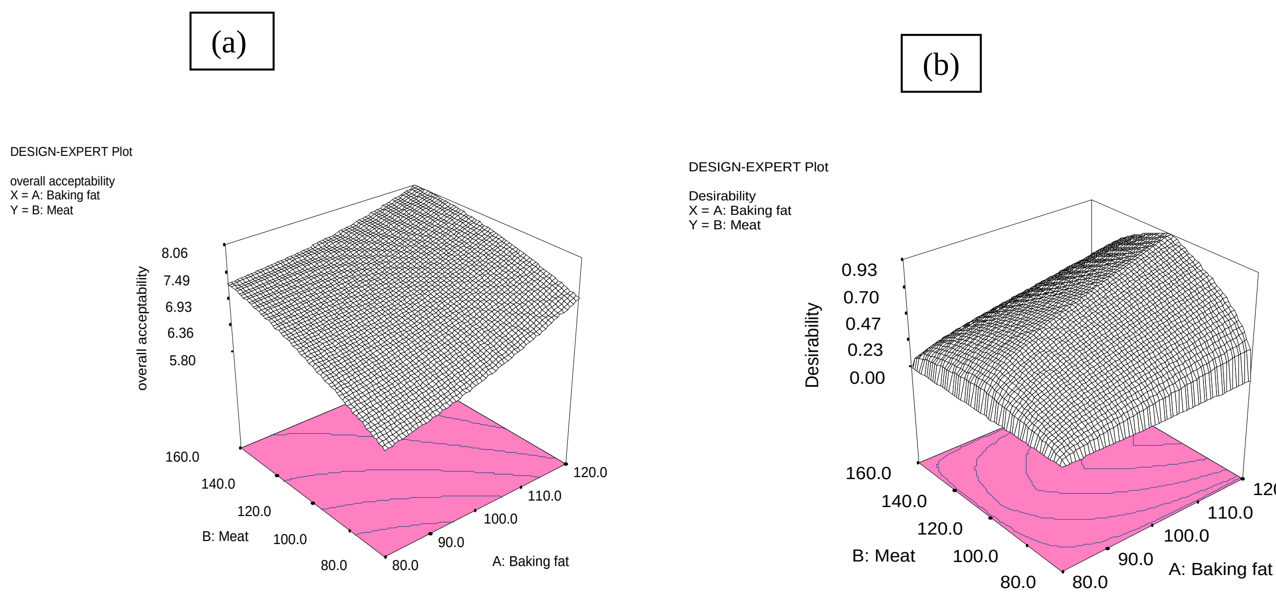

Figure 2. (a) 3D-plot depicting effect of ground meat and baking fat on overall acceptability of Chicken biscuit and (b) 3D-plot depicting effect of ground meat and baking fat on desirability of Chicken biscuits

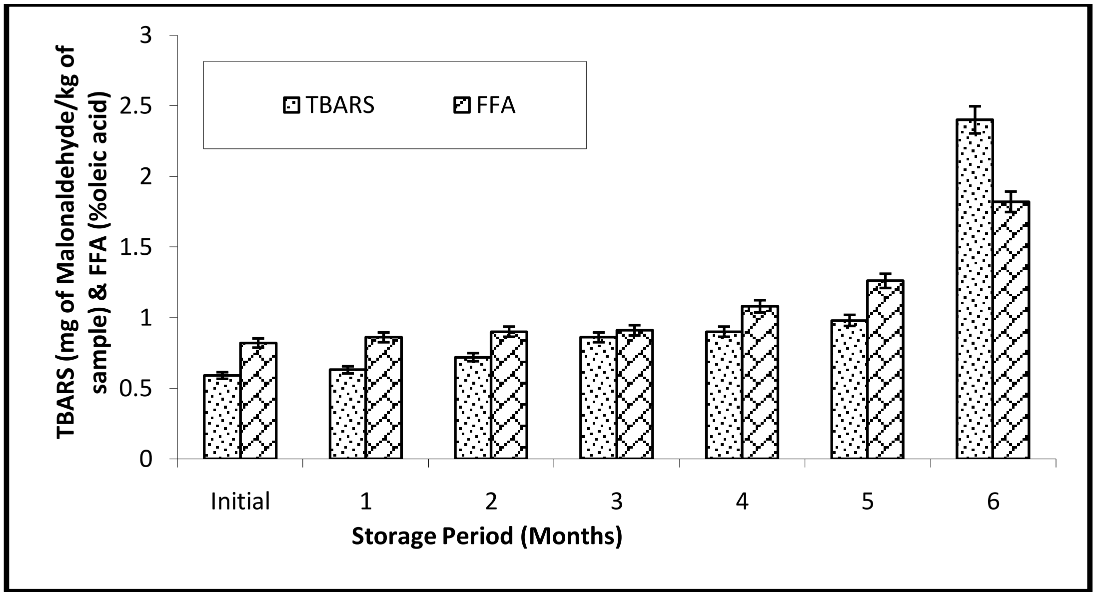

Figure 3. Evaluation of oxidative and hydrolytic rancidity profile of chicken biscuits during storage (mean±SD, n=3).

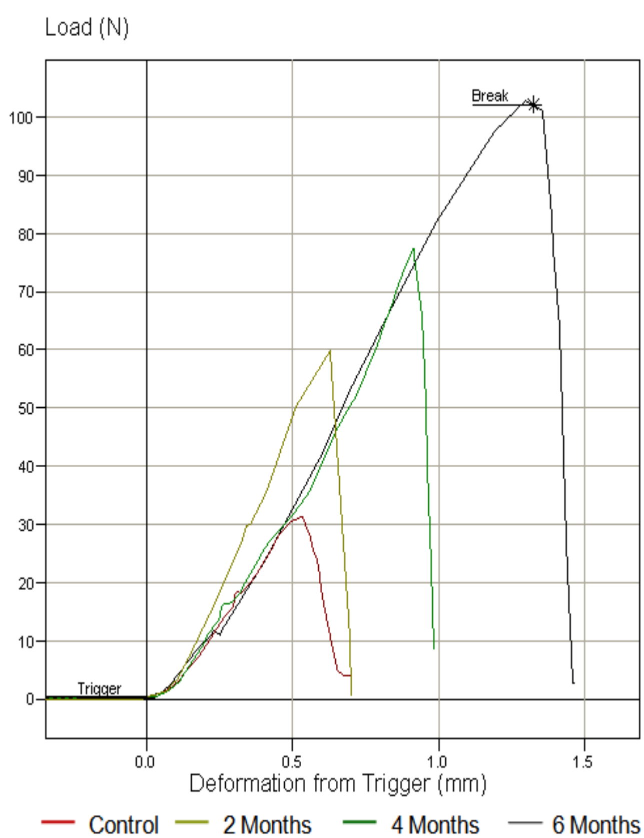

Figure 4. Typical force-deformation graph representing snap strength evaluation of chicken biscuits during storage

Table I. Experimental design for chicken biscuits

Run	Factor1 A:Baking fat	Factor 2 B:Meat	Response Overall acceptability*
1	100.00	176.57	7.42
2	71.72	120.00	6.60
3	80.00	160.00	7.43
4	100.00	120.00	7.22
5	128.28	120.00	8.28
6	100.00	120.00	7.15
7	120.00	80.00	7.02
8	100.00	120.00	7.21
9	120.00	160.00	8.12
10	80.00	80.00	5.72
11	100.00	120.00	7.00
12	100.00	63.43	6.23
13	100.00	120.00	7.00

*9 point hedonic scale

Table II. Proximate composition (g/100g dry sample, except for moisture) of Chicken solids fortified biscuits

Components (%)	Composition (g/100g)
Moisture	4.78±0.95
Fat	20.34±0.28
Protein	25.39 ±1.36
T. Carbohydrate	43.86 ±1.95
T. Ash	4.08±0.82
Energy (kcal)	456

Note: Results are mean± SD of three determinations

Table III. Total fatty acid profile of chicken biscuits during storage at ambient temperature (24-34°C) (as methyl ester-% of total fatty acids)

Fatty acid as methyl ester	Initial	6 months
Lauric ($C_{12:0}$)	0.38±0.04	0.36±0.03
Myristic ($C_{14:0}$)	3.98 ±0.36	3.92±0.31
Palmitic ($C_{16:0}$)	18.46±0.94	18.52 ±1.29
Stearic ($C_{18:0}$)	12.56 ±0.89	12.49 ±0.98
Oleic ($C_{18:1}$)	38.99 ±1.20	31.08 ±1.98
Linoleic ($C_{18:2}$)	11.16 ±0.14	8.82 ±0.18
Linolenic ($C_{18:3}$)	3.18 ±0.09	2.02 ±0.04
Arachidonic ($C_{20:4}$)	2.43 ±0.11	1.34 ±0.08

Note: Values are mean±SD (n=3)

Table IV. Effect of incorporation of ground chicken on the textural characteristics of biscuits during storage

	Three Point Breaking Test	
Storage period (months)	Firmness (N)	Deformation (mm)
Initial	45.92±2.19	0.80±0.03
2	59.79±3.84	0.83±0.02
4	77.52±3.05	0.91±0.04
6	102.82±4.89	1.30±0.24

Note: All data are the mean ± SD of three determinations

Table V. Changes in sensory parameters of Chicken Biscuits packed in PFP pouches and stored under ambient temperature (24˚C - 34˚C) conditions.

Storage Period (months)	Colour	Flavour	Texture	Taste	OAA
1	8.39 + 0.16	7.88 + 0.10	8.15 + 0.17	8.16 + 0.14	8.28 + 0.18
2	8.25±0.19	7.72 + 0.14	8.29 + 0.15	8.11 + 0.18	8.21±0.19
3	8.11 + 0.14	7.66 + 0.12	7.96 + 0.13	7.94 + 0.10	8.18±0.17
4	8.03 ± 0.27	7.58 ± 0.14	7.70 ± 0.18	7.90 ± 0.12	7.93 ± 0.15
5	7.88 + 0.19	7.50 + 0.11	7.68 + 0.15	7.82 + 0.19	7.80±0.14
6	6.45 + 0.11	6.58 + 0.17	6.54 + 0.14	6.87 + 0.15	6.61±0.11

Note: Results are mean± SD of fifteen determinations

Table VI. Microbiological profile of chicken biscuits during storage at ambient temperature (24˚C - 34˚C) (cfu/g)

Storage period (months)	SPC	Coliforms	Y&M	*E.coli*
Initial	$2.2x10^2$	ND	ND	ND
1	$4x10^2$	ND	ND	ND
2	$4x10^2$	ND	ND	ND
3	$3x10^2$	ND	ND	ND
4	$4x10^2$	ND	ND	ND
5	$3x10^3$	ND	ND	ND
6	$4.2x10^3$	ND	ND	ND

Note: Results are mean± SD of three determinations; ND=none detected

Conclusion

Protein energy malnutrition is one of the most serious health problems in many parts of the world especially in developing countries like India. Development of biscuits or snack items supplemented with meat proteins may definitely improve the nutritional status of vulnerable groups like pregnant women, nursing mothers, elderly, school going & young children and help in reducing the incidence of malnutrition. The studies revealed the feasibility of incorporating chicken meat in the production of Biscuits with improved nutritional and sensory quality without adversely affecting the textural characteristics. Application of statistical design approach by Response Surface Methodology for the optimization of ingredients was found to be suitable to obtain a sensorily acceptable product. The biscuits with chicken meat are a good source of quality protein, fat and carbohydrate and provide a calorific value of 456 kCal per 100g of the product. The physico-chemical, microbiological and sensory profile during storage established a shelf life of 5 months at ambient temperature.

Chicken biscuits not only fulfil the protein requirement but also help to overcome the biological problems due to high altitude. The product has got good scope as a snack item in day care centers, schools and emergency rations. Consumption of protein rich chicken biscuits would also help to raise the nutritional status of the population. It can cater to the needs of Armed forces and civilian sectors also.

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