Microbiological Analysis of Chicken Meat Cutlets Incorporated with Functional Ingredients

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ABSTRACT
The objective of this study was to assess the microbiological quality chicken meat cutlets by incorporating functional ingredients like mushrooms, sesame seeds and wheat gluten at acceptable levels and their combined effect on the shelf life of frozen chicken meat cutlets. The TVC count of conventional packed uncooked control and MSW chicken meat cutlets decreased significantly (p≤0.05) from 4.65 to 4.29 log cfu/g and 4.25 to 3.96 log cfu/g respectively with the increase in the frozen storage period. The TVC count in control and MSW, vacuum packed uncooked chicken meat cutlets decreased significantly (p≤0.05) from 4.65 to 4.16 log cfu/g and 4.25 to 3.86 log cfu/g respectively. The average TVC of fresh conventional packed cooked chicken meat cutlets was 2.28 log cfu/gm of control and 2.13 log cfu/gm of the cooked MSW chicken meat cutlets. It decreased significantly (p≤0.05) to 2.07 and 1.96 log cfu/gm respectively after two months of frozen storage period. The average TVC of vacuum packed control cooked chicken meat cutlets decreased significantly (p≤0.05) from 2.28 to 2.00 log cfu/gm and of MSW cooked chicken meat cutlets from 2.13 to 1.90 log cfu/g. The decrease was more in the MSW chicken meat cutlets as compared to control. Also, lower counts were observed in vacuum packed control MSW chicken meat cutlets as compared to conventional packed.

Keywords: Chicken Meat Cutlets, Microbiological quality, TVC count and Functional ingredients.

INTRODUCTION
Chicken meat and its products are very popular. Chicken meat is highly preferred over other meats in India and is accepted by the society because there are no religious reservations. Chicken meat is leaner than beef, lamb and goat meat and contains less marbling and subcutaneous fat. Being lean, it is an excellent source for the preparation of low fat chicken meat products. Chicken meat Cutlets are flat croquette of minced meat, flour, pulse, nuts, potato, condiments, spices and often coated with bread rusk crumbs and are one of the most popular snack based products (Singh, et al. 2014).
Meat is not only highly susceptible to spoilage, but also frequently implicated in the spread of food borne illness. Contaminated raw meat is one of the main sources of foodborne illness (Bhandare et al., 2007; Podpecan et al., 2007). Heredia et al. (2001) reported that the microbial quality of ground meat analyzed was unsatisfactory, and the product was important cause of food poisoning. Similarly, Duffy et al. (2001) reported that the mean log APC and TCC were highest in store-ground pork and pork sausages. Astorga et al. (2002) also reported that chicken products (especially those made with ground meat) represented notable hazards to humans and were a cause of public health concern.

Mushrooms are generally considered to be a good source of protein, dietary fibres and vitamins especially vitamin D and vitamin B complex. They provide little calories, fats, sugars and are commonly recommended in healthy diets. Partial substitution of meat and meat products with mushrooms would result in reduced intake of calories, fats, cholesterol and increased levels in dietary fibres, copper, potassium and riboflavin.

Sesame seeds are widely considered healthful foods. The seeds are especially rich in mono-unsaturated fatty acid oleic acid, which comprise up to 50% fatty acids in them. The sesame seed is used whole in cooking for its rich nutty flavour.

Wheat gluten is the water-insoluble protein portion contained within the endosperm of wheat. It can either act as a binder, extender or restructurer for meat products (e.g., turkey, beef, pork or lamb) or as an ingredient in the production of simulated or imitation meats (e.g., hamburger, sausage or crab analogs).

Chicken meat cutlets were prepared in the Department of Food Science and Technology, College of Agriculture, Punjab Agricultural University, Ludhiana. Development of chicken meat cutlets by incorporating functional ingredient such as mushrooms, sesame seeds and vital wheat gluten known for their health promoting properties and to investigate the Microbiological analysis of chicken meat cutlets.

MATERIALS AND METHODS
The present investigation was conducted in the Department of Food Science and Technology, College of Agriculture, Punjab Agricultural University, Ludhiana. This chapter contains relevant information pertaining to design and methodological steps used in the investigation.

2.1 Raw materials
2.1.1 Chicken meat
Frozen minced chicken meat of Republic of Chicken brand (400gms unit), was purchased from their outlet in Ludhiana and stored at -20±2°C in deep freezer, till its use in the preparation of chicken cutlets. The chicken meat was thawed using microwave oven (Batliboi Eddy Co. Model No. ER-5054 D) for 2 minutes at power level 4.

2.1.2 Mushrooms
The button mushrooms (Agaricus bisporus) in shrink packages (200gms) was purchased from the local market. The mushrooms were ground in food processor to form paste (Kenstar Karishma Multi Processor, Model no.MF0808) before its incorporation into the batter for the preparation of chicken cutlets.

2.1.3 Sesame seeds
Sesame seeds was procured from the local market and roasted under low flame for 2 minutes. After roasting they are incorporated into the batter for the preparation of chicken meat cutlets.

2.1.4 Wheat gluten
Wheat gluten of edible food quality was procured from DKSH India Private Limited, New Delhi.

2.1.5 Spice mix and Additives
A pre-standardized formulation of dry spices was prepared. Cinnamon, coriander, cumin powders of MDH brand and black pepper and red pepper of Catch brand were procured from the local Ludhiana market for the preparation of dry spice mix.
Table 1: Dry Spice Mix (120g)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black pepper</td>
<td>20</td>
</tr>
<tr>
<td>Cumin</td>
<td>40</td>
</tr>
<tr>
<td>Coriander</td>
<td>40</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>12</td>
</tr>
<tr>
<td>Red pepper</td>
<td>8</td>
</tr>
</tbody>
</table>

2.1.6 Green Curry Stuff (GCS)

For the preparation of Green Curry Stuff Onion, Peas, Coriander, Potatoes were purchased from the local market. Ginger and Garlic paste of Smith and Jones brand was also obtained from local market. Onions were washed, peeled and paste was prepared using food processor (Kenstar Karishma Multi Processor, Model no. MF0808). Green Curry Stuff (GCS) was prepared by blending onion paste, garlic paste and ginger paste (2:1:1 respectively).

Peas were taken out from pods and coarsely grinded using pestle and mortar for their incorporation in the cutlets. Coriander leaves were manually cut into pieces with the help of knife. Potatoes were washed, boiled and mashed manually for their incorporation into chicken meat cutlets.

Food grade sodium alginate, calcium carbonate, sodium nitrite, sodium nitrate was procured from the local market. Refined Groundnut oil (Fortune) and Iodized salt (Tata) were used in the formulation.

2.1.7 Packaging material

Co-extruded plastic film (200 guage) suitable for conventional and vacuum packaging under freezing conditions was used for the packaging of chicken meat cutlets.

2.2 Standardization of product formulation

The recipe of chicken meat cutlets was standardized by consulting literature and by taking the opinion of taste panel members during product standardization. Trials were conducted using different levels of salt and spices for the standardization of the recipe. The standardized recipe of chicken meat cutlets is given in Table. The standardization process also helped in the training of the taste panel members.

After standardizing the recipe, the trials were conducted incorporating mushrooms (0, 5, 10, 15 and 20 % levels), sesame seeds (0, 1.5, 2.5, 3.5 and 4.5 % levels) and wheat gluten (0, 0.5, 1.0, 1.5 and 2 % levels) in the products for their optimization in formulation of chicken meat cutlets. On the basis of sensory evaluation, best levels of mushrooms (15.0%), sesame seeds (2.5%) and wheat gluten (1.5%) were selected for incorporation in the final product i.e. chicken meat cutlets. The chicken meat cutlets were used for the analysis of proximate composition, cooking characteristics, organoleptic and microbiological quality at interval of 15 days upto 2 months of frozen storage.

2.3 Preparation of cutlets

The chicken meat mince, salt and sodium nitrite were mixed in Hobart mixer Model N-50 for five minutes. Mushrooms (15%), sesame seeds (2.5%) and wheat gluten (1.5%) were added followed by dry spices mix and other additives as per the formulation to form uniform batter. After uniform mixing of all the ingredients, the batter was moulded into cutlets with the help of oval shaped metallic moulds 10 cm long and 6.5 cm maximum width. The cutlets (approximately 80gm each) were precooked in hot air oven at 200°C for 12 minutes. The precooked cutlets were removed from the moulds and packed conventionally using heat sealer (Ambala Associates) and under vacuum using vacuum packaging machine (Teknik Industrial Traders, Ambala city Model D2Q400-2D) in co-extruded plastic films. The packed cutlets were frozen stored at (-20± 2°C) in commercial freezer upto two months.
2.4 Total viable count (TVC)
The microbiological analysis (TVC) of the raw and cooked products was conducted by standard pour plate method (APHA 1984). 25g of sample was aseptically mixed with 225 ml of sterile distilled water (pH 7 and 0.8% NaCl) and blended for 4 hours. This provided 10⁻¹ dilution, further dilutions were prepared under aseptic conditions in the same manner. One ml of diluted sample was poured in each petri plate and mixed with Nutrient agar gently. The plate was rotated clockwise and anticlockwise for uniform distribution of inoculum in Nutrient agar. After solidification of the medium in the plates, the plates were incubated at 37°C in inverted position for 48 hours. After incubation, colonies were counted and expressed as:

\[
\text{TVC/g = Mean colony count \times dilution factor}
\]

### Table 2: Composition of Nutrient Agar (Log cfu/g)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>5.00</td>
</tr>
<tr>
<td>Peptone</td>
<td>5.00</td>
</tr>
<tr>
<td>Beef extract</td>
<td>3.00</td>
</tr>
<tr>
<td>Agar</td>
<td>20.00</td>
</tr>
<tr>
<td>pH</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

3.1 Microbiological analysis of chicken meat cutlets

The average total viable count (TVC) for fresh minced chicken meat was 5.57 log cfu/gm and for fresh batter for raw chicken meat cutlets after mixing with other ingredients was 5.82 log cfu/gm. The TVC of uncooked and cooked, control as well as chicken meat cutlets containing mushroom, sesame seed and wheat gluten (MSW), packed in conventional and vacuum pack were conducted after every one month interval up to two months of frozen storage period.

The average TVC of fresh conventionally packed uncooked chicken meat cutlets (after short heating 180°C for 10 minutes) was 4.65 log cfu/gm of control and 4.25 log cfu/gm of chicken meat cutlets containing mushroom, sesame seed and wheat gluten (MSW). TVC values of cutlets decreased significantly (p≤0.05) to 4.29 log cfu/gm and 3.96 log cfu/gm respectively after two months of frozen storage period. The average TVC of vacuum packed control cooked chicken meat cutlets decreased significantly (p≤0.05) from 2.28 to 2.0 log cfu/gm and the average TVC of MSW cooked chicken meat cutlets decreased significantly (p≤0.05) from 2.13 to 1.90 log cfu/gm at the end of two months frozen storage (-20±2°C) period.

The lower counts in cooked MSW chicken meat cutlets might be due to antimicrobial properties of ginger. Inhibition of pathogens by ginger treatment has been reported by Salzer (1982) and lower counts in treated samples with spices was also reported by Negbenebor et al. (1995) and Syed Ziauddin et al. (1996). Pranoto et al. (2005) examined that enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin and seen the effect against *E.coli, S. aureus, Salmonella typhimurium, Listeria monocytogenes* and *Bacillus cereus*. 
Table 3: Effect of packaging and frozen storage on TVC (log cfu/gm) of uncooked and cooked chicken meat cutlets (n=3)

<table>
<thead>
<tr>
<th>Storage Period (Days)</th>
<th>Uncooked</th>
<th>Cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional Packaging</td>
<td>Vacuum packaging</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>MSW</td>
</tr>
<tr>
<td>0</td>
<td>4.65 ± 0.01</td>
<td>4.25 ± 0.01</td>
</tr>
<tr>
<td>30</td>
<td>4.56 ± 0.02</td>
<td>4.16 ± 0.01</td>
</tr>
<tr>
<td>60</td>
<td>4.29 ± 0.01</td>
<td>3.96 ± 0.01</td>
</tr>
</tbody>
</table>

Uncooked
C.D.(p≤0.05)
Treatment = 0.012; Packaging = 0.012; Storage = 0.014; Treatment × Packaging = NS;
Treatment × Storage =0.02; Packaging × Storage = 0.02; Treatment × Packaging × Storage = NS

Cooked
C.D.(p≤0.05)
Treatment = .014; Packaging = 0.014; Storage = 0.017; Treatment × Packaging = NS;
Treatment× Storage=0.025; Packaging× Storage= 0.025; Treatment× Packaging × Storage = .035;

The TVC values of chicken meat cutlets were within the maximum standard limits (below log 10^6 cfu g^-1) up to two months of frozen storage period. The reduction in microbial load might be due to the destructive effect of freezing on bacteria. This is in accordance with the results of Anand et al. (1989) in frozen chicken, Bentley et al. (1989) in frozen beef patties, Anand et al. (1991) in frozen chicken patties and Panda et al. (1993) in frozen quail meat.

Third (1995) reported that TVC of cooked frozen patties (buffalo, chevon and chicken) ranged between 7.36-8.96×10^3/g, which decreased to a minimum of 4.52×10^3/g and 5.84×10^3/g when cooked after two months of frozen storage.
Summary
Development of chicken meat cutlets by incorporation of functional ingredients like mushrooms, sesame seeds and wheat gluten increased the nutritional value by increasing protein and fiber content. The TVC count of conventional packed uncooked control and MSW chicken meat cutlets decreased significantly (p≤0.05) from 4.65 to 4.29 log cfu/g and 4.25 to 3.96 log cfu/g respectively with the increase in the frozen storage period. The decrease was more in the MSW chicken meat cutlets as compared to control. Also, lower counts were observed in vacuum packed control MSW chicken meat cutlets as compared to conventional packed.

REFERENCES