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Research Article

Edible oil marination in broiler meat for short term preservation

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Abstract

The aim of this study was to evaluate the quality and shelf life of raw broiler meat incorporated with soybean, mustard and flax seed oil under refrigerated storage at 4±1ºC. Meat samples were divided into four different batches i.e. T_0 = (Control group), T_1= (1% Soybean oil), T_2= (1% mustard oil), T_3= (1% flax seed oil). After 0, 7 and 14 days of storage, the samples were tested for physicochemical characteristics (pH, water holding capacity), oxidative stability (TBARS), sensory properties (color) and microbiological counts (TVC, TCC, and TYMC), proximate analysis (DM, EE, CP, Ash). When compared to control samples, the addition of oils had a significant ($P<0.05$) effect on physicochemical characteristics, oxidative stability, microbiological and sensory quality. During the whole storage process, the pH and water holding capacity in batches of T_1, T_2 and T_3 were considerably lower ($P<0.05$) than in the control group. Among all the treatment batches, the mustard oil (T_2) had significantly lower ($P<0.01$) TBARS values during storage. The T_2 treatment showed comparatively lower values of viable count, coliform count and yeast-mold count throughout the storage period. The color of the T_0 sample was far superior than other treatments. The T_2 treatment had the most preferred good odor, whereas the control group had the least. Based on the findings of this study, it is possible to conclude that mustard oil may be used for meat marination and preservation and extending the shelf life of stored meat rather than soybean and flax seed oil. According to the findings of this comparative study of different types of oil marination, mustard oil could be used in the preservation of raw broiler meat at refrigerated storage.

Introduction

Bangladesh is mainly an agricultural country which is adorned with different agricultural and livestock products. In our country lion share of the people are directly or indirectly concerned with livestock rearing. The livestock population in Bangladesh consists of 24.54 million cattle, 26.604 million goat, 3.607 million sheep and 304.106 million chickens (DLS, 2021). Most of the farmers are interested in rearing poultry mainly for meat purpose. Meat is recognized as a highly nutritious food, being an excellent source of high quality protein (Akter et al., 2009). It also contains essential amino acids which is essential for any healthy diet. It contributes to human nutrition by delivering a wide range of micro and macro nutrients. Poultry meat is preferred for consumption over other meats throughout the world, since it is cheap, easily available and has no religious taboos (Prabakaran, 2012). Chicken meat is favored by consumers around the world because of its desirable nutritional qualities, such as a low fat content and a relatively high concentration of polyunsaturated fatty acids. Fresh meat is also highly perishable product due to its biological composition (Yu et al., 2005). In addition, meat and poultry products have frequently been found to be contaminated with microorganisms during the butchering and manufacturing process. These microorganisms produce undesirable quality changes in meats, especially in relation to lactic acid bacteria, a major bacterial group associated with meat spoilage (Doulgeraki et al., 2012). Meat or meat products typically spoil due to one of the two major causes; microbial growth or chemical deterioration. In chemical deterioration, lipid oxidation is important in the processed meat industry because it is one of the major causes of quality deterioration. Lipid oxidation can impart adverse effects not only on sensory attributes such as color, texture, odor, and flavor but also on nutritional quality of the products (Nunez and Boleman, 2008). Lipid per oxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and poly unsaturated fatty acids (Verma et al., 2009).

Recently, researchers had tried to increase the shelf life of meat and meat products through different processes such as refrigeration (Akhter et al., 2022; Rahman et al., 2017), curing (Woods et al., 2019), Drying (Akhter et al., 2009), irradiation (Sadakuzzaman et al., 2021; Haque et al., 2017; Rima et al., 2019; Islam et al., 2018, 2019 and 2021), through adding electrolyzed water (Azad et al., 2021) and by adding natural antioxidants (Ali et al., 2022; Hossain et al., 2021; Bithi et al., 2020; Disha et al., 2020; Saba et al., 2018; Jahan et al, 2018; Siddiqua et al., 2018). Now-a-days different preservation methods of meat have been developed for short time preservation in the world. For centuries, people have refrigerated meat to extend its shelf life, although most improvements in refrigeration technologies have occurred in the past century. The application of refrigeration for the preservation of meat has been practiced widely to maintain their quality and

safety during storage, distribution and marketing. For this reason, the practice of freezing meat in Bangladesh has experienced a dramatic increase over the last two decades. Marination was originally invented by chefs as a way to improve the flavor, juiciness, texture, and overall enjoyment of a product. Food companies have taken full advantage of marination. By integrating staged ingredient addition into the process, marination can improve product flavor and juiciness but more importantly overall yield. Marinated meat products are consumed increasingly. In addition to taste, marinating has been considered to increase product safety and shelf life. Limited research has been conducted on marinating chicken meat using oils in terms of physical, chemical, organoleptic, and microbiological characteristics.

Only a few researches have done to see the effect of oil marination on the shelf life in chicken meat (Fratianni et al., 2010; Matan et al., 2010). There was not more research so far conducted before this experiment on chicken breast meat with different types of oil in Bangladesh. When meat is enriched with different types of oil, we can recommend this as natural preservative. The aim of preservation is not only to retard the food spoilage but also to control undesirable changes of wholesomeness, nutritive value and growth of microorganisms. Based on the above discussion the present study was conducted to investigate the effect of oil on quality and safety of raw chicken meat during refrigerated preservation.

Materials and methods

The present experiment has been conducted in accordance with the following systematic programs:

Collection of Raw materials

Boneless chicken broiler meat of 9 kg obtained by slaughtering of poultry by halal method was procured from KR Market, Bangladesh Agricultural University, Mymensingh. The meat samples were immediately transferred to the Animal Science Laboratory.

Sample preparation

About 4 kg of fresh meat sample was taken for the preparation of chicken. At first, the chicken meats were properly cleaned with fresh water and all the body fat, tendons, skin and as well as separable connective tissues were trimmed off from the boneless meat with sharp knife. Then the meat was properly mixed with 1% of different types of oil properly as per experimental design. There were four treatment groups, such as T_0= (Control group), T_1= (1% Soybean oil), T_2= (1% mustard oil), T_3= (1% flax seed oil). Then the meat was separately packed in a zipper bag, keep the required sample for experiment and rest are transferred to refrigerator.

Instrumental color measurement

Instrumental color measurement was carried out on meat from longissimus muscle. Color was measured at 24 h post-slaughter using Konica Minolta Chroma Meter (CR 410, Konica Minolta Sensing, Inc., Osaka, Japan), a Miniscan Spectro colorimeter programmed with the CIE Lab, (International Commission on Illumination) L*, a*, and b* system, where L* represents lightness, a* redness and b* yellowness (CIE Lab, 2014). The analysis was carried out on the medial surface (bone side) of the meat at 24 h post-mortem (Rahman et al., 2020). The colorimeter was calibrated using the specific whiteboard before measurement began. Each value was an average of three measurements from an area of the meat between 4–5 cm^2 to get a representative evaluation of the samples. The l* value is the lightness component, which ranges from 0 to 100 (from black to white); a* and b* both range from −60 to +60 with a∗ ranging from green if negative to red if positive and b* ranging from blue if negative to yellow if positive. All samples were served in the petri dishes. Measurement of color was accomplished at 0 day and repeated at, 7th days and 14th days; up to the end of the refrigerated storage at 4ºC.

Proximate Composition

Proximate composition such as Dry Matter (DM), Ether Extract (EE) and Crude Protein (CP) were carried out according to the methods (AOAC, 1995). All determination was done in triplicate and the mean value was reported.

Dry matter

Dry matter content determination was done by drying the sample. The differences in weight between the fresh and dried samples represent the water content. A microwave oven will be used for the experiment.

$$\%\ \text{of DM} = \frac{\text{Weight before drying} - \text{Weight after drying}}{\text{Weight befo re drying}} \times 100$$

Crude Protein (%)

The CP was determined by micro kjeldahl method. Total nitrogen content of each sample was determined in triplicate by using kjeldahl apparatus. In this case total nitrogen was determined by digestion the samples with 20 ml concentrated sulphuric acid (H_2SO_4) in presence of K_2SO_4, $CuSO_4$ and selenium powder followed by distillation of ammonia liberated by alkali (NaOH) into boric acid and titrated with standard HCl. The nitrogen values thus obtained were converted to total crude protein by multiply with a factor of 6.25.

The calculation is as follows:

$$\frac{\text{Titrate required (ml)} \times\ 0.014\ \text{(milli equivalent of N)} \times\ \text{Strength of HCl}}{\text{weight of sample}} \times 100$$

% of CP = % of nitrogen × conversion factor (6.25)

Ether Extract (%)

EE content was determined by Soxhlet apparatus using diethyl ether. At first empty flask weight was taken. Then 5g sample was taken in a thimble and added 200 ml acetone in a Soxhlet. Extraction was done at 40-45°C which took about 7-8 hours. After extraction the flask were taken out and dried in oven for 30 minutes at 100°C. The flask containing ether extract was cooled in desiccators and weighed. The calculated value for ether extract content was obtained as percent of the sample.

The formula is mentioned below:

$$\% \text{ of ether extract} = \frac{\text{Weight of the sample}}{\text{Weight of the Ether extract}} \times 100$$

Ash (%)

Weighed samples were taken in porcelain crucibles and pre-ashed at 100°C in an electric oven. The crucibles were then placed in a muffle furnace and heated at 550°C for 6 hours. The crucibles were then cooled in desiccators. The average weight in percentage of each sample of the remaining material was taken as ash.

The formula is mentioned below:

$$\% \text{ of Ash content} = \frac{E}{C} \times 100$$

Where,

E = Weight of ash

C = Weight of sample

Physicochemical properties measurement

Raw pH measurement

Meat pH value was measured 24 h after slaughter (ultimate pH) using a pH meter. The pH was measured by inserting electrode at three different points of the meat which was calibrated prior to use at pH 7.0 by pH meter (Hanna HI 99163, USA). Triplicate measurements at 1 cm depth on the medial portion of meat were averaged.

Water holding capacity (WHC)

WHC was measured according to the methodology of Choi et al. (2018). Thawed samples (1 g each) were wrapped in absorbent cotton and placed in a 1.5 ml eppendorf tube. The tubes with samples were centrifuged in a centrifuge separator (H1650-W Tabletop high speed micro centrifuge) at 10,000 RPM for 10 min at 4º C temperature and then the samples were weighed. The WHC% of the sample is expressed as the ratio of the sample weight after centrifugation to the initial sample weight, using the following formula:

$$\text{Water holding capacity (\%)} = \frac{(\text{Weight of sample after centrifugation})}{(\text{Weight of sample before centrifugation})} \times 100$$

Biochemical analysis

Thiobarbituric Acid Values (TBARS) (mg-MDA/kg)

Lipid oxidation was assessed in triplicate using the 2-thiobarbituric acid (TBA) method described by (Schmedes and Holmer, 1989). Chicken breast piece meat samples (5 g) were blended with 25 ml of 20% trichloro acetic acid solution (200 g/l of tricholoro acetic acid in 135 ml/l phosphoric acid solution) in a vortex machine for 60s. The homogenized sample was filtered with Whatman filter paper number 4 and 2 ml of the filtrate was added to 2 ml of 0.02 M aqueous TBA solution (3 g/l) in a test tube. The test tubes were incubated at 100°C for 30 min and cooled with tap water. The absorbance was measured at fixed wavelength of 532 nm using a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan). The TBA value was expressed as mg malonaldehyde per kg of chicken breast piece meat sample.

Equipment and reagents

2-thiobarbituric acid (TBA), 25 mL of 20% trichloro acetic acid solution (200 g/l of tricholoro acetic acid in 135 ml/ phosphoric acid solution), Whatman filter paper number 4, 2 ml of 0.02 M aqueous TBA solution (3 g/L), 532 nm using a UV-VIS spectrophotometer (UV-1200, Shimadzu, Japan), pestle and mortar, beaker, test-tube, graduated cylinder, temperature-controlled water-bath, centrifuge machine, vortex machine, cold water, spectrophotometer, and distilled water.

Microbial assessment

Total viable count, total coliform count and total yeast-mold count were performed for microbiological assessment. The procedures that were used to determine these parameters are described below:

Preparation of samples for TVC, TCC and Yeast-Mold count

A quantity of 10 g of chicken meat sample was aseptically excised from stored stock sample. Each of the stored chicken breast piece samples was thoroughly and uniformly macerated in a mechanical blender using a sterile diluents (0.1% peptone water) as per recommendation of International Organization for Standardization (ISO, 1995). A quantity of 10 g of the minced chicken breast piece sample was taken aseptically transferred into a sterile container containing 90 ml of 0.1% peptone water. A homogenized suspension was made in a sterile blender. Thus 1:10 dilution of the samples was obtained. Later on using whirly mixture machine different serial dilutions ranging from 10-2 to 10-6 were prepared according to the instruction of the standard method (ISO, 1995).

Media and reagent employed for bacteriological study

Solid media and reagents

The media employed for these bacteriological analysis included plate count agar (PCA), Macconkeyagar (MA) and potato dextrose agar (PDA). The commercial media were prepared according to the direction of the manufacturers. The diluent used during the study was 0.1% peptone water.

Glasswares and other appliances

Different types of glasswares and appliances were used during the course of the experiment. These included test tubes (with or without Durham's fermentation tube and stopper), pipette, a conical flask, Petri dishes (1 ml, 5 ml, 10 ml and 25 ml volumes), a glass rod mixer, a test tube holder, a pestle and mortar, a spiny mixture machine, blender machine, water bath, incubator, refrigerator, sterilizing instruments, hot air oven, ice boxes, electronic balance, electronic pH meter etc.

Preparation of media

In three separate conical flasks, 8.75 g of PCA agar, 27.54 g of MA agar and 19.5 gm of PD agar were dissolved in three separate 500 ml of cold distilled water, respectively, and then heated to boiling for dissolving the components thoroughly. After boiling, the mixture was sieved using clean cheesecloth to disintegrate the components thoroughly. The media were then sterilized in an autoclave at 121°C (6.795 kg pressure/sq. inch) for 30 minutes. The pH of the final reaction was set at 7.0 ± 0.1. The agar was now ready to be poured. The medium was maintained at 45°C in a water bath before pouring.

Enumeration of total viable count (TVC) (CFU/g)

To determine TVC, 0.1 ml of each ten-fold dilution was transferred and spread on triplicate PCA agar using a sterile pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. One sterile spreader was used for each plate. The plates were then kept in an incubator at 37°C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. Colonies were counted with the aid of a colony counter. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The TVC was calculated according to (ISO, 1995). The results of the total bacterial count were expressed as the number of organism of colony forming units per gram (CFU/g) of chicken breast piece meat samples.

Enumeration of total coliform count (TCC)

0.1 ml was sent and distributed across a triple dilution of every ten fold. The MA agar was used with a sterile pipette for each dilution to determine total coliform counts. The samples were diluted as soon as possible with a sterile glass spreader over the surface of the plate. Each plate was fitted with a sterile spreader. They were then maintained for 24-48 hours in an incubator at 37°C. Following incubation, plates exhibiting 30-300 colonies were counted. The samples were diluted as soon as possible with a sterile glass spreader over the surface of the plate. Each plate was fitted with a sterile spreader. They were then maintained for 24-48 hours in an incubator at 35°C. The total coliform count was calculated according to ISO (1995). The results of the total coliform count were expressed as the number of organism of colony forming units per gram (CFU/g) of chicken breast piece meat samples.

Enumeration of Yeast-Mold count

For the calculation of the number of yeasts and molds, a sterile pipette was used to transfer 0.1 ml of each tenfold dilution to duplicate PDA agar. The samples were diluted as soon as possible with a sterile glass spreader over the surface of the plate. Each plate was fitted with a sterile spreader. The plates were maintained for 48-72 hours in an incubator at 37°C. Following incubation, plates exhibiting 30-300 colonies were counted. With the use of a colony counter, the colonies were numbered. The dilution factor for yeast and mold count increased the average number of colonies at a specific dilution. The yeast and mold count was calculated according to ISO (1995). The results of the yeast and mold count were expressed as the number of organism of colony forming units per gram (CFU/g) of chicken breast piece meat samples.

Statistical model and analysis

The proposed model for the planned experiment was factorial experiment with two factors A (Treatments) and B (Days of Intervals) is:

$y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijk}$ $i = 1,\ldots,a$; $j = 1,\ldots,b$; $k = 1,\ldots,n$

Where:

y_{ijk} = observation k in level i of factor A and level j of factor B

μ = the overall mean

A_i = the effect of level i of factor A

B_j = the effect of level j of factor B

Data were statistically analyzed using SAS Statistical Discovery software, NC, USA. DMRT test was used to determine the significance of differences among treatments means.

Results and discussion

Sensory Evaluation

The total breast meat samples were divided into four groups. These were treated as T_0 = (Control group), T_1 = (1% Soybean oil), T_2 = (1 % mustard oil), T_3 = (1% flax seed oil).

Instrumental color value

In case of lightness (l*) of fresh broiler meat, most preferable color was observed from T_2 (56.45) and less preferable color was observed from T_0 (57.269) group among all four treatments. The most preferable color was observed at T_2 at 0 day (60.674) and less preferable color was found at 14^{th} day (52.02). The l* values were significantly differed at different treatment groups ($P < 0.01$), days intervals ($P < 0.01$) and the interaction between treatments and days interval ($P < 0.01$). In case of redness (a*) of fresh broiler meat, most preferable color was observed from T_2 (1.67) and less preferable color was observed from T_0 (0.338) group among all four treatments. The most preferable color was observed at T_2 at 14^{th} day (2.57) and less preferable color was found at 0 day (1.3). The a* values were significantly differed at different treatment groups ($P < 0.01$), days intervals ($P < 0.01$)

and the interaction between treatments and days interval (P < 0.01). In case of yellowness (b*) of fresh broiler meat, most preferable color was observed from T_2 (9.938) and less preferable color was observed from T_0 (7.588) group among all four treatments. The most preferable color was observed at T_2 at 0 day (11.49) and less preferable color was found at 14th day (7.71). The b* values were significantly differed at different treatment groups (P < 0.01), days intervals (P < 0.01) and the interaction between treatments and days interval (P < 0.01).

The meat color is the qualitative trait that most influences the choice of the consumer to purchase or reject the product. L*, a* and b* value of T_2 treatment were found higher compared to T_1 and T_3 treatments. All these values were found significantly differed (p<0.01). L*, a* and b* value decreased of increasing storage period. Gradual decline in color scores of meat stored at refrigeration conditions at 4ºC might be due to pigment and lipid oxidation resulting in non-enzymatic browning between lipids and amino acids. A similar result was reported by Kumar and Tanwar (2011) in ground mustard incorporated chicken meat nugget. A decrease in appearance and color scores of meat products with increase in storage period was also reported by Singh et al. (2011), Kandeepan et al. (2010) and Chidanandaiah and Sanyal (2009). Among four treatments, significantly higher color score was observed in 12% carrot group than other treatments which was similar to the findings Zargar et al. (2014).

Table 1. Effect of different types of oil on instrumental color value (Mean ± SE) in marinated chicken breast piece meat at 4±1°C temperature

Parameters	DI	Treatments					Level of significance		
Color		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T×DI
	0	64.090±3.471	59.733±4.587	60.674±1.601	54.183±4.530	59.670^{a}±3.533			
	7	54.370±2.981	56.566±2.601	59.656±1.693	56.236±2.370	56.708^{a}±2.412			
l*	**14**	53.346±3.412	51.863±6.294	52.020±3.010	45.616±0.913	50.712^{b}±3.407	**	**	**
	Mean	**57.269^{a}±3.288**	**56.054^{a}±4.494**	**56.450^{a}±2.101**	**52.012^{b}±2.604**				
	0	0.496±0.903	0.946±0.243	1.303±0.378	1.226±0.809	0.993^{c}±0.583			
	7	1.186±1.110	1.403±0.274	1.156±0.718	0.843±0.334	1.147^{a}±0.609			
a*	**14**	0.666±1.350	0.993±0.703	2.576±0.378	1.150±1.126	1.013^{b}±2.422	**	**	**
	Mean	**0.338^{c}±1.121**	**1.114^{b}±0.406**	**1.678^{a}±0.491**	**1.073^{b}±0.756**				
	0	5.636±1.614	6.280±0.587	11.496±2.718	7.720±1.800	7.783^{a}±1.694			
	7	9.290±0.371	9.460±0.318	10.606±1.971	8.823±1.360	9.545^{a}±1.005			
b*	**14**	7.836±0.276	**7.110±0.697**	**8.906±2.398**	7.310±1.087	8.091^{a}±1.114	**	**	**
	Mean	**7.588^{a}±0.753**	**8.416^{a}±1.101**	**9.938^{a}±1.735**	**7.591^{a}±1.415**				

Same superscripts in different treatments groups and days of interval did not differ significantly, whereas different superscripts in different treatments groups and days of interval differ significantly. T_0 = (Control group), T_1 = (1% Soybean oil), T_2 = (1 % mustard oil), T_3 = (1% flax seed oil), DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Interval. ** means significant at 1% level of probability.

Proximate analysis

There are four types of chicken meat sample were made for the determination of proximate components. These were treated as T_0 = (Control group), T_1 = (1% Soybean oil), T_2 = (1 % mustard oil), T_3 = (1% flax seed oil). In 0 day, DM, CP, EE, ash were deterrmined and then all samples were stored at 4±1 ºC for 14 days and analyzed on 0, 7th and 14th days. The values of proximate components are shown in table 4.2 shows that there were not significant in case of DM and CP and significant (p<0.05) in case of EE and ash, days of interval and interaction between treatment and days of interval for all sensory parameters (DM, CP, EE and Ash). The range for DM, CP, EE and Ash were 25.63 to 26.71, 21.56 to 21.89, 2.64 to 2.68, 1.35 to 1.45 respectively for all groups. The range values for days of interval for DM, CP, EE and Ash were 2.34 to 2.54, 21.54 to 21.97, 0.62 to 0.72 and 1.32 to 1.44 respectively.

Dry Matter (DM)

Table 2 shows that there were no significant difference in all treatments, days of interval and interaction between treatment and days of interval for DM parameter. The ranges for mean value of DM were 25.63 to 26.71 for all groups. Among these four treatments, most preferable DM content was observed at T_3 group. The lowest amount DM content indicates this product is most preferable. The highest amount of DM content indicates this product is less preferable. Less preferable DM content was observed at control group. The DM content was increased with the increase storage period because moisture loss was decreased with the increase storage period. The most preferable DM content was observed from 0 day and less preferable DM content from 14th day but in terms of consumers view it was accepted. Similar results were reported for Indonesian traditional meatballs with a DM content ranged from 56.17 to 60.32% (Purnomo and Rahardiyan, 2008). Naveena et al. (2008) also reported an increase in the DM content with the increase storage period for pomegranate peel extract and pomegranate rind powder extract respectively.

Crude Protein (CP)

Table 2 shows that there were no significant difference in all treatments, days of interval and interaction between treatment and days of interval for CP parameter. The ranges for mean value of CP were 21.56 to 21.89 for all groups. Among four treatment groups, the lowest amount of CP content was observed from T_3 group. The CP content was decreased with the increase storage period. The most preferable CP content was observed at 0 day and less preferable CP content at 14th day but in terms of consumers view it was accepted. Suradkar et al. (2013) reported a decrease in the protein content of chicken nuggets containing bread crumbs. Similar findings were also reported by Ali et al. (2022) in spent chicken nuggets incorporated with bee honey. Protein content decreased significantly in wheat bran and dried carrot pomace incorporated chicken sausage (Yadav et al., 2018) which is similar to the present findings.

Ether Extract (EE)

Table 2 shows that there were significant difference in all treatments, days of interval and interaction between treatment and days of interval for EE parameter. The ranges for mean value of EE were 2.64 to 2.68 for all groups. Among four treatment groups, the most preferable EE content was observed from T_2 group. The lowest amount of EE content indicates this product is most

preferable for consumers' health. Less preferable EE content was observed from T_2 group. The EE content was decreased with the increase storage period. The data shows that the EE content was decreased to 2.66% in all treatments after 14 days of storage. Verma et al. (2013) observed a decrease in the fat content of mutton nuggets by the incorporation of guava powder. Suradkar et al. (2013) also reported similar results in different meat products. Ether extract content of the products showed significantly ($p<0.05$) decreasing trend with increasing levels of incorporation of pumpkin in chicken sausages reported by Zargar et al. (2014).

Ash

Table 2 shows that there were significant difference in all treatments, days of interval and interaction between treatment and days of interval for Ash parameter. The ranges for mean value of Ash were 1.35 to 1.45 for all groups. Among these four treatments, the most preferable ash content was observed from T_2 group. The lowest amount of ash content indicates this product is most preferable for consumers' health. Less preferable ash content was observed at control group. The ash content was significantly increased with the increase storage period. The most preferable Ash content was observed at 0 day and less preferable ash content at 14th day but in terms of consumers view it was accepted. The data showed that the highest amount of ash content was increased to 1.47% in all treatments after 14th days of storage. Zargar et al. (2017) reported that the ash content of the products showed significant ($P<0.05$) decreasing trend with increasing levels of incorporation of carrot in chicken sausages. Bhosale et al. (2011) found a decrease in the ash content for ground carrot and mashed sweet potato incorporated chicken nuggets which are similar to this findings.

Table 2 Effect of different types of oil on proximate parameters (Mean ± SE) in marinated chicken breast piece meat at 4±1°C temperature

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T×DI
DM (%)	**0**	25.20±0.041	26.46±0.038	26.45±0.044	26.66±0.057	26.34±0.047	NS	NS	NS
	7	25.79±0.058	26.63±0.034	26.52±0.057	26.69±0.049	26.47±0.049			
	14	25.89±0.063	26.89±0.021	26.71±0.047	26.78±0.055	26.54±0.046			
	Mean	**25.63±0.054**	**26.66±0.031**	**26.56±0.049**	**26.71±0.053**				
CP (%)	**0**	22.61±0.011	21.69±0.034	21.88±0.011	21.70±0.011	21.97±0.016	NS	NS	NS
	7	21.57±0.017	21.59±0.010	21.75±0.012	21.53±0.012	21.61±0.013			
	14	21.49±0.017	21.53±0.012	21.69±0.011	21.47±0.015	21.54±0.014			
	Mean	**21.54±0.015**	**21.60±0.018**	**21.77±0.011**	**21.56±0.012**				
EE (%)	**0**	2.52±0.003	2.62±0.005	2.63±0.005	2.61±0.005	0.62^{c}±0.004	*	*	*
	7	2.61±0.003	2.66±0.005	2.66±0.004	2.66±0.004	0.65b±0.004			
	14	2.68±0.006	2.70±0.003	2.76±0.005	2.69±0.005	0.72^{a}±0.004			
	Mean	**2.64^{c}±0.007**	**2.66^{b}±0.003**	**2.68^{a}±0.003**	**2.66^{b}±0.001**				
Ash (%)	**0**	1.17±0.017	1.32±0.014	1.31±0.011	1.35±0.026	1.32^{c}±0.017	*	*	*
	7	1.36±0.015	1.46±0.010	1.43±0.017	1.42±0.022	1.44^{a}±0.0.16			
	14	1.52±0.015	1.58±0.008	1.57±0.015	1.57±0.016	1.47^{a}±0.013			
	Mean	**1.35^{b}±0.015**	**1.45^{a}±0.011**	**1.43^{a}±0.015**	**1.44^{a}±0.021**				

Different superscripts in different treatments groups and days of interval did not differ significantly. T_0 = (Control group), T_1 = (1% Soybean oil), T_2 = (1 % mustard oil), T_3 = (1% flax seed oil), DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Intervals.*means significant at 5% level of probability.

Physicochemical Quality

For the physicochemical study, four types of chicken meat samples were prepared. These T_0 = (Control group), T_1 = (1% Soybean oil), T_2 = (1 % mustard oil), T_3 = (1% flax seed oil). After determining the pH on the 0 day, all samples were kept at 4°C for 14 days and tested on the 0, 7th and 14th days.

pH Value

The pH changes in chicken meat treated with different types of oil during refrigerated (4°C) storage are shown in Table 3. At varied treatment levels, the overall observed mean pH of the chicken samples ranged from 6.27-6.44. Throughout the storage periods, the pH of chicken samples indicated a significant difference ($P<0.001$) between treatments. The various superscripts seen on the 0, 7th, and 14th days of observation revealed a substantial difference. Throughout the storage period, T_2 maintained lowest pH values than control, T_1 and T_3 samples. In compared to the control group, T_2 had the most preferred pH during the storage period. The pH value of meat in all treatments gradually decreased as the storage period extended. The range of overall observed mean pH value was 6.22 to 6.50 at different days of interval. The initial pH value of the control sample was 6.70 and decreased to 6.25 after 14 days of storage, significantly higher than other treatments (Table 3). The accumulation of lactic acids from microbial secretions and thaw loss of chicken meat were likely to blame for the lowering pH trend. Bacteria and mold have a tendency to diminish as storage duration increases, and they release pH lowering components. Similar findings were observed by Singh et al. (2011). The rise in the pH ($P<0.05$) of the control samples may be caused by bacterial consumption of acids produced during the breakdown of proteins due of the depletion of the stored glucose. The last increase in pH levels might have been caused by release of ammonia molecules from endoprotease or proteolytic microbial flora in the raw meat (Sarker et al., 2021; Ali et al., 2022).

Water holding capacity (WHC)

Table 3 shows the WHC of chicken meat combined with various oils as well as the control group after 14 days of refrigerated storage. On days 0, 7 and 14, there was a substantial variation between the different treated batches. The range of overall observed WHC from the meat was 91.00 to 93.66 at different treatment levels. The range of overall observed of different days of intervals of WHC value was 91.66 to 93.58. Among four treatments, the WHC in the control sample was significantly higher

than in the samples treated with different types of oil respectively. The WHC was gradually declined during storage in various treatments as storage days increased. Among these four treatments, the most preferable WHC value was observed from T_2 group. The lowest amount of WHC value indicates the product is most preferable for consumer's health.

Several researchers have demonstrated that a significant negative correlation exists between breast meat lightness color values and breast meat pH (Allen et al., 1997). Poultry meat with low pH has been associated with low water-holding capacity (WHC), which results in increased cook-loss and driploss (Froning, 1991).

Table 3. Effect of different types of oil on physicochemical parameters (Mean ± SE) in marinated chicken breast piece meat at 4±1°C temperature

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T×DI
	0	6.70±0.035	6.47±0.023	6.32±0.016	6.52±0.017	6.50^{a}±0.022			
	7	6.37±0.023	6.30±0.014	6.27±0.006	6.36±0.008	6.33^{b}±0.013			
pH	**14**	6.25±0.023	6.18±0.008	6.23±0.008	6.24±0.010	6.22^{c}±0.011	**	**	**
	Mean	**6.44^{a}±0.026**	**6.32^{b}±0.015**	**6.27^{c}±0.01**	**6.37^{b}±0.011**				
Water	**0**	94.33±0.333	93.66±0.333	92.66±0.333	93.66±0.566	93.58^{a}±0.391			
holding	**7**	93.00±0.577	92.33±0.333	90.66±0.881	91.66±0.881	91.91^{b}±0.668			
capacity	**14**	93.66±0.023	91.66±0.008	89.66±0.666	91.66±0.333	91.66^{b}±0.257	*	*	*
	Mean	**93.66^{a}±0.311**	**92.55^{b}±0.224**	**91.00^{c}±0.626**	**92.33^{b}±0.593**				

Same superscripts in different treatments groups and days of interval did not differ significantly, whereas different superscripts in different treatments groups and days of interval differ significantly. T_0 = (Control group), T_1 = (1% Soybean oil), T_2 = (1 % mustard oil), T_3 = (1% flax seed oil), DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Intervals. ** means significant at 1% level of probability; *means significant at 5% level of probability.

Biochemical properties

Thiobarbituric Acid Value (TBARS)

Table 4 shows that there were significant difference in all treatments, days of interval and interaction between treatment and days of interval for TBARS parameter. The ranges for mean value of TBARS were 0.165-0.174 for all groups. Among these four treatments, the most preferable TBARS value was observed from T_2 group. The lowest amount of TBARS value indicates the product is most preferable for consumer's health. The TBARS values increased significantly ($p<0.001$) during storage in all treatments. Similar findings were reported by Chidanandaiah and Sanyal (2009) in meat patties during refrigerated storage. Yadav et al. (2018) found a significant increase in TBARS value of control and fiber enriched sausage with an increase in storage period. Similar findings were reported by Sarker et al. (2021) in goat meat sausage during refrigerated storage.

Table 4. Effect of different types of oil on biochemical parameters (Mean ± SE) in marinated chicken breast piece meat at 4±1°C temperature

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T×DI
	0	0.083±0.003	0.099±0.005	0.101±0.004	0.109±0.001	0.098^{c}±0.004			
TBARS	**7**	0.105±0.001	0.116±0.002	0.128±0.001	0.123±0.001	0.118^{b}±0.001			
(mgMDA/	**14**	0.314±0.012	0.293±0.003	0.266±0.005	0.296±0.003	0.292^{a}±0.005	**	**	**
kg)	**Mean**	**0.167^{c}±0.007**	**0.170^{b}±0.003**	**0.165^{c}±0.003**	**0.174^{a}±0.001**				

Different superscripts in different treatments groups and days of interval differ significantly. T_0 = (Control group), T_1 = (1% Soybean oil), T_2 = (1 % mustard oil), T_3 = (1% flax seed oil), DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Intervals. ** means significant at 1% level of probability. TBARS = Thiobarbituric acid reactive substances.

Microbiological assessment

The present study observed the presence of micro-flora (TVC) and food borne pathogens (Coliform and Yeast-Mould) on control and different treatment groups at different days of intervals and at different treatment levels. After 0 days of observation, four types sample was preserved at 4°C for the observation at 7^{th} and 14^{th} days. Table 5 shows that there were significant difference in all treatments, days of interval and interaction between treatment and days of interval for all parameters (TVC, TCC and TYMC). The range for TVC, TCC and TYMC were 5.26 to 5.47, 2.39 to 2.81 and 2.47 to 2.84 respectively for all groups. The range values for days of interval for TVC, TCC and TYMC were 5.22 to 5.44, 2.36 to 2.62 and 2.45 to 2.77 respectively.

Total viable count (TVC)

The total viable count in the control sample was significantly higher than in the samples treated with all group among four treatments. The most preferable TVC was found in T_2 at 0 day and minimum in 14 days. Table 4 shows the total viable count of chicken meat combined with various oils as well as the control group after 14 days of refrigerated storage. On days 0, 7 and 14, there was a substantial variation between the different treated batches. The range of overall observed TVC from the meat was 5.26 to 5.47 (log10CFU/g), at different treatment levels. The range of overall observed of different days of intervals of TVC value was 5.22 to 5.44 (log10CFU/g). Among four treatments, the viable count in the control sample was significantly higher than in the samples treated with different types of oil respectively. TVC was gradually raised during storage in various treatments as storage days increased. The less amount of TVC value indicates the product is most preferable for consumer's health (T_2 group). However, a number of studies have demonstrated that compounds existing in many spices also possess antimicrobial activity (Zheng et al., 2000). Mixtures of cinnamon and clove oil were able to suppress the growth of major spoilage microorganisms in intermediate moisture foods (Matan et al., 2006). It was reported by Bithi et al. (2020), Disha et al. (2020) and Hossain et al. (2021) that the plant extracts such as garlic, ginger and roselle provided antioxidant and antimicrobial benefits to raw chicken products during cold storage. Microbial load was reduced in treated samples than the control.

Total coliform count (TCC)

The total coliform count in the control sample was significantly higher than in the samples treated with all group among four treatments. The most preferable TCC was found in T_2 at 0 day and minimum in 14 days. Table 4 shows the total coliform count of chicken meat combined with various oils as well as the control group after 14 days of refrigerated storage. On days 0, 7 and 14, there was a substantial variation between the different treated batches. The range of overall observed TCC from the meat was 2.39 to 2.81 (log10CFU/g), at different treatment levels. The range of overall observed of different days of intervals of TCC value was 2.36 to 2.62 (log10CFU/g). Among four treatments, the coliform counts in the control sample were significantly higher than in the samples treated with different types of oil respectively. TCC was gradually raised during storage in various treatments as storage days increased. The less amount of TCC value indicates the product is most preferable for consumer's health (T_2 group).Similar findings were observed by Singh and Immanuel (2014) of raw chicken meat emulsion incorporated with clove powder, ginger and garlic paste at refrigerated storage (4±1ºC). Reddy et al. (2017) observed a significantly ($P<0.05$) lower coliform count in chicken meat patties incorporated with natural antioxidant extracts i.e., rosemary (RE) and green tea (GTE).

Total yeast-mould count (TYMC)

The total yeast-mould count in the control sample was significantly higher than in the samples treated with all group among four treatments. The most preferable TYMC was found in T_2 at 0 day and minimum in 14 days. Table 4 shows the total yeast-mold count of chicken meat combined with various oils as well as the control group after 14 days of refrigerated storage. On days 0, 7 and 14, there was a substantial variation between the different treated batches. The range of overall observed TYMC from the meat was 2.47 to 2.84 (log10CFU/g), at different treatment levels. The range of overall observed of different days of intervals of TYMC value was 2.45 to 2.77 (log10CFU/g). Among four treatments, the yeast and mold counts in the control sample were significantly higher than in the samples treated with different types of oil respectively. TYMC was gradually raised during storage in various treatments as storage days increased. The less amount of TYMC value indicates the product is most preferable for consumer's health (T_2 group). The antibacterial action of CP inhibited fat deterioration and prevented bacteria from metabolizing fat. Asha et al. (2014) found that essential spice oil also increased the lifetime of minced beef to six days when kept at a temperature of 4±1°C. In Buffalo meat, treated with essential clove oil maintained at cooling temperature. Fernandes et al. (2016) reported on the results of a research study related to antimicrobials in beef meatballs. They noted that the presence of mould and yeasts was not detected in any cooked meatball samples. The lower TYMC of the treated meat sample may be attributed by the antifungal properties of oil.

Table 5. Effect of different types of oil on microbial parameters (Mean ± SE) in marinated chicken breast piece meat at 4±1°C temperature

Parameters	DI	Treatments					Level of significance		
		T_0	T_1	T_2	T_3	Mean	Treat.	DI	T×DI
	0	5.34±0.023	5.20±0.014	5.16±0.018	5.18±0.023	5.22^{c}±0.019			
	7	5.46±0.011	5.23± 0.017	5.27±0.024	5.27±0.040	5.31^{b}±0.023			
TVC	**14**	5.62±0.011	5.39±0.020	5.36±0.022	5.43±0.057	5.44^{a}±0.028	**	**	**
(logCFU/g)	**Mean**	**5.47^{a}±0.015**	**5.27^{c}±0.017**	**5.26^{c}±0.021**	**5.29^{b}±0.047**				
	0	2.69±0.057	2.27±0.030	2.28±0.051	2.37±0.011	2.36^{c}±0.038			
	7	2.81±0.011	2.33±0.029	2.41±0.057	2.41±0.057	2.53^{b}±0.038			
TCC	**14**	2.98± 0.018	2.64±0.034	2.49±0.053	2.50±0.057	2.62^{a}±0.041	**	**	**
(logCFU/g)	**Mean**	**2.81^{a}±0.029**	**2.41^{c}±0.031**	**2.39^{c}±0.052**	**2.44^{b}±0.042**				
	0	2.69±0.057	2.39±0.057	2.30±0.057	2.32±0.057	2.45^{e}±0.057			
	7	2.87±0.057	2.51±0.014	2.51±0.023	2.53±0.017	2.59^{b}±0.027			
TYMC	**14**	2.98±0.014	2.80±0.023	2.61±0.017	2.64±0.057	2.77^{a}±0.027	**	**	**
(logCFU/g)	**Mean**	**2.84^{a}±0.048**	**2.56^{b}±0.023**	**2.47^{c}±0.027**	**2.49^{c}±0.052**				

Different superscripts in different treatments groups and days of interval differ significantly. T_0 = (Control group), T_1 = (1% Soybean oil), T_2 = (1 % mustard oil), T_3 = (1% flax seed oil), DI=Day Intervals, Treat= Treatment, T×DI=Interaction of Treatment and Day Intervals. ** means significant at 1% level of probability. TVC = Total viable count, TCC = Total coliform count, TYMC =Total yeast and mould count.

Conclusion

It reveals from the study that chicken breast meat can be preserved for 10 days using mustard oil. It may be an effective and cheap solution to prolong the shelf life of broiler breast meat. It also reveals that mustard oil added at 1 % in chicken meat sample evinced better results upon its assessment on the basis of certain physicochemical properties, proximate composition, anti-oxidative properties and sensory attributes of chicken meat over other levels as well as control. Therefore, it can be recommended for marination of chicken meat sample as prolong storage. Therefore, it can be concluded that level of 1% mustard oil for marination of chicken breast meat to extend the storage of meat

Conflicts of Interest

The authors declare that there is no potential conflict of interests.

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