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[1]Department of Animal Science
Bangladesh Agricultural University
Mymensingh- 2202, Bangladesh
[2]Department of Poultry Science
Bangladesh Agricultural University
Mymensingh-2202, Bangladesh

***Corresponding author:**

Professor Dr. Md. Mukhlesur Rahman
Department of Animal Science
Bangladesh Agricultural University
Mymensingh- 2202, Bangladesh
Email: mmrahman.as@bau.edu.bd

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Food grade vinegar acts as an effective tool for short-term meat preservation

MIA Sarker[1], MA Hashem[1], MAK Azad[1], MS Ali[2] and MM Rahman[1]*

Abstract

The study was designed to evaluate the physico-chemical, microbial and sensorial quality of beef incorporated with different levels of vinegar at refrigerated storage (4±1ºC). Fresh beef samples were divided into three different batches i.e. T_0 = control (without vinegar), T_1 = 5% vinegar and T_2 = 10% vinegar. The samples were evaluated for sensory properties (color and flavor), physico-chemical properties (pH, cooking loss, FFA, POV and TBARS) and microbial counts (TVC, TCC and TYMC) on 0, 7 and 14 days of storage at 4ºC. The obtained results showed that addition of different levels of vinegar significantly ($p<0.05$) influenced on sensory, physico-chemical and microbiological properties compared to control samples. Comparatively better color and flavor were found in T_1 and T_2 respectively among the treatments ($p<0.05$). Lower pH was observed in T_1 and T_2 compared to control treatment. POV, FFA and TBARS values were found better in T_1, whereas the other treatments fluctuated slightly. The lowest microbial counts were significantly lower in higher vinegar treated groups along with at different day's intervals in TVC, TCC and TYMC ($p<0.01$). It might be stated from the experiment that vinegar is a means of fresh beef preservation for a short time. From this study, it also concluded that 10% vinegar is effective for short term preservation of beef satisfactorily at refrigerated condition.

Introduction

Beef is a widely available source of animal protein in Bangladesh. Meat is the edible part of the skeletal muscle of an animal and is a highly perishable product due to its biological composition (Akter et al., 2009). In Bangladesh there are 402.56 million livestock and poultry from which 7.51 million metric ton meat is produced in 2019 (DLS, 2019). Meat was the first important food that met the hunger of ancient people living in caves. It plays a very vital role in keeping the human body strong in order to provide energy, health and vigor (Baset et al., 2003; Rahman, 2000). Meat is the most concentrated and easily accessible nitrogenous food and is a good source of high quality protein that contains those amino acids, essential for human life. Polyunsaturated fatty acids of meat are important for brain development especially during the fetal state. Meat can be regarded as an important source of dietary vitamins and minerals. A vegetable diet compared with a meat diet is usually incomplete in respect of essential amino acids. Moreover, the vegetable proteins are less easily digested and remain in the stomach for a shorter period than meat protein with the result that a feeling of hunger recurs more readily (Gracey, 1992).

Meat is an excellent source of many important nutrients; however, it is subject to a high rate of deterioration. It is also an ideal environment for bacteria to thrive due to its high protein and moisture contents. Microbial deterioration of meat begins soon after exsanguinations. The quantity of spoilage microorganism present in fresh meat at the time of processing has an impact on product's shelf life. Bacteria, mould and yeast are the three common microorganisms found in meat. The moulds and yeast growing on meat are aerobic whereas bacteria thriving in meat could be aerobic, anaerobic or facultative (Jay et al., 2008). Several researchers had tried to increase the shelf life of meat and meat products through different processes such as refrigeration (Rahman et al., 2017), curing (Woods et al., 2009), Drying (Akhter et al., 2009), irradiation (Haque et al., 2017; Islam et al., 2018, 2019 and 2021; Rima et al., 2019) and by adding natural antioxidants (Bithi et al., 2020; Disha et al., 2020; Saba et al., 2018; Jahan et al, 2018; Siddiqua et al., 2018; Hashem et al., 2021). The process of freezing may decrease the number of microorganisms during storage. However, some species of bacteria found during refrigerated storage such as Pseudomonas, *Brochothrix thermosphacta* and lactic acid bacteria (LAB) can survive this process and resume growth (Labadie, 1999; Ellis and Goodacre, 2001; Signorini et al., 2006). Freezing with vinegar has been an excellent preserving technique for meat in which meat can be preserved in a condition similar to that of normal state and can be kept satisfactory for six months to one year but with poor procedures the quality of meat deteriorates within a few days (Crist et al., 2014; Theron and Lues, 2007). The quality and safety of frozen meat depends upon rapid freezing, continuous electricity supply, temperature stability, good freezer management, proper packaging and cleanliness before freezing. Unfortunately, most of those points have not been followed in

Bangladesh because of ignorance, unawareness and unavailable materials/techniques. Fresh meat remains almost the same food value and flavor after proper freezing with vinegar.

Vinegar is one of the few products around today that has been in grandma's kitchen for centuries, making it a clean-label ingredient. Often described as sour to the taste buds, vinegar ingredients have long been appreciated by meat and poultry processors for their contribution to tenderizing, preserving, enhancing flavor and even influencing color. Although acetic acid is the primary constituent of vinegar after water, acetic acid is not vinegar. Vinegar contains many vitamins and other compounds inherent to the substrate material. Some of these are responsible for imparting color and unique flavors to vinegar. Some vinegars further develop color and flavor from being fermented or aged in wood barrels (Park et al., 2014a). There are many varieties of commercial vinegar, which are often used in meat and poultry marinades (Crist et al., 2014; Karam et al., 2020). The quality of meat is generally determined by appearance, texture, flavor, color, microbial activity and nutritive value. All these parameters are influenced by freezing and frozen storage. But now a day, most of the people of Bangladesh want to consume fresh and safe meat. The demand for meat and meat products is increasing continuously in Bangladesh, due to the higher income of food consumers. A limited research has been done in Bangladesh to investigate the meat quality and shelf life with vinegar application on raw meat. Therefore, present study has aimed to investigate and carryout to observe the effect of vinegar on sensory, physicochemical and microbiological quality of beef during refrigerated storage.

Material and Methods

Place of experiment

The research was conducted at Meat Research Lab., Department of Animal Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Experimental samples

The samples of beef were collected from 'Tarakanda-bazar' in Mymensingh district. The samples were obtained from hind limbs of a bull which was above 2 years (age determined through dentition; 2 teeth) and weighing live weight of 250 kg. After removing the fat, ligaments, bone and tendons from the muscles, they were randomly divided into nine (9) parts. A quantity of 2 kg of beef samples were taken from the forelimbs and packed in a zipper bag separately. The parameters were studied for three types of properties from these samples. The samples were refrigerated at a temperature around 4ºC for 14 days. The sensory properties like color, smell, juiciness, tenderness and overall acceptability were observed. The physico-chemical properties like moisture loss, pH, lipid oxidation as per-oxide value, free fatty acids value and TBARS value were analyzed.

Preparation of sample

The samples were observed to study the sensory parameters. About 1 kg raw beef sample was evaluated for color and odor at different days. These were accomplished at 0, 7 and 14 days of refrigerated storage at 4ºC. After that beef samples from three thawing methods were prepared for determining water holding capacity (WHC), pH, peroxide value, free fatty acids (FFA) value and thiobarbituric acid reacting substances (TBARS). The equipment required is a balance of sufficient accuracy (± 0.05 g), beef meat samples, petridis, sensory evaluation sheet, Knife, chopping board, filter paper, pH meter, weighing machine, grinding machine, conical flask, beaker, funnel and aluminum foil paper.

Sensory evaluation

Raw beef samples were analyzed for their color and odor according to the American Meat Science Association guidelines (AMSA, 1995). Sensory evaluation was carried out in individual booths under controlled conditions of light, temperature and humidity. Sensory evaluation was accomplished at 0 day and repeated at 7 and 14day; up to the end of refrigerated storage at 4ºC.

pH measurement

The pH was determined (Trout et al., 1992) with a digital pH meter (Seven Easy pH, Mettler-Toledo GmbH, Switzerland). For this, 10 g of sample was homogenized with 50 ml of distilled water using a vortex machine for 1min and the electrode was dipped into the suspension to note down the pH.

Cooking Loss

To determine cooking loss, weighed 15g sample and wrapped it in a heat-stable foil paper and kept in a water bath at 80°C for 30 minutes. The internal temperature was not measured, but from a previous study it was estimated that the optimum internal meat temperature (75-80°C) would be gained by 30 minutes. Samples surface were dried and weighed. Cooking loss was practiced at 0 day, 14 days and 28 days. Cooking loss was calculated after draining the drip came out the cooked meat as follows:

$$\text{Cooking loss (\%)} = \frac{(\text{Weight of sample} - \text{weight after cooking at 71°C for 30 min})}{\text{Weight of sample}} \times 100$$

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 centigrade temperature, following which 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:

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

Free fatty acid value

Free fatty acids (FFA) were determined according to (Koniecko, 1979). Five (5) g of the sample was weighed in a 250 ml glass stopper Erlenmeyer flask with 30 ml of chloroform, and then thoroughly agitated with a vortex machine for 1 min. The sample was filtered under vacuum through Whatman filter paper number 1 to remove meat particles. Five drops of 1 percent phenolphthalein indicator was added with the filtrate and titrated against 0.1N alcoholic KOH to get the end point (pink color).

Percent FFA content was calculated as:

FFA (%) = ml titration × Normality of KOH × 28.2/g of sample

Peroxide value (POV)

Peroxide value (POV) was determined according to (Sallam et al., 2004). The sample (3 g) was weighed in a 250 ml glass stopper Erlenmeyer flask and heated in a water bath at 60°C for 3 min to melt the fat, then thoroughly agitated with vortex machine for 3 min with 30 ml acetic acid-chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered under vacuum through Whatman filter paper number 1 to remove meat particles. Saturated potassium iodide solution (0.5 ml) was added to the filtrate and continued with addition of starch solution. The content was titrated against 0.01N sodium thiosulphate to get the end point (non-aqueous layer turned to colorless). POV was calculated and expressed as milliequivalent peroxide per kilogram of sample:

POV (meq/kg) = (S×N/W) × 1000

Where, S= Titration value (ml), N= Strength (0.01), W= Sample weight (g)

Thiobarbituric acid reactive substances (TBARS)

TBARS value was determined as per the extraction method described by Witte et al. (1970). For this, 5 g of ground sample was mixed with 25 ml of pre-cooled 20% trichloroacetic acid (TCA) solution using a vortex machine for 1 min. Then the content was filtered through Whatman filter paper No. 1 to get TCA extract. Two (2) ml of this TCA extract was mixed with 2 ml of TBA solution (orthophosphoric acid 4.05 ml, TBA0.09g, DW 30 ml, for 34.5 ml TBA solution) in small beaker and placed in an oven (100°C) for 30 minutes. Then take it to a normal temperature. After reducing the temperature TBARS value was measured at fixed wavelength of 532 nm with a scanning range of 531 nm to 533 nm using UV-VIS spectrophotometer (Elico SL-159, Mumbai, India).

Microbial assessment

For microbial assessment total viable count, total coliform count and total yeast-mould count were undertaken. To determine these parameters the procedures which were followed are described below:

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

Total viable count (TVC), Total coliform count and Yeast and Mould count of the samples were enumerated following the methods as described by American Public Health Association (APHA, 1984). About 10 g of sample was blended with 90 ml of sterile 0.1% peptone water in a pestle and mortar and serial dilutions were prepared as per recommendation of International Organization for Standardization (ISO, 1995). Thus 1:10 dilution of the samples was obtained. Later on using whirly mixture machines different serial dilutions ranging from 10-2 to 10-6 were prepared according to the instruction of the standard method.

Solid media and reagents

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

Glassware and other appliances

Different types of glassware and appliances were used during the course of the experiment. These were as follows: Test tubes (with or without Durham's fermentation tube and stopper), petri dishes, conical flask, pipette (1 ml, 5 ml, 10 ml and 25 ml capacities), glass rod spreader, test tube stand, mortar and pestle, whirly mixture machine, blender machine, water bath, incubator, refrigerator, sterilizing instruments, hot air oven, ice boxes, electronic balance, electronic pH meter etc.

Enumeration of total viable count (TVC)

For the determination of total bacterial counts, 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 35°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 total viable count was calculated according to ISO (1995). The results of the total bacterial count were expressed as the number of organisms of colony forming units per gram (CFU/g) of beef samples.

Enumeration of total coliform count (TCC)

For the determination of total coliform counts, 0.1 ml of each ten-fold dilution was transferred and spread on triplicate MA 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 35°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 coliform count. The total coliform count was calculated according to ISO (1995). The results of the total coliform count were expressed as the number of organisms of colony forming units per gram (CFU/g) of beef samples.

Enumeration of yeast and mould count

For the determination of yeast and mould counts, 0.1 ml of each ten-fold dilution was transferred and spread on triplicate PDA 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 25°C for 48-72 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 yeast and mould count. The yeast and mould count was calculated according to ISO (1995). The results of the yeast and mould count were expressed as the number of organisms of colony forming units per gram (CFU/g) of beef samples.

Experimental design and statistical analysis

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

$yijk= \mu + Ai + Bj+(AB)ij+ \varepsilon ijki = 1,\ldots,a; j = 1,\ldots,b; k = 1,\ldots,n$
Where, yijk= observation k in level i of factor A and level j of factorB
B μ = the overall mean
Ai = the effect of level i of factor A
Bj= 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.

Results and discussions

Effect of vinegar on the sensory evaluation of beef

Samples of each group were evaluated by the eight honorable judges. Sensory evaluation was carried out in individuals both under control conditions of light, temperature, and humidity. Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair, and 1 for poor. All samples were served in the Petri dishes and were returned for further physicochemical analysis. Experimental data of sensory evaluation are shown in Table 1.

Color

It was found a positive effect of vinegar on the juiciness during the refrigerated storage of beef. The color was significantly influenced byvinegar treated groups compared with control groups. The range of color score among all three treatments was 3.55 to 4.88. The highest initial color score was found in T_0 (4.88) but, after 14^{th} days of refrigerated storage T_1 showed the maximum color score (4.50). The color scores were significantly differences among the treatments, days interval and interaction between the treatment and days interval ($p<0.05$). Most preferable color was observed from T_1 and less preferable color was observed from T_0 during the 14^{th} days of refrigerated storage. A decrease in appearance and color scores of meat products with increase in storage period was also reported by Kandeepan et al. (2010). Some authors reported that lightness values in meat and meat products are related to surface water, water vapor exchanges between the products and the environment and modifications of the different states of the home pigments (Fernandez-Lopez et al., 2005).

Flavor

The results of beef flavor at different vinegar treated groups are presented in Table 1. The range of odor score among three treatments was 4.37 to 4.47. The range of flavor among different days of intervals was 4.08 to 4.77. The flavor scores were significantly differences among the treatments, days interval and interaction between the treatment and days interval ($p<0.05$). Flavors are generated from a complex interaction of tastes, tactile senses and aromas taken collectively throughout the tongue, nasal, sinus and oral cavities. The science of consumer acceptance, cluster analyses and drawing relationships among all flavor determinants is a relatively new discipline in beef flavor. Consumers rate beef that has lipid degradation products generated from a low degree of doneness and Maillard flavor products from fast, hot cookery the highest in overall liking, and current research has shown that strong relationships exist between beef flavor and consumer acceptability, even more so than juiciness or tenderness. Similar value was observed in flavor is one of the major causes of quality deterioration (Raghavan et al., 2007) because it can negatively affect sensory attributes such as color, texture, and odor as well as the nutritional quality of the product (Nunez and Boleman, 2008). Deterioration of flavor during storage occurred due to microbial growth, formation of FFA and oxidative rancidity (Irshad et al., 2016).

Table 1. Effect of vinegar on color and odor of beef stored at 4±1°C (mean value)

Parameter	DI	Treatment				Level of Significance		
		T_0	T_1	T_2	Mean	D	T	D×T
Color	0	4.88^{a}	4.84^{a}	4.66^{b}	4.79			
	7	4.38^{b}	4.72^{a}	4.62^{a}	4.24	*	*	*
	14	3.55^{b}	4.50^{a}	4.45^{a}	4.17			
	Mean	4.27	4.67	4.58				
Flavor	0	4.56^{b}	4.88^{a}	4.88^{a}	4.77			
	7	4.35^{b}	4.74^{a}	4.76^{a}	4.62	*	*	*
	14	3.33^{c}	4.50^{b}	4.66^{a}	4.16			
	Mean	4.08	4.71	4.77				
Juiciness	0	4.66^{b}	4.88^{a}	4.88^{a}	4.81			
	7	4.12^{b}	4.66^{a}	4.70^{a}	4.49	*	*	*
	14	3.54^{c}	4.33^{b}	4.52^{a}	4.13			
	Mean	4.11	4.62	4.70				

Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair, and 1 for poor. 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 = 5 % level of vinegar, T_2 = 10 % level of vinegar, D=Days of Intervals, T= Treatment, T×D=Interaction of treatment and days of intervals.*means significant at 5% level of probability.

Juiciness

It was found a positive effect of vinegar on the juiciness during the refrigerated storage of beef. The range of juiciness score among three treatments was 4.54 to 4.88. Higher vinegar level (T_2) showed the maximum juiciness (4.88) among all three treatments and the lowest juiciness (3.54) was found in T_0 treatment. The juiciness scores were significantly differences among the treatments, days interval and interaction between the treatment and days interval ($p<0.05$). Meat juiciness is also an attribute valued by most consumers which increases flavor, softness, easier to chew, and stimulates saliva production in the mouth. Although consumers routinely pay more for cuts of meat that are typically tenderer, there is some expectation that the meat will also be juicy. Moreover, meat juiciness plays a key role in meat texture, probably contributing to its variability (Thompson, 2004). In order to obtain a tender and juicy meat, there is a complex interplay between the animal's pasture, age, species, breed, protein intake, calcium status, stress before and at killing, and how the meat is treated after slaughter (Morgan et al., 2002).

Effect of vinegar on the physic-chemical properties of beef

Raw pH evaluation

The pH changes in beef treated with vinegar during refrigerated (4°C) storage are shown in Table 2. The pH values were generally decreased in T_1 and T_2 samples after 0 days of refrigerated storage but these values were gradually increased with the increased storage period. The range of overall observed mean pH from the meat samples was 4.45–4.82 at two different levels of vinegar. The initial pH value of the control group was 5.54 and increased to 5.72 after 14th days of refrigerated storage. All the values were significantly higher than that of vinegar treated groups. The different superscripts observed from three treatment groups indicates there were significant differences ($p<0.05$) in pH among the treatments. The data showed a slight increase in the pH values for all samples up to 14th days of experiment. Among these three treatments, the most preferable raw pH was observed from the T_1treatment. A much lower pH was found at higher vinegar level (T_2). The pH followed an increasing trend throughout the refrigerated storage period in all the samples, similar with the findings of Sallam et al. (2004). They observed a significant increase in the pH of both control and treated batches with the advancement of the storage period.

Cooking loss

The range of overall observed cooking loss at different treatments was 26.31 to 35.23% during the refrigerated storage of beef at different levels of vinegar. Among all the treatments, the highest cooking loss was observed at control group (no vinegar) than vinegar treated groups. The lowest amount of cooking loss was found in T_2indicated this product is most preferable for consumers' choices than other treatment groups. The average cooking losses were 33.69, 29.25 and 28.36% respectively in T_0, T_1 and T_2 and these were statistically significant ($p<0.05$). Significant differences were also found in different days' intervals (0, 7 and 14) along with interaction between treatments and days interval regarding cooking loss. Cooking loss indicates the reduction in weight of raw beef during refrigerated storage. Major components of cooking losses are thawing, dripping and evaporation.

Water holding capacity

A lower water holding capacity was found in T_0 (8.16%) compared to T_1 (8.87%) and T_2 (9.93%) during 14 days of refrigerated storage period. It was found that addition of vinegar in two different levels on raw meat increase its WHC and the values were significantly different ($p<0.05$). The range of overall WHC at different treatments was 7.22 to 10.44% during the refrigerated storage of beef at different levels of vinegar. Among all the treatments, the highest WHC was observed at T_2 group (10.44%) at the 1st day of experiment. Significant differences in WHC were also found in different days intervals (0, 7 and 14th) along with interaction between treatments and days interval regarding cooking loss ($p<0.05$). Water holding capacity (WHC) is the ability of meat to hold its inherent and added moisture during fabrication, processing, and storage. Poor water holding capacity in meat results in diminished visual appeal and inferior palatability traits for consumers as well as reduced ingredient retention, protein functionality, and product yields for processors. Raw beef muscle fibers are susceptible to undergoing a rapid *postmortem* pH decline and exhibiting inferior WHC characteristics. In pork and turkey, low WHC is closely linked to excessive *postmortem* protein denaturation, particularly that of myosin. Other reports found minimal differences in protein denaturation between pale breast meat and normal or dark colored meat. Furthermore, limited data have been reported on changes in WHC in chicken meat with postmortem time. It has been reported that cook and drip loss increased in broiler breast fillets from 1 to 6 days postmortem, but the mechanism for this increase was not explored. Using a salt-induced water uptake method, it has been observed that WHC is greater at 24 h than at 2 h postmortem in broiler breast meat (Bowker and Zhuang, 2016).

Table 2. Effect of vinegar on pH and cooking loss of beef stored at 4±1°C (mean value)

Parameter	DI	Treatment				Level of Significance		
		T_0	T_1	T_2	Mean	D	T	D×T
pH	0	5.54^{a}	5.12^{b}	4.64^{c}	5.10			
	7	5.67^{a}	5.18^{b}	4.82^{c}	5.43	*	**	*
	14	5.72^{a}	5.22^{b}	4.96^{c}	5.30			
	Mean	5.64	5.17	4.81				
Cooking loss	0	35.23^{a}	32.44^{b}	31.65^{c}	33.11			
	7	33.38^{a}	28.42^{b}	27.12^{c}	29.64	**	**	*
	14	32.45^{a}	26.88^{b}	26.31^{c}	28.55			
	Mean	33.69	29.25	28.36				
Water holding capacity	0	8.92^{c}	9.33^{b}	10.44^{a}	8.16			
	7	8.35^{c}	9.05^{b}	10.13^{a}	9.18	**	**	*
	14	7.22^{c}	8.24^{b}	9.23^{a}	8.23			
	Mean	8.16	8.87	9.93				

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 = 5 % level of vinegar, T_2 = 10 % level of vinegar, D=Days of Intervals, T= Treatment, T×D=Interaction of treatment and days of intervals. ** means significant at 1% level of probability; *means significant at 5% level of probability.

Free fatty acid value (FFA %)

Table 3 shows the effects of different levels of vinegar with the control group on free fatty acid (FFA) value. The FFA value of the control sample remained significantly higher on 0, 7 and 14th day of refrigerated beef storage compared to vinegar treated groups. The range of overall observed FFA (%) of different days of intervals was 0.33 to 0.58%. The different superscripts at 0, 7 and 14th days of observation indicated that there were significant ($P<0.01$) differences among these three days of observation. The most preferable FFA was observed from 0 day at T_2 and less preferable FFA was observed from the 14th day at T_0 treatment. Higher vinegar level might be inhibited the oxidation of fats and that reduced the FFA value. During the refrigerated storage of beef with vinegar, the FFA values were also significantly differed between the interaction of treatment and days interval ($p<0.05$). This may be due to possible low lipolysis and lipolytic enzyme activity in vinegar treated leading to low production of free fatty acids (Aguirrezabal et al., 2000). The significant ($p<0.05$) increase in FFA content of the products during storage might be due to growth of lipolytic microorganisms (Das et al., 2008). The FFAs are products of the enzymatic or microbial degradation of lipids. It gives information about stability of fat during storage.

Peroxide value (POV)

Table 3 shows the effects of different levels of vinegar with the control group on peroxide value (POV). The POV of the control sample remained significantly higher on 0,7 and 14th day of refrigerated beef storage compared to vinegar treated groups. Within the vinegar treated groups the POV was also vary significantly ($p<0.01$). T_0 showed lower POV than T_1 and T_2. Among these three treatments, the most preferable peroxide value was observed in T_0. The lowest amount peroxide value indicates this product is most preferable for consumer's health. The highest amount of peroxide value indicates this product is less preferable. Peroxide value of control and treatments showed a significant difference ($p<0.01$) in between the storage period. Similar results were reported by Singh et al. (2014). The lowest amount peroxide value indicates this product is most preferable for consumers' health. During refrigerated storage with vinegar the POV increased in all treatments, but the increasing pattern in vinegar treated groups were slower. However, antioxidant with treatments generally could minimize POV in meat sample during storage compared with the control.

Thiobarbituric acid reactive substances (TBARS)

The highest TBARS value was found in T_0 compared to vinegar treated groups and the lowest TBARS value was found in T_2 treatment. The average TBARS values were 0.35, 0.13 and 0.09 meq-MA/kg in T_0, T_1 and T_2, respectively. The range of overall observed TBARS value of different days of intervals was 0.07 to 0.42meq-MA/kg. The values were significantly differed among all the treatment ($p<0.01$) along with the different days interval ($p<0.05$). During the refrigerated storage of beef with vinegar, the FFA values were also significantly differed between the interaction of treatment and days interval ($p<0.05$). Among these three treatments, the most preferable TBARS value was observed in higher level of vinegar treatment (T_2). The lowest amount of TBARS value indicates the product is most preferable for consumer health.

Vinegar is a liquid solution and one of the most typical pickling agents with 5-10% acetic acid and it preserves food by altering water activity or pH (Vaishali et al., 2019). It provides flavor to the product and also vitamins. The pH 4.6 is a distinguishing and characteristic feature of the process which doesn't allow the bacteria to grow and proliferate. Vinegar can be synthesized by alcoholic fermentation or the acetic acid fermentation. Vinegar provides an acidic medium to the food for preservation and improves the shelf life of the food product. Park et al. (2014b) stored the blanched tea leaves for 4 days at 30ºC in pickling solutions as mixture of soy sauce, water and vinegar in different concentrations. Jang et al. (2006) conducted a study on Korean seasoned beef to examine vinegar and sake as preservation hurdles and detect their effect of sensory quality and microbial stability. They found that the combination of vinegar and sake did not improve the sensory quality however; microbial stability was improved both at 8ºC and 20ºC. Acetic acid has been used for decontamination of carcass to increase shelf life. Laboratory studies showed significant reduction of E. coli on rib-eye steaks treated with an acetic acid dip.

Table 3. Effect of vinegar on the physic-chemical properties of beef stored at 4±1°C (mean value)

Parameter	DI	Treatment				Level of Significance		
		T_0	T_1	T_2	Mean	D	T	D×T
FFA (%)	0	0.41^{a}	0.38^{b}	0.33^{c}	0.37			
	7	0.50^{a}	0.39^{b}	0.35^{c}	0.41	*	**	*
	14	0.58^{a}	0.43^{b}	0.39^{c}	0.47			
	Mean	0.50	0.40	0.36				
POV (meq/kg)	0	4.26^{a}	3.04^{b}	2.21^{c}	3.17			
	7	4.66^{a}	3.11^{b}	2.25^{c}	3.34	*	**	*
	14	4.84^{a}	3.17^{b}	2.33^{c}	3.45			
	Mean	4.59	3.11	2.26				
TBARS (meq-MA/kg)	0	0.28^{a}	0.11^{b}	0.07^{c}	0.15			
	7	0.35^{a}	0.14^{b}	0.09^{c}	0.19	*	**	*
	14	0.42^{a}	0.15^{b}	0.12^{c}	0.23			
	Mean	0.35	0.13	0.09				

Different superscripts in different treatments groups and days of interval differ significantly. T_0 = Control group, T_1 = 5 % level of vinegar, T_2 = 10 % level of vinegar, D=Days of Intervals, T = Treatment, T×D=Interaction of treatment and days of intervals. ** means significant at 1% level of probability; *means significant at 5% level of probability. FFA = Free fatty acids, POV = Peroxide value, TBARS = Thiobarbituric acid reactive substances

Effect of Vinegar on the Microbiological properties

The presence of total micro-flora (Total Viable Count or TVC) and food borne pathogens (Coliform and Yeast-Mold) were identified in two levels of vinegar along with control treatment during the refrigerated storage of beef. The experimental data is shown in Table 4.

Total viable count (TVC)

The average TVC in T_0 group (6.27 logs CFU/g) was significantly ($p<0.01$) higher than the vinegar treated samples. Average TVC value was 5.11 logs CFU/g in T_1 and 3.31 logs CFU/g in T_2 after 14th days of refrigerated beef preservation. Higher level of vinegar effectively reduced the microbial load during the refrigerated storage of beef. It was found that the level of vinegar was proportional to the suppression of microbial growth. The less amount of TVC value indicates this product is most preferable for consumers' health (T_2 group). The Differences of TVC values among the different vinegar treated groups along with control differed significantly ($p<0.01$). Significant differences were also found in different days intervals (0, 7 and 14th) along with interaction between treatments and days interval ($p<0.05$).

Total coliform count (TCC)

The highest TCC value was found in T_0 group (1.75 logs CFU/g) and the lowest TCC value was found in T_2 (0.67 logs CFU/g) and the values were significantly differed among different treatments ($p<0.01$). Average TCC value was 1.70, 1.14 and 0.84 logs CFU/g in T_0, T_1 and T_2 after 14th days of refrigerated beef preservation. Higher level of vinegar effectively reduced the growth of coliform bacteria during the refrigerated storage of beef. It was found that the level of vinegar was proportional to the suppression of the growth of coliform bacteria. The less amount of TCC value indicates this product is most preferable for consumers' health (T_2 group). The Differences of TCC values among the different vinegar treated groups along with control differed significantly ($p<0.01$). Significant differences were also found in different days intervals (0, 7 and 14th) along with interaction between treatments and days interval ($p<0.05$).

Total yeast and mold count (TYMC)

The highest TYMC value was found in T_0 group (1.95 logs CFU/g) and the lowest TYMC value was found in T_2(1.07 logs CFU/g) and the values were significantly differed among the different treatment groups ($p<0.01$). The average TYMC value was 1.91, 1.13 and 1.11 logs CFU/g in T_0, T_1 and T_2 respectively, after 14th days of refrigerated beef. Higher level of vinegar effectively reduced the growth of yeast and mould count during the refrigerated storage of beef. It was found that the level of vinegar was proportional to the suppression of the growth of yeast and mould count. The less amount of TYMC value indicates this product is most preferable for consumers' health (T_2 group). The Differences of TYMC values among the different vinegar treated groups along with control differed significantly ($p<0.05$). Significant differences were also found in different days intervals (0, 7 and 14th) along with interaction between treatments and days interval ($p<0.05$).

The vinegar or a combination of the cultured sugar/vinegar blend and commercial seasoning may be valuable ingredients to control *Clostridium jejuni* and *Salmonella typhimurium* in poultry products during refrigerated storage. Additionally, the 3% cultured sugar and vinegar blend prevented texture change in frozen chicken breasts and increased tenderness of refrigerated, ready-to-eat chicken breasts, which is often cited as the most important quality of both refrigerated and frozen meat (Birk et al., 2010; Park et al., 2014a; Theron and Lues, 2007).

Table 4. Effect of vinegar on the microbial properties of beef stored at 4±1°C (mean value)

Parameter	DI	Treatment				Level of Significance		
		T_0	T_1	T_2	Mean	D	T	D×T
TVC (log CFU/g)	0	6.22^a	5.07^b	3.25^c	4.85	*	**	*
	7	6.25^a	5.12^b	3.27^c	4.88			
	14	6.35^a	5.13^b	3.40^c	4.96			
	Mean	6.27	5.11	3.31				
TCC (log CFU/g)	0	1.67^a	1.05^b	0.67^c	1.13	*	**	*
	7	1.68^a	1.15^b	0.88^c	1.24			
	14	1.75^a	1.23^b	0.97^c	1.32			
	Mean	1.70	1.14	0.84				
TYMC (log CFU/g)	0	1.88^a	1.11^b	1.07^c	1.35	*	**	*
	7	1.89^a	1.12^b	1.13^c	1.38			
	14	1.95^a	1.16^b	1.14^c	1.42			
	Mean	1.91	1.13	1.11				

Different superscripts in different treatments groups and days of interval differ significantly. T_0 = Control group, T_1 = 5 % level of vinegar, T_2 = 10 % level of vinegar, D=Days of Intervals, T = Treatment, T×D=Interaction of treatment and days of intervals. ** means significant at 1% level of probability; *means significant at 5% level of probability. TVC = Total viable count, TCC = Total coliform count, TYMC = Total yeast and mould count

Conclusion

Vinegar is widely used as preservative and marinating agent in culinary industry. It has acidic properties along with strong shelf life quality. The present research reveals that 10% vinegar can improve all physico-chemical attributes and also act as anti-microbial agents irrespective of storage durations. Undoubtedly, 10% vinegar fortification in fresh meat for preservation at 4°C is very much effective.

Conflicts of Interest

The authors declare that there are no potential conflicts of interests.

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