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Impact of *Moringa oleifera* Leaf and Flaxseed on Lipid Oxidation and Microbiological Characteristics of Chicken Burger During Cold Storage

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Abstract

Background: Practice of making burgers out of chicken instead of red meat is gaining popularity. because of their high fat substance and on account of no social or strict limitations to the utilization of poultry. The present study aimed to assess effect of adding *Moringa oleifera* leaf and flaxseed combinations on lipid oxidation and microbiological traits of chicken burger during cold storage.

Methods: In this study the pH, peroxide value, thiobarbituric acid (TBA) as well as microbiological characteristics of chicken burger formulated by various levels of *Moringa oleifera* leaf and flaxseed powder were evaluated. Samples were as follows: control=0%FS+0%MLP; T1=20%FS+0%MLP; T2=15%FS+5%MLP; T3=10%FS+10%MLP; T4=5%FS+15%MLP; and T5=0%FS+20%MLP.

Results: The results showed that the pH value of burger samples supplemented with *Moringa oleifera* and flaxseed was decreased ($P \leq 0.05$) with an increasing period of storage and ranged between 3.5–5.1 and 3.3–4.9 when stored for 15 and 30 days, respectively.

Conclusion: Peroxide values of T2, T3, T4 and T5 as well as the value of TBA within the MLP-treated and FS-treated samples ($P \leq 0.05$) decreased with the progression of the storage period. Microbiological characteristics ($P \leq 0.05$) were affected by the incorporation of MLP and FS in chicken burger treatments.



Introduction

Moringa oleifera is a species of the monogenic family: It is native to the semi-Himalayan regions of India, Pakistan, Bangladesh and Afghanistan. The plant has great nutritional and medicinal value and contains many minerals, vitamins and proteins of high biological value [1,2].

Despite the nutritional properties found in flaxseeds, many people are not aware of the health benefits of flaxseeds and their nutritional applications. Flaxseed content of omega-3acids, dietary fiber and lignans; attracting food manufacturers to be used in commercial food manufacturing [3].

One of the main causes of the decomposition of meat and meat products is lipid oxidation. Additionally, it has an impact on low-density lipoprotein (LDL) cholesterol and many other lipid compounds [4]. The final byproducts of this process can degrade the organoleptic qualities of the meat and standardized goods, including their flavor, color, and scent. As a result, their nutritional value is reduced [5].

Other than nutritional degradation, lipid oxidation produces cytotoxic and genotoxic mixtures that are harmful to people's health [6]. Products made from meat that have been oxidatively damaged have issues such tissue damage, putrefaction, loss of complement, an increase in free radicals, and the creation of malonaldehyde, which lowers the antioxidant capacity of the products. [7]. The values of TBA and peroxide can be used as quality indicators of fat in chicken meat within 4 days of storage in the refrigerator [8].

Due to the growth of bacteria and oxidation of lipids during storage, the flavor and nutritional value of chicken burgers are both negatively impacted [9]. By preventing and delaying oxidation, the expansion of cellular improvements has come to be known as a method for extending the time period for the consumption of food goods and lowering waste and nutritional losses [10]. In meals, they serve as cell reinforcements. However, toxicologists and nutritionists have long recognized that many substances used in food preparation have a toxic nature [9].

Moringa leaves contain a variety of useful compounds making it a nutritious source of natural antioxidants [11]. The use of Moringa leaf meal was found to expand the crude protein and reduce the fat content of frankfurter sausage where a maximum of 6 g/kg of meat was used [12]. In addition, beef burger patties were used to a level of 12%. Several plausible explorations have additionally proven their various restorative states to be true [13].

The burger consists of minced meat usually in the form of a disk with diameters of 80-150 mm and thickness of 5-20 mm. In cheap food outlets, the

common name is hamburger or basically a burger. Initially, burgers were made with beef (ideally lean beef), but as of late chicken and lamb burgers are increasingly becoming more natural. Depending on the type and nature of the products, additional animal tissues, such as lipids or connective tissues/ligaments, may be included in the mixture. The burger is frequently served with green salad, mayonnaise, mustard, and cheese bits on bread rolls or buns [14].

At the slaughter stage, meat is contaminated from many sources. Controlling the microbiological quality of meat and meat products is crucial to achieving the best quality and safety since the health of the animal has an impact on the final product. Various meat products should be checked for the presence of toxins such mycotoxins, clostridia, and *Staphylococcus aureus* [15].

The chicken may have previously undergone a number of processing steps before being transported to the market or to additional processing facilities for cooking. The microbiological quality of chickens depends on certain processes, such as immersion and irradiation [16].

The presence of pathogenic bacteria such as coliform bacteria in meat and meat products may indicate contamination with pathogenic bacteria such as salmonella and staphylococcus bacteria, which causes significant effects on the consumer. To verify this, some pathogens were detected in the chicken burger samples. The current study aimed to investigate the effect of *Moringa oleifera* and flaxseed combinations on fat oxidation and the microbiological properties of chicken burgers.

Methods

Materials

Raw chicken meat purchased from a general store in Abha, Saudi Arabia, carefully cleaned, boned, and ground with a grinder, then refrigerated for one day prior being used to prepare chicken burgers, flaxseeds, and *Moringa oleifera*, salt, white pepper, black pepper, garlic and onions were sourced from the local market, and used to prepare chicken burgers. Treatments are as follows: control = 0% FS + 0% MLP; T1 = 20 % FS + 0% MLP; T2 = 15% FS + 5% MLP; T3 = 10% FS + 10% MLP; T4 = 5% FS + 15% MLP; and T5 = 0% FS + 20% MLP.

Chicken burger processing

As stated by [17], fresh chicken burger samples were set up. All components were twice minced, and then, using a burger maker, 50g baffles were made into circular discs of burger. The products were cooked for 20 minutes to a temperature of 75°C within a hot air oven that had been preheated to 180°C. The burgers were

rotated over every 10 minutes to ensure uniform cooking.

Determination of pH

The pH of different chicken burger samples was measured using a digital pH meter (ON9410.GL.Britain) according to [18].

Peroxide value determination

The official standard method was used to measure the peroxide value [18]. After being steeped in 200 ml of chloroform for eight hours, 50g of chicken burger samples were filtered. Next, a saturated potassium iodide solution is reacted with in the dark with a mixture of the filtrate and chloroform/acetic acid (2:1.5, v/v). The amount of peroxide in the released iodine was then determined by titrating it against a sodium thiosulfate solution and calculating the milliequivalents of active oxygen per kilogram of sample (meqO₂/kg).

Determination of TBA value

According to the procedure outlined by [19], the TBA values of raw chicken burgers and treatments at 0, 15, and 30 days of storage were calculated and reported as milligrams of malonaldehyde per kilogram of the sample.

Microbiological characteristics

Microbiological characterization was performed using the method described by [20]. To determine the different microbiological characteristics of chicken burger samples, a series of dilutions were prepared using 30g of each sample and sterile distilled water to give a 10⁻¹ dilutions. 1 ml of the suspension was added to the first tube of the dilution series, which consisted of six tubes each holding 9 ml of sterile distilled water. This was done several times up to dilution 10⁻⁷.

Agar plate count was used to estimate the total viable number. 1ml of aliquots of the appropriate dilutions were transferred to sterile Petri dishes. 10-15ml of thawed and cooled agar is added to each dilution. The inoculums were mixed well with the medium and left to solidify. The total viable number was expressed as (cfu/g) for the sample. Potato dextrose agar (PDA) medium with 40 ppm chloramphenicol was used to count yeast and mold number. 0.1ml of samples were plated onto PDA medium and incubated at 25°C-28°C for 48h. Counts were expressed as (cfu/g) of samples.

Coliform counts were estimated by coating one ml sample on MacConkey Agar medium. The plates were incubated at 37°C for 48h and the numbers were expressed as (cfu/g) of samples. Brilliant green bile lactose broth was used as a confirmatory test. The test tubes were then incubated at 44°C for 48hours. Each confirmed positive tube was cultured in E. To

determine the number of staphylococci 0.1ml of the appropriate dilutions were plated onto Baird Parker Agar medium. The plates were then incubated at 37°C for 48h and the numbers were presented as (cfu/g) of the sample.

Statistical analysis

To evaluate several chicken burger samples, the findings were examined using analysis of variance (ANOVA), Duncan's multiple range test, and 5% probability significance.

Results

Peroxide value

The peroxide value of the T2, T3, T4 and T5 chicken burger samples was decreased with the increase storage period (Fig.1). The peroxide values of control and T1 significantly ($p \leq 0.05$) increased with storage with a maximum value of 1.88 and 1.82, respectively after 30 days.

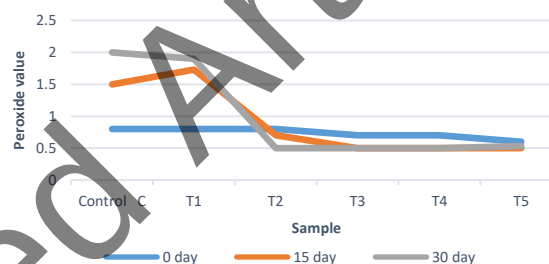


Figure 1: Peroxide value of various samples of chicken burger. Control = 0% Flaxseed (FS) + 0% Moringa Leaf powder (MLP); T1 = 20% FS + 0 % MLP; T2 = 15 % FS + 5% MLP; T3 = 10% FS + 10% MLP; T4 = 5% FS + 15% MLP; and T5 = 0% FS + 20% MLP

TBA (Thiobarbituric acid) value

TBA value of the various chicken burger samples are illustrated in (Fig.2). Significant differences were found in stored burger samples. The TBA values of control and T1, T2, T4 and T5 were significantly ($p \leq 0.05$) increased with storage with a maximum value of 2.54mg/kg for T1 after 15days, while a significant decrease on TBA value (1mg/kg) was noticed in T3 with (10%FS+10%MLP).

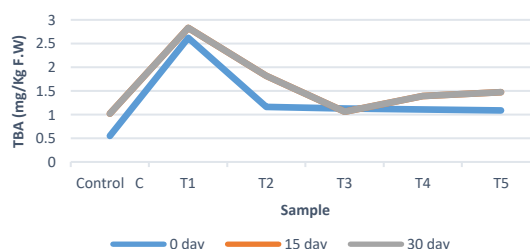


Figure 2: TBA value of the various chicken burger samples. Control = 0% Flaxseed (FS) + 0% *Moringa oleifera* (MLP); T1

= 20% FS + 0 % MLP; T2 = 15 % FS + 5% MLP; T3 = 10% FS + 10% MLP; T4 = 5% FS + 15% MLP; and T5 = 0% FS + 20% MLP

pH values

The pH values of the various chicken burger samples are illustrated in (Fig.3). The pH of the burger samples decreased slightly throughout the cold storage period with increase of Moringa leaf powder and decrease of flaxseed powder. The initial pH of control fresh chicken burger was 5.9, however, the initial pH values were varied from (3.5-5.5) in the various chicken burger samples supplemented with Moringa leaf and flaxseed powder. The pH of burger samples stored for 15 and 30 days ranged between 3.5–5.1 and 3.3–4.9, respectively.

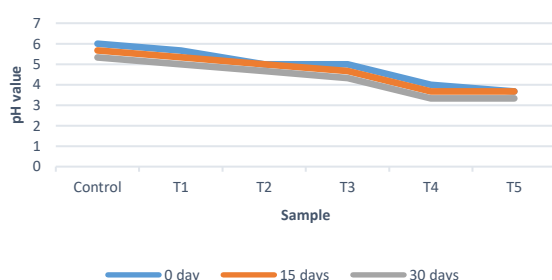


Figure 3: pH value of various samples of chicken burger. Control = 0% Flaxseed (FS) + 0% Moringa Leaf powder (MLP); T1 = 20% FS + 0 % MLP; T2 = 15 % FS + 5% MLP; T3 = 10% FS + 10% MLP; T4 = 5% FS + 15% MLP; and T5 = 0% FS + 20% MLP

Microbiological characteristics of chicken burger

As shown in (Fig. 4), The highest viable count was found in T1 burger sample which was formulated with 20% FS, while the lowest count was found in T5 burger sample which was formulated with 20% MLP (Moringa leaf powder).

Within samples treated with MLP and FS, total coliform count of T1 (20%FS+0%MLP) was higher than T4(5%FS+15%MLP) throughout storage period. Within samples treated with M.L.P. and F.S., the total coliform count of T1 (20%FS+0%MLP) was higher than T4(5%FS+15%MLP) throughout the storage period.

Escherichia coli was found in the control and T1 burger samples without significant difference as a result of storage for 15 and /or 30days. On the other hand, *E. coli* was devoid in T2, T3, T4 and T5 burger samples. Also, *Staphylococcus aureus* was detected in the control burger samples as well as T1, T2 and T3 burger samples on 0 day, while *Staphylococcus aureus* was devoid for the entire of the storage period in T3, T4 and T5 burger samples.

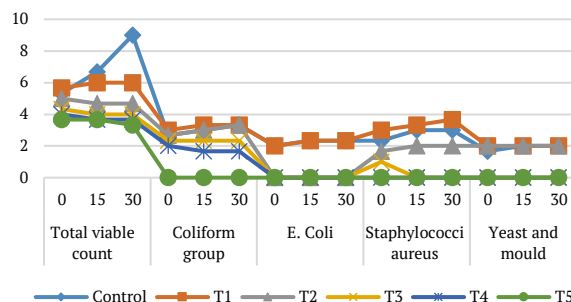


Figure 4: Effect of flaxseed flour (FS) and moringa oleifera leaf powder (MLF) on the microbiological properties of chicken burgers. Control = 0% Flaxseed (FS) + 0% Moringa Leaf powder (MLP); T1 = 20% FS + 0 % MLP; T2 = 15 % FS + 5% MLP; T3 = 10% FS + 10% MLP; T4 = 5% FS + 15% MLP; and T5 = 0% FS + 20% MLP

Discussion

During storage at both refrigeration and freezing temperatures, lipid oxidation of fresh chicken burgers experiences significant unfavorable alterations. Peroxide and TBA value were selected in this investigation to reflect primary and secondary lipid oxidation, respectively. In general, burger samples containing MLP and FS shown postponed oxidation of lipids in comparison to the controls on all storage days. The use of MLP and seed extracts in meat products was also shown in multiple studies to greatly reduce lipid oxidation [21, 22]. The reduction in peroxide readings may be caused by MLP's ability to prevent lipid peroxidation because it includes antioxidants such polyphenols and flavonoids [23].

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The incorporation of burger with MLP and FS resulted in a slight decrease in the total viable count of burger samples when compared with the total viable count of control sample, this reduction of the total viable count may be due to antimicrobial properties of *Moringa oleifera* leaves [30]. Similar results were obtained with [31, 32, 22]. The results present in (Fig. 4), reveals that coliform was found in the control and in all chicken burger treatment with exception to T5 sample, which was devoid of this microbial group.

According to the findings in (Fig. 4), MLP plays a protective role in chicken burgers. [33] have shown that

moringa leaves can prevent the growth of a variety of dangerous bacteria, including *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, and *Enterobacter aerogenes* [21] further suggested that the MLP may have contributed to the decrease these microorganisms found in chicken patties treated with 50 and 100 g/kg moringa during the course of the storage period. Intriguingly, the elimination of *Staphylococcus aureus* by integration of (10%FS+10%MLP) in chicken burgers highlights the protective effect of MLP and FS that may improve the safety of these meals during storage. Both the T1 and T2 burger samples as well as the control chicken burger contained small amounts of yeast and mold and absent in other samples. In addition, storage for 15 or 30 days had no significant effects on yeast and mould count.

The study showed that the lipid oxidation and microbial characteristics of chicken burger could be enhanced through the addition of *Moringa oleifera* leaf and flaxseed powder. Based on TBA evaluation conducted, T3 with (10%FS+10%MLP) was found to be the most generally acceptable with low microbial load. Therefore, preparation of burger with 10% FS+10% MLP may improve the oxidative stability and microbiological quality of chicken burger during the cold storage.

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