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Evaluation quality chicken meat during frozen storage

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Abstract

The comet assay was carried out (Deoxyribonucleic acid (DNA) was found in the breast and the thigh), and the degradation of DNA in the chicken sample may be an indicator that the storage conditions were not suitable. It is an indication for the quality of meat called malondialdehyde (MDA). The total volatile basic nitrogen, also known as TVB-N, is an indicator of the degradation of proteins, and this test is used to evaluate the quality of meat. a total of forty frozen carcass samples were collected at random from different Iraqi local markets in Baghdad. Twenty of these samples were extracted from the breast and thigh flesh of the same carcass and subjected to analysis with a comet assay, spectrophotometer, and keldjal equipment. The DNA comet assay revealed the lowest value in frozen breast DNA damage (9.85±1.88), but the result of the malondialdehyde MDA revealed no significant differences between the groups. The findings of the total volatile basic nitrogen TVB-N test showed that frozen thigh meats had a value of (9.19± 0.30) whereas breast meats had a value of (7.81± 0.58).

Keywords: DNA; MDA; TVB-N; Chicken meat; Quality

1. Introduction

Frozen chicken is mostly used to extend the product's shelf life and keep its quality up until it is eaten. There are many ways that fresh chicken meat can be changed by both chemical and biological processes. Simple ways to lessen the damage that has been done to chicken are to freeze and cure it [1]. However, if the process of freezing is well-managed and adheres to health regulations, bacteria present in frozen chicken meat will not grow if it is substituted for fresh chicken meat [1]. Skins of imported chicken femurs produced high level of contamination [2]. Bacteriological growth and chemical or enzymatic deterioration reactions can be slowed by freezing food, making it possible to keep food for a longer period of time [3].

Chicken has a higher concentration of monounsaturated and polyunsaturated fatty acids (MUFA and PUFA) than pork or red meat (PUFA). Alterations to poultry's lipid profile may have an effect on the meat's taste and nutritional value. [4-5]Poultry production is a growing and economically an important industry, and therefore, the interest in improving the production results through improved health of the poultry [6].

The DNA comet assay is a simple, rapid, and inexpensive technique that can be used to monitor the achievement of an unbroken cold chain by official control agencies. Using the comet assay, researcher demonstrate how to assess DNA damage in frozen chicken that has been subjected to temperature abuse and extended storage, respectively [7].

The amount of TVB-N present in chicken meat is one of the most important chemical reference indices used to determine the freshness of the meat [8].The TVBN (total volatile basic nitrogen) content of meat is an important indicator of the likelihood of meat spoilage occurring. The freshness of the meat is critical in determining consumer acceptance, as it is

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for their health-related requirements. During the storage period, the amount of nitrogen in the meat product (in the form of TVBN) will increase. Some volatile amines, such as ammonia, dimethylamine (DMA), and triethylamine (TMA), will accumulate and increase over time, causing the meat to lose its freshness and flavor [9-10].

Malondialdehyde (MDA) is a toxic byproduct of polyunsaturated fatty acid peroxidation that has received the most attention additionally, MDA reacts with DNA in human cells to form highly mutagenic adducts [11].Lipid oxidation can induce chemical spoilage, pigment degradation, lipid, essential fatty acid, protein, and fat-soluble vitamin breakdown, and energy loss. It can also create health concerns by generating carcinogenic compounds (such as malondialdehyde MDA) and possibly poisonous substances in meat and food items (such as aldehydes, ketones, and alkanes) [12].

2. Material and methods

2.1. Sample collection

A forty frozen carcass samples were taken randomly from Iraqi local markets (Baghdad) , Twenty samples were taken from the breast and thigh meat have taken from the same carcass**.**

2.2. DNA comet assay

2.2.1. Cell suspensions

A surgical blade was used to slice about 1 g of chicken tissue from the chest and thighs before the skin was removed. 5 cc of ice-cold phosphate buffered saline (PBS) was poured into a measuring glass containing the tissue pieces, using a magnetic stirrer at 500 rpm for 5 minutes, a syringe was filled with ice and the tissue was separated. To further refine the homogenate, it was run through a 400-mm mesh screen and then a 200-mm nylon filter. The investigation was aided by the suspension in this manner.

2.2.2. Comet assay

The method described by [13]was employed in this case. After drying, the slides were coated with 50–100 ml of a 5 g/l low gelling temperature (LGT) agarose solution and examined. This was done to ensure that the cell-agarose blend was firmly attached to the slide. To make this mixture, we added 8ml of the cells to 300ml PBS at 40C, or 50ml cell suspension and 500ml 8 g/L LGT agarose at 45C [14]. In order to allow the agarose to gel, the slides were placed on ice for 2–3 minutes. Using tweezers or a needle, the cover slide was pushed out of the way. Each sample resulted in the production of two slides. The lysis solution (25 g/l sodium dodecyl sulfate) was placed on these slides for 15 minutes at room temperature in 45 mM TRIS-borate, 1 mM ethylene diamine tetraacetic acid, disodium salt dehydrate buffer (TBE), and then flushed for 5 minutes in TBE. Electrophoresis was carried out in a submarine-mode electrophoresis chamber with TBE at 2V/cm for 2 minutes. After that, the slides were air-dried and cleaned for 5 minutes in cold (4C°) distilled water.

2.2.3. Staining

The slides were recolored for five to ten minutes with ethidium bromide at a concentration of ten milligrams per milliliter [13].They were given a final cleaning in distilled water before being mounted under cover glass.

2.2.4. Evaluation

A fluorescence microscope fitted with a red excitation filter set was utilized with an amplification of 400×. The degree of the DNA relocation was resolved with the picture examination framework, Komet 3.0 from (Kinetic Imaging Ltd., Liverpool). The tail moment of the comet (tail length×% DNA in tail/100) was utilized as a proportion of DNA movement. On both of the two slides arranged for each sample, 50 haphazardly chosen cells were estimated, in this manner giving 100 comets for every sample. Computations and charts were made utilizing the computer programs Statistica 6.0 and Excel 5.0.

2.3. Total volatile basic nitrogen (TVB-N)

The Kjeldahl method was used to estimate the percentage of Total volatile basic nitrogen in the samples and based on the method mentioned by [15].and others by taking a known weight of the sample within (0.2 g) placed in a beaker, and adding to the sample (5 ml) of concentrated sulfuric acid An appropriate amount of a mixture of potassium sulfate and copper sulfate was added, and the digestion process was carried out by heating the contents. Intensive distillation ends with a test tube immersed in a receiving flask containing a known volume of boric acid (20%) in addition to drops of red methyl guide and dye (bromocresol blue). Then the liquid collected with hydrochloric acid (0.1) is scavenged and a

control solution (Planck) was prepared from the chemicals in the above except for the model. The Total volatile basic nitrogen percentage is calculated according to the following equation.

$$\text{TVB} - \text{N mg \%(mg/100g sample)} = (\text{a} - \text{b}) \times \frac{\times\ 28.014\ \times 100}{\text{S}}$$

Where S is the meat sample weight in grams, b is the volume of added H2SO4 in blank in ml, a is the volume of added H2SO4 in the sample in ml, and f is the standard factor of H2SO4 [16].

2.4. Determination of Malondialdehyde (MDA)

The concentration of MDA in serum was determined according to Buege and Aust method. MDA formed from breakdown of polyunsaturated fatty acids serves as a convenient index of peroxidation reaction. the thiobarbituric acid method was used to estimate the MDA, which reacts with thiobarbituric acid (TBA) giving pink color read at λ max 535 nm as recorded by [17].

2.5. Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Two and One-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. Also, independent t test was used to assess the difference between two means. $P < 0.05$ is considered statistically significant [18].

3. Results and discussion

3.1. DNA comet assay

The result in (figure 1) revealed that there were a significant ($P<0.05$) differences between frozen thigh and frozen breast in %DNA in head and tail length.

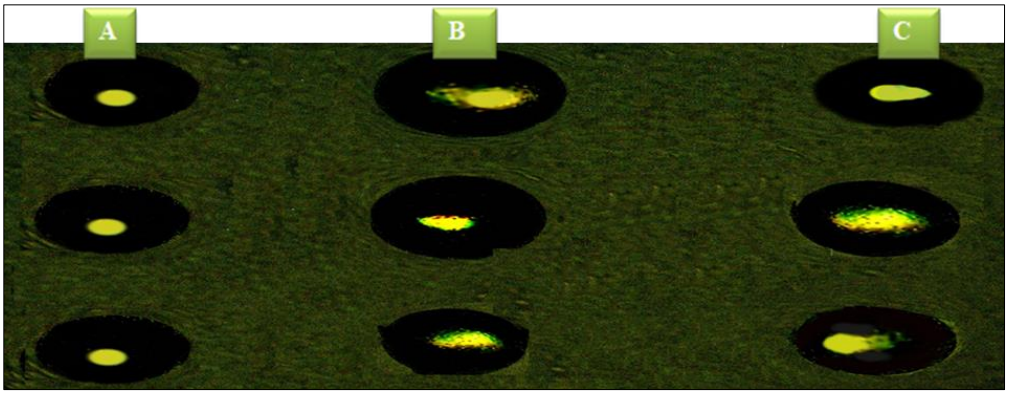

Figure 1 Comet of chicken sample (breast and thigh) (A= normal, B= thigh, C= breast)

The table (1) recorded lowest value in comet length , tail DNA damage in frozen breast compared with the value of frozen thigh (9.85±1.88 and 12.59±2.11) respectively.

Table 1 Relative damage index (RDI) in frozen chicken breast and thigh

Samples	DNA damage (%)
Breast	9.85±1.88b
Thigh	12.59±2.11a
P-value	0.34

Means with a different small letter in the same column are significantly different ($P<0.05$)

The detection limit of the comet assay was applicable to samples that were still considered to be of good quality in terms of shelf life, demonstrating the assay's high sensitivity as a rapid test for evaluating the quality of frozen chicken [7].

The increasing DNA damage during refrigerated storage was owing to activation of lysosomal nucleases in muscle cells. About 50 degradative enzymes that can hydrolyze proteins, DNA, RNA, polysaccharides, and lipids are known to be contained in lysosomes, all of which are acid hydrolases optimally active near pH 5[19].

The result of this study disagree with [20] who showed that the higher significant values (P<0.01) in DNA damage of frozen breast than those values in the thigh.

The obtain result agree with [21] they noted that the unirradiated samples of chicken, if not exposed to other DNA-fragmenting treatments such as blanching or cooking, always show a number of intact cells without "comets".

3.2. Total volatile basic nitrogen (TVB-N)

Frozen thigh meats showed significant (P<0.05) increased (9.19±0.30) in TVB-N compared with breast meats (7.81±0.58) table (2).

Table 2 Level of total volatile basic nitrogen (TVB-N) in breast and thigh of frozen chicken

Samples	TVB-N (mg/100gm)
Breast	7.81±0.58b
Thigh	9.19±0.30a
P-value	0.05

Means with a different small letter in the same column are significantly different (P<0.05)

Total volatile basic nitrogen values (TVB-N) were increased as the storage period progressed in all the freezing periods. It's possible for a variety of reactions to take place during frozen storage of various meat components [22]. This result disagree with [23] showed that the control sample recorded 2.84 mg N2/100g sample, while the samples treated by aerobic packaging recorded TVBN values from 2.41 to 2.57 mg/100g and the samples treated by vacuum packaging recorded values from 2.21 to 2.54 mg/100g under the same conditions.

The results agree with [24] they noted that the TVBN value of chicken meat samples have significant effects with respect to treatments, packaging, and storage interval Higher value of TVBN (5.26 ± 0.39 mg/100 mL) was observed in chicken meat in 14^{th} day of storage.

3.3. Malondialdehyde (MDA)

There was no significant (P<0.05) differences was noticed in MDA value between thigh and breast.

Table 3 Level of malondoaldyhede (MDA) in breast and thigh of frozen chicken

Samples	MDA (µmol/L)
Breast	1.61±0.11
Thigh	1.13±0.22
P-value	0.06

Malondialdehyde (MDA) is the final product of the decomposition of lipid peroxidation induced by free radical. MDA concentration can be used to assess the extent of lipid peroxidation and the accumulation of ROS in an organism [25].

This result was disagree with [26] the dark muscle contains higher concentrations of lipids, haeme proteins, and microsomal enzymes than white muscle, making it more vulnerable to lipid oxidation from prooxidants. Due to their high concentrations of oxidation catalysts (such as myoglobin and iron), fish and poultry meats are vulnerable to

oxidative reactions. Stored muscle food is deteriorated primarily by lipid oxidation. It is the oxidative reactions in meat that have the greatest impact on the quality of the meat.

(11) revealed that the least concentration of MDA obtained in his study was 27 mmoles/g fresh weight of chicken. This is equivalent to 27,000 μmolar MDA/g. Thus if an adult human consumes 100 g of frozen muscle in a meal, this would amount to an oral exposure of 2,700,000 μmolar of MDA, a very high dose indeed. Consequently, it can be reasonably concluded that the frozen meat and fish samples analyzed have very unacceptable levels of MD.

4. Conclusion

In this particular investigation, DNA damage in frozen breast and thigh meat was identified using a comet assay. During its time in commercial storage, the meat from the thighs was significantly damaged. According to the findings of the study, the amount of malondoaldyhede (MDA) that was present in the frozen breast meat was relatively high, whereas the level of total volatile basic nitrogen (TVB-N) that was present in the frozen thigh meat was relatively high.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared that there was no conflict of interest.

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