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Microbial counts and eating quality of breast broilers meat subjected to different freezing and refreezing storage periods

ABSTRACT

This study aimed to assess the effect of the frozen and refrozen storage durations on the sensory and microbial quality of broiler chickens' meat. A total of 40 breast muscle of broilers meat were dividing into two treatments (freezing and refreezing treatments). The meat in the first treatment was subdivided into three equal parts and subjected into 0 (without freezing), 1-, 2- and 3-months freezing storage periods while the meat samples in the second treatment was subjected into 1, 2 and 3 months freezing then thawing overnight at 4°C and refreezing for 1, 2, and 3 months before evaluation microbial count and sensory quality of meat. Results showed that refreezing storage duration had affected undesirably on flavor, juiciness, and tenderness significantly (P≤ 0.05) at (2 and 3), (1 and 3) and 3 months of refrozen storage respectively, whereas the data of overall acceptability were significantly (P≤ 0.05) reduced when the duration of frozen and refrozen at (1, 2 and 3) months storage increased compared to fresh samples (without freezing). The data of microbial counts were significantly (P ≤0.05) increased with prolonging the duration of frozen and refrozen storage in comparison with control treatment. In conclusion, the significant differences between fresh, thawed and refrozen meat in this study should give consumers concerns about buying frozen meat or consuming thawed meat.

INTRODUCTION

The consumption of chicken meat has been increasing over the decades throughout the world because of its high-protein and low-fat content which are important characteristics for a healthy diet compared with beef, lamb and pork meat, chicken meat is cheap, easily available, and acceptable to all communities (Jayasena et al. 2013). However, chicken meat can rotten rapidly if it is not stored, processed, packaged or distributed correctly (EFSA, 2013). It can be spoiled by microbial activity or through the oxidative processes due to the high content of polyunsaturated fatty acids resulting in undesirable changes which make this meat unfit for human consumption (Bosco et al. 2016). Fresh meat often treated by a number of preservation techniques to increase their shelf-life (Hammad et al., 2017). Freezing technique is the common methods used to protect meat by preventing the microorganism growth that cause food-borne illnesses (Albrecht et al., 2019). Freezing has been an excellent preserving technique for meat and meat products for long time in which meat and meat products can be preserved in a condition similar to that of normal state and can be kept satisfactory for six months or one year according to type of meat. After proper freezing, meat remains almost same nutritional value and palatability traits. The quality of meat is

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generally determined by technological traits, palatability traits, microbial activity and nutritive value. Freezing commercially at -18°C to -20°C is standard of eating quality compared to fresh meat and this freezing temperature is effective for both preservation of meat manufacturing of meat (Soyer et al., 2010). Freezing delay, the microbial growth, metabolic activities and chemical reactions, and preserve the meat quality until it reaches the consumer. However, the correct thawing practice should also be selected in order to ensure the quality of the final product (Akhtar et al. 2013). Moreover, sensory traits of meat can affected by the freezing- thawing processes in relation to color, texture (including juiciness) or flavor (rancidity) characteristics. Texture would be affected since ice crystals could cause tissue or cell disruption producing damage and the loss of water-holding capacity (Farouk and Price, 1994; Farouk and Swan, 1998). During thawing and refreezing processes, moisture lift from muscle cells to the spaces between cells (Charoenrein, 2018). Freezing, thawing and refreezing cause damage to cell walls, leading purge to be released more easily from the meat and moisture lost from the muscle cells not re-absorbed upon thawing (Leygonie et al., 2012). However, the storage conditions of freezing meat may be just as important as the freezing rate and, perhaps more significant, in determining the ultimate quality of frozen meat (Charoenrein, 2018; Leygonie et al., 2012). Although a few studies have also reported the effect of freezing and refreezing on meat quality (Zhang & Ertbjerg, 2019), microbial activities (Mohammed et al., 2021). Thus, the present study will be to investigate the effects of freezing and refreezing on sensory quality characteristics and microorganism count of broiler meat.

MATERIAL AND METHODOLOGIES

Around of 40 chicken meat samples were bought from Taza and Ehtimad slaughter house in Erbil city then transferred in sterilized polyethylene bags to Lab, Animal resource department laboratories, College of Agricultural and Engineering Science, Salahaddin university. The samples of broiler meat were dividing into two portions which were randomly assigned into freezing and refreezing treatments. The first portion was further subdivided into three equal parts and subjected into 0 (without freezing), 1.0, 2.0 and 3.0 months freezing storage periods. The second portion was subjected into 1.0, 2.0 and 3.0 months freezing (-20°C) storage periods then thawing for 12h at 4°C and refreezing The freezing treatment group was frozen at (-20°C) for 1, 2, and 3months before thawing and testing. The refrozen group was frozen at (-20°C) then thawed overnight at 4 ˚C for 12h in their original packages then refreezing at (-20°C) for 1.0, 0.2, and 3.0 months. All microbial count estimation (total bacterial count, proteolysis bacteria, lipolysis bacteria and coli form bacteria) and eating quality values (tenderness, juiciness, flavor and acceptance) of broiler chicken meats were evaluated in both treatments).

Microbiological count estimation

Total Plate Counts:

One g of broiler meat was drawn aseptically and transferred to a test tube containing 9 ml of distils water. In order to determine the microbial counts 0.1 ml samples of serial dilutions (1:10 diluent, and distil water) of chicken breast homogenates were spread on the surface of dry media. Ten fold dilutions were spread on petri dishes in duplicate for enumerations of Total Aerobic Counts (TAC) on Standard Methods Agar (LAB) following 3 days incubation at 32 °C following the procedure of Ghollasi Mood et al. (2017). The data (growth counts) were transformed to log10 values.

Coliforms bacteria Counts: Serial dilutions were prepared as above, using MacConky agar medium (LAB) for plating. The inoculated plates were incubated at 37°C for 48 hr. After incubated estimating the number of dark red colonies, according to the procedure recommended by American Public Health Association (APHA, 1992).

Proteolytic bacteria Counts: Serial dilutions were prepared as above, with the exception of using skim milk (10%) nutrient agar medium (LAB) for plating. The inoculated plates were incubated at 37°C for 48 hr. then immersed in 1% hydrochloric acid then colonies coated with clear zones which are visible are calculated, according to the procedure recommended by American Public Health Association (APHA, 1992).

Lipolytic bacteria Counts: Serial dilutions were prepared as above, with the exception of using olive oil (1%) nutrient agar medium (LAB) for plating and incubated at 37°C for 48 hr then flooding the plate with 8-10 ml of saturated Copper sulphate solution and allowed to stand for 10-15 min. The reagent was poured off, the plates was washed gently in running water for one hour to remove the excess of copper sulphate. After that, the colony while a bluish-green colored zone appeared was measured according to the procedure recommended by American Public Health Association (APHA, 1992).

Panel tests

The sensory evaluation scores (panel test) of flavor, juiciness, tenderness and overall acceptance were measured according to (Baker and Drafler, 1975). Ten panelists who have enough experience participated in the sensory evaluation test, they also supported with adequate information in detail respect to the nature of the evaluation of each character, Evaluate of panel test were doing at 11 am with left a period of time between assessment and the last with saved a drink water between assessment.

STATISTICAL ANALYSIS

The experiment was a Complete Randomized Design (CRD). Statistical analysis was performed by using the General Linear Models (GLM) procedure of Statistical Analysis System (SAS) package Version 9.2 software. When significant effects were found, comparison among means was made by Duncan's multiple range tests. The statistical significance was set at ($P \leq 0.05$).

RESULTS AND DISCUSSION:

Sensory evaluation of broilers breast meat

The results presented in Table (1) indicate that meat samples during freezing and refreezing storage duration had effected on flavor non-significantly except at 2 and 3 month of refrozen storage which increased significantly ($P \leq 0.05$) on flavor of breast meat samples the data of flavor were non-significantly when the duration of frozen (1, 2 and 3) month and refrozen at 1 month storage increased excluding at 2 and 3 month of refreezing decreased significantly when compared with fresh samples (without freezing), it may be due to the effect of storage conditions, where oxidation of lipid is limited, breast chicken meats odour intensity can be enhanced and other odours can be reduced or might to be due to the individual differences of the panelists evaluation of this trait, while, a reduction in flavor value is obtained when the storage period increased. A similar trend in sensory changes was proofed in studies conducted by (Śmiecińska et al. 2015; Santosh kumar et al. 2014 and Augustyńska et al., 2019), where the prolongation of the deep freeze storage was associated with the deterioration of the flavor of broiler chickens meat. The juiciness was observed that the values of juiciness non- significantly differ ($P \leq 0.05$) with extending the storage period (1, 2 and 3) month of freezing and during 2.0 month of refreezing while during 1.0 and 2.0 month of refreezing decreased significantly ($P \leq 0.05$) this decrease in juiciness might be due to slight dehydration (loss of moisture) of the breast meat samples during the extended period of storage otherwise the reason is due to the high cooking loss which affects the juiciness. Similarly, sensory changes were observed in studies by Santosh Kumar et al. (2014), who proofed that prolonging the freezing storage duration caused in deteriorated juiciness of broiler breast meat. A similar trend of sensory changes was demonstrated in studies conducted by Śmiecińska et al. (2015), Santosh kumar et al. (2014), and Augustyńska et al., (2019) where the prolongation of the deep freeze storage was associated with the deterioration of the juiciness of broiler chickens meat.

The results of tenderness did not show any significantly differ ($P \leq 0.05$) during frozen and refrozen storage excluding at 3.0 month of refreezing the tenderness value reduced significantly ($P \leq 0.05$). This sensory result was due to the thawing loss that resulted in less water available to hydrate the muscle fibres; thus, a greater quantity of fibres per surface area seemed to increase the toughness as perceived by the sensory panel. Whereas an improvement in tenderness value during freezing period when compared with other frozen storage period is thought to be due to the effect of a combination of the breakdown of the muscle fibres by enzymatic action during proteolysis, ageing, and the loss of structural integrity caused by ice crystal formation The formation of large, extracellular ice crystals disrupts the physical structure, largely breaking myofibrils and resulting in

tenderization. However, the formation of small intracellular ice crystals increases the rate of ageing probably by the release of protease enzymes (Vieira et al., 2009). Finally the acceptability values of chicken breast meats decreased significantly (P≤ 0.05) during frozen and refrozen storage may possibly be due to several changes affecting sensory qualities may take place in meat and its products stored frozen. This changes explained by way of the physical (recrystallization, denaturation, freeze-thawing burns), chemical (hydrolysis, auto-oxidation) as well as microbiological and enzymatic (hydrolysis, oxidation, dehydration) transformations. The extent of such transformations depends on the temperature and duration of freezing, including the conditions of storage (Akhtar et al., 2013; Gambuteanu et al., 2013).

Table (1): The effect of frozen and refrozen storage and storage period (months) (means ± standard error) on the sensory evaluation of chickens breast meats

Treatment / Period	Fresh	Freezing			Refreezing		
	24 h	1 month	2 month	3 month	1 month	2 month	3 month
Flavor	1.600 ± 0.22 b	2.100 ± 0.23 ab	2.100 ± 0.23 ab	2.100 ±0.17 ab	1.500 ± 0.22 b	2.400 ± 0.22 a	2.700 ± 0.30 a
Juiciness	1.900 ± 0.10 c	2.100 ± 0.10 cb	2.300 ± 0.15 cb	2.200 ± 0.24 cb	2.300 ± 0.16 ab	2.600 ± 0.20 cb	2.900 ± 0.17 a
Tenderness	1.700 ± 0.15 b	2.000 ± 0.25 ab	2.100 ± 0.17 ab	1.900 ± 0.10 ab	1.800 ± 0.20 b	1.900 ± 0.17 ab	2.400 ± 0.16 a
Acceptance	1.200 ± 0.13 c	1.900 ± 0.23 b	1.900 ± 0.17 b	1.800 ± 0.13 b	1.800 ± 0.20 b	2.000 ± 0.14 b	2.500 ± 0.16 a

*a- f different letters in the same row indicate significant differences (P≤ 0.05).

Note: the sensory evaluation degrees ranged for each of the flavor (1=Very good …. 5 = reject) Juiciness (1= Very juicy …. 5 = dry) Tenderness (1=Very tender …. 5 = dry) O. Acceptance (1= V. Acceptable …. 5= Reject)

Microbial count of broilers breast meat

The results in Table (2) showing that the data of total bacterial count, lipolytic, proteolytic and coliform bacteria were significantly (P≤ 0.05) increased with extending the duration of frozen and refrozen storage compared with fresh samples (control). The higher value of (Total Bacterial Count, lypolytic, proteolytic and coliform bacteria in fresh chicken may due to the high natural contamination of chicken meat during handling (Hammad et al., 2020). Or may be due to high drip loss resulting from long time of thawing 12hrs. which considered good media for growth of microorganism. Cutting carcass, packaging and through the bad storage condition leads to prepare a suitable condition for microbial growth the microbes reached to the meat products (berry, 2001). Several studies have indicated that high moisture content is one of several factors quite conducive to microbial growth (Berger et al., 2018; Hammad, Ma, Jin, et al., 2019). When compared mean log values (log10 CFU/g) of microorganisms (total bacterial count, lipolytic, proteolytic and coliform bacteria) in frozen breast chicken samples at 1 month freezing storage with 2 and 3 month freezing appeared that the mean values were significantly (P≤ 0.05) decreased with increased storage period at 2 and 3 month of freezing storage, this may be due to lower pH and no available nutrients favorable for microbial growth (berry, 2001). Inversely, in refrozen breast chicken samples, all mean log values (log10 CFU/g) of (total bacterial count, lipolytic, proteolytic and coliform bacteria) increased significantly (P≤ 0.05) at 1, 2 and 3 months of storage compared with the control and frozen samples may be due to the cell wall disruption of breast broiler after thawing of meat samples and during continual refreezing storage which leads to make a suitable condition for growth several kind of microbes. All microbial counts of broiler meat samples determined during frozen storage were low in number and can be categorized as satisfactory and within the acceptable

values, agreed with that of standardization and quality control by Dempster (1986), who concluded that the total counts must be within the range10^3 -10^7 CFU/g of meat.

Table (2): The effect of frozen and refrozen storage and storage period (months) (means ± standard error) on the bacterial count (Log10 CFU/g) of chickens breast meats

Treatment / Period	Fresh	Freezing			Refreezing		
	24 h	1 month	2 month	3 month	1 month	2 month	3 month
Total Bacterial.Count	3.746± 0.01 g	4.886± 0.01 d	4.831± 0.01 e	4.784± 0.01 f	4.961± 0.01 c	5.000± 0.01 b	4.784± 0.01 a
Lipolytic bacteria	2.270± 0.07 d	3.896± 0.03 b	3.765± 0.05 b	3.606± 0.05 c	4.111± 0.02 a	4.244± 0.01 a	3.606± 0.05 a
Proteolytic bacteria	2.240 ± 0.07 f	4.014± 0.02 d	3.911± 0.02 de	3.829± 0.02 e	4.211± 0.02 c	4.355± 0.02 b	3.829± 0.02 a
Coliform bacteria	2.215 ± 0.09 e	3.753± 0.04 d	3.617± 0.03 c	3.542± 0.04 cb	4.037± 0.02 cb	4.122± 0.03 ab	4.042± 0.04 a

*a- f different letters in the same row indicate significant differences ($P < 0.05$).

CONCLUSION

We concluded that the number of repeated freeze-thaw cycles increased it affected the sensory evaluation (decreased the flavor, juiciness, tenderness and overall acceptance) and microbiological quality of breast chicken samples, causing the deterioration of meat quality. Whereas, increased the Total Microbial Count, lipolytic, proteolytic and coli form bacteria with prolonging frozen and refrozen storage then, repeated freeze-thaw cycles should be minimized in terms of sensory quality breast broilers meat and microbial quality.

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التعداد الميكروبي والصفات الحسية للحم صدور فروج اللحم الخاضعة لفترات مختلفة من التخزين بالتجميد واعادة تجميده

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الكلمات المفتاحية:
لحم الفروج، التعداد الميكروبي، تقيم الحسي، خزن بالتجميد، اعادة التجميد.

الخلاصة

تم استخدام إجمالي 40 عضلة صدر من فروج اللحم وقسمت الى معمالتين (معاملة التجميد ومعاملة إعادة التجميد). تم تقسيم اللحوم في المعاملة الأولى إلى ثلاثة أجزاء متساوية ومن ثم تم تعريضها إلى فترات تخزين 0 (بدون تجميد) ، 1 ، 2 و 3 أشهر للتجميد بينما تعرضت عينات اللحوم في المعاملة الثانية إلى 1 ، 2 و 3 أشهر للتجميد ثم الاذابة حتي صباح اليوم التالي (12 ساعة) عند 4 درجات مئوية وإعادة التجميد ثانية لمدة 1 ، 2 و 3 أشهر قبل تقييم العد الميكروبي والحسية للحوم. اظهرت النتائج هذه الدراسة أن مدة التخزين في حالة إعادة التجميد لم تؤثرعلى النكهة, العصيرية والطراوة وكانت الفروقات معنوية ($P\leq0.0$) عند 2 و 3 , 1 و 3 و 3 أشهر من فترة الخزن بالتجميد واعادة التجميد على التوالي .في حين أن بيانات المقبولية انخفضت بشكل معنوي ($P\leq0.0$) عند زيادة مدة التجميد وإعادة التجميد عند الخزن لفترات 1 ، 2 ، 3 اشهر مقارنة بالعينات الطازجة (بدون تجميد). أما بالنسبة لبيانات لاعداد البكتريا فأنها زادت معنويا ($P\leq0.0$) مع إطالة مدة الخزن بالتجميد وإعادة التجميد مقارنة بعينات اللحم الطازج (بدون تجميد). هدفت هذه الدراسة إلى تقييم تأثير فترات التخزين بالتجميد وإعادة تجميدها على التقيم الحسي والعد الميكروبي للحم فروج اللحم لذلك، الاختلافات الكبيرة بين صفات الميكروبية والحسية بين اللحوم الطازجة والمعاد تجميدها في هذه الدراسة اعطاء مخاوف للمستهلك بشأن شراء اللحوم المجمدة أو استهلاك اللحوم المذابة والمعاد تجميده.