

**Original Paper****Effects of different repeated freeze-thaw cycle processes on the physicochemical quality of chicken meat cuts**Shaimaa, A. El-Barody^{*1}, Dalia, F. Khater², Amani, M. Salem¹¹Department of Food Control, Faculty of Veterinary Medicine, Benha University, Egypt.²Animal Health Research Institute, Tanta branch, Egypt**ARTICLE INFO****Keywords**

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ABSTRACT

The study was undertaken to assess physicochemical quality changes in chicken meat cuts caused due to repeated freeze-thaw cycles by four different thawing methods. In total, 48 random samples of freshly slaughtered chicken samples were collected from local commercial retail shops in Tanta city (Gharbia Governorate, Egypt) and submitted to freezing at -18°C for 1 week then. Four varying thawing methods were done in microwave oven for 1 minute, in the refrigerator at 3°C for 22 hours, packed in low-density polyethylene bags and placed under tap water for 4 hours and at room temperature for 4 hours. The obtained results revealed that pH and TVN decreased in all samples with different ratios. The lowest TBA values (mg malonaldehyde/ kg) in breast and thigh meat cut samples thawed and re-thawed in the microwave were 0.56 ± 0.03 , 0.73 ± 0.01 , 0.86 ± 0.05 and 0.80 ± 0.02 , respectively. While protein % in breast and thigh meat samples thawed and re-thawed in refrigerators showed the best values (17.53 ± 0.35 , 17.40 ± 0.10 , 17.26 ± 0.20 and 17.33 ± 0.15), however, the fat % were 3.70 ± 0.10 , 3.86 ± 0.15 , 3.56 ± 0.05 and 3.66 ± 0.15 , respectively. Overall, the pH and TVN values decrease in all samples by different ratios. Accurately, the samples thawed in microwave showed the lowest TBA values while in refrigerators method showed best protein and fat values.

1. INTRODUCTION

The consumption of poultry meat has increased and has reached high levels around the world (Benli, 2016). Poultry meat is considered an excellent source of high-quality protein required for the nutrition of infants, young children, and adults. Also, vitamins especially B complex and minerals are present in considerable amounts in poultry meat (Cahe *et al.*, 2002). Due to the high increase in consumer demand for safe poultry meat, chicken meat quality control has become a necessity (Carvalho *et al.*, 2015). Freezing is the most accepted way of food preservation to make the safety of meat products in the meat export market (Leygonie *et al.*, 2012). The way and techniques used in freezing and thawing processes are considered an important role in the preservation of the quality of frozen foods (Mandigo and Osburn 1996). The process of thawing has been ignored. Thawing has risks such as oxidation of lipids because of prolonged thawing time (Otto *et al.*, 2004).

During frozen storage, there are important biochemical changes, such as lipid and protein oxidation which can affect the quality of frozen chicken meat (Soyer, *et al.*, 2010) and increase in protein denaturation, oxidation of lipid and protein. The pH value is an indicator of the keeping quality of chicken meat where the pH value

is used to measure the shelf life and quality as pH plays an important role for the microbiological growth which affect the shelf life of meat (Hathout-Aml and Ali-Soher, 2010). Lipid oxidation is responsible for the reduction in nutrition quality and changes in flavor, the quality of chicken meat during frozen storage depends greatly on TBA value (Boast, 1985).

Therefore, the present study has been undertaken to assess the changes in the physicochemical qualities such as pH, TVN, TBA, protein, and fat value of chicken breast and thigh meat under repeated freeze-thaw cycles by four different thawing methods.

2. MATERIAL AND METHODS**2.1. Collection of samples**

Forty-eight random samples of freshly slaughtered chicken samples were collected from local commercial retail shops in Tanta city and kept in separate plastic bags, transferred directly to the laboratory in an insulated icebox under complete aseptic conditions without any delay. The chicken samples were divided into 2 groups, breast, and thigh meat samples, then all samples were individually frozen at (-18°C) for (1 week) then. Four different thawing methods were done in microwave oven for 1 minute; in the refrigerator at around 3°C for 22 hrs.; Under tap water for 4 hrs.; at room temperature for 4 hrs.

* Corresponding author: Shaimaa, ahmed.eissa19855@gmail.com

After thawing, the different examination was adopted by the same methods and reexamined. All prepared samples were subjected to the following investigations:

2.2. Determination of pH (Pearson, 2006)

About 10 g of sample were blended in 10 ml of neutralized distilled water in a blender. The homogenate was left at room temperature for 10 minutes and shaking continuously. Then an electrical pH meter (Bye model 6020, USA) was applied to measure the pH value. It is of great concern to report that the pH meter was calibrated using two buffer solutions (7.01 and 4.01).

2.3. Total Volatile Nitrogen "TVN" (FAO, 1980)

Accurately, 10 g of the sample were added to 30 ml of distilled water and mixed for 2 minutes in a clean dry beaker. Thus, 2 drops of 0.02 M HCl were added to render the pH value of 5.2. The homogenate was slowly heated to 70°C and cooled to room temperature and filtered into the inner compartment of Conway dish then added 2 ml of 0.01 M HCL. The outer ring was filled with 2 ml of the sample extract and 1 ml of saturated potassium carbonate. The Conway unit was rotated gently, and the dish was covered and incubated for 2 hours at 36 °C, HCL in the inner ring was titrated against 0.01 M NaOH by using a methyl red indicator (T1 ml)

$$\text{TVN}/100\text{g} = 26.88 \times (2 - T1)$$

Where T1 = the volume of NaOH consumed in the titration.

2.4. Thiobarbituric acid (TBA) number (mg malonaldehyde/kg of the sample): (Kirk and Sawyers 1991)

Ten grams of samples were blended with 48 ml of distilled water, then added 2 ml of ammonium chloride in a warring blender for 2 minutes and left at room temperature for 10 minutes. The mixture was transferred into Kjeldahl flasks by washing with additional 50 ml distilled water, followed by an antifoaming preparation and a few glass beads. The Kjeldahl distillation apparatus was assembled, and the flask was heated using an electric mantle. Fifty ml of distillate in 10 minutes from the time of boiling were collected. The distillate was mixed, and then 5 ml were pipetted into a glass-Stoppard tube and 5 ml of TBA reagent were added. The tubes were stoppered, shaken, and put in a boiling water bath for 35 minutes. Prepared the blank by using 5 ml distilled water with 5 ml TBA reagent and treated like the sample. After heating, the tube was cooled under tap water for 10 minutes. A portion was carried to a cuvette and the optical density (OD) of the sample was read against the blank using spectrophotometer at a wavelength of 538 nm.

TBA value (mg malonaldehyde / kg of poultry sample) = $D \times 7.8$,
Where, D: the read of the sample against blank.

2.5. Determination of protein content (AOAC, 2000)

The Kjeldahl method was carried out using two grams of the examined samples in the digestion flask. Fifteen grams of potassium sulfate (K₂SO₄), 0.5 gm of metallic mercury, and 40 ml of sulphuric acid (H₂SO₄) were added to the samples. The flask was placed in an inclined position and gently heated until frothing ceases, then boiled until the solution was cleared for 30 minutes. The flask was cooled below 25 °C and then 25 ml of sodium thiosulphate (Na₂S₂O₃) was added to prevent pumping, then enough 50% NaOH (90 ml) was added without shaking.

$$\text{Nitrogen \%} = \frac{[(\text{ml of acid} \times \text{N of acid}) - (\text{ml of NaOH} \times \text{N of NaOH})]}{(\text{Weight of sample})} \times 1.4007$$

$$\text{Protein \%} = \text{Nitrogen \%} \times 6.22$$

2.6. Determination of fat content (AOAC, 2000)

The Soxhlet technique was applied to evaluate fat content. Five grams of heat-dried samples were weighted in a thimble with porosity permitting rapid passage of ether and placed in the Soxhlet extractor which was connected to the condenser. Soxhlet flask containing petroleum ether was connected to the extractor and electrically heated. The extraction was continued for 6 hrs., then petroleum ether was evaporated in a boiling water bath and the flask was dried in the oven at 100°C for 30 minutes, then cooled in desiccators and weighed. The fat % was calculated from the weight of the flask before and after extraction.

2.7. Statistical analysis

Statistical data analysis for the three independent replicates was treated by one-way ANOVA using the SPSS program according to Ronser (2002).

3. RESULTS

The results presented in table (1) revealed that the pH value of breast and thigh meat samples thawed in the microwave was 6.03±0.05 and 6.30±0.10. While in breast and thigh samples thawed in refrigerators, under tap water, and at room temperature were 6.96±0.05, 7.00±0.20, 6.30±0.10, 6.10±0.10, 5.73±0.30, and 5.66±0.15, respectively. Moreover, the pH value of re-thawed breast and thigh meat samples in the microwave was decreased to 5.93±0.11 and 6.03±0.05. But, in breast and thigh meat samples were decreased to 6.86±0.11, 6.76±0.25, 5.80±0.34, 5.93±0.11 and 5.26±0.05, 5.43±0.11 which re-thawed in refrigerators, under tap water, and at room temperature, respectively.

Table 1 Effect of various thawing processes on pH of the examined chicken meat cut samples (n=48).

Method	In microwave		In refrigerators		Under tap water		At room temperature	
	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh
Thawed	6.03±0.05 ^b	6.30±0.10 ^b	6.96±0.05 ^a	7.00±0.20 ^a	6.30±0.10 ^b	6.10±0.10 ^b	5.73±0.30 ^c	5.66±0.15 ^c
Re-thawed	5.93±0.11 ^b	6.03±0.05 ^b	6.86±0.11 ^a	6.76±0.25 ^a	5.80±0.34 ^b	5.93±0.11 ^b	5.26±0.05 ^c	5.43±0.11 ^c

*Mean values with different superscripts in the same rows are significantly different at (P<0.05).

Table (2) indicated that the TVN values (mg/100g) in breast samples that thawed in the microwave and refrigerators were 13.43 ± 0.05 ; 13.83 ± 0.30 while,

12.56 ± 0.15 and 12.33 ± 0.25 for breast meat samples thawed by under tap water and at room temperature respectively.

Table 2 Effect of various thawing processes on TVN of the examined chicken meat cut samples (n=48).

Method	In microwave		In refrigerators		Under tap water		At room temperature	
Status	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh
Thawed	13.4 ± 0.05^b	13.4 ± 0.25^b	13.8 ± 0.30^a	13.7 ± 0.10^{ab}	12.6 ± 0.15^d	13.4 ± 0.00^c	12.3 ± 0.25^d	12.4 ± 0.10^d
Re-thawed	13.1 ± 0.10^b	13.2 ± 0.25^b	13.6 ± 0.15^a	13.7 ± 0.10^a	12.5 ± 0.15^c	12.9 ± 0.26^b	12.1 ± 0.32^d	12.4 ± 0.11^{cd}

*Mean values with different superscripts in the same rows are significantly different at (P<0.05).

On other hand, TVN (mg/100g) in breast meat samples after rethawing under tap water and at room temperature still decrease to 12.53 ± 0.15 and 12.13 ± 0.32 respectively.

Also, thigh meat samples that were re-thawed under tap water and at room temperature were 12.90 ± 0.26 and 12.43 ± 0.11 respectively.

Table 3 Effect of various thawing processes on TBA (mg of malonaldehyde /kg) of the examined chicken meat cut samples (n=48).

Method	In microwave		In refrigerators		Under tap water		At room temperature	
Status	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh
Thawed	0.56 ± 0.03^d	0.73 ± 0.01^c	0.91 ± 0.02^b	0.90 ± 0.02^b	1.24 ± 0.19^a	1.00 ± 0.01^b	1.01 ± 0.13^b	0.89 ± 0.01^b
Re-thawed	0.86 ± 0.05^d	0.80 ± 0.02^d	0.92 ± 0.02^{cd}	0.93 ± 0.03^{cd}	1.30 ± 0.10^a	1.27 ± 0.06^a	1.05 ± 0.13^{bc}	1.16 ± 0.20^{ab}

*Mean values with different superscripts in the same rows are significantly different at (P<0.05).

Data in the table (3) showed results of TBA value (mg malonaldehyde/ kg) which increased especially in the samples thawed at room temperature and under tap water. While samples thawed in microwave showed best results. Breast meat samples thawed in the microwave was 0.56 ± 0.03 while breast meat samples were 0.91 ± 0.02 , 1.24 ± 0.19 , and 1.01 ± 0.13 which thawed in refrigerators, under tap water, and at room temperature, respectively. Also, on re-thawed breast meat samples which thawed under tap water still have the highest value of 1.30 ± 0.10 while the lowest value was 0.86 ± 0.05 which thawed in the microwave. However, the thigh meat samples thawed in microwave showed the best result 0.73 ± 0.01 compared to thigh meat samples thawed under tap water and at room temperature were 1.00 ± 0.01 and 0.89 ± 0.01 mg malonaldehyde/ kg.

The results achieved in table (4) revealed that the protein % value of breast and thigh meat samples thawed in refrigerators was 17.53 ± 0.35 and 17.40 ± 0.10 . While in breast and thigh meat samples

thawed in the microwave, under tap water, and at room temperature were 16.76 ± 0.25 , 16.86 ± 0.15 , 16.20 ± 0.20 , 16.33 ± 0.15 and 15.56 ± 1.20 , 15.90 ± 0.10 respectively. Moreover, protein % of re-thawed breast and thigh meat samples in refrigerators were decreased to 17.26 ± 0.20 and 17.33 ± 0.15 . But, in breast and thigh meat samples were decreased to 16.53 ± 0.25 , 16.86 ± 0.15 , 16.13 ± 0.15 , 16.23 ± 0.25 and 15.20 ± 0.26 , 15.76 ± 0.05 which rethawed in the microwave, under tap water and at room temperature, respectively.

The results in table (5) declared that the fat content in Breast and thigh meat samples. On the first thawing, the breast and thigh meat samples which thawed in refrigerators showed the highest value 3.70 ± 0.10 and 3.86 ± 0.15 while thawed samples were 3.40 ± 0.10 , 3.10 ± 0.10 , 3.20 ± 0.10 , 3.40 ± 0.10 and 3.00 ± 0.00 , 3.10 ± 0.10 which thawed in the microwave, under tap water, and at room temperature, respectively. On other hand, breast and thigh meat samples which re-thawed in refrigerators still showed the highest value 3.56 ± 0.05 and 3.66 ± 0.15 compared to breast and thigh meat samples re-thawed at room temperature 3.00 ± 0.00 and 3.00 ± 0.10 .

Table 4 Effect of various thawing processes on TVN of the examined chicken meat cut samples (n=48).

Method	In microwave		In refrigerators		Under tap water		At room temperature	
Status	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh
Thawed	16.8 ± 0.25^b	16.9 ± 0.15^b	17.5 ± 0.35^a	17.4 ± 0.10^a	16.2 ± 0.20^{cd}	16.3 ± 0.15^c	15.6 ± 1.20^e	15.9 ± 0.10^{de}
Re-thawed	16.5 ± 0.25^{bc}	16.8 ± 0.25^b	17.3 ± 0.20^a	17.3 ± 0.15^a	16.1 ± 0.15^d	16.2 ± 0.25^{cd}	15.2 ± 0.26^f	15.8 ± 0.05^e

*Mean values with different superscripts in the same rows are significantly different at (P<0.05).

Table 5 Effect of various thawing processes on fat content (%) of the examined chicken meat cut samples (n=48).

Method	In microwave		In refrigerators		Under tap water		At room temperature	
Status	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh
Thawed	3.40 ± 0.10^b	3.10 ± 0.10^{cd}	3.70 ± 0.10^a	3.86 ± 0.15^a	3.20 ± 0.10^f	3.40 ± 0.10^b	3.00 ± 0.00^d	3.10 ± 0.10^{cd}
Re-thawed	3.30 ± 0.10^b	3.00 ± 0.00^c	3.56 ± 0.05^a	3.66 ± 0.15^a	3.00 ± 0.00^e	3.16 ± 0.05^b	3.00 ± 0.00^c	3.00 ± 0.10^c

*Mean values with different superscripts in the same rows are significantly different at (P<0.05).

4. DISCUSSION

The obtained result in Table (1) indicated that the pH value decreased in all samples where the freezing lead to resale of exudate and loss of water from the meat which cause an increase in the concentration of the solutes, so the pH of thawed meat was decreased (Leygonie et al.,2012). These results agree with Abd

El-Baki et al. (1983) who concluded that during frozen storage of breast and leg muscles the pH value decreased. Also agree with Lampra et al. (2021) which found the pH value decreased in broiler meat samples subjected to many cycles of freezing and thawing by different methods, from 6.02 to 5.97 to 5.89 in room temperature, from 6.02 to 5.93

to 5.81 in chiller temperature and from 6.02 to 5.82 to 5.82 in the microwave. Changes in the pH were directly affecting the amount of drip loss which altered the electrostatic repulsion between the thick and thin filaments in the meat (Yu *et al.*, 2005).

The results achieved in Table (2) revealed that the TVN decreased in all samples and these results agree with those recorded by Abd El Wahed (1986) who reported that during frozen storage of breast and thigh samples the TVN decreased from 12.42% to 11.06% and from 11.08% to 10.16%, respectively. As well as loss of nitrogenous compounds either as volatile substances caused by microbial or separated in drip during thawing (Moris *et al.*, 1975). Moreover, TVN decreased in samples thawed under tap water and at room temperature as protein decreased in these samples due to increasing APC This result agree with Edris *et al.* (2012) who recorded when TVN 11.29 protein recorded 21.06 in breast samples, while in the thigh when TVN 8.10 protein recorded 20.13 in thigh samples.

As shown in Table (4), TBA value in the samples thawed in microwave showed the lowest value while samples thawed under tap water showed the highest value this result agrees with (Lampira *et al.*, 2021) which found that the highest TBA value at room temperature thawing method which increase from 0.13 to 0.019 to 0.21. TBA increased during frozen storage could be due to oxidation of unsaturated fatty acid as well as lipolysis (Davidkova and Khan 1967). Lipid oxidation can occur at lower temperatures even frozen (Hanenian *et al.*, 1989). Also, samples thawed in microwave showed the best results may be due to inactivation effect of microwave on bacteria by energy-dependent phenomenon (Lu *et al.*, 2011) as well as enzymatic alterations may explain the electroporation and metabolic breakdown of the bacterial cell (Dreyfuss and Chipley 1980).

Table (5) showed the decrease in protein value in all samples, the best value showed in samples thawed in refrigerators. Loss of protein could be attributed to denaturation (matsumoto 1980) and the breakdown of protein by the proteolytic enzyme (Eun *et al.*, 1994). This result agrees with (Xia *et al.*, 2012) who found that thawed in refrigerator the pork samples showed physicochemical characteristics nearly to fresh samples and the least quality losses compared to at room temperature, underwater immersion, and in a microwave. Furthermore, Table (5) showed a decrease in fat value in all samples after thawing, samples thawed in refrigerators showed the best result. These results agree with (Hammed *et al.*, 2019) who found that fat% decrease in poultry samples which freeze and refreeze from 5.12 to 4.80 and 3.00 as well as protein% from 17.35 to 16.70. Loss of fat content to the formation of ice crystals which caused mechanical damage to the tissue and the possible escape of some fat with the escaped fluids (Fahmy *et al.*, 1981).

5. CONCLUSION

pH and TVN values decrease in all samples by different ratios. Samples thawed in microwave showed the lowest TBA values while in refrigerators method showed best protein and fat values.

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