Int.J.Curr.Microbiol.App.Sci (2021) *10*(03): 141-149

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 10 Number 03 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1003.020

Effects of Different Thawing Methods on the pH, Water Holding Capacity, Extract Release Volume and TBA value of Broiler Chicken under Repeated Freeze-thaw Cycles

Lampra Debbarma[1], V. Chandirasekaran[1], A. Irshad[1*], S. Sureshkumar[2] and T. R. Gopala Krishna Murthy[3]

[1]Department of Livestock Products Technology, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University, India
[2]Centre of Excellence in Meat Science and Technology, CVAS, Mannuthy, Kerala Veterinary and Animal Sciences University, India
[3]Poultry Disease Diagnosis and Surveillance Laboratory, Veterinary College and Research Institute, Namakkal, India

**Corresponding author*

Keywords

Broiler chicken meat, Freeze-thaw cycles, Meat pH, Water holding capacity, Extract release volume, TBA value

Article Info

Accepted:
04 February 2021
Available Online:
10 March 2021

A B S T R A C T

The present study was undertaken to assess broiler chicken breast fillets' physicochemical quality characteristics changes caused due to repeated freeze-thaw cycles by three different thawing methods. Meat samples were subjected to slow freezing at -18℃ for seven days and thawed under various thawing methods viz. room temperature (RT) at (31-34℃), chilling temperature (CT) at (1-4℃) and microwave thawing (MT) at 2450 MHz (Model No. BMO-700T). Six freeze-thaw cycles were carried out with each cycle containing one week for a total number of 6 weeks (i.e. 42 days) for a total number of 3 trials. As the number of freeze-thaw cycles increased pH, water holding capacity and extract release volume decreased significantly ($p<0.01$), whereas Thiobarbituric Acid Value (TBA Value) increased significantly ($p<0.01$) with repeated freeze-thaw cycles.

Introduction

Freezing of meat has been practiced for several decades to prolong its shelf-life and maintain the quality during storage, distribution and marketing (Hanenian and Mittal, 2004). The freezing temperature of -18℃ to -20℃ is the effective preservation temperature for raw meat and further processing of stored meat. Poultry meat is frozen at-18℃ or below should be consumed within 12 months of storage (FSSAI, 2020). The rate of freezing determines the size and formation of ice crystals. Small intracellular

ice crystals are formed in fast freezing with minimal damage to meat components. Whereas large intercellular ice crystals are formed in slow freezing (Grujic *et al.*, 1993). These large extracellular ice crystals cause rupture of muscle cells and exudation of fluid. These exudates are called drip or weep, which possess numerous important nutrients (Sen and Sharma, 2004).

Due to the formation of ice crystals during freezing muscle, cells are mechanically damaged. With the increase in freezing and thawing cycles, thawing loss and cooking loss also increase (Farouk and Swan, 1998). Changes in meat quality brought by repeated freezing and thawing are critical to the meat industry since their goal is to produce high quality, attractive, pleasant products and sale to consumers which can earn them profits. Parameters like appearance, texture, flavour, colour, nutritive value and microbial activity determine the shelf-life of meat affected by freezing and thawing cycles. Research has been conducted on effect of repeated freeze-thaw cycle on beef and pork but barely on chicken. Studies on freezing and thawing solely on chicken breast fillets up to 6 cycles and effect of repeated freeze-thaw cycle under Indian conditions could not be traced in the literature much. Therefore, the present study has been under taken to assess the changes in the physicochemical qualities such as pH, water holding capacity, extract release volume and TBA value of broiler chicken breast fillets under repeated frreze-thaw cycles by three different thawing methods.

Materials and Methods

Broiler meat samples

Breast fillets from the commercial broiler weighing approximately 2.0 kg were collected hygienically from retail meat shop and brought to the Department of Livestock Products Technology (Meat Science), Veterinary College and Research Institute, Namakkal – 637002, in a closed ice-chilled container. The breast fillets were packed in HDPE bags and frozen at -18ºC in a commercial deep freezer for seven days. Meat samples taken from breast fillets were subjected to analysis of physicochemical parameters viz. pH, water holding capacity, extract release volume and thiobarbituric acid value(TBA) value.

Freezing and thawing

The breast fillets packed in HDPE bags were slow frozen at -18℃ in acommercial freezer for a total number of 42 days with weekly six freeze-thawing cycles. The temperature was checked regularly and the fillets were thawed every seven days interval using three different thawing methods viz. room temperature thawing (RT) (25±1℃), chiller thawing (CT) (4±1℃) and microwave thawing (MT) (2450 MHz) (Model No. BMO-700T). Time taken to reach 4℃ for different thawing methods such as RT, CT and MT were 5 hours, 16 hours and 2 minutes, respectively. In MT the breast fillets were thawed for 1 minute on one side and then turned on the other side for another 1 minute. The first batch i.e. 3 fillets, one each from RT, CT and MT methods were studied for the following parameters in duplicate. The remaining breast fillets were subjected to freeze again after thawing. Likewise, the breast fillets were frozen and thawed every week for 6 cycles and the experiment repeated for six times during the study period.

PH value

The estimation of breast muscle pH was measured using a digital pH meter (Model 361, Systronics, India). About 5g of meat was homogenized in a tissue homogenizer with 45 ml of distilled water for about one minute.

The pH was recorded by immersing the combined glass electrode of digital pH meter in the homogenate.

Water holding capacity (WHC)

Water holding capacity of breast fillet was measured by filter paper press method suggested by Hamm (1960) with slight modification. 500 mg of meat was pressed between folded filter papers by applying 2.8 kg weight for 5 mins. Weight of meat flake and filter paper after pressing was recorded. WHC was expressed as percent water retained by the meat sample.

Formula = WHC (% water retained) = B-F/B X 100

Where, B= Weight of meat sample (weight of meat)

F = Weight of meat sample after pressing + weight of difference in the weight of filter paper)

Extract release volume (ERV)

Extract release volume (ERV) was estimated by adopting the method recommended by Pearson (1968). 60 ml of extraction reagent was added to 15g of meat sample and homogenized in a tissue homogenizer. Filtrate was filtered through Whatman No. 1 filter paper in a measuring cylinder and measured as ml of extract release volume filtered in 15 minutes.

Thiobarbituric acid value (TBA)

Thiobarbituric acid value was determined by the extraction method of Witte *et al.,* (1970). 50 ml of chilled 20% TCA was added to 20g of meat sample. It was homogenized for 20 seconds at 20000rpm and filtered through Whatman No. 1 filter paper. Filtrate was collected in a measuring cylinder and made the volume upto 100 ml using distilled water. 5 ml of filtrate or extract was mixed with 5 ml of 0.01M TBA. Tightly closed test tubes and a blank containing 5 ml of 10 percent TCA and 5 ml of 0.01M TBA solution were placed in a boiling water bath (100ºC for 30 minutes. Test tubes were cooled under running water for 10 minutes. Absorbance value was measured at 532 nm using spectrophotometer. TBA value was expressed as mg malonaldehyde /Kg of meat. TEPP was used as standard in the concentration of Standard 1µg/ ml to 5 µg/ ml)

Results and Discussion

Changes observed in pH, water holding capacity (WHC), extract release volume (ERV) and Thiobarbituric acid value (TBA) are shown in table 1 and 2 along with graphical representation (Fig. 1 to 4)

pH value

As the number of freeze-thaw cycles increased pH was decreased significantly ($p<0.01$) irrespective of the thawing methods. Thawing methods had no significant impact on the pH value of meat. The pH of the samples decreased significantly from 0 day to the 6^{th} cycle. The decrease in pH during repeated freezing and thawing can be attributed to the loss of minerals and proteins as exudates during the three different thawing processes. Hence, the free hydrogen ions (H^+) in a solution decreases which results in decreased pH (Rahman *et al.,* 2014). Exudation of fluid from the muscle tissue lead to an increased concentration of solutes which may have resulted in reducing pH in the present study as reported by (Leygonie *et al.,* 2012). Denaturation of proteins due to the action of microbes and enzyme releases H^+ which in turn attributes to decreased pH (Ali *et al.,* 2015).

Table.1 Effect of multiple freeze-thaw cycles (room temperature, chiller temperature and microwave thawing) on the pH and Water holding capacity of chicken breast muscle (Mean±S.E.)#

Thawing Methods	Raw Meat	Freeze-thaw cycles						Sig. Level
	0	C1	C2	C3	C4	C5	C6	
pH value								
Room Temperature	6.02±0.04^{d}	5.97±0.07^{cd}	5.89±0.02^{bc}	5.78±0.03^{ab}	5.74±0.03^{a}	5.86±0.03^{abc}	5.94±0.04^{cd}	**
Chiller Temperature	6.02±0.04^{b}	5.93±0.01^{a}	5.81±0.04^{ab}	5.83±0.05^{a}	5.81±0.04^{a}	5.90±0.05^{ab}	5.96±0.06^{b}	**
Microwave	6.02±0.04^{c}	5.82±0.05^{ab}	5.82±0.05^{ab}	5.77±0.05^{a}	5.76±0.01^{a}	5.92±0.04^{abc}	5.98±0.07^{bc}	**
Overall	6.02±0.04^{d}	5.91±0.03^{bc}	5.84±0.02^{ab}	5.79±0.03^{a}	5.77±0.02^{a}	5.89±0.02^{bc}	5.96±0.03^{cd}	**
Sig. level	NS	NS	NS	NS	NS	NS	NS	
Water Holding Capacity (%)								
Room Temperature	83.00±1.29^{de}	84.70±0.62^{eB}	82.50±0.46^{cdAB}	81.12±0.53^{cd}	80.62±0.31^{cAB}	78.16±0.55^{bB}	75.94±0.42^{a}	**
Chiller Temperature	83.00±1.29^{bc}	84.95±0.72^{cB}	83.80±1.00^{bcB}	82.95±0.68^{bc}	81.49±0.87^{bB}	76.49±0.96^{aB}	76.26±0.84^{a}	**
Microwave	83.00±1.29^{c}	82.48±0.79^{cA}	81.04±0.93^{bcA}	80.84±0.89^{bc}	78.52±1.09^{bA}	73.32±0.83^{aA}	73.37±1.46^{a}	**
Overall	83.00±1.29^{cd}	84.04±0.47^{d}	82.45±0.53^{cd}	81.63±0.45^{bc}	80.21±0.54^{b}	75.99±0.65^{a}	75.19±0.63^{a}	**
Sig. level	**NS**	*	*	**NS**	*	**	**NS**	

#Mean±S.E. with different superscripts row-wise (small alphabet) and column-wise (capital alphabet) differ significantly (P<0.05) n=6 for each treatment at each thawing cycle; NS- Non Significant; *p<0.05; **p<0.01

Table.2 Effect of multiple freeze-thaw cycles (room temperature, chiller temperature and microwave thawing) on the physical properties of chicken breast muscle (Mean±S.E.)#

Thawing Methods	Raw Meat	Freeze-thaw cycles						Sig. Level
	0	C1	C2	C3	C4	C5	C6	
Extract Release Volume (ml)								
Room Temperature	24.33±0.42^{c}	21.17±0.95^{b}	20.33±0.49^{abA}	19.67±0.49^{ab}	20.00±0.37^{abAB}	20.00±0.58^{ab}	19.00±0.52^{aA}	**
Chiller Temperature	24.33±0.42^{c}	22.83±0.70^{bc}	22.83±0.60b^{cB}	20.67±0.61^{a}	21.50±0.56^{abB}	19.83±0.79^{a}	20.33±0.49^{aB}	**
Microwave	24.33±0.42^{c}	21.83±0.48^{b}	19.83±0.70^{abA}	21.33±1.45^{b}	19.67±0.56^{abA}	19.50±0.67^{ab}	18.67±0.21^{aA}	**
Overall	24.33±0.42^{e}	21.94±0.43^{d}	21.00±0.46^{cd}	20.56±0.54^{bc}	20.39±0.33^{abc}	19.78±0.38^{ab}	19.33±0.29^{a}	**
Sig. level	NS	NS	**	NS	*	NS	*	
TBA (mg malonaldehyde / kg)								
Room Temperature	0.13±0.01^{a}	0.19±0.01^{bB}	0.21±0.02^{bc}	0.22±0.01^{bcdB}	0.23±0.01^{cdeB}	0.27±0.00^{e}	0.25±0.01^{de}	**
Chiller Temperature	0.13±0.01^{a}	0.14±0.01^{aA}	0.16±0.01^{a}	0.17±0.01^{abA}	0.21±0.01^{bcAB}	0.22±0.03^{c}	0.21±0.02^{bc}	**
Microwave	0.13±0.01^{a}	0.17±0.01^{bB}	0.20±0.01^{c}	0.19±0.01^{cAB}	0.20±0.01^{cA}	0.23±0.01^{d}	0.25±0.01^{d}	**
Overall	0.13±0.01^{a}	0.17±0.01^{b}	0.19±0.01^{bc}	0.19±0.01^{cd}	0.22±0.01^{d}	0.24±0.01^{e}	0.24±0.01^{e}	**
Sig. level	**NS**	**	**NS**	**	*	**NS**	**NS**	

#Mean±S.E. with different superscripts row wise (small alphabet) and column wise (capital alphabet) differ significantly (P<0.05) n=6 for each treatment at each thawing cycle; NS- Non Significant; *p<0.05; **p<0.01

Fig.1 Effect of multiple freeze-thaw cycles (room temperature, chiller temperature and microwave thawing) on the pH Values of chicken breast muscles (Mean±S.E.)

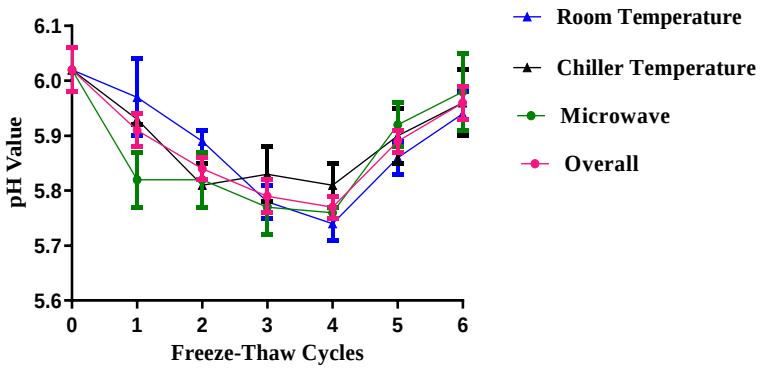


Fig.2 Effect of multiple freeze-thaw cycles (room temperature, chiller temperature and microwave thawing) on the Water Holding Capacity of chicken breast muscles (Mean±S.E.)

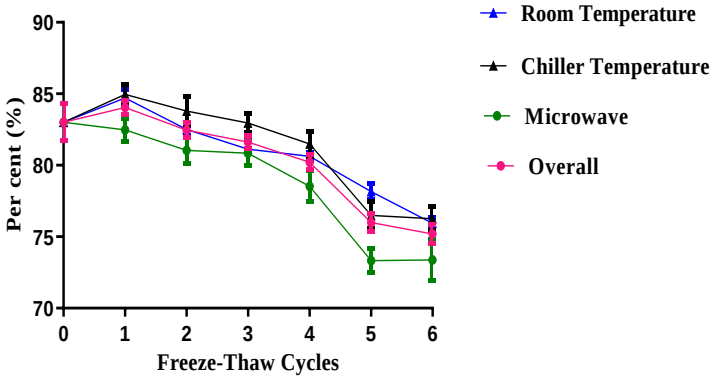


Fig.3 Effect of multiple freeze-thaw cycles (room temperature, chiller temperature and microwave thawing) on the Extract Release Volume of chicken breast muscles (Mean±S.E.)

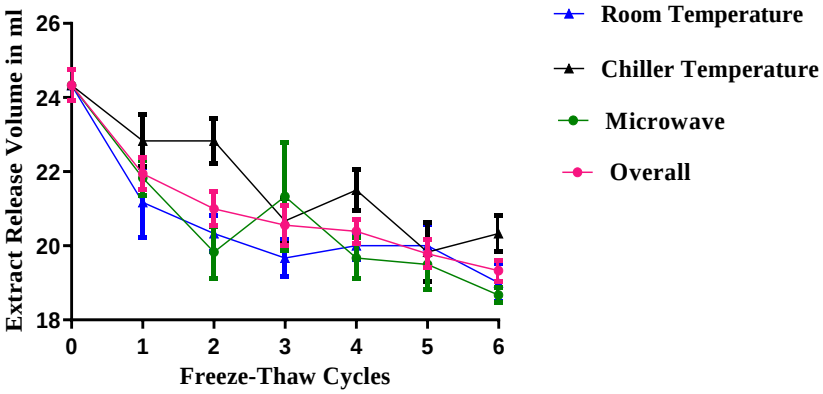
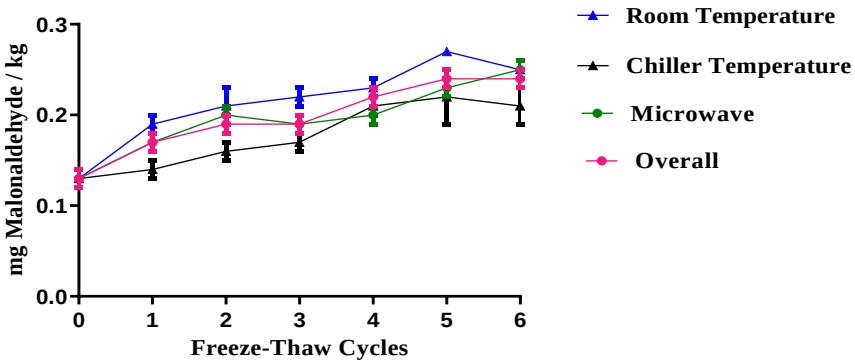


Fig.4 Effect of multiple freeze-thaw cycles (room temperature, chiller temperature and microwave thawing) on the TBA Value (mg Malonaldehyde / kg) of chicken breast muscles (Mean±S.E.)



Water holding capacity (WHC)

As the number of freeze-thaw cycles increased, WHC was decreased significantly ($p<0.01$) irrespective of the thawing methods. Significant difference ($p<0.01$) was observed amongst the thawing methods in all the cycles except 3 and 6. The ability of meat to retain its water when external forces such as cutting, grinding, heating or pressing is called WHC. The present findings can be explained by the fact that repeated free-thaw cycles leads to reformation of ice crystals which damages the cell membranes leading to loss of WHC (Srinivasan *et al.*, 1997).

WHC is directly related to pH and moisture. Decrease in pH will lead to decrease in WHC. The present study, WHC and pH both decreased significantly ($p<0.01$) from 0 day to the 6^{th} cycle. Furthermore, proteolysis and cell membrane degradation occurs due to repeated freeze-thaw cycles enhancing oozing out of fluids and low water binding capacity of the meat contributing to decreased WHC. Vieira *et al.*, (2009) reported that WHC is reduced by both frozen storage and thawing methods. Huff-Lonergan and Lonergan (2005) reported that WHC is dependent on myofibrillar protein structure. Myofibrillar protein and muscle cells' ability to bind and trap water is directly affected by pH, ionic strength, and oxidation.

Extract release volume (ERV)

Extract release volume (ERV) decreased significantly ($p<0.01$) irrespective of the methods of thawing as the number of repeated freeze-thaw cycles increased. The significant difference was observed amongst the thawing methods in cycle 2, 4 and 6, respectively.

Extract release volume is an indicator of the freshness of meat, and it decreases in the stored meat due to bacterial growth. The limit of ERV which indicate spoilage is 17ml as reported by Pearson (1968). ERV is useful to predict shelf-life of meat. Better sensory quality and lower microbial load release more ERV than poor quality meat (Swami *et al.*, 2015). The decrease in ERV values can be attributed to proteolysis of meat during storage as well as thawing (Jaiswal *et al.*, 2018). Similar trends of decline in ERV during storage period were also reported in chicken meat by Reddy and Varadarajulu (1981). Though ERV values decreased significantly ($p<0.01$) in different thawing methods, the meat cannot be categorized as spoiled as values were still above 17ml.

Thiobarbituric acid value (TBA) (mg malonaldehyde/kg)

As the number of freeze-thaw cycles increased Thiobarbituric acid value (TBA) (mg malonaldehyde/kg) also increased significantly ($p<0.01$) irrespective of the methods of thawing. Significant difference was observed among the thawing methods in cycle 1, 3 and 4.

TBA value is used as an indicator of food quality and is the most popular test for measuring the oxidative deterioration of lipids in muscle. Threshold value of TBA is 1-2 mg malonaldehyde/kg, which is indicative rancidity of meat (Swami *et al.,* 1962). Significant increase ($p<0.01$) in TBA value from zero day to 6th cycle was observed in the present study. Similar result was obtained by (Soyer *et al.,* 2010) in chicken breast meat frozen at -18 ºC for six months. In the present study, oxygen permeability during storage period might have encouraged bacterial activity leading to lipid oxidation, resulting in increased TBA value (Sen, 1996)). Besides, lipids, haeme proteins and microsomal enzymes also promote lipid oxidation which is evident as TBA values increased during the six freeze-thaw cycles. The values of TBARS increased with the increase in number of freeze-thaw cycles and storage period. Freeze-thaw cycles attribute to the loss of muscle integrity due to accelerated oxidation. The breakage of the cell due to ice crystals releases pro-oxidants for lipid oxidation (Benjakul and Bauer, 2001).

In conclusion: the present study on the effects of different thawing methods on broiler chicken's physico-chemical properties under repeated freeze-thaw cycles can be concluded that the parameters like pH, WHC and ERV declined significantly. On the other hand, TBA value increased considerably with an increased number of freeze-thaw cycles. It can also be concluded that chiller thawing had minimal affect on the meat quality followed by room temperature thawing, whereas microwave thawing had the maximum.

References

Ali S., N. Rajput, M. A. Khan, C. B. Li and G. H. Zhou, 2015. Effect of multiple free-thaw cycles on the quality of chicken breast meat. *Food Chem.,* 173: 808-814.

Benjakul, S. and F. Bauer, 2001. Biochemical and physicochemical changes in catfish (*Silurus glanis Linne*) muscle as influenced by different freeze-thaw cycles. *Food Chem.,* 72(2): 207-217.

FSSAI, 2020. Training Manual on Food Safety Supervisor Course, Special – (Level 3) – Manufacturing, Poultry Meat and Poultry Meat Products and Eggs. Available at: https://fostac.fssai.gov.in/fostac/doc/Training%20Manual%20-%20Poultry%20Meat%20and%20Poultry%20Meat%20Productsy_22.09.2018.pdf

Farouk, M. M. and J. E. Swan, 1998. Effect of muscle condition before freezing and simulated chemical changes during storage on the pH and colour of beef. *Meat sci.*, 50(2): 245-256.

Grujic, R., L. Petrovic, B. Pikula, and L. Amidzic, (1993). Definition of the optimum freezing rate—1. Investigation of structure and ultrastructure of beef *M. longissimusdorsi* frozen at different freezing rates. *Meat Sci.*,33(3): 301–318.

Hamm, R., 1960. Determination of water holding capacity in meat, Hamm Press method.

Hanenian, R. and G. S. Mittal, 2004. Effect of freezing and thawing on meat quality. *Journal of Food, Agri. and Environment*, 2(3 and 4): 74-80.

Huff- Lonergan, E. and S. M. Lonergan,

2005. Mechanism of water holding capacity of meat: the role of postmortem biochemical and structural changes. *Meat Sci.*,71(1): 194-204

Jaiswal, R. K., S. K. Mendiratta, S. Talukdar, A. Soni and G. B. Bomminayuni, 2018. Comparative evaluation of dye reduction tests for assessment of microbial quality of chevon. *Int. J. Livestock Research*, 8(3): 133-139.

Lawrie, R. A., 1991. *In Meat Sci.*, 5th edition, R. A. Lawrie. Pergamon press, Oxford.

Leygonie, C., T. J. Britz and L. C. Hoffman, 2012. Impact of freezing and thawing on the quality of meat: Review. *Meat Sci.*, 91(2): 93–98.

Pearson, D., 1968. The correlation of the extract release volume of stored beef with other spoilage values. *J. of Food Tech.*, 3: 207.

Reddy, P. and P. Varadarajuli, 1981. Relationship among microbial pH in refrigerated chicken. *Indian J. Poultry. Sci.*, 16(1): 22-27

Rahman, M. H., M. M. Hossain, S. M. E. Rahman, M. A. Hashem and D. H. Oh, 2014. Effect of repeated freeze-thaw cycles on beef quality and safety. *Korean J. Food Sci. An.* 34(4): 482-495.

Sen, A. R, 1996. Quality changes in buffalo meat during storage in dry ice pack. Ph. D. thesis submitted to IVRI, Izatnagar.

Sen, A. R. and N. Sharma, 2004.Effect of freezing and thawing on the histology and ultrastructure of buffalo muscle. *Asian-Aust.J.Anim. Sci*. 17(9):1291-1295

Soyer, A., B. Ozalp, U. Dalmis and V. Bilgin, 2010. Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. *Food chem.*, 120(4): 1025-1030

Srinivasan, S., Y. L., Xiong and S. P. Blanchard, 1997. Effects of freezing and thawing methods and storage time on thermal properties of freshwater prawns (*Macrobrachium rosenbergii*). *J. of the Sci. of Food and Agri.*, 75(1): 37–44

Swami, J. N., S. S. Raut and S. N. Rindhe, 2015. Effect of freeze-thaw cycle on quality of rabbit meat. *Haryana Vet.* 54(2): 160-163

Vieira, C., M. T. Diaz, B. Martinez and M. D. Garcia-Cachan, 2009. Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. *Meat Science*, 83(3): 398–404

Hamm, R., 1960. Determination of water holding capacity in meat, Hamm Press method.

Witte, V. C., G. F. Krause, and M. E. Bailey, 1970. A new extraction method for determining 2-Thiobarbituric acid values of pork and beef during storage. *J. Food Sci.*, 35(5): 582-585.

How to cite this article:

Lampra Debbarma, V. Chandirasekaran, A. Irshad, S. Sureshkumar and Gopala Krishna Murthy, T. R. 2021. Effects of Different Thawing Methods on the pH, Water Holding Capacity, Extract Release Volume and TBA value of Broiler Chicken under Repeated Freeze-thaw Cycles. *Int.J.Curr.Microbiol.App.Sci.* 10(03): 141-149.
doi: https://doi.org/10.20546/ijcmas.2021.1003.020