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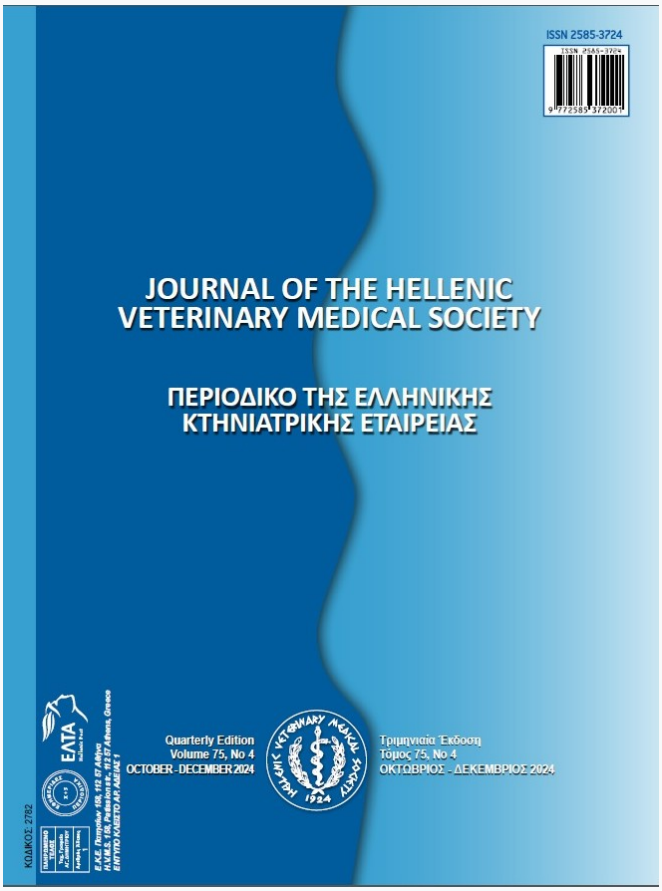

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Research article
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Effects on beef microstructure using fractal dimension and ANN modelling

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ABSTRACT: The freezing time of beef has been predicted using an artificial neural network (ANN), which relies on data obtained from the microstructure of meat. For this reason, cross-sectional images of beef meat were captured during six periods of frozen storage (2, 4, 6, 8, 10, and 12 months after slaughter). The equivalent diameter and ratio of area of the ice crystals relative to the cell were determined, and the fractal dimension was chosen to describe the porous microstructure due to the crystallization of ice in frozen beef. As a result, when meat has been frozen for a long time, larger ice crystals form. In contrast, the fractal dimension decreased with the change in the microstructure of muscle tissue during storage. Artificial neural network analysis (ANN) revealed a high accuracy of prediction performance for each morphological attribute. These results show that the fractal dimension can be used as an effective method to characterize the structure of beef during frozen storage, and ANN models can successfully describe structural changes in beef meat during frozen storage.

***Keywords*:** Frozen beef meat; ice crystal diameter; fractal dimension; artificial neural network

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INTRODUCTION

Freezing is one of the most effective ways to prolong the shelf life of frozen foods (Kiani and Sun, 2011; Zequan et al., 2019). This method of preservation concerns both intermediate products intended for manufacturing, like pre-cut vegetables, partially cooked meats, dough or batter for bakery products, sauces, and various food components, as well as those available to consumers. The principle of this method is based on the solidification of water into ice, thus causing a decrease in the activity of the water in the food (Luan et al., 2018). This induces both a reduction in microbiological activity and a slowdown in biochemical reactions, which are responsible for the degradation of quality (Kim et al., 2013). In previous studies, many researchers have shown the effect of freezing and frozen storage on meat quality through several factors that focus on differences in microbiological, physical, chemical, and sensory properties (Medić et al., 2018; Choi et al., 2018; Zhang et al., 2019b; Holman et al., 2018; Alonso et al., 2016; Seong et al., 2017). However, the expansion of ice crystal volume during frozen storage and its influence on microstructure has been rarely reported.

During the freezing and frozen storage of meat, the formation of ice crystals can produce a porous form in muscle tissue. The appearance of the meat microstructure is difficult to determine by available methods (He et al., 2015). Fractal dimension is a geometric parameter that measures the complexity or irregularity of a shape or structure (Mandelbrot, 1982). It has been widely used in various fields to quantify the structural properties of objects, including the characterization of food during storage (Kono et al., 2017). This was demonstrated by Wang et al. (2022) when they used fractal dimension to explore an effective quality assessment system based on the microstructural changes of fish stored at different temperatures for an extended period.

Artificial neural networks (ANNs) have proven to be a valuable tool in modelling and prediction (Liu et al., 2013). ANN modelling is a computer system that mimics the functioning of biological neurons, specifically the brain and nervous system (Quaglio et al., 2020; Gonzalez-Fernandez et al., 2019; Zhang et al., 2016).

In the context of computer applications, an artificial neural network (ANN) exhibits a high learning capacity and the ability to identify and model complex and nonlinear relationships between input and output in a system (Nourbakhsh et al., 2014). The application of neural networks has been reported as useful in predicting changes that occur in meat quality and evaluation (Huang et al., 2007). For example, Zhu et al. (2021) used a neural network to predict various qualities of dry-cured ham based on protein degradation. Similarly, Kaczmarek and Muzolf-Panek (2022) utilized the ANN modelling technique to simulate variations in TBARS levels in the intramuscular lipid fraction of raw beef enriched with plant extracts and also employed predictive models to monitor changes in thiol (SH) group levels in raw and thermally processed ground chicken meat enriched with selected plant extracts during storage at different temperatures (Kaczmarek and Muzolf-panek, 2021). Microstructure research focusing on describing the fractal dimension of meat during long-term frozen storage, along with prediction modelling, has not been fully explored. Therefore, the aim of this study is to develop a model for accurate prediction of beef frozen storage period using an artificial neural network (ANN) based on meat microstructure. The main variables studied are ice crystal equivalent diameter, ice crystal surface-to-cell ratio and fractal dimension.

MATERIALS AND METHODS

In order to collect a homogeneous batch of samples (sex, age, and race), twenty samples were selected from slaughtered beef of a local breed known as “Brune de l’ Atlas” of less than two years old at the municipal slaughterhouse in Batna city, north-eastern Algeria. Samples were taken from the m. biceps femoris 24 h after slaughter. Each sample was divided into six sections with an approximate weight of 700 g, corresponding to periods of frozen storage (2, 4, 6, 8, 10, and 12 months). Meanwhile, images of fresh meat samples were captured on the same day in order to compare the change in the microstructure after having been frozen for a year at limited intervals. The samples were placed inside polythene bags, where they were exposed to a controlled temperature of -40°C. The freezing process was conducted using an air freezer (CRF-NT64GF40, Condor, Algeria), equipped with an electric fan that maintained a ventilation air of 0.6 m/s. A digital thermometer was inserted into the meat samples to continuously monitor the temperature. The freezing procedure was concluded once the internal temperature of the muscle samples had reached -23°C. After that, all samples were vacuum packed and frozen at -23 °C ± 0.6. Throughout the frozen storage period, which extended for a total

of one year, the muscle temperature was monitored (three times per day) using an infrared thermometer to avoid any temperature fluctuations. Temperature monitoring throughout the frozen storage period was carried out using a thermometer (TIA 101, China). Typically, the infrared thermometer measures the ambient temperature of the freezer. However, to obtain the temperature of a specific sample, it was necessary to place the thermometer in direct contact with the sample. The consequent average freezing storage temperature was -23 °C ± 0.6 °C, which is indicative of a minor fluctuation associated with freezer defrosting. These meticulous procedures were implemented to ensure accurate analysis of the meat samples at different stages of freezing and storage.

Preparation of histological sections

Cryosubstitution was used to investigate the voids left in muscle tissue by ice crystals, Su et al. (2014), who used this method to compare the formation of ice crystals between pressure shift freezing and the traditional method on different meats (freezing porcine liver and shrimp). Clarke's solution (ethanol and acetic acid, 3:1) was used at -23 °C to fix the samples for 24 h. The fresh samples (at the start of the experiment) were fixed in a Clarke solution upon their arrival at the laboratory in order to achieve subsequent histological sections. Tissue samples were then returned to room temperature. After fixation, pieces of about 1 to 2 cm in length by 1 cm in width are cut from the meat muscles. This cutting is done in a way that traverses the muscle fibers perpendicularly to their natural orientation, in order to obtain a cross-sectional slice. Subsequently, these pieces are arranged in numbered cassettes. Next, samples were dehydrated in an ethanol gradient using the protocol and technique defined by Lakehal et al. (2019), followed by immersion in xylene and paraffin at 59 °C. Each sample is then embedded in paraffin and trapped in a paraffin block. These sections were then sliced 7 µm thick with a microtome (Leica, Jung-histocut 820, Germany), placed on glass slides, deparaffinized with xylene, and stained with Calleja's solution, (a mixture of 100 ml of 1% indigo-carmine solution and 200 ml of saturated acid picric solution) for subsequent microscopic analysis (Hildebrandt and Hirst, 1985). Using this staining method, the muscle proteins stain green and collagen blue. A microscope (Axioscope Zeiss, Germany) equipped with a digital camera (SLS-Mvision, China) was used to inspect all the prepared slides.

Microscopic analysis

Five cross-sectional images were collected for each sample during each storage period and transformed into binary images. For further analysis, three parameters were selected: equivalent diameter of ice crystals, the ratio of the area of ice crystals relative to that of cells, and the fractal dimension. The diameter of a circle region calculated by ImageJ is called the "equivalent diameter. The areas of the cells were determined after the program automatically drew the cell outline. Additionally, the computerized database (Excel, 2021) was utilized to calculate the ratio of the area of ice crystals relative to that of cells, and the fractal dimension. The measurements were taken three times. In the majority of studies, the fractal dimension has been obtained using the box counting method (He et al., 2017; Luan et al., 2018). However, in our study, we performed a fractal analysis of ice crystal particle images using the Area/Perimeter method, which primarily relies on measuring the perimeter length (L) in µm for ice crystals of different sizes and the surface area (S) in μm^2 bounded by the contour (Hagiwara et al., 2002). ImageJ software (version 1.52a) was used to examine the morphology of ice crystals. The perimeters (L) of the outlines in the photographs, as well as the areas (S) enclosed by them, were measured for ice crystals. In order to calibrate the spatial dimensions of the analyzed objects, initially expressed in pixels, a standard micrometer slide was used to calibrate the image.

The equation that follows (Eq. 1) was used to calculate *Fd* of ice crystals (Mandelbrot, 1982).

$$S \propto L^{2/Fd} \qquad (1)$$

Where *Fd* is a fractal dimension.

$Ln\ S = 2/Fd\ ln\ L + A$, where A is constant.

Fd= 2/slope. it is necessary to plot the curve of *ln S* as a function of *ln L* that corresponds to the ice crystal particle outline.

For normal forms like circles and squares, the fractal dimension assumed the value between 1 and 0. Each unit of image data had a minimum of 50 ice crystal particles counted.

According to the procedures of Lakehal et al. (2015), ANN modelling was used to define the relationship between storage time and equivalent ice crystal diameters, fractal dimensions, and the ratio of area ice crystals relative to the cell. The main appli-

cation of neural networks is to discover optimal solutions for complex, non-linear systems. The multilayer perceptron (MLP) is the most frequently used type of neural network. The most basic MLP architecture comprises three layers, as illustrated in Fig. 1. This study utilized a multilayer perceptron (MLP) with one hidden layer, the next stage involved the validation of the trained MLP using a set of data that had not been used previously. To identify the optimal number of neurons. The network was trained until the maximum epoch limit of 10,000 was reached, while utilizing a learning rate of 0.01 and a momentum constant of 0.9. To train the database, a BP "Back propagation" neuronal network algorithm is utilized, which involves the training, test, and validation bases, as well as optimal architecture parameters like the number of layers and neurons and transfer function type. The input layer receives data from external sources. The hidden layers comprise numerous unobservable neurons that receive input data from the input layer. Finally, the output layer generates a response that conforms to the system's desired output. During the design and optimization phase, the MATLAB interface was used. To measure the fit between the model prediction and the data points, the R^2 value was determined. Meanwhile, RMSE is used as an evaluation metric in prediction tasks to assess the accuracy of the predictions.

Statistical analysis

The current study's results were statistically evaluated using the SPSS software version 20 (IBM SPSS Statistics v22). Analysis of variance (one-way ANOVA) techniques, as well as Tukey multiple comparison tests, were employed to examine differences in the data acquired in the experimental study. The information was presented as a mean value with a standard deviation.

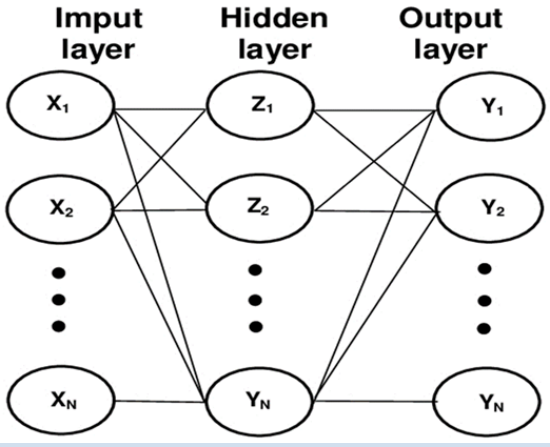

Figure 1. Schematic of the training process of the ANN

RESULTS AND DISCUSSION

Microscopic analysis

The structure of meat changes dramatically during freezing due to the change in the relationship between protein and water molecules (Nakazawa et al., 2020). Fig. 2 (A-G) depicts transverse sections of muscle fiber from both fresh meat (Fig. 2A) and frozen meat during different storage times (Fig 2B-G). The structural organization of the muscle of fresh meat was well seen in cross-section was well maintained as a whole. The muscle fibers were bundled together with little space between them, similar to that reported by Zhu et al. (2004), but many extremely fine microfissures in the muscle fibers are interpreted as artifacts during the preparation of histological sections (Fig. 2A). Hou et al. (2020) substantiated the significance of the microstructural integrity of frozen foods, emphasizing that it primarily relies on the size and placement of ice crystals formed during the freezing process.

During the second month of storage (Fig. 2B), small voids that were considered to be imprints of intracellular ice crystals were observed in the meat samples. As the shelf life increased, intracellular ice crystals appear larger. In some spaces, extracellular ice crystals have shrunk muscle fibers. Other areas of muscle tissue are completely destructured and deformed, with no trace of the fiber outlines visible (Fig. 2C, 2D, 2E, 2F, and 2G). In general, preserving meat and meat products in good condition for a long time may be as simple as reducing the storage temperature (Zhang et al., 2019a). Even so, during frozen storage, water crystallization within the tissue could lead to a higher solute concentration, which could result in endomysium separation and perimysium expansion. These changes manifest as a reduction in cell size and an increase in extracellular space (Luan et al., 2018). In addition, at the start of recrystallization during freezing, a slight melting of small extracellular ice crystals leads to the formation of large ice crystals, thus causing pressure on the cell (Kono et al., 2017).

For quantitative analysis of long-term stored ice crystals, the equivalent diameter and the ratio of the area of ice crystals relative to that of cells were measured and presented in Fig. 3. The equivalent diameter of ice crystals increased significantly, going from 29.09 μm after 2 months to approximately 39.34 μm after 12 months. Similarly, the ice crystal/cell surface

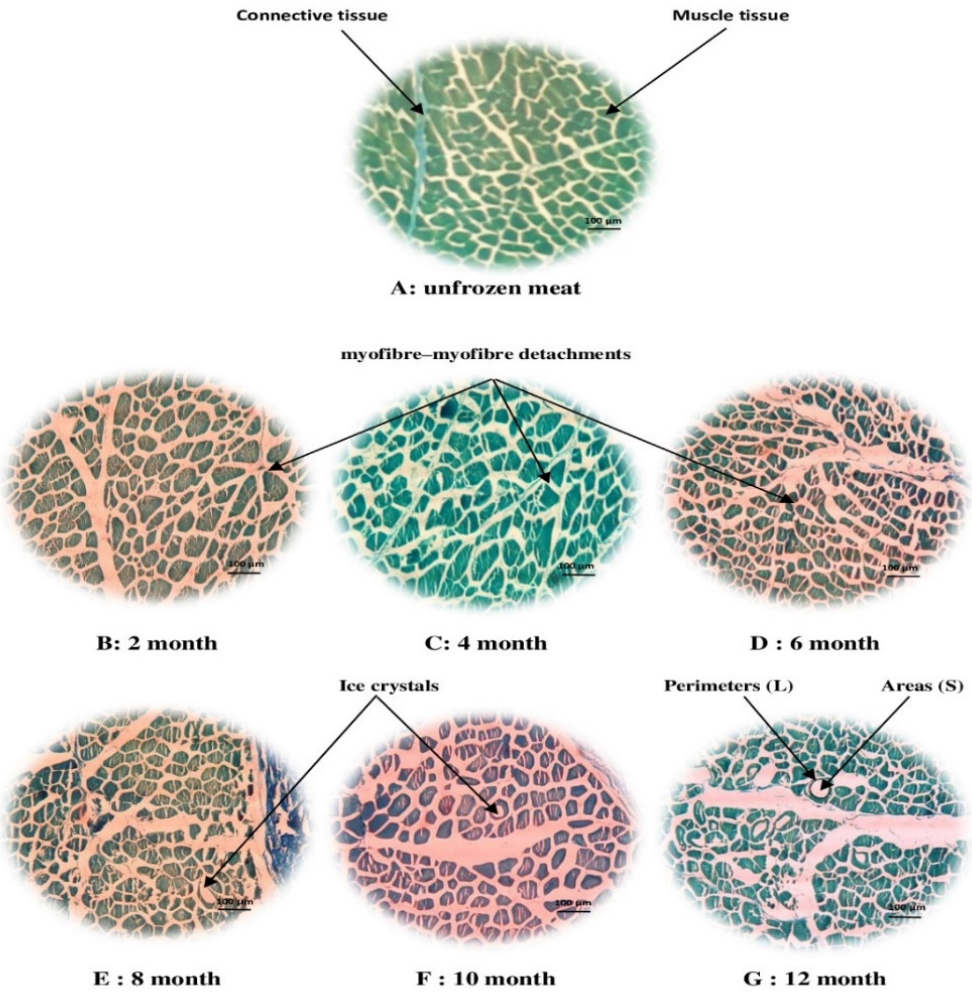

Figure 2. Changes in microstructural cross section of frozen beef muscles.

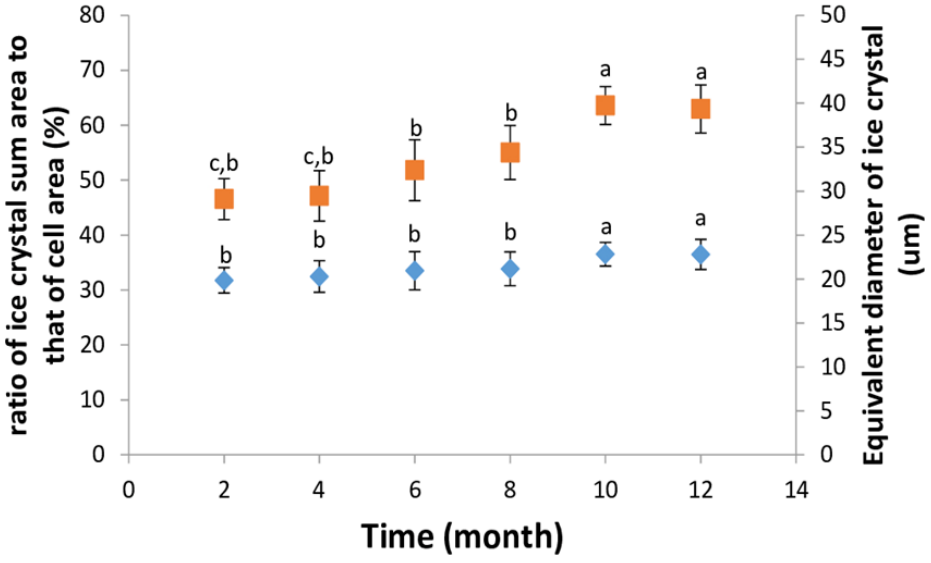

Figure 3. Effect of frozen storage duration on the equivalent of ice diameter and the ratio of ice crystal sum area to that of cell area of frozen beef muscle. ■: Mean of Equivalent diameter of ice crystal, ◆: Mean of ration of ice crystal sum area to that cell area.

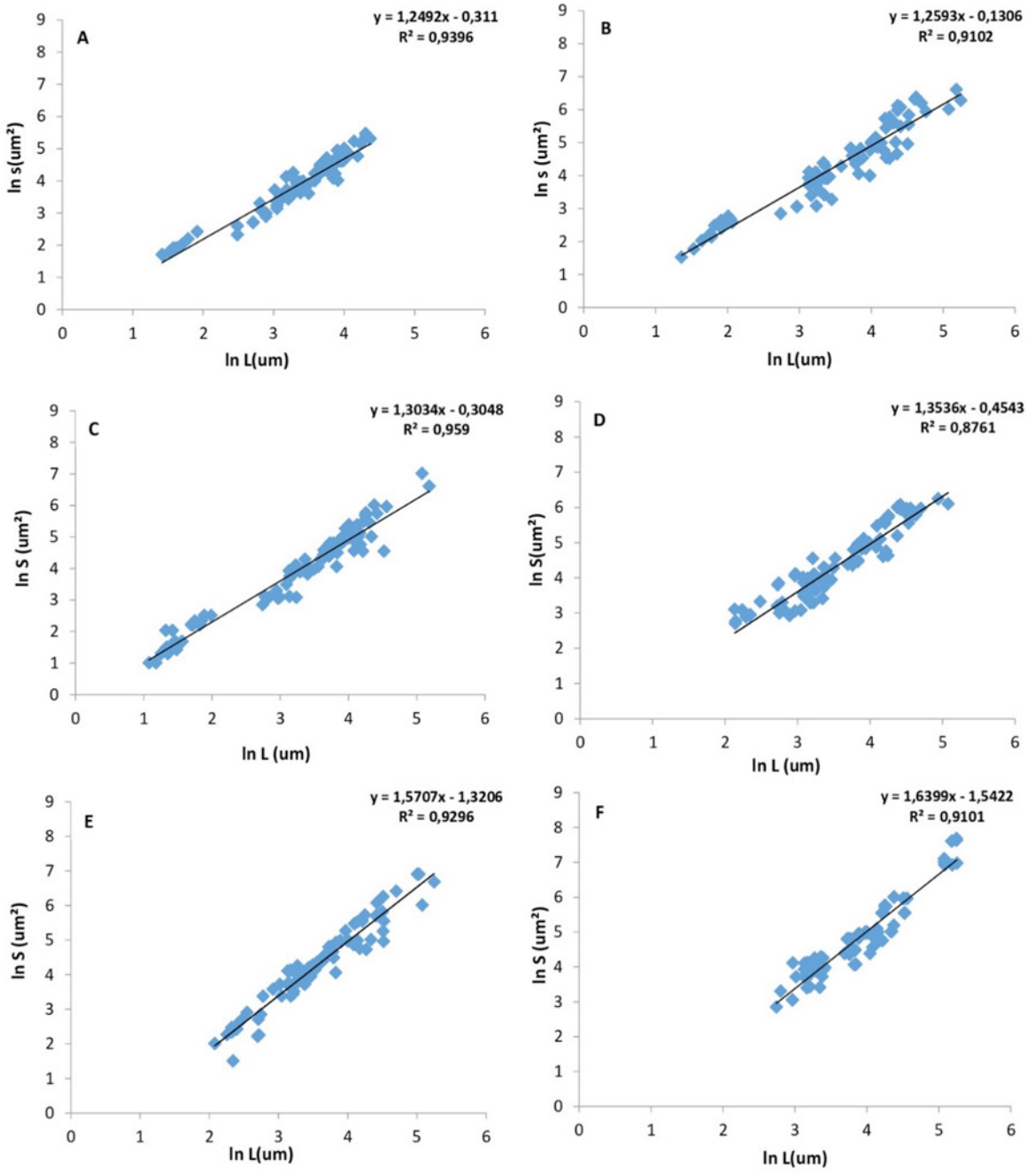

Figure 4. Plots of log S vs log L for different period of storage. (A) freezing for 2 months, (B) freezing for 4 months, (C) freezing for 6 months, (D) freezing 8 months, (E) freezing for 10 months, (F) freezing for 12 months.

ratio for all frozen samples increased significantly ($p < 0.05$) in percentage during frozen storage, reaching an average of 31.77-36.46 %. Ice crystal growth results from recrystallization during frozen storage (Payne et al., 1994). Therefore, the slight melting of small ice crystals can result in the creation of new large ice crystals during storage. As a result, the number of ice crystals and the ratio of surface ice crystals to cells increased slightly (Fig. 3). An increase in the volume of ice crystals can cause significant mechanical damage to the meat, leading to an increase in the concentration of unfrozen liquid inside and outside the cell, leading to an increase in thawing loss (Jiang et al., 2020; Shi et al., 2018).

Fractal analysis has become a quantitative analysis tool for evaluating a variety of disordered and complex shapes with enough sensitivity to detect small

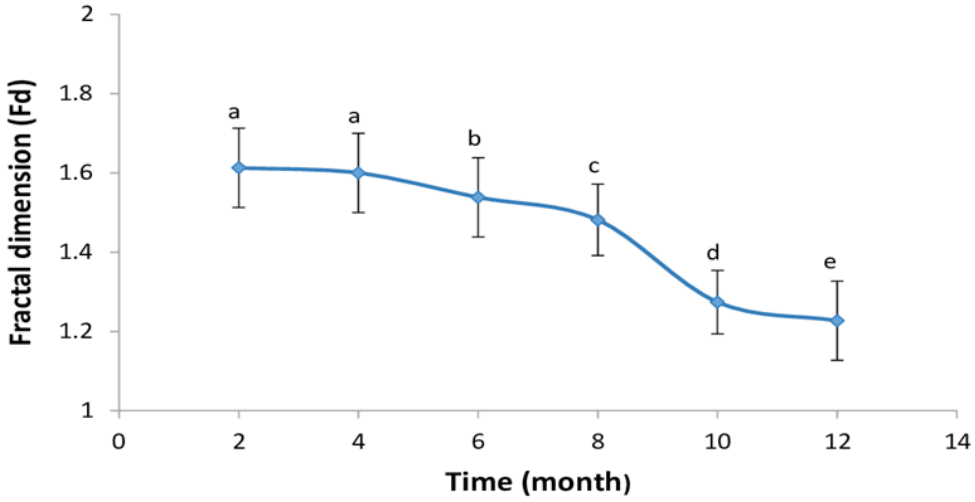

Figure 5. Dependence of the fractal dimension (Fd) on storage time. [a,b,c,d,e] Letters above the error bars express the significance levels among Fd values, respectively, with different treatments ($p < 0.05$).

changes in shape (He et al., 2017). The morphology of ice crystal particles has been studied using this method. Fig. 4 illustrates typical *ln(S)* and *ln(L)* relationship of ice crystals in a single histological image after different storage periods. The coefficients of determination ranged between 0.87 and 0.95. Plots for samples stored for 6 months (Fig. 4C) showed that the fitting line was comparatively more accurate (R^2=0. 95).

Fig. 5 represents the beef meat's fractal dimension values at various storage durations. This result is consistent with that of Luan et al. (2018), who found that the fractal dimension of frozen Trichiurus (*Trichiurus haumela*) samples decreased with increasing storage time. We have observed that the decrease in fractal dimension is accompanied by a higher degree of microstructural irregularity as the volume of the ice crystals increases. Large ice crystals can physically disturb cells, resulting in mechanical damage and a decrease in fractal dimension (He et al., 2017). Furthermore, Kono et al. (2017) stated that the smoothing of the rough surfaces of ice crystals due to recrystallization is indicated by a decrease in fractal dimension values during frozen storage.

Accuracy of ANN modelling

Based on the calculated data for the equivalent diameter of ice crystals, the fractal dimension, and the ratio of ice crystal area relative to the cell, an artificial neural network analysis model was developed to create a prediction model for the time beef had been frozen. The results of our proposed model are shown in Table 1. The R^2 values for the training dataset and the verification dataset were 0.943 ($RMSE_c$= 0.0310) and 0.945 ($RMSE_v$= 0.0227), respectively, indicating that the fractal component of frozen beef meat can be predicted with high accuracy. The R_c^2 and R_v^2

Table 1 Architecture of ANN models and prediction accuracies for ration of icecrystal sum area to that cell area, equivalent diameter and fractal dimensions

ANN models	Architecture			Prediction accuracy			
	Input	hidden layer	Output	R2c	RMSEc	RMSEv	R2v
ration of ice crystal sum area to that cell area	2	2	1	0.945	0.425	0.017	0.877
Equivalent diameter of ice crystal	2	3	1	0.894	0.510	0.010	0.739
fractal dimension	2	3	1	0.943	0.0310	0.0227	0.945

R^2c: Determination coefficient of calibration.
RMSEc; Root mean square error of calibration.
RMSEv : Determination coefficient of cross validation.
R^2v: Root mean square error of cross validation

values for the ratio of the ice crystal area relative to the cell ranged from 0.945 (RMSE= 0.425) to 0.877 ($RMSE_v$= 0.017). The model reasonably predicted the equivalent diameter of the ice crystal with R_c^2 (calibration) and R_v^2 (cross validation) values of 0.894 and 0.739, respectively, as well as 0.510 and 0.010 for $RMSE_c$ and $RMSE_v$. These findings showed that artificial neural network models indicated prediction performances with higher accuracies for each morphological attribute.

CONCLUSION

The present research examined structural changes in beef tissues that had been stored for a long time. The study of the microstructure of the beef samples revealed a constant increase in the size of ice crystals, indicating structural alterations of the frozen muscles with increasing storage time. The equivalent diameter of the ice crystal as well as the ratio of ice crystal surface area to cell surface area increased. The obtained experimental results indicate that, in general, the fractal dimension, which quantifies the pore structure formed in tissue samples, shows a decreasing trend as storage time increases. Therefore, from this experimental research, we can say that the fractal dimension of muscle tissue might be a useful tool for identifying changes in the microstructures of beef samples. Thus, a neural network model was successfully applied to predict differences in ice crystal size. They were built on the basis of a data set based on characteristics and experimentally obtained results in terms of ice crystal properties and time storage. The proposed model, which employs artificial intelligence (ANN), can be expanded and can be useful as an identification tool in the field of food preservation through further research to explore its applicability beyond the current scope and to evaluate its performance under diverse circumstances.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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