Journal of Food Measurement and Characterization
https://doi.org/10.1007/s11694-024-02791-5

ORIGINAL PAPER

In-pack sonication of chicken breast: effects of ultrasound conditions on physicochemical and microbiological properties, and shelf-life of optimally processed meat during frozen storage

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Received: 28 February 2024 / Accepted: 28 July 2024

Abstract
Conventional ultrasound (US) has been used to incorporate active components into packaging material, but in-pack sonication and its effect on food quality and shelf-life need further exploration. This study aims to examine how US treatment conditions and packaging materials affect aged chicken breast's shelf-life, microbiological, sensory, and physicochemical characteristics, including peroxide value (PV), free fatty acids (FFA), cooking loss (CL), and muscle fiber index (MFI). Samples were sonicated either without packaging (WP) or packed inside low-density polyethylene (LDPE). The results were then compared with untreated and conventionally treated samples. Response surface methodology (RSM) optimized the specific energy consumption (SEC), acoustic power, and product characteristics based on sonication power (4.4, 35.5, and 66.0 W) and time (10, 20, and 30 min). The quality attributes of optimally processed samples were then assessed during 60 days of -18 °C frozen storage. The optimal sonication conditions were identified as 15.46 min of 66 W, corresponding to 5.16 kJ/kg SEC and 17.03 W acoustic power. In-pack sonication was superior to conventional sonication in terms of product quality attributes. Optimized in-pack sonication reduced PV, FFA, and total bacteria count by 15.0, 17.6, and 37.4% but increased CL, MFI, and shelf-life by 4.1, 107.8, and 64.2%, respectively. The proposed in-pack ultrasonics approach could contribute to achieving sustainable development goals (SDGs) and sustainable food production, considering the low energy consumption and food waste reduction through delaying food spoilage.

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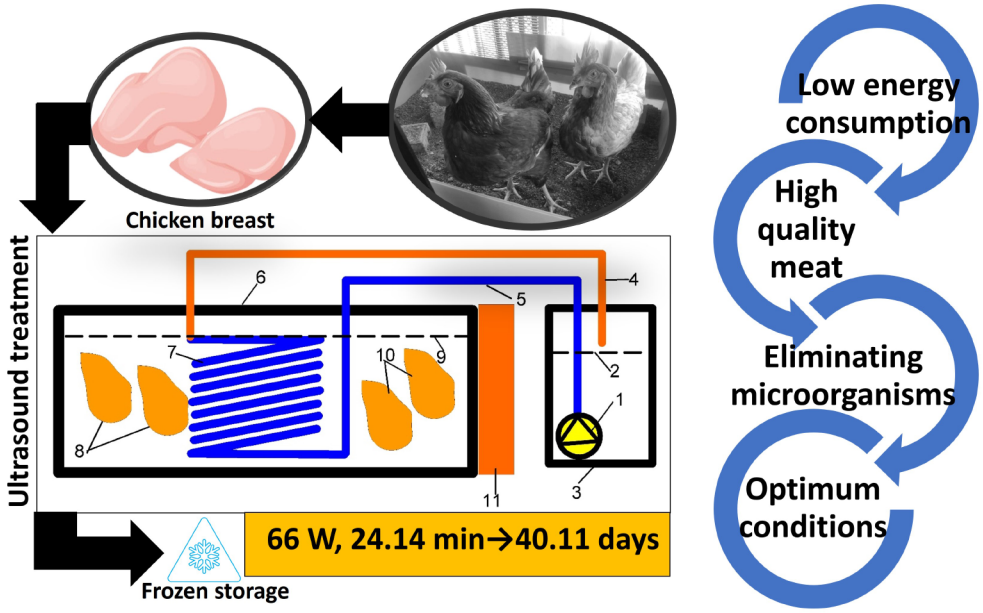
Published online: 16 August 2024

Graphical Abstract



Keywords Ultrasound · Meat processing · Emerging technologies · Shelf-life · Optimization

Introduction

The consumer's perception of the meat's quality is influenced by its freshness and other physicochemical attributes [1]. Flavor, juiciness, and appearance are critical qualities in meat that affect the physical, chemical, and organoleptic properties of meat products such as poultry [2]. Increased demand for high-quality meat products is expected [3]. Also, variations in seasonal production and needs for transportation necessitate poultry's extended shelf-life, which is commonly achieved by frozen storage. However, the quality characteristics may decline during storage, necessitating technological advances to address this issue. In this concept, emerging technologies, such as US, are among the candidates that may provide solutions, considering the promising results reported in recent studies [4].

US waves with 20–1000 kHz frequencies and energy intensities of 10–1000 W/cm^2 are usually used for food processingPressure waves and refraction in the medium are created when the sound reaches a specific medium. As a result, cavities or bubbles grow in successive US cycles until they become unstable, collapse, and generate elevated temperatures and pressure [5]. The micro and macro levels of biological materials and tissues may be affected by such phenomena, which may positively impact the quality and safety of foods [6].

Considering the sonication-caused impacts, this technology has been explored as an alternative to conventional meat processing technology, such as beef meat processing [7]. Also, it has been used to enhance the quality of low-salt meat products [8]. Besides, combined US-enzymatic treatment was used to treat hen breast meat [9]. Literature also highlighted the potential of sonication in improving chicken physicochemical properties [10] and assisting meat marination and tenderization [6]. Previous reports also mentioned that the US improved meat's thawing and cooking loss (CL) [11], slowed down protein structural damage and oxidation during frozen storage [12], and assisted the meat curing process [13].

Despite numerous research studies exploring conventional sonication of in meat, there is limited information on in-pack ultrasonication to extend the packaged meat shelf-life since the in-pack sonication technique has been introduced to the food industry [14]. At the same time, there is limited data on developing a US-based process to improve the physicochemical properties of aged chicken breasts during frozen storage. Accordingly, this study aimed to determine the effects of in-pack and conventional sonication on aged chicken breast and to assess the quality parameters of the optimally processed product during cold storage.

Materials and methods

Sample preparation

Aged egg-laying hens (1.5 years with average weight of 1500 g) were considered in this study to assess the impact of US. These aged hens cannot lay significant eggs, and their meat has low market value due to low quality (e.g., low tenderness and unpalatability) compared to broilers. The aged hens were purchased from the Basrah province market and originated from the same farm. The slaughter processing was performed per animal slaughtering's ethics and welfare policy [15, 16]. The scientific/ethical committee of the College of Agriculture (University of Basrah) reviewed and approved all the procedures aligned with the approval number AW62902202-1-1. A manual cut with a sharp knife was performed on the left artery of the lower jaw of the chicken neck (15–20 mm from the head gland and about 15 mm from the end of the hyoid bone). After exsanguination, the slaughtered chicken was cleaned, and the breasts were taken. Packaging materials used in this study were low-density polyethylene (LDPE) bags with a density of 0.93 g\cm^3 and a thickness of 40 µm (Fig. S1).

US equipment and in-pack/conventional sonication process

A 5 L bath US device (model LUC-405, Daihan Labtech Co. Ltd., Korea) with internal dimensions of 150 × 155 × 300 mm and a power of 350 W was used in this study. The device operated at an input voltage of 220 V and a frequency of 50 Hz and output a US frequency of 40 kHz. The sonication bath was equipped with a tubular heat exchanger consisting of a 10 L water tank, submersible pump (Anchor, UK), plastic tube, and ice water to maintain a constant temperature of 30 °C.

In all the sonication treatments, four pieces of breast were used each time, i.e., four chickens were used in each replication, and the experiments were conducted in triplicate. For the conventional sonication, samples without packaging (WP) were placed in the sonication bath. At the same time, samples packed in LDPE were put in the sonication bath to perform in-pack sonication. Afterward, the effects of 4.4–66.0 W US power and 10–30 min treatment time (UTT) on breasts' quality parameters were studied. Then, samples were stored at a -18°C freezer (model 1500, LG, China) to assess the changes in physicochemical and microbiological parameters during frozen storage.

Conventional and control treatments

Electrical stimulation, as a conventional method of tendering chicken breast [17] was used by applying 3.67 V/cm for 1 min to treat samples before packaging according to [18]. Chicken breast meat without any additional process, named raw chicken breast meat (RM), is considered a control treatment.

US parameters analysis

It is vital to analyze ultrasonic characteristics to improve comprehension of the effects of processing parameters on dependent parameters. Analysis of ultrasonic parameters and related calculations were performed at the "emerging food processing laboratory" of the National Pingtung University of Science and Technology.

Consumption of specific energy

The following equation was used to determine the SEC. [19]:

$$SEC = \frac{P}{m} \times 100 \quad (1)$$

SEC is the consumption of specific energy (kJ/kg), and m is the chicken meat mass (kg).

Electric power consumption (P) was calculated as follows:

$$P = U \times I \times t \quad (2)$$

Where t is time (s), and U and I represent voltage (V) and current (A) measured by a voltmeter and ammeter, respectively.

Acoustic power

The acoustic power was calculated by the increase in water temperature under the influence of the US using the following equation:

$$P_{aco.} = \frac{m\, C_p\, (T_o - T_1)}{t} \quad (3)$$

$P_{aco.}$: acoustic power (W), m: mass of water (kg), cp.: specific heat (J/kg m), T_0: final water temperature (m) and, T_1: Initial water temperature (m) and t: time (s).

Physical characteristics

CL

CL was calculated according to [20] after grilling samples (treated breast meat) at 200 °C for 30 min (Eq. 4):

$$CL\,(\%) = \frac{W_{bc} - W_{ac}}{W_{bc}} \times\ 100 \tag{4}$$

Where, CL is the cooking loss (%),W_{bc} is the weight before cooking (g), and W_{ac} is the weight after cooking (g).

MFI

The MFI was determined using the approach described by [21]. Briefly, 5 g of meat was taken and cut into cubes. Then, in a 30:20 ratio, it was mixed with 50 ml of a solution containing 0.25 M sucrose and 0.02 M potassium chloride. Pieces were left for 5 min after thawing, then ground for 40 min and filtered. Precipitate was collected and dried at 40 °C for 40 min. The muscle fibers index was calculated according to Eq. 5 as follows:

$$MFI\ =\ W_p \times\ 100 \tag{5}$$

Wherein, *"MFI"* is the muscle fibers index, and "W^{p}" is the precipitate weight (g).

Chemical properties

The peroxide value (PV) and free fatty acid (FFA) were estimated according to [22]. Briefly, for PV determination, 5 g of breast meat was added to 25 mL of diether ether solvent, filtration, glacial acetic acid, chloroform, and 5 5 5 mL of saturated potassium iodide. The starch index was prepared by adding 1 g starch to 100 mL distilled water, and the peroxide value was calculated. FFA was determined by combining breast meat with diether ether, 98% ethyl alcohol, and phenolphthalein index, then filtering and adding NaoH to clean the filtrate.

Microbiological tests

Microbiological tests were performed as indicated by [23]. In brief, 1 ml of the milk sample was mixed with 9 ml of sterile peptone water (0.1% peptone), after which decimal dilutions of the milk samples were prepared under sterile conditions. Microbiological tests included a total count of bacteria (TCB), psychrophilic, and proteolytic bacteria. The culture medium, Nutrient Agar, prepared by the company (Himedia, India), was used and prepared by dissolving 28 g of it in one liter of distilled and sterilized water in the incubator at a temperature of 121 °C for 15 min. Then, the TCB petri dishes were incubated at 37 °C for 24–48 h, and the growing bacterial colonies were counted. As for the psychrophilic plates, the plates were incubated at 7 °C for 10 days, after which the ever increasing colonies were counted. As for the proteolytic bacteria, skim milk (10% concentration, pH = 7.4 ± 0.02) was incorporated into Nutrient Agar (Hemidia, India), after which the culture was sterilized, and the plates were incubated at ambient temperature [24].

Kinetic model for PV and half-life during storage

A kinetic model for PV increase during storage was used to calculate the half-life. The initial kinetics models were utilized to compute the increased kinetic PV (mEq/kg) during the storage period, as shown in Eq. 6 [25].

$$PV = PV_0\, exp.(+k_1 t) \tag{6}$$

Where PV is the peroxide value at any given time, PV_0 is peroxide value at zero time, k_1 is the constant rate of the first kinetic model (1/day), t is the time (day), and the positive sign refers to increased PV during storage. The half-life to an increment of PV in chicken meat was calculated from the following equation [25]:

$$t_{1/2} = \frac{-\ln\,(0.5)}{k_1} \tag{7}$$

k_1 is the constant rate (1/day).

Sensory attributes

A trained panel of twenty-five judges was assigned to evaluate the chicken patties based on a nine-point hedonic scale (9 is like extremely, 5 is neither like nor dislike, and 1 dislikes extremely), taking into account sensory qualities including color, tenderness, juiciness, flavor, and overall acceptability. The methodology used was outlined by [26].

Experimental design, optimization, and statistical analysis

Two independent factors, power (4.4, 35.5, 66 W) and UTT (10, 20, 30 min), were used to optimize the qualitative qualities of aged chicken breast meat using Design Expert V13. Utilizing a central composite design (CCD) response surface methodology was used. The regression analysis of the independent variables (responses) was conducted using a quadratic polynomial regression model to predict the qualitative qualities of aged chicken breast meat. The significant

level was $p < 0.05$. Version 13 of Design-Expert was used for statistical analysis to achieve a significant $p < 0.05$ for the data.

Results and discussion

Data summary

Table 1 summarizes the independent variables (power, US treatment time (UTT)) obtained from response surface methodology and experimental data of the responses (SEC, Acoustic power, CL, MFI, PV, FFA, TCB, psychrophiles, and proteolytic bacteria) for different conditions.

SEC

The RSM-obtained central composite design and responses experimental data (SEC, acoustic power, physicochemical and microbial properties of the US-treated breast meat) are presented in Table 1. According to this data, SEC ranged between 0.406 kJ/kg to 11.00 kJ/kg for UTT treatments at 4.4 W power (10 min) and 66 W (30 min), respectively. This observation highlighted that elevated sonication power for an extended time could result in higher SEC. Data presented in Table S1-S2 indicated that the effects of the reduced cubic model (RCM), power, UTT, and their interaction were significant ($p < 0.05$), but execute squared UTT was not significant ($P > 0.05$). Moreover, fit statistics parameters indicated the RCM could be used to well-predict SEC because the R^2 and predicted R^2 were considerably close to 1. Also, the predicted R^2 reasonably agrees with the adjusted R^2, i.e., the difference is less than 0.2. These could meet the suggested criteria mentioned in the literature; for example, model adequacies should be checked by lack of fit > 0.1; $R^2 > 0.95$; (Adj-R^2–Pre. R^2) < 0.2; pre. $R^2 > 0.7$; Adeq. Precision > 4 [27]. The results in Fig. 1a demonstrated that SEC increased with power and UTT. An SEC of 0.342 kJ/kg was obtained for 10 min of 4.4 W UTT. Increasing the power (~66 W), and the UTT (~30 min) enhanced the SEC (~10.93 kJ/kg). These observations highlighted the significant impacts of both power and UTT on SEC.

Acoustical power

Table 1 depicts the maximum value of acoustic power, which was 18.33 W at 66 W power and 30 min of UTT, and the minimum was 1.76 W at the power of 4.4 W and 20 min of UTT. Elevating input power and UTT increased temperature, consequently increasing acoustical power according to Eq. 3.

As presented in Table S1, quadratic model (QM), power, and squared power have a significant effect ($p < 0.05$). Still, UTT, UTT^2, and interaction between power, UTT, and Lack of Fit were not significant ($p > 0.05$). Therefore, QM can be used to predict acoustic power in line with the coefficients of QM (Table S2). Also, the model adequacy was verified by the $R^{2,}$ which was up to 0.996, Adj $R^2 = 0.993$, Predicted $R^2 = 0.975$, and Adequate Precision = 59.92 for the model proposed for acoustical power. According to Fig. 1b, power and UTT were the crucial factors affecting the acoustic power. For example, when the input power increased from 4.4 to 66 W, the acoustic power increased from 2.10 to 16.34 W within 10 min sonication. Also, acoustic power increased from 1.76 to 17.91 W when UTT increased by

Table 1 Central composite design obtained from response surface methodology and experimental data of the responses (physicochemical and microbial properties of the ultrasound-treated breast meat) for the deferent conditions

Run	Independent variables						Dependent variables				
	P	UTT	SEC	AP	CL	MFI	PV	FFA	TCB	Psy.	Pro
1	35.2	20	5.33	13.3	46.92	102.34	0.110	0.156	280	40	29
2	66	30	11	18.33	64.76	140.45	0.082	0.112	167	21	12
3	66	20	6.46	16.16	59.05	137.34	0.102	0.125	252	28	26
4	4.4	10	0.406	2.00	31.82	92.56	0.171	0.191	515	50	50
5	35.2	20	5.33	13.27	47.46	100.12	0.185	0.16	283	38	38
6	35.2	20	5.6	13.7	51.52	103.72	0.121	0.151	325	40	35
7	35.2	10	2.6	13.00	41.73	100.61	0.132	0.172	350	36	40
8	4.4	20	0.72	1.80	42.23	96.82	0.153	0.181	450	43	45
9	4.4	30	0.80	1.76	49.13	99.69	0.131	0.153	380	39	40
10	66	10	3.33	16.66	46.81	134.54	0.12	0.14	316	35	30
11	35.2	30	8.06	13.43	55.41	111.76	0.105	0.154	440	30	28
12	35.2	20	5.46	13.72	49.22	107.77	0.131	0.16	300	35	37
13	35.2	20	5.33	13.00	41.92	109.87	0.14	0.141	350	42	30

P: power (W), UTT: ultrasound treatment time (min.), SEC: specific energy consumption (kJ/kg), AP: Acoustic power(W), CL: Cooking loss (%), MFI: Muscle fiber index, PV: Peroxide value (mEq/kg), FFA: Free fatty acids (%), TCB: Total count Bactria (CFU/g), Psy: Psychrophiles (CFU/g), Pro: Proteolytic Bacteria (CFU/g)

30 min. Moreover, the results illustrated that the power has a more considerable effect on the acoustic power than UTT.

Physical properties

CL

Table 1 illustrated that the higher CL was 64.76% at 66 W power and 30 min UTT, and the lowest value was 31.82% at 4.4 W power and 10 min UTT. Table S1 showed that the QM, power, and UTT have a significant effect ($p < 0.05$), but the squared power, squared UTT, the interaction between power, and Lack of Fit were insignificant ($p > 0.05$). According to the empirical model's statistical parameters ($R^2 = 0.922$, Adj $R^2 = 0.866$, Predicted $R^2 = 0.766$, Adequate Precision = 15.42), the QM can predict the CL. The regression coefficients are presented in Table S2. Data presented in Fig. 1c shows the relationship between power, UTT (two independent factors), and CL (dependent variable). The findings revealed that CL increased significantly ($p < 0.05$) with US power; CL increased from 32.85% at 4.4 W within 10 min of UTT to 48.35% at 66 W at 10 min UTT. When the

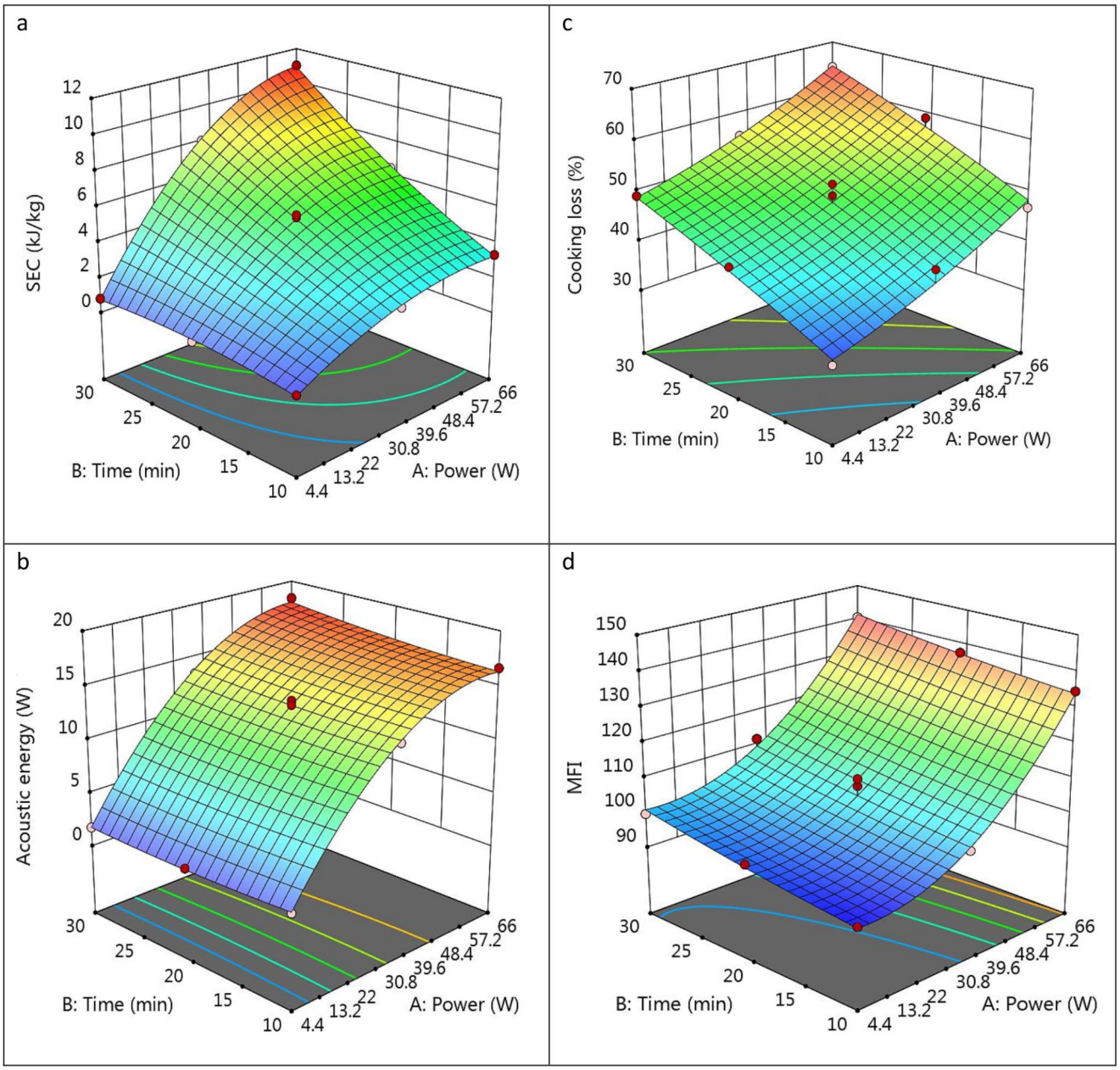


Fig. 1 Response surface and contour plots for **a**: specific energy consumption (SEC), **b**: acoustical energy, **c**: cooking loss, and **d**: Muscle fiber index (MFI)

UTT increased from 10 to 30 min at 4.4 W power, CL also increased from 32.85 to 48.85%, respectively, and increased from 48.35 to 64.98%, respectively, at 66 W power. Depending on the conditions applied, US treatment of chicken meat can affect cooking loss [28]. Increasing cooking time and US power reduced the cooking loss of chicken actomyosin Zou et al. [29].

MFI

The higher MFI was 140.45 at 66 W power and 30 min UTT and reduced to the lowest value (92.56) at 4.4 W power and 10 min UTT (Table 1). This observation is because the US has led to the breaking of meat fiber and weakening protein structure. Three-dimensional graphs in Fig. 1d illustrate the effect of the power and the UTT on the MFI. Results showed that MFI increased from 92.22 at 4.4 W and 10 min UTT to 141.37 at 66 W and 30 min UTT and increased to 133.91 at 66 W and 10 min UTT. In contrast to the control, US treatments enhanced MFI. These observations indicated that the development of myofibrillar particles and the conversion of F-actin to G-actin have been demonstrated to be significantly impacted by US treatment, which can enhance the tenderness of meat. Besides, the quadratic model, power, UTT, and squared power have a significant effect ($p < 0.05$), but the overlap, squared UTT, and Lack of Fit were not significant ($p > 0.05$) (Table S1). R^2, adjusted R^2, and predicted R^2 were higher than 0.94, and the predicted R^2 is in reasonable agreement with the adjusted R^2, i.e., the difference between adjusted and predicted R^2 was lower than 0.2. According to these observations and data modeling, the Quadratic model can be used to predict MFI. Furthermore, the coefficients of QM are presented in Table S2.

According to a previous investigation, high-intensity US can disrupt the actin-myosin complex and relax the firmly coupled myofibrils [30]. This results in smaller myofibrillar particles and an increase in MFI. Increased MFI indicates enhanced meat tenderness since it represents the degree of disturbance to the myofibrillar structure [31]. It has been explained in the literature that US-assisted brining increased the amount of free-/peptide-bound alpha-amino-nitrogen (α-NH2-N) in the muscle, resulting in a higher level of proteolysis and enhanced muscle fiber fragmentation compared to static brining treatment [13]. The study also documented that US-assisted brining yielded myofibrillar protein opening configurations with higher exposed hydrophobic groups. Also, it was reported that US alters protein structure and interaction, making protein complex systems more flexible while expanding protein internal structure, increasing binding sites, and improving saturation. Selective interactions can change the driving force of new binding sites [32]. Another study explained how US increased meat tissue fragmentation, highlighting sonication's ability to disrupt protein networks weakening the reticular structure of proteins [33]. The above-mentioned mechinisms could also be involved in the present study on in-pack sonication of aged chicken meat. Overall, US is assumed to be a safe technology for food treatment in terms of effects on proteins and amino acids, especially compared to harsh chemcial-based modification [32]. However, further safety experiments might be needed for its practical application in chicken meat processing, which can be considered in future studies.

Chemical indicators

PV

The results in Table 1 clarified that the experimental PV ranged between 0.082 mEq/kg at a power of 66 W and UTT of 30 min and 0.185 mEq/kg at a power of 35.2 W and UTT of 20 min. According to Table S3, the 2FI model, power, and UTT significantly affect FFA ($p < 0.05$), but the Lack of Fit was not significant ($p > 0.05$). R^2=0.673, the difference between adjusted R^2 and predicted R^2 was lower than 0.2, and Adeq Precision was higher than 4. Therefore, the 2FI model can be used to predict the PV. The Fig. 2a visualizes the correlation between power, UTT, and PV. The highest predicted PV by RSM (0.17 mEq/kg) was observed at 10 min of 4.4 W, while the lowest predicted PV by RSM (0.09 mEq/kg) was achieved at 30 min of 66 W sonication. This observation indicated that increasing UTT and power reduced predicted PV from 0.17 to 0.09 mEq/kg. This observation could be attributed to improving meat quality due to intensified US shocks on meat. US (25 kHz and 128 W) in producing dry fermented sausages might influence proteolysis and the development of chemicals resulting from lipid oxidation during storage [24].

FFA

According to Table 1, the FFA reached the highest value of 0.191% after 10 min of 4.4 W sonication. In comparison, the lowest value of 0.112% was achieved at 30 min of 66 W. Linear model, power, and UTT significantly impacted the FFA ($p < 0.05$). Besides, the lack of fit was not insignificant ($p > 0.05$). R^2 was 0.8815, the difference between adjusted and predicted R^2 was lower than 0.2, and Adeq. Precision > 4 (19.989) (Table S3). Therefore, the linear model can describe the FFA, and the regression coefficients are given in Table S2. The outcomes in Fig. 2b illustrated that the lowest value of FFA was 0.11% at 66 W power and the UTT of 30 min. This observation indicates that increasing power and UTT could decrease FFA. Samples that were sonicated

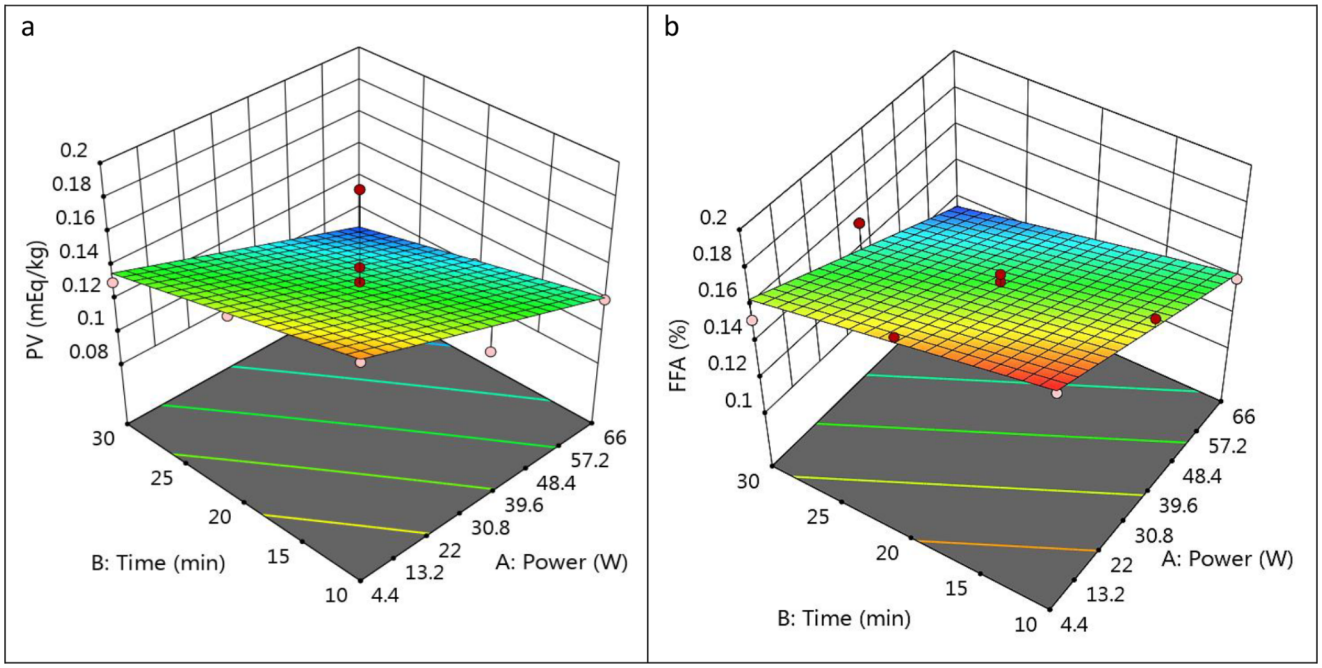


Fig. 2 Response surface and contour plots for **a**: Peroxide value (PV), **b**: Free fatty acids (FAA)

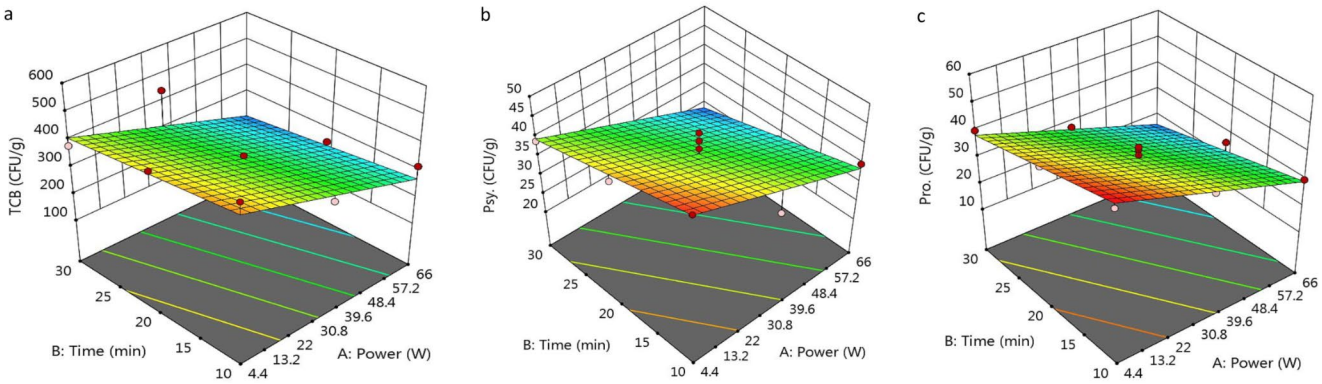


Fig. 3 Response surface and contour plots for, **a**: Total count Bactria (TCB), **b**: Psychrophiles (Psy), and **c**: Proteolytic (Pro.)

for 3 and 6 min had increased total free amino acid contents and reduced after 9 min [24].

The effect of US on microorganisms

TCB

According to Table 1, the TCB reached 515 CFU/g at 4.4 W and 10 min UTT. This value was reduced to 167 CFU/g at 66 W and 30 min UTT. Total bacteria count was significantly affected by linear model and power but not significantly affected by UTT. In addition, lack of fit was not significant ($p > 0.05$). R^2=0.673, the difference between adjusted R^2 and predicted R^2 was lower than 0.2, and Adeq Precision was 9.689 (Table S4). Therefore, A linear model was proposed to predict TCB during US treatment. Moreover, Table S2 presents the coefficients of the linear model. The 3D Fig. 3a demonstrated that the greatest TCB was 473.07 CFU/g at 10 min of 4.4 W. The lowest value was 205.07 CFU/g at 30 min of 66 W. Figure 3b shows that both factors substantially impact reducing the TCB. The results showed that the TCB decreased from 473.7 to 269.70 CFU/g when power increased from 4.4 to 66 W at 10 min of UTT [34]. reported that increasing US power led to the inactivation of microorganisms. The external US cavitation force, which corresponds to the pressure amplitude of the shock waves produced on the collapse of the cavitation bubble, is related to the impact of US power [35]. The pressure produced by bacteria makes them more susceptible to sonication treatments because of the cavitation phenomenon on their surface [36]. With higher US intensities, further irreversible cell damage and microbial inactivation in the meat could

be expected. High-intensity US treatment disrupts the cell membranes of microorganisms, leading to cell death and a reduction in the microbial load [36].

Psychrophiles

Psychrophiles decreased from 50 CFU/g at 4.4 W power and the UTT of 10 min to 21 CFU/g at 66 W and 30 min UTT (Table 1). Mechanical effects of sonication and US -US-generated slightly electrolyzed water could inactivate microorganisms [24]. The statistical analysis illustrated a significant ($p < 0.05$) effect for the linear model, power, and UTT (Table S3). Moreover, the lack of fit was not significant ($p > 0.05$). It has been noted that R^2, Adjusted R^2, and Predicted R^2 were 0.8413, 0.8096, and 0.7573, respectively. These parameters clarified that the linear model can be used to calculate Psychrophile count, and the linear model coefficients are presented in Table S2. The results in the 3D Fig. 3b drawn by the RSM showed that the psychrophile bacteria reached 50 CFU/g at 4.4 W and 10 min UTT. This value was decreased to 33.85 CFU/g at 66 W and 10 min UTT. The minimum predicted psychrophile bacteria was 23.52 CFU/g at 66 W power by the UTT of 30 min. US leads to inactivating microorganisms significantly with increased power and treatment time [37]. The power and UTT have the same importance in the reduction of Psychrophiles.

Proteolytic bacteria

The results in Table 1 clarified that the lowest count of proteolytic bacteria was 12 CFU/g at 66 W power and the UTT of 30 min, which increased with decreasing power and UTT. Also, the linear model, power, and UTT significantly affect the proteolytic bacteria. In addition, the lack of fit was insignificant ($p > 0.05$) (Table S4). R^2=0.8023, Adjusted R^2=0.8003, Predicted R^2=0.7305. According to statistical parameters, a linear model was proposed to predict the Proteolytic bacteria count, and the coefficients of this model are depicted in Table S2. Figure 3c shows the effect of interference between power and UTT in the proteolytic bacteria. Sonication power and time affected proteolytic bacteria count. For example, at a power of 4.4 W and a UTT of 10 min, the proteolytic bacteria was 51.67 CFU/g, which was decreased to 29.34 CFU/g when a power of 66 W was applied for the same UTT. Similarly, the number of proteolytic bacteria was reduced to 16.01 CFU/g at 4.4 W power and 30 min UTT. High-power ultrasound can damage and disrupt biological cell walls, facilitating the destruction of living cells. [38].

Optimization process

The results of the optimization process for the physical, chemical, and microbial characteristics of the US-treated breast meat are depicted in Table 2. The results illustrated that the optimal conditions for improving physical, chemical, and microbial properties were at 15.36 min of 66 W. The results also showed no significant differences ($p > 0.05$) between the predicted and actual values and all attributes. The results clarified significant differences ($p < 0.05$) between the experimental values of US treatment, conventional method, and raw chicken breast meat. The cooking loss of the meat treated by US was insignificantly higher ($p < 0.05$) than the conventional treatment of raw chicken breast.

US has outperformed conventional treatment, and the MFI value was significantly different ($p < 0.05$) for US, conventional, and control treatment. According to the MFI, the results showed that the MFI was significantly higher ($p < 0.05$) for sonicated samples than conventionally treated and raw meat. US can disrupt cell membranes and increase meat tenderness by breaking down muscle fibers [36]. US has demonstrated its effectiveness in inactivating a wide range of microorganisms in meat products, making it a promising non-thermal technology for enhancing food safety and quality [39].

The effects of high-energy US, also known as high-intensity US, on permeability, solubility, and O_2 diffusion

Table 2 The results of the optimization process for the performance of the ultrasound device, conventional method and the physicochemical and microbial properties of the ultrasound-treated breast meat

P (W)	UTT (min.)	Dependent variables	Predicted	Experimental	Conventional method	RM
66	15.36	SEC (kJ/kg)	5.61	5.16	308.57	-
		Acoustic power(W)	16.69	17.03	-	-
		Cooking loss (%)	53.38	52.80	50.74	47.100
		Muscle fiber index	136.09	136.91	65.871	76.430
		Peroxide value (meq/kg)	0.11	0.13	0.15	0.10
		Free fatty acids (%)	0.13	0.14	0.17	0.120
		TCB (CFU/g)	250.00	251.00	401.00	621.00
		Psychrophiles (CFU/g)	30.00	32.00	38.00	56.00
		Proteolytic Bacteria (CFU/g)	24.00	25.00	41.00	67.00

P: power, UTT: ultrasound treatment, SEC: specific energy consumption, TCB: total count Bactria; RM: raw chicken breast meat

coefficients alter physical and chemical properties [6]. According to Fig. 4d, US significantly reduced TCB, psychrophiles, and proteolytic bacteria ($p < 0.05$). TCB, Psychrophiles, and Proteolytic Bacteria in breast meat treated by US were reduced from 621 – 251, 56 – 32, and 67 – 25 CFU/g, respectively. Moreover, these parameters were decreased by 37.40, 15.78, and 39.02%, respectively, compared to C. The inactivation of microorganisms by the US is due to the formation of free radicals, the thinning of cell membranes, and the extrusion of the intracellular matrix, ultimately leading to cell death [40].

In-pack sanitation *vs.* conventional sanitation of quality treated chicken meat by the US

Figure 4 shows the effect of WP, LDPE, US, C, and RM on the CL, PV, MFI, FFA, and TCB. According to the results, LDPE Packaging had lower CL, PV, FFA, and TCB values

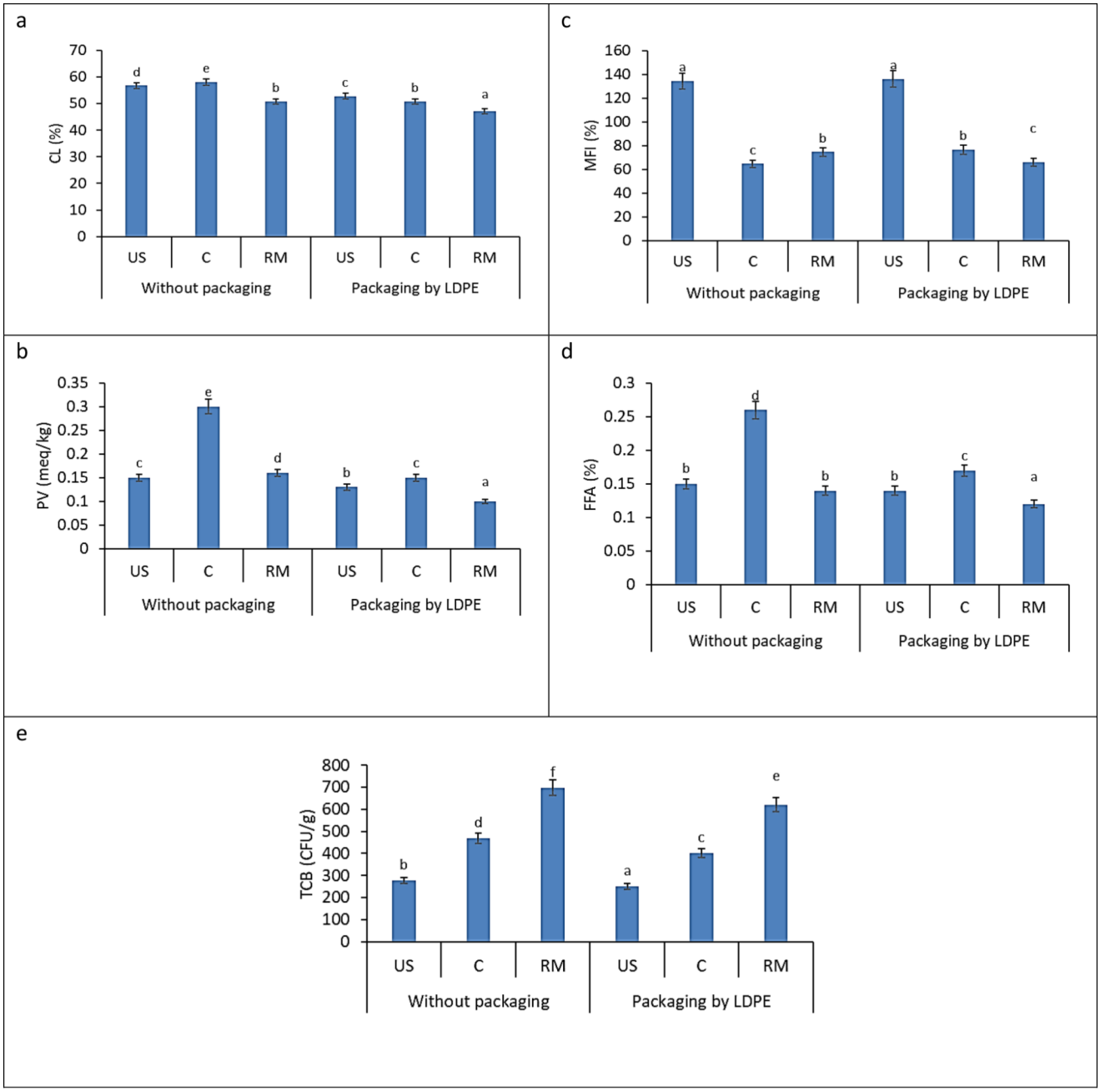


Fig. 4 Effect of packaging and without packaging sonication on (**a**): cooking loss (CL); (**b**): peroxide value (PV); (**c**): muscle fiber index (MFI) (**d**): free fatty acids (FFA); (**e**): total count of bacteria (TCB). LDPE: low-density polyethylene packaging, US: sonicated at optimal conditions, C: conventional treatment, RM: raw meat. Deferent letters refer to significant differences among treatments at level $p < 0.05$

at all treatments (US, C, and RM). The highest values of CL were 56.71, 58.07, and 50.8% observed for WP samples of US, C, and RM. On the other hand, LDPE yielded the lowest CL values of 52.80, 50.74, and 47.10% for US, C, and RM, respectively. For PV, packaged meat treated by the US gave lower PV (1.14 meq/kg) than WP (0.15 meq/kg). In general, the PV of packaged meat was lower than without packaging meat at all treatments (Fig. 4b). As for MFI, meat in-packed had higher MFI than without packaging for all treatments. That is, meat inpacked treated by US has higher MFI (140%) than without packaging (134%), as illustrated in Fig. 4c. According to Fig. 4d, the FFA was lower in meat packaging by LDPE than without packaging. For instance, in-LDPE packed and without packaging sonicated breasts were 0.14 and 15.2%, respectively. Also, the results revealed that FFA in LDPE-packed sonicated breast was lower than without packaging by 48.14%.

Also, the US decreased TCB by 147.4% and 59.76% compared to LDPE-packed RM and C. Moreover, TCB of in-LDPE packed sonicated breasts was reduced by 10.75% compared to WP. In a previous study, Meléndez-Pérez et al. [41] found that the water vapor transmission of LDPE was 1.433×10^{-4} g/cm^{2}s. The literature has reported that LDPE materials could well-preserved the meat surface [42]. More recently, Katsara et al. [43] stated that LDPE-based packaging increased the shelf-life of other meat products (bacon, salami, and mortadella).

Besides, our results showed that sonication provided a higher MFI than C and RM. For example, sonicated samples represented an MFI of 136.09%, while C and RM had lower values of 76.43 and 65.87%. The reason behind this observation is the breaking down of breast structure, mainly due to the physical impacts of sonication, such as cavitation in localized pressurized zones.

Effect of frozen storage period on quality of aged chicken meat

Figure 5 shows the quality attributes of aged chicken meat treated by US, conventional, and raw meat *vs.* frozen storage periods (FSP). CL values of meat samples after treatment (zero days) by US, conventional, and raw meat were 52.8, 50.74, and 47.1%, respectively. The results illustrated that the CL increased as FSP increased (Fig. 5a). That is, when FSP increased from 0 to 60 days, the CL increased from 52.8 to 55.8%, respectively, using US, and increased from 50.74 to 59.74%, respectively using conventional. Moreover, the increase in the CL of raw meat was lower than the other treatments at all FSPs. CL reached 52.08, 50.74, and 47.10% after treatment by US, C, and RM, respectively, and increased to 55.80, 79.54, and 53.1% at FSP of 60 days. Similarly, CL using US immerse-free porcine longissimus muscle samples at zero days was 6.80% and increased to 10.07% at 180 days of FSP [44]. High CL can result in lower cooked weight, reducing the product's profitability and cost-effectiveness by reducing the number of servings from the same raw material. High cooking loss can lead to a drier, less tender product, making it less appealing to consumers who prefer moist and tender food items [45].

For MFI, the results revealed that MFI was not significant ($p < 0.05$) increased as FSP increased for all treatments (Fig. 5b). MFI increased by 1.48, 2.94, and 3.40% for US, conventional, and raw meat when FSP increased from 0 to 60 days. Lee et al. [46] demonstrated that MFI increased as FSP increased. The results revealed a significant ($P < 0.05$) effect between treatments on the MFI. It has been explained that increased tenderness results from increased proteolysis by calpains, increasing MFI [46].

According to Fig. 5c, the PV of US-treated chickens was 50% lower than conventionally treated chickens but 23% higher than that of raw meat after treatment (on day 0 of FSP). At day 60 of FSP, the PV of chicken treated by US was reduced by 20.1 and 6.6% compared to conventional and raw meat. A previous study [24] reported increased PV of sonicated packed dry fermented sausages. High PV values, typically above 10 meq/kg, may indicate that the product is unfit for human consumption and should be rejected [47]. The results showed that the PV increased as the storage period increased for all treatments, which can be attributed to the oxidation and hydrolysis reactions in the oil. These changes can significantly impact the quality and shelf life of the meat.

Besides, the FFA was lower than conventional by 17.64% at 0 FSP (after treatment by US) but higher than raw meat by 16.66 at 0 days of FSP (Fig. 5d). Moreover, FFA at 60 days of FSP was lower than the conventional and raw meat by 13.15, and 2.94%, respectively. After US treatment, enzymatic oxidation was discovered to be a crucial metabolic pathway that contributed to the development of flavor characteristics [20]. This finding was possible due to the increased activity of lipases and lipoxygenase. The increased activity of lipases can negatively affect the quality properties of food products by promoting lipid hydrolysis (hydrolysis of glycerides, glycolipids, and phospholipids, releasing free fatty acids that can undergo further oxidation), oxidation, and the formation of undesirable compounds that can affect color, flavor, and nutritional value. Proper control and inactivation of these enzymes are crucial for maintaining the quality and shelf-life of food products [48]. High levels of FFAs in lipids can lead to the development of off-flavors and off-odors, making the product less appealing to consumers [49]. The results depicted that FFA levels tend to increase during FSP of meat. This increase in FFA is attributed to hydrolytic reactions that occur during storage.

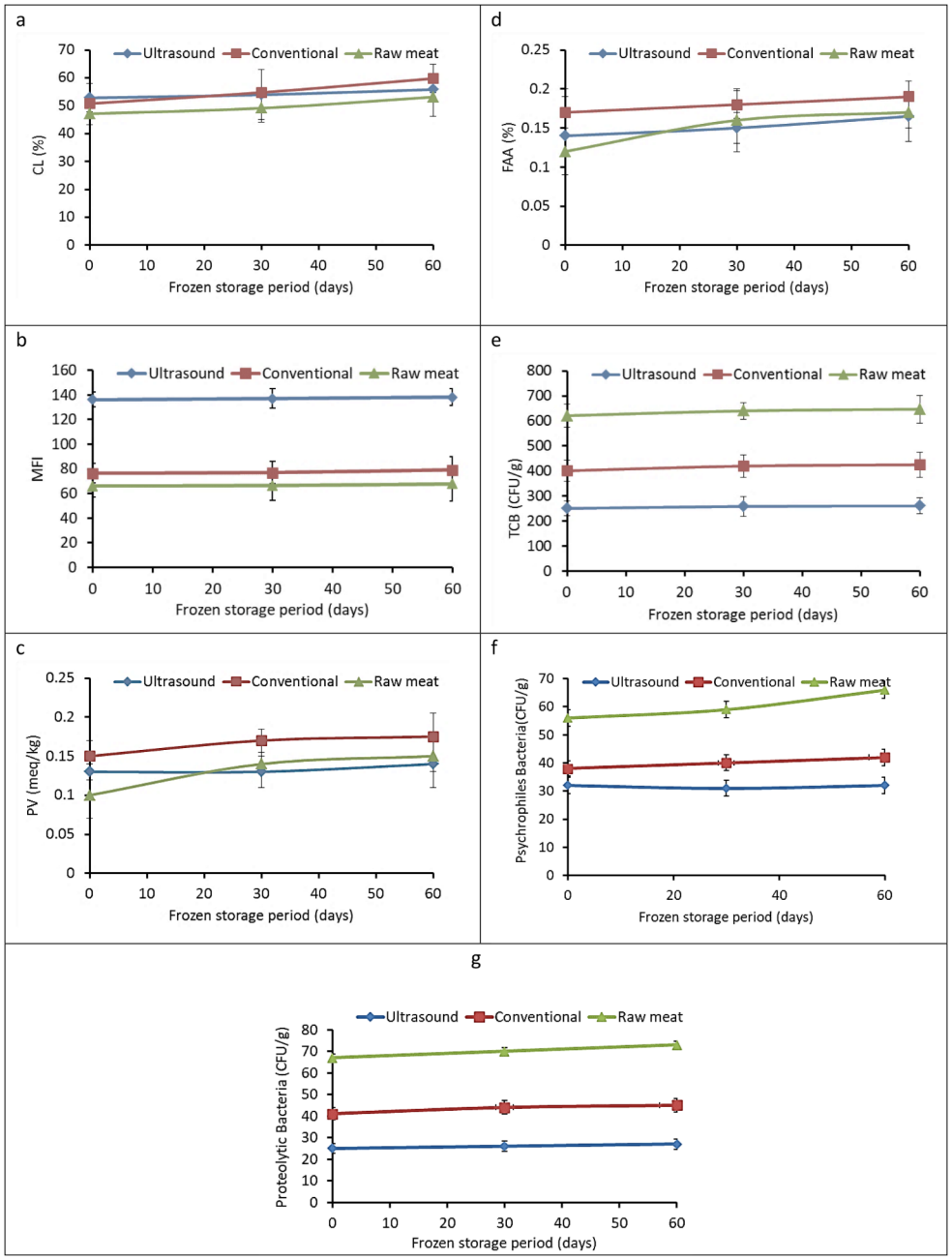


Fig. 5 Quality attributes of aged chicken meat treated by ultrasound, conventional, and raw meat versus frozen storage period

TCB of aged chicken meat at 0 days of FSP (after treatment) was reduced by 37.40 and 59.58% compared to the conventional and raw meat, respectively, and increased by 38.58 and 59.65% at 60 days of FSP, respectively (Fig. 5e). The results also, revealed that the TCB increased insignificantly ($p > 0.05$) by 3.98, 5.98, and 4.18%, when the FSP increased from 0 to 60 days for US, C, and RM, respectively. TCB was raised during refreezing treatment with increased FSP in chicken and beef meats [50]. Since *Salmonella* and *Escherichia coli* are sensitive to US, this methodology can be considered a revolutionary green technology for decontaminating and tenderizing poultry meat [51]. Studies suggested that frozen storage can reduce the number of bacteria in meats over time, but complete elimination, especially of spore-forming bacteria, is challenging. The low-temperature treatment can somewhat suppress the survival and reproduction of microorganisms [50, 52]. Excessive TCB can produce off-flavors, off-odors, discoloration, and slime, making the product less appealing to consumers [53]. Psychrophiles are cold-loving microorganisms that thrive at low temperatures, including those encountered during frozen storage. These bacteria are adapted to survive and even grow in refrigerated and frozen environments. Frozen storage can influence the proteolytic activity of bacteria.

Table 3 Reaction rate constants of first orders kinetic model, and half-life

Treatments	First order model			$t_{1/2}$
	k_1	RMSE	*R*	
Ultrasound	0.0015	5.92E-05	0.999915	461.3471
Conventional	0.00247	0.000195	0.999772	280.8861

k_1: reaction rate constants, RMSE: root mean square error, R: correlation coefficient, and $t_{1/2}$: half-life

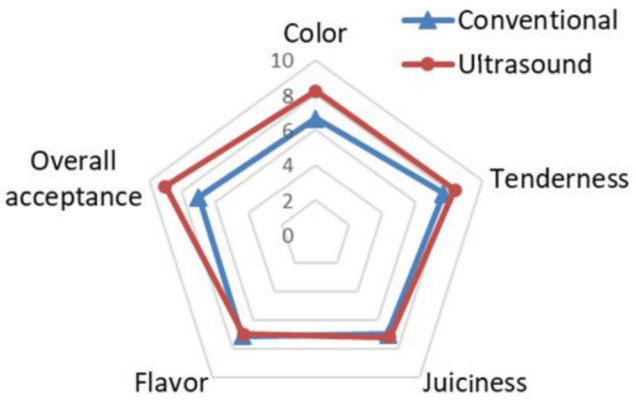


Fig. 6 Sensory characteristics of chicken breast treated by ultrasound and conventional method

Studies suggested that freezing and frozen storage may have minimal impact on the proteolysis of certain food products.

Figure 5e-f illustrates that Psychrophiles and Proteolytic Bacteria in the US-treated samples were lower than in the conventionally-treated samples and raw meat. This is because US treatment can disrupt microorganisms' cell walls and membranes through the physical effects of cavitation and shear forces. The application of ultrasound can help inhibit the growth of psychrophilic bacteria in meat [54]. The results indicated that the Psychrophiles and Proteolytic bacteria increased from 32 to 33 and 25–27 CFU/g during 60 days of FSP. Some microorganisms, particularly psychrophilic (cold-loving) bacteria, can adapt and grow at low temperatures during frozen storage [55].

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Mathematical modeling of PV development and half-life

The results presented in Table 3 revealed that the US rate constant reached 0.0015 1/day from 0.00247 1/day (conventional method). Therefore, developing PV for chicken meat treated by US treatment requires longer than conventional. The half-life of US-treated chicken meat was higher than conventional by 64.24%, indicating the enhanced quality of sonicated samples. The PV measures the degree of oxidation in lipids, while the half-life describes the required time for the concentration of a reactant to decrease by half. As oxidation progresses in lipids, the PV will increase while the half-life of the oxidation reaction decreases, providing complementary information about the stability and shelf-life of the product [56].

As highlighted in the literature [57, 58], US could be further explored to discover other potential applications, and the present study has contributed to food sonication. The findings of this study introduced in-pack sonication of meat as a novel processing technology to extend the shelf life and enhance physiochemical and microbiological properties.

Sensory attributes

The results in Fig. 6 showed that the highest scores were 8.23, 8.34, and 9.00 for the qualities of color, tenderness, and overall acceptance, while the flavor and juiciness did not differ significantly in US from the conventional method, reaching 7.20 and 7.00 respectively. The lowest color value was 6.66 at the conventional method. The sensory qualities of US showed a significant improvement compared to the sensory attributes in the conventional method. The improvement in color, suppleness, and general acceptance may be due to US accelerating overall changes in color, limiting oxymyoglobin formation, and slowing metmyoglobin formation [6].

US-induced cavitation generates shock waves that alter the nature of the muscular structure, which is why many studies have tried in recent years to demonstrate the ability of US to disrupt cell membranes, increasing meat tenderness by breaking down muscle fibers [6]. US has noticeable effects on the juiciness and flavor of meat that has received higher degrees of treatment but is highly dependent on power and time [59].

Conclusions

A sonication-based processing way was developed to preserve the quality properties of chicken breasts during FSP. Technical information revealed in this study can facilitate the development of US-based industrial units. The US device's specific energy consumption and acoustic power have increased while increasing the power and time of US treatment. Besides, findings demonstrated that the ideal conditions were 15.36 min of 66 W. There were no significant differences ($p > 0.05$) between the predicted and actual values of the independent variables and all attributes. Moreover, the conventional method, raw chicken breast meat, and US treatment time differ significantly. Sonication could affect CL, MFI, psychrophiles, proteolytic, and total bacteria count, producing a product with better quality parameters. The number of microbes in chicken samples decreased by increasing power and US treatment time. A significant

difference has appeared between the in-pack US treatment and the conventional method. According to the findings, ultrasonics is a promising pretreatment for frozen chicken with a possibility of up-scaling and commercialization that must be explored in future studies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11694-024-02791-5.

Acknowledgements The authors of the Department of Animal Production and Food Sciences, College of Agriculture, University of Basrah appreciated the availability of labs and instruments. MG contributed to the study design, calculation, and experiments related to determining system performance, validation of technical parameters, system design guidance, and manuscript writing; therefore, appreciating the facilities provided by NPUST, Taiwan.

Author Contributions Asaad Rehman Al-Hilphy: Investigation, Validation, Formal analysis, Methodology, Resources, Conceptualization; Majid H. Alasadi: Conceptualization, Writing – original draft, Software, Supervision; Murtadha Kareem AlLami, Methodology, Data curation. Brijesh K. Tiwari: Writing - review & editing; Mohsen Gavahian: Investigation, Validation, Conceptualization, Writing - review & editing, Supervision, Visualization.

Funding No fund to be declared.

Data availability Authors elected not to share data.

Code availability Not applicable; there is no code to be shared in this study.

Declarations

Consent to participate Not applicable/this research does not need consent to participate.

Competing interests The authors declared that there are no conflicts of interest.

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