

Maintaining functional properties of shell eggs by ultrasound treatment

Cengiz Caner* and Muhammed Yuceer*

Abstract

BACKGROUND: Ultrasonic treatment is an emerging technique that could be an alternative to existing thermal processing techniques in foods. Ultrasonic treatments may also be used to extend the shelf life of egg during storage period in ambient temperature. The effectiveness of ultrasound treatment with different power levels (200 W, 300 W, 450 W) and treatment times (2 min and 5 min) was evaluated for enhancing the functional properties of eggs during storage at 24 ° C for 6 weeks.

RESULTS: Ultrasound treatment power and treatment time had significant effects on Haugh unit, yolk index, albumen pH, dry matter, relative whipping capacity, and albumen viscosity resulting in extended shelf life. Attributes such as yolk index, Haugh unit, pH, whipping capacity, dry matter for 300 W and 450 W treatments were better than control and 200 W treatments. Longer treatment time and power showed a significant influence on functional properties.

CONCLUSION: Power levels of 300 W and 450 W of ultrasound treatments had improved internal quality of fresh eggs during storage, but negative effect on shell strength. The study showed that ultrasound treatment could be an alternative and effective technique for maintaining the internal qualities of fresh eggs during long-term storage while Fourier transform near infrared spectroscopy could be used as a new tool for the assessment of freshness.

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Keywords: ultrasound; shell eggs; egg quality; shelf life; functional properties

INTRODUCTION

Fresh eggs are a natural and inexpensive source of high-quality protein in the human diet. Eggs are also one of the most consumed foods worldwide due to their multi-functional properties.¹ In 2011, around 70 million tons of egg were produced around the world.² Shell eggs undergo considerable quality changes resulting in losses during storage due to the fact that eggs are highly perishable. Thus, it is necessary for eggs to reach the final consumer market without a considerable time lapse^{3,4} or, if a time lapse is unavoidable, it is important to improve the egg freshness and extend the shelf life using effective treatment techniques during storage and beyond.

Development of non-thermal food preservation methods is necessary for maintaining the qualities of perishable foods. Non-thermal methods allow processing of foods below the temperatures used during thermal pasteurisation. Several non-thermal technologies, including high hydrostatic pressure,^{5–7} ultrasound,^{8,9} pulsed electric fields,^{10–13} and ozonation^{14–17} have been developed and studied in recent years for perishable food. Ultrasonic treatment is one of these emerging techniques that could be the alternative to existing thermal processing techniques. It is based on the transmission of ultrasonic sound waves (20–100 kHz frequency) through a medium. It enhances convective heat transfer as well as generates bubble explosions, which produce local hot spots that could cause inactivation of microorganisms and destruction of enzymes by cavitation.^{18,19} High-power ultrasonic treatment is also known to damage or disrupt biological cell walls, which will result in the destruction

of living cells.^{9,19–21} The use of ultrasound in fresh produce as a non-thermal technique is a relatively recent endeavour and considered as a food preservation method to enhance food quality, safety and stability. Recently, numerous ultrasound studies have been published on fruits, vegetables, fruit juices and dairy products.^{9,19,22} Similarly, using ultrasonic waves may improve the overall characteristics of shell eggs that will result in significant savings for the egg industry.

A considerable amount of published work on ultrasonic treatment has dealt with microbiological effects on perishable food rather than functional and chemical properties even though it has a broad range of use in food industry.^{19,22–29} Thus, it is necessary to investigate the use of ultrasound in the protection of the internal qualities of shell eggs. To the best of the authors' knowledge, no other studies have been conducted or published to determine the effects of ultrasound on functional properties of shell eggs. For this reason, the objectives of this work were to investigate the effects of different ultrasound power and treatment times on interior qualities of shell eggs including functional properties [relative whipping capacity (RWC), Haugh units (HU), viscosity and pH of

* Correspondence to: Cengiz Caner and Muhammed Yuceer, Canakkale Onsekiz Mart University, Engineering Faculty, Department of Food Engineering, 017020-Canakkale, Turkey. E-mail: ccaner@comu.edu.tr; myuceer@comu.edu.tr

Department of Food Engineering, Canakkale Onsekiz Mart University, 017020, Canakkale, Turkey

Table 1. Effect of the ultrasound treatment (200, 300 or 450 W) on Haugh unit (HU) and egg grade during 6 weeks of storage

Treatment	Storage time (weeks)						
	0	1	2	3	4	5	6
CNT	78.81 (AA) [†] ± 0.80 ^{Aa}	75.94 (AA) ± 0.23 ^{Ba}	72.93 (A) ± 0.36 ^{Ca}	67.79 (A) ± 0.56 ^{Da}	65.81 (A) ± 0.42 ^{Ea}	58.00 (B) ± 0.34 ^{Fa}	46.77 (B) ± 0.62 ^{Ga}
200 W	79.35 (AA) ± 0.35 ^{Aa}	76.55 (AA) ± 0.35 ^{Ba}	73.70 (AA) ± 0.35 ^{Cab}	71.30 (A) ± 0.35 ^{Db}	68.89 (A) ± 0.72 ^{Eb}	62.58 (A) ± 0.69 ^{Fb}	51.68 (B) ± 0.70 ^{Gb}
300 W	80.53 (AA) ± 0.66 ^{Ab}	76.60 (AA) ± 0.27 ^{Bab}	73.87 (AA) ± 0.48 ^{Cb}	71.65 (A) ± 0.39 ^{Db}	68.92 (A) ± 0.26 ^{Eb}	62.49 (A) ± 0.58 ^{Fb}	52.27 (B) ± 0.58 ^{Gb}
450 W	81.27 (AA) ± 0.54 ^{Ac}	76.89 (AA) ± 0.32 ^{Bb}	73.98 (AA) ± 0.38 ^{Cb}	71.77 (A) ± 0.32 ^{Db}	69.16 (A) ± 0.52 ^{Eb}	62.67 (A) ± 0.50 ^{Fb}	53.38 (B) ± 0.68 ^{Gc}

Data are expressed as mean ± standard deviation.

^{a-c}Means in the same column with different superscript lowercase letters are significantly different ($P \leq 0.05$).

^{A-G}Means in the same row with different superscript upper case letters are significantly different ($P \leq 0.05$).

[†]Uppercase letters in brackets are egg grades, which are given as: AA, HU > 72; A, HU = 71–60; B, HU = 59–31; C, HU < 30.

CNT, control.

Table 2. Effect of the ultrasound treatment (200, 300 or 450 W) on yolk index during 6 weeks of storage

Treatment	Storage time (weeks)						
	0	1	2	3	4	5	6
CNT	0.45 ± 0.01 ^{Aa}	0.43 ± 0.01 ^{Ba}	0.39 ± 0.01 ^{Ca}	0.33 ± 0.01 ^{Da}	0.30 ± 0.01 ^{Ea}	0.27 ± 0.01 ^{Fa}	0.25 ± 0.01 ^{Ga}
200 W	0.46 ± 0.01 ^{Aa}	0.43 ± 0.01 ^{Bab}	0.40 ± 0.01 ^{Cb}	0.35 ± 0.01 ^{Db}	0.34 ± 0.01 ^{Eb}	0.30 ± 0.01 ^{Fb}	0.28 ± 0.01 ^{Gb}
300 W	0.47 ± 0.01 ^{Aa}	0.44 ± 0.01 ^{Bb}	0.40 ± 0.01 ^{Cb}	0.36 ± 0.01 ^{Dbc}	0.34 ± 0.01 ^{Eb}	0.30 ± 0.01 ^{Fb}	0.28 ± 0.01 ^{Gb}
450 W	0.48 ± 0.01 ^{Aa}	0.44 ± 0.01 ^{Bb}	0.40 ± 0.01 ^{Cb}	0.37 ± 0.01 ^{Dc}	0.34 ± 0.01 ^{Eb}	0.31 ± 0.01 ^{Fc}	0.29 ± 0.01 ^{Gc}

Data are expressed as mean ± standard deviation.

^{a-c}Means in the same column with different superscript lowercase letters are significantly different ($P \leq 0.05$).

^{A-G}Means in the same row with different capital letters are significantly different ($P \leq 0.05$).

CNT, control.

albumen and yolk] during storage at 25 °C as well as to investigate Fourier transform near infrared (FT-NIR) spectroscopy as the assessment tool.

MATERIALS AND METHODS

White shell (Lohmann White laying hen breed, 41 weeks of age), unwashed, large size (AA), unfertile, freshly laid (1-day-old) clean (faeces-free) eggs supplied by A.B. Foods Inc. (Bandirma, Turkey) were used in the study.

Ultrasonic equipment and treatments

For ultrasonic treatment, an Industrial Ultrasonic Processor UIP1000hd (Hielscher Ultrasonics GmbH, Teltow, Germany) with 1000 W of power with BS2d18 probe was used. During the experiment five shell eggs at ambient temperature were sunk into an ultrasonic bath filled with water of 24 °C and treated with ultrasonic power at 200 W, 300 W and 450 W for 2 and 5 min in each time. The water for the ultrasonic unit was changed between treatments. Control eggs were handled in water without ultrasonic treatment of the holding time (2 and 5 min). The treatments were control, 200 W for 2 min (200–2), 200 W for 5 min (200–5), 300 W for 2 min (300–2), 300 W for 5 min (300–5), 450 W for 2 min (450–2), and 450 W for 5 min (450–5). After the treatments, all eggs screened for cracks and leakage, and all cracked eggs were removed from the experiment and replaced with another treated intact egg. The eggs were subsequently placed in open moulded plastic egg trays for storage at 24 °C for 6 weeks until tested. Ten eggs per treatment were taken at each storage interval for the evaluation.

Haugh unit and yolk index

Haugh units (HU) were measured on 10 eggs using a digital calliper (CD-15CP; Mitutoyo Ltd, Andover, UK) based on the equation:³⁰ $HU = 100 \times \log_{10}(h - 1.7G^{0.37} + 7.6)$, where h is the height of the thick albumen (mm) and G is the weight of the whole egg (g). The parameter h was estimated by averaging three measurements carried out at different points of the thick albumen at a distance of 10 mm from the yolk using the digital calliper (CD-15CP; Mitutoyo Ltd). The eggs were graded as follows:³⁰ AA, where HU > 72; A, where HU = 71–60; B, where HU = 59–31; and C, where HU < 30.

Yolk index was calculated as yolk height/yolk width. Yolk height and width were measured with digital calliper (CD-15CP; Mitutoyo Ltd).

Albumen viscosity

The eggs per treatment were broken individually, chalazae were separated and then the albumen was collected for measuring viscosity. Albumen viscosity (mPa s) measurements were carried out at 20 ± 0.5 °C using a Brookfield viscometer (Model DV II + Pro D 220, TC-502) with Rheocalc software (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). Silicone oil standard solution was used to calibrate the viscometer.³⁰ The spindle (UL adapter, 30 rpm) was selected and result was recorded after a 20 s.

pH measurements

After the eggs had been broken, the albumen and yolk were separated from each other and then firm and thin albumen were homogenised for 20 s in a blender (Model 32 BL 80; Waring, Torrington, CT, USA) for measurement of the pH (pH 210 meter; Hanna Instruments, Woonsocket, RI, USA).^{3,30}

Table 3. Effect of the ultrasound treatment (200, 300 or 450 W, each for 2 and 5 min) on albumen pH during 6 weeks of storage

Treatment	Storage and treatment times																													
	0 weeks			1 week			2 weeks			3 weeks			4 weeks			5 weeks			6 weeks											
	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min												
CNT	8.26 ± 0.06 ^{Aa,i}	8.30 ± 0.02 ^{Aa,i}	8.45 ± 0.02 ^{Ba,i}	8.44 ± 0.02 ^{Ba,i}	8.61 ± 0.02 ^{Ca,i}	8.61 ± 0.02 ^{Ca,i}	8.73 ± 0.02 ^{Da,i}	8.75 ± 0.02 ^{Da,i}	8.88 ± 0.05 ^{Ea,i}	8.88 ± 0.05 ^{Ea,i}	9.02 ± 0.04 ^{Fa,i}	9.01 ± 0.02 ^{Fa,i}	9.10 ± 0.06 ^{Ga,i}	9.11 ± 0.06 ^{Ga,i}	8.26 ± 0.04 ^{Aa,i}	8.26 ± 0.03 ^{Aa,i}	8.36 ± 0.05 ^{Ba,i}	8.34 ± 0.02 ^{Ba,i}	8.47 ± 0.03 ^{Ca,i}	8.47 ± 0.03 ^{Ca,i}	8.56 ± 0.03 ^{Da,i}	8.56 ± 0.03 ^{Da,i}	8.67 ± 0.02 ^{Ea,i}	8.67 ± 0.02 ^{Ea,i}	8.82 ± 0.03 ^{Fa,i}	8.82 ± 0.03 ^{Fa,i}	8.93 ± 0.04 ^{Ga,i}	8.93 ± 0.04 ^{Ga,i}		
200 W	8.28 ± 0.04 ^{Aa,i}	8.26 ± 0.03 ^{Aa,i}	8.43 ± 0.02 ^{Ba,i}	8.39 ± 0.02 ^{Ba,i}	8.56 ± 0.03 ^{Ca,i}	8.56 ± 0.03 ^{Ca,i}	8.67 ± 0.02 ^{Da,i}	8.64 ± 0.02 ^{Da,i}	8.80 ± 0.04 ^{Ea,i}	8.80 ± 0.04 ^{Ea,i}	8.93 ± 0.03 ^{Fa,i}	8.87 ± 0.02 ^{Fa,i}	9.03 ± 0.04 ^{Ga,i}	9.03 ± 0.03 ^{Ga,i}	8.26 ± 0.03 ^{Aa,i}	8.16 ± 0.02 ^{Ab,ii}	8.36 ± 0.05 ^{Ba,ii}	8.34 ± 0.02 ^{Ba,ii}	8.41 ± 0.03 ^{Ca,ii}	8.41 ± 0.03 ^{Ca,ii}	8.48 ± 0.06 ^{Da,ii}	8.45 ± 0.02 ^{Ca,ii}	8.63 ± 0.03 ^{Da,ii}	8.63 ± 0.03 ^{Da,ii}	8.86 ± 0.02 ^{Ea,ii}	8.86 ± 0.02 ^{Ea,ii}	8.93 ± 0.03 ^{Fa,ii}	8.93 ± 0.03 ^{Fa,ii}	9.03 ± 0.04 ^{Ga,ii}	9.03 ± 0.04 ^{Ga,ii}
300 W	8.26 ± 0.03 ^{Aa,i}	8.16 ± 0.02 ^{Ab,ii}	8.36 ± 0.05 ^{Ba,ii}	8.34 ± 0.02 ^{Ba,ii}	8.47 ± 0.03 ^{Ca,ii}	8.47 ± 0.03 ^{Ca,ii}	8.56 ± 0.03 ^{Da,ii}	8.52 ± 0.05 ^{Da,ii}	8.69 ± 0.04 ^{Ea,ii}	8.69 ± 0.04 ^{Ea,ii}	8.82 ± 0.03 ^{Fa,ii}	8.80 ± 0.03 ^{Fa,ii}	8.93 ± 0.03 ^{Ga,ii}	8.93 ± 0.03 ^{Ga,ii}	8.26 ± 0.03 ^{Aa,i}	8.14 ± 0.02 ^{Ab,ii}	8.09 ± 0.02 ^{Aa,iii}	8.33 ± 0.03 ^{Ba,ii}	8.41 ± 0.03 ^{Ca,ii}	8.45 ± 0.02 ^{Ca,ii}	8.65 ± 0.02 ^{Ea,iii}	8.75 ± 0.03 ^{Fa,ii}	8.75 ± 0.02 ^{Fa,ii}	8.86 ± 0.02 ^{Ga,ii}	8.86 ± 0.02 ^{Ga,ii}	8.93 ± 0.03 ^{Fa,iii}	8.93 ± 0.03 ^{Fa,iii}	9.03 ± 0.04 ^{Ga,iii}	9.03 ± 0.04 ^{Ga,iii}	
450 W	8.14 ± 0.02 ^{Aa,ii}	8.09 ± 0.02 ^{Aa,iii}	8.33 ± 0.03 ^{Ba,ii}	8.34 ± 0.05 ^{Ba,iii}	8.41 ± 0.03 ^{Ca,ii}	8.41 ± 0.03 ^{Ca,ii}	8.48 ± 0.06 ^{Da,ii}	8.45 ± 0.02 ^{Ca,ii}	8.65 ± 0.02 ^{Ea,iii}	8.65 ± 0.02 ^{Ea,iii}	8.75 ± 0.03 ^{Fa,ii}	8.75 ± 0.02 ^{Fa,ii}	8.86 ± 0.02 ^{Ga,ii}	8.86 ± 0.02 ^{Ga,ii}	8.14 ± 0.02 ^{Aa,ii}	8.09 ± 0.02 ^{Aa,iii}	8.09 ± 0.02 ^{Aa,iii}	8.33 ± 0.03 ^{Ba,ii}	8.41 ± 0.03 ^{Ca,ii}	8.45 ± 0.02 ^{Ca,ii}	8.65 ± 0.02 ^{Ea,iii}	8.75 ± 0.03 ^{Fa,ii}	8.75 ± 0.02 ^{Fa,ii}	8.86 ± 0.02 ^{Ga,ii}	8.86 ± 0.02 ^{Ga,ii}	8.93 ± 0.03 ^{Fa,iii}	8.93 ± 0.03 ^{Fa,iii}	9.03 ± 0.04 ^{Ga,iii}	9.03 ± 0.04 ^{Ga,iii}	

Data are expressed as mean ± standard deviation.

A–G Different superscript upper case letters denote significant differences between storage periods in same power of ultrasound treatment and treatment time ($P < 0.05$).a–b Different superscript lowercase letters denote significant differences between treatment times in same power of ultrasound treatment and storage period ($P < 0.05$).I–IV Different Roman numbers denote significant differences between storage periods in same treatment time and power of ultrasound treatments ($P < 0.05$).

CNT, control.

Total solids (dry matter) of albumen and yolk

Total solids (dry matter) (% w/w) of the egg albumen and yolk were determined using an Abbe refractometer with a peltier system (DR-A1; Atago Co. Ltd, Tokyo, Japan) at $20 \pm 1^\circ\text{C}$.³⁰

Foaming properties

Foam stability of the egg albumen and whole egg samples and RWC were measured at 20°C . Foam was obtained by whipping 75 mL of egg albumen/whole egg in a Hobart mixer (N50CE; Hobart Foster Scandinavia A/S, Aalborg, Denmark) at speed 2 for 90 s and then at speed 3 for 90 s at room temperature. Foam stability was measured with a graduated cylinder after the foam had been allowed to rest for 1 h. Volume (mL) was used as a measure of this property. Foam stability was measured in the same vessel as the volume of released fluid at the bottom 1 h after whipping. The experiment was repeated three times and means values were calculated as $\text{RWC} = \frac{\text{volume of prepared foam} - \text{volume of liquid drainage}}{\text{original volume of liquid}} \times 100$.

Eggshell breaking strength

Eggshell breaking strength (puncture strength) was determined using a texture analyser (TA.XT2; Texture Technologies Corp., Scarsdale, NY, USA). The eggshell was punctured at the top (small end) and bottom (large end) using a 3 mm die probe at 5 mm s^{-1} constant speed with a 30 kg load cell in compression mode. The force required to puncture the shell was recorded as eggshell breaking strength (kg_f).^{4,30}

Fourier transform near infrared measurements

Spectral measurements were taken on the egg yolks and albumen in reflectance and transmittance modes using an FT-NIR spectrometer using a Bruker multi-purpose analyser FT-NIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) equipped with InGaAs detectors according to Aday and Caner.³¹ A fibre optic probe was placed directly on the equatorial surface of the albumen or yolk during measurements. Thirty-two scans were performed for each reflectance spectrum in about 15.32 s, and 128 scans for transmittance in about 62 s. Resolution was 8 cm^{-1} for both spectral measurement modes. For finding the OH band, we transformed from wavenumber (cm^{-1}) to wavelength (nm) by using the OPUS software (Bruker Optik).³¹

Data analysis

Statistical procedures were performed using LSM-PROG GLM of the SAS program (SAS Institute, Cary, NC, USA). Differences in samples as a result of ultrasonic treatment and time were tested statistically. Analysis of variance was carried out on all measured parameters among the treatment to determine any significant differences during or after storage. Statistical significance was defined at $P \leq 0.05$.

RESULTS**Haugh unit and egg grade**

The Haugh unit (HU) is a measure of egg protein quality based on the height of the albumen. The higher the number, the better the quality.^{4,30}

Table 1 shows the HU values of eggs with different treatments during storage. HU decreased with increasing storage time

Table 4. Effect of the ultrasound treatment (200, 300 and 450 W) on egg yolk pH during 6 weeks of storage

Treatment	Storage time (weeks)						
	0	1	2	3	4	5	6
CNT	6.04 ± 0.02 ^{Aa}	6.08 ± 0.03 ^{Aa}	6.16 ± 0.04 ^{Ba}	6.21 ± 0.02 ^{BCa}	6.24 ± 0.02 ^{CDa}	6.31 ± 0.03 ^{DEa}	6.36 ± 0.03 ^{Ea}
200 W	6.02 ± 0.03 ^{Aa}	6.06 ± 0.02 ^{Aa}	6.14 ± 0.04 ^{Bab}	6.17 ± 0.02 ^{BCab}	6.20 ± 0.04 ^{Cab}	6.25 ± 0.03 ^{DB}	6.30 ± 0.03 ^{Eb}
300 W	5.97 ± 0.02 ^{Aa}	6.04 ± 0.03 ^{Ba}	6.11 ± 0.03 ^{Cbc}	6.14 ± 0.02 ^{CDb}	6.16 ± 0.02 ^{Dbc}	6.21 ± 0.02 ^{Ec}	6.26 ± 0.02 ^{Fb}
450 W	5.93 ± 0.02 ^{Aa}	5.98 ± 0.02 ^{Bb}	6.07 ± 0.03 ^{Cc}	6.12 ± 0.02 ^{Db}	6.15 ± 0.02 ^{DEc}	6.17 ± 0.02 ^{EFc}	6.21 ± 0.06 ^{Fc}

Data are expressed as mean ± standard deviation.

^{a-c}Means in the same column with different superscript lowercase letters are significantly different ($P \leq 0.05$).

^{A-F}Means in the same row with different superscript uppercase letters are significantly different ($P \leq 0.05$).

CNT, control.

in all groups, in agreement with previous investigation during storage.^{28,30,32–34} A decrease in HU was induced by egg albumen³⁰ thinning due to ageing related to changes occurring in ovomucin. Thinning was due to a destruction of the lysozyme–ovomucin complex when loss of CO₂ resulted in an increase of pH.^{30,35}

During storage, the structure of the firm albumen fraction deteriorates, a part of ovomucin, which is bound in a complex is released.^{1,36} The control eggs had significantly lower ($P < 0.05$) HU than that of the ultrasound treated eggs during storage. Data from this study indicated that HU of control eggs decreased much faster over time. Control and 200 W eggs had significantly lower HU than 300 W and 450 W treated eggs until 3 weeks. However, after 6 weeks of storage, eggs treated at 450 W were different to the control group, and other treatments had higher HU values of 53.38 at 6 weeks (Table 1). Eggs treated at 200 W and 300 W had significantly higher values than control eggs (Table 1), but those treated at 450 W did not. Treatments at 450 W were more effective than the others in preserving albumen quality during storage. Eggs treated with 450 W remained at 53.38 HU after 6 weeks of storage, compared with 52.27 (300 W) and 51.68 (200 W), and also 46.77 for control eggs (Table 1). This indicated that various ultrasonic treatments preserved the albumen and yolk quality, while eggs treated with 200, 300 and 450 W maintained a grade AA (HU > 72) through 2 weeks. There were significant increases in albumen height, HU and yolk height of the eggs depending on the time of ultrasound power. The present study showed that the shelf life of eggs was prolonged by at least 1 week by ultrasonic treatment, especially 300 W and 450 W. We can conclude that ultrasound power between 200 W and 450 W could be used to maintain the interior quality of shell eggs and extend the shelf life of fresh eggs.

Sert *et al.*³² reported 35 kHz ultrasound for 5, 15 and 30 min treatment, and showed that 30 min applications on shell eggs were significantly different, although the control and applications at 5 min were not significantly different. However, 15 and 30 min applications were significantly different from each other and had higher HU than the control and 5 min applications. This study demonstrated that various ultrasound treatments preserved albumen quality during long-term storage. These results agreed with those obtained by Sert *et al.*³² and Bhale *et al.*³⁷

Yolk index

During storage, the yolk index value (YI), the ratio of yolk height to yolk width, an indicator of egg freshness, decreased as a result of a progressive weakening of the vitellin membranes and liquefaction of the yolk.^{1,30,38} The YI of the control eggs was significantly lower

than that of all ultrasound-treated eggs after 1 week of storage. The storage time has a significant effect on YI. After 6 weeks of storage, the YI of control eggs decreased from 0.45 to 0.25, while ultrasonic treatment with 450 W, 300 W and 200 W were 0.29, 0.28 and 0.28, respectively. YI values of ultrasonic treatment with 450 W eggs at 6 weeks were similar to the YI of control eggs at 4 weeks (Table 2). YI of ultrasonic treatment with 450 W had significantly higher YI values than 300 W and 200 W after 4 weeks.

Applications of ultrasonic treatment were significantly able to preserve the yolk quality for at 2 weeks longer than in the control at room temperature, in agreement with Sert *et al.*^{28,32} Sert *et al.*³² reported that the results obtained for 35 kHz ultrasound and 30 min application were significantly different compared to the control and 5 min application, while the 15 and 30 min applications were significantly different from each other and had higher YIs than the control and 5 min applications. According to Yuceer and Caner,³⁰ Caner³³ and Bhale *et al.*³⁷ ultrasonic treatments effectively inhibited albumen liquefaction and water uptake by the yolk by reducing the rate of water and CO₂ loss from the albumen through the eggshell and then minimised yolk quality loss.

pH measurement

Egg storage systems must also ensure that the interior quality of the egg is maintained, as indicated by a good proportion of a thick egg white, a firm yolk and albumen. Results of the effects of ultrasound power and application time are presented in Table 1 and Table 2. Power and time had significantly affected pH of albumen but not yolk. Albumen pH is measure of indicator for egg freshness.^{30,33,34} Freshly laid eggs have an albumen pH value of 7.5–8.5,^{30,39} and contain 1.44–2.05 mg CO₂ g⁻¹ of albumen.^{30,39} During storage of eggs, the pH of the albumen increases; this is thought to be related to the deterioration of albumen quality. After an egg has been laid, the pH of the albumen increases from near neutral (pH 7.0) to as high as 9.5 owing to the release of CO₂ from the breakdown of carbonic acid in egg white, resulting in changes in the bicarbonate buffer system.^{30,39,40} The escape of CO₂ through the pores of the shell causes a rapid increase in alkalinity, especially in the albumen. The enhancement effects of ultrasonic treatment in maintaining albumen freshness are due to the stability and sealing characteristics of the pores.

Statistical analyses showed that interactions of three factors (week × power × time) ($P < 0.05$) were significantly important. In this study, overall the pH of albumen in all the eggs significantly increased during storage times. The albumen pH values were higher for control eggs than all ultrasonic treatment during storage (Table 3 and Table 4). The albumen pH for control eggs ranged

Table 5. Effect of the ultrasound treatment (200, 300 and 450 W, each for 2 and 5 min) on albumen viscosity (20 s) during 6 weeks of storage

Treatment	Storage and treatment times													
	0 weeks		1 weeks		2 weeks		3 weeks		4 weeks		5 weeks		6 weeks	
	2 min	5 min	2 min	5 min	2 min	5 min								
CNT	72.05 ± 0.47 ^{Aa,l}	72.45 ± 0.50 ^{Aa,l}	66.25 ± 0.41 ^{Aa,l}	65.51 ± 0.30 ^{Aa,l}	57.40 ± 0.34 ^{Ba,l}	57.23 ± 0.44 ^{Ba,l}	48.50 ± 0.48 ^{Ca,l}	48.72 ± 0.41 ^{Ca,l}	37.60 ± 0.42 ^{Da,l}	38.13 ± 0.51 ^{Da,l}	23.40 ± 0.57 ^{Ea,l}	23.94 ± 0.4 ^{Ea,l}	11.40 ± 0.79 ^{Fa,l}	11.86 ± 0.77 ^{Fa,l}
200 W	72.59 ± 0.44 ^{Aa,l}	72.69 ± 0.37 ^{Aa,l}	68.46 ± 0.78 ^{Aa,l}	68.52 ± 0.5 ^{Aba,l}	62.47 ± 0.61 ^{Ba,l}	63.08 ± 0.53 ^{Ba,l}	55.07 ± 0.57 ^{Ca,l}	55.30 ± 0.50 ^{Ca,l}	41.34 ± 0.67 ^{Ca,l}	42.65 ± 0.40 ^{Da,l}	32.11 ± 0.39 ^{Da,l}	32.54 ± 0.7 ^{Da,l}	19.74 ± 0.45 ^{Ea,l}	21.04 ± 0.4 ^{Ea,l}
300 W	73.31 ± 0.61 ^{Aa,l}	73.41 ± 0.40 ^{Aa,l}	67.60 ± 0.48 ^{Aa,l}	67.95 ± 0.54 ^{Aa,l}	63.60 ± 0.72 ^{Aa,l}	62.28 ± 0.62 ^{Ba,l}	55.42 ± 0.51 ^{Ba,l}	55.10 ± 0.62 ^{Ca,l}	42.62 ± 0.37 ^{Ca,l}	42.47 ± 0.33 ^{Da,l}	32.40 ± 0.65 ^{Da,l}	35.85 ± 0.7 ^{Da,l}	21.38 ± 0.7 ^{Ea,l}	22.47 ± 0.7 ^{Ea,l}
450 W	73.61 ± 0.5 ^{Aa,l}	73.96 ± 0.5 ^{Aa,l}	66.32 ± 0.6 ^{Ba,l}	66.65 ± 0.5 ^{Ba,l}	61.11 ± 0.6 ^{Ba,l}	60.30 ± 0.7 ^{Ca,l}	54.73 ± 0.5 ^{Ca,l}	53.88 ± 0.6 ^{Da,l}	42.26 ± 0.8 ^{Da,l}	42.42 ± 0.4 ^{Da,l}	35.24 ± 0.4 ^{Ea,l}	32.52 ± 0.4 ^{Ea,l}	23.43 ± 0.7 ^{Fa,l}	22.28 ± 0.7 ^{Fa,l}

Data are expressed as mean ± standard deviation.

A–F Different superscript uppercase letters donate significant differences between storage periods in same power of ultrasound treatment and treatment time ($P < 0.05$).

a–f Different superscript lowercase letters donate significant differences between treatment times in same power of ultrasound treatment and storage period ($P < 0.05$).

I–II Different Roman numbers donate significant differences between storage periods in same treatment time and power of ultrasound treatments ($P < 0.05$).

CNT, control.

from 8.26 initially to 9.11 at the end of storage (Table 3). For ultrasound treated eggs, albumen pH values reached 8.84–8.86 (450 W), 8.88–8.93 (300 W), and 8.96–9.03 (200 W). Eggs treated with ultrasound for 2 and 5 min had significantly different (Table 3).

It can be seen that there was a significant difference between 450 W and other treatments for 5 min. In the application of 300 W and 450 W except the 2- and 3-week periods, the difference in the weekly change was statistically significant. There were no significant differences among 300 W and 450 W treated eggs. The albumen freshness of 450 W and 300 W at 5 min treated eggs after 6 weeks was comparable to that of control eggs after 4 weeks and 200 W at 2 min eggs after 5 weeks (Table 3). These findings showed that 450 W and 300 W ultrasound treatments were most effective in maintaining albumen during storage. The significantly lower albumen pH found in ultrasound-treated eggs during storage indicated that the ultrasound power and time treatment were effective in preventing the rate of albumen liquefaction, helping to maintain albumen quality by controlling the albumen pH. The results agree with those of Yuceer and Caner,³⁰ Caner,³³ Scott and Silversides,³⁹ Walsh *et al.*⁴¹ and Ahn *et al.*⁴²

Yolk pH also increased by storage time. The pH of yolk in freshly laid eggs is generally about 6.0, but during storage of eggs, the pH gradually increases to between 6.4 and 6.5. For ultrasound-treated eggs, yolk pH values reached 6.21 (450 W), 6.26 (300 W), and 6.30 (200 W). Eggs treated with 450 W had significantly lower yolk pH values than those treated by 300 and 200 W after 5 weeks (Table 4). At the end of storage, 450 W had lower yolk pH and significantly different than 200 and 300 W. All treatments had significantly lower yolk pH than the control. These findings showed that 450 W and 300 W, for 5 min, ultrasound treatments were effective in maintaining pH of shell eggs during storage.

Albumen viscosity

Viscosity of the egg albumen is the most functional properties such as emulsification, whipping ability and gelling properties of the eggs.^{43–45} The albumen that surrounds the yolk, which is the thick albumen, progressively liquefies and thins with time, transforming itself into thin albumen due to the changes in the lysozyme–ovomucin complex caused by the increase of pH during the storage. Any adverse effects on the viscosity of albumen will affect these properties and would make eggs unsuitable for use in the food industry and reduce the shelf life of the eggs.^{43,46–48} During storage, the physical and chemical characteristics of the gelatinous structure of thick albumen change and gradually break down into a clear liquid, losing consistency.

Several authors determined the shear viscosity of liquid egg white.^{48–50} The viscosity values found by different authors vary between 10 and a few hundred millipascals. Variations between measurements are most probably caused by discrepancies in sample preparation and measurement protocol. Liquid egg white is a thixotropic material for which the viscosity is time-dependent.⁵¹ Egg albumen is a pseudoplastic fluid and its viscosity depends on the shear force. The albumen viscosity values measured in this study during storage are given in Table 5. The viscosity of the albumen decreased during storage, during which a decrease in viscosity was observed, confirming earlier results obtained by Kannan *et al.*⁴³ and Kemps *et al.*⁴⁶ The albumen viscosity depends on the ovomucin–lysozyme complex. When lysozyme is present in the complex, it becomes stronger and its destabilisation is due to a pH increase during storage.^{46–51} The liquefaction of albumen due to the increase in pH is influenced by the ovomucin–lysozyme complex, which leads to changes in viscosity of the albumen during

Table 6. Effect of ultrasonic treatments (200, 300 and 450 W) on albumen total solids (% w/w dry matter) during 6 weeks of storage

Treatment	Storage time (weeks)						
	0	1	2	3	4	5	6
CNT	11.24 ± 0.04 ^{Aa}	11.59 ± 0.14 ^{Aa}	12.23 ± 0.19 ^{Ba}	13.13 ± 0.27 ^{Ca}	14.19 ± 0.41 ^{Da}	15.48 ± 0.25 ^{Ea}	17.45 ± 0.62 ^{Fa}
200 W	11.24 ± 0.06 ^{Aa}	11.47 ± 0.23 ^{Aa}	12.07 ± 0.16 ^{Ba}	12.73 ± 0.24 ^{Cb}	13.53 ± 0.46 ^{Db}	14.13 ± 0.22 ^{Eb}	15.52 ± 0.63 ^{Fb}
300 W	11.24 ± 0.07 ^{Aa}	11.40 ± 0.27 ^{Aa}	12.03 ± 0.13 ^{Ba}	12.49 ± 0.34 ^{Cb}	13.17 ± 0.33 ^{Dc}	13.89 ± 0.23 ^{Eb}	14.70 ± 0.34 ^{Fc}
450 W	11.26 ± 0.09 ^{Aa}	11.37 ± 0.12 ^{Aa}	12.00 ± 0.23 ^{Ba}	12.47 ± 0.14 ^{Cb}	13.13 ± 0.25 ^{Dc}	13.85 ± 0.13 ^{Eb}	14.41 ± 0.15 ^{Fc}

Data are expressed as mean ± standard deviation.

^{a-c}Means in the same column with different lowercase letters are significantly different ($P \leq 0.05$).

^{A-F}Means in the same row with different capital letters are significantly different ($P \leq 0.05$).

CNT, control.

Table 7. Effect of treatment time (2 and 5 min, at overall treatment power) on albumen total solids (% w/w dry matter) during 6 weeks of storage

Storage time (weeks)	Treatment time (min)	
	2	5
0	11.24 ± 0.11 ^{Aa}	11.25 ± 0.12 ^{Aa}
1	11.45 ± 0.18 ^{Aa}	11.43 ± 0.17 ^{Aa}
2	12.07 ± 0.19 ^{Ab}	12.06 ± 0.19 ^{Ab}
3	12.67 ± 0.29 ^{Ac}	12.59 ± 0.25 ^{Ac}
4	13.42 ± 0.47 ^{Ad}	13.40 ± 0.46 ^{Ad}
5	14.20 ± 0.61 ^{Ae}	14.15 ± 0.54 ^{Ae}
6	15.41 ± 0.87 ^{Af}	15.09 ± 0.82 ^{Bf}

Data are expressed as mean ± standard deviation.

^{a-f}Means in the same column with different superscript lowercase letters are significantly different ($P \leq 0.05$).

^{A-B}Means in the same row with different superscript uppercase letters are significantly different ($P \leq 0.05$).

storage.^{49–51} According to these results, the ultrasound treatment minimises changes in carbohydrate and protein moieties involved in the formation of the ovomucin complex, resulting in a loss of the gel-like structure during storage, and minimises changes in pH and maintains albumen quality.¹ The less viscous the fluid is, the greater its ease of movement (fluidity). The statistical analyses showed that interactions of three factors (week × power × time) ($P < 0.05$) were significantly important. The albumen viscosity for control eggs ranged from 72.05 initially to 11.40 at the end of storage (Table 5). For treated eggs, albumen viscosity values decreased 23.43–22.28 (450 W), 21.38–22.47 (300 W), and 19.74–21.04 (200 W) (Table 5).

Total solids (dry matter) of albumen and yolk

The total solid (dry matter) of albumen⁵² index, measuring the liquid concentration of albumen, has also been used as an indicator of egg freshness which liquefaction of albumen occur through protease enzymes at increasing pH.⁵³ Albumen consists primarily of about 90% water into which is dissolved 10% proteins (including albumens, mucoproteins and globulins). The water in albumen permeates the yolk and yolk can permeate albumen. Weak and watery albumen permits the yolk lead to changes in albumen and yolk concentrations. During long storage time, the drymatter (DMA) increases owing to mixing of the yolk into the albumen. Albumen DMA values for control eggs were higher than those for ultrasound treated eggs (Table 6 and Table 7). The increase

in DMA during storage has been attributed to liquefaction of the yolk and subsequent mixing into the albumen. This liquefaction process occurs as the result of interaction between the lysozyme–ovomucin complex as the pH increases during storage. In general, liquefaction would result in an increase of fluidity in egg albumen and associated with deterioration in egg quality.^{53,54} Statistical analyses revealed that interactions of three factors (week × power × time) were not significantly important, although the main effects of storage time and ultrasound power were statistically significant. The DMA of control egg albumen ranged from 11.24 initially to 14.41–17.45 at the end of storage (Tables 6 and 7). In contrast, the 450 W and 300 W treatments maintained DMA values at 14.41 and 14.7, respectively, at the end of storage. From the data in Tables 6 and 7, it can be seen that there was a significant difference between 450 W and 300 W than 200 W during storage. Ultrasound treated 450 W and 300 W had significantly lower yolk DMA values than 200 W and control samples. Significant differences in albumen DMA were observed between control and ultrasound treated samples ($p < 0.05$). The albumen freshness of 450 W and 300 W ultrasound treated eggs after 6 weeks was comparable to that of control eggs after 4 weeks and 200 W after 5 weeks.

Yolk DMA values decreased significantly during storage. The statistical analyses showed that interactions of three factors (week × power × time) ($P < 0.05$) were significantly important. Yolk total solids decreased during storage from a maximum of 42.57% at 0 weeks to 41.02–42.10% at 6 weeks storage and agree with Yuceer and Caner,³⁰ and Jones.⁵⁵ Significant differences in DMA yolk values were observed between control and ultrasound treatments ($P < 0.05$) (Table 8). Control eggs had significantly lower yolk DMA values than all others (Table 8). 450 W and 300 W ultrasound treated eggs after 6 weeks was comparable to that of control eggs after 4 weeks and 200 W after 5 weeks.

Relative whipping capacity (foaming properties)

The whipping ability of egg white can be assayed by measurement of foam volume and foam stability (amount of liquid released from the foam in a given time). Foam stability is determined by measuring the loss of liquid resulting from destabilisation, i.e. leakage, measuring volume decrease or density increase with time. Foam stability reflects the water holding capacity of the foam.⁴⁶ The stability or drainage volume of foam is influenced by the thickness of the interface, foam size distribution, interface permeability, and surface tension. This interferes with the formation of a cohesive film at the air/water interface, causing a decrease in foam stability.^{49,56} The pH in the aqueous phase determines the magnitude and nature of protein charges and therefore affects the foaming properties.^{46,49,55–57}

Table 8. Effect of the ultrasound treatments (200, 300 and 450 W, each for 2 and 5 min) on total solids (dry matter) of egg yolk during 6 weeks of storage

Treatment	Storage and treatment times																														
	0 weeks			1 weeks			2 weeks			3 weeks			4 weeks			5 weeks			6 weeks												
	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min	2 min	5 min													
CNT	42.57 ± 0.05 ^{Aa,i}	42.61 ± 0.09 ^{Aa,i}	42.46 ± 0.11 ^{Ba,i}	42.44 ± 0.23 ^{Ba,i}	42.35 ± 0.24 ^{Bca,i}	42.35 ± 0.35 ^{Bca,i}	42.24 ± 0.26 ^{Ca,i}	42.26 ± 0.21 ^{Ca,i}	42.03 ± 0.32 ^{Da,i}	42.00 ± 0.36 ^{Da,i}	41.68 ± 0.20 ^{Ea,i}	41.70 ± 0.24 ^{Ea,i}	41.06 ± 0.16 ^{Fa,i}	41.02 ± 0.23 ^{Fa,i}	42.58 ± 0.06 ^{Aa,i}	42.57 ± 0.08 ^{Aa,i}	42.46 ± 0.27 ^{Aba,i}	42.47 ± 0.28 ^{Aba,i}	42.37 ± 0.24 ^{Bca,i}	42.38 ± 0.32 ^{Bca,i}	42.24 ± 0.46 ^{Ca,i}	42.02 ± 0.23 ^{Da,i}	41.87 ± 0.26 ^{Da,i}	41.93 ± 0.34 ^{Da,i}	42.02 ± 0.22 ^{Ea,i}	42.00 ± 0.22 ^{Ea,i}	42.10 ± 0.33 ^{DEa,i}	42.12 ± 0.36 ^{DEa,i}	42.00 ± 0.22 ^{Ea,i}	42.08 ± 0.27 ^{Ea,i}	42.10 ± 0.13 ^{Ea,i}
200 W	42.58 ± 0.06 ^{Aa,i}	42.57 ± 0.08 ^{Aa,i}	42.46 ± 0.27 ^{Aba,i}	42.47 ± 0.28 ^{Aba,i}	42.37 ± 0.24 ^{Bca,i}	42.38 ± 0.32 ^{Bca,i}	42.34 ± 0.22 ^{Bca,i}	42.35 ± 0.32 ^{Bca,i}	42.24 ± 0.46 ^{Ca,i}	42.24 ± 0.34 ^{CDa,i}	42.02 ± 0.23 ^{Da,i}	42.06 ± 0.24 ^{Da,i}	41.87 ± 0.26 ^{Da,i}	41.93 ± 0.34 ^{Da,i}	42.58 ± 0.06 ^{Aa,i}	42.57 ± 0.08 ^{Aa,i}	42.46 ± 0.27 ^{Aba,i}	42.47 ± 0.28 ^{Aba,i}	42.37 ± 0.24 ^{Bca,i}	42.38 ± 0.32 ^{Bca,i}	42.24 ± 0.46 ^{Ca,i}	42.02 ± 0.23 ^{Da,i}	41.87 ± 0.26 ^{Da,i}	41.93 ± 0.34 ^{Da,i}	42.02 ± 0.22 ^{Ea,i}	42.00 ± 0.22 ^{Ea,i}	42.10 ± 0.33 ^{DEa,i}	42.12 ± 0.36 ^{DEa,i}	42.00 ± 0.22 ^{Ea,i}	42.08 ± 0.27 ^{Ea,i}	42.10 ± 0.13 ^{Ea,i}
300 W	42.58 ± 0.06 ^{Aa,i}	42.58 ± 0.07 ^{Aa,i}	42.49 ± 0.34 ^{Aba,i}	42.50 ± 0.19 ^{Aba,i}	42.38 ± 0.42 ^{Bca,i}	42.39 ± 0.23 ^{Bca,i}	42.36 ± 0.42 ^{Bca,i}	42.37 ± 0.34 ^{Bca,i}	42.24 ± 0.42 ^{CDa,i}	42.25 ± 0.43 ^{CDa,i}	42.10 ± 0.33 ^{DEa,i}	42.12 ± 0.36 ^{DEa,i}	42.00 ± 0.22 ^{Ea,i}	42.02 ± 0.23 ^{Ea,i}	42.58 ± 0.06 ^{Aa,i}	42.57 ± 0.08 ^{Aa,i}	42.46 ± 0.27 ^{Aba,i}	42.47 ± 0.28 ^{Aba,i}	42.37 ± 0.24 ^{Bca,i}	42.38 ± 0.32 ^{Bca,i}	42.24 ± 0.46 ^{Ca,i}	42.02 ± 0.23 ^{Da,i}	41.87 ± 0.26 ^{Da,i}	41.93 ± 0.34 ^{Da,i}	42.02 ± 0.22 ^{Ea,i}	42.00 ± 0.22 ^{Ea,i}	42.10 ± 0.33 ^{DEa,i}	42.12 ± 0.36 ^{DEa,i}	42.00 ± 0.22 ^{Ea,i}	42.08 ± 0.27 ^{Ea,i}	42.10 ± 0.13 ^{Ea,i}
450 W	42.58 ± 0.09 ^{Aa,i}	42.58 ± 0.06 ^{Aa,i}	42.52 ± 0.24 ^{Aba,i}	42.53 ± 0.12 ^{Aba,i}	42.39 ± 0.34 ^{Bca,i}	42.40 ± 0.21 ^{Bca,i}	42.37 ± 0.24 ^{Bca,i}	42.40 ± 0.15 ^{Bca,i}	42.27 ± 0.12 ^{CDa,i}	42.29 ± 0.13 ^{CDa,i}	42.15 ± 0.22 ^{DEa,i}	42.16 ± 0.12 ^{DEa,i}	42.08 ± 0.27 ^{Ea,i}	42.10 ± 0.13 ^{Ea,i}	42.58 ± 0.06 ^{Aa,i}	42.57 ± 0.08 ^{Aa,i}	42.46 ± 0.27 ^{Aba,i}	42.47 ± 0.28 ^{Aba,i}	42.37 ± 0.24 ^{Bca,i}	42.38 ± 0.32 ^{Bca,i}	42.24 ± 0.46 ^{Ca,i}	42.02 ± 0.23 ^{Da,i}	41.87 ± 0.26 ^{Da,i}	41.93 ± 0.34 ^{Da,i}	42.02 ± 0.22 ^{Ea,i}	42.00 ± 0.22 ^{Ea,i}	42.10 ± 0.33 ^{DEa,i}	42.12 ± 0.36 ^{DEa,i}	42.00 ± 0.22 ^{Ea,i}	42.08 ± 0.27 ^{Ea,i}	42.10 ± 0.13 ^{Ea,i}

Data are expressed as mean ± standard deviation.
A–F Different superscript uppercase letters denote significant differences between storage times in same treatment and time ($P < 0.05$).
a–f Different superscript lowercase letters denote significant differences between treatment times in same treatment and storage time ($P < 0.05$).
I–III Different superscript Roman numbers denote significant differences between treatments in same treatment time and storage time ($P < 0.05$).
CNT, control.

A highly significant interaction between the ultrasound power and storage time was observed for albumen foaming properties ($P < 0.05$). During storage, a decrease in RWC was observed during storage, confirming earlier results obtained by Yuceer and Caner.³⁰ Albumen RWC values significantly decreased 1129–1075 at 0 weeks to 291 (control), 475 (200 W), 612 (300 W), and 645 (450 W) at end of storage (Table 9 and Table 10). There were significant differences in the RWC of albumen control (475) and treatments. There were also significant differences in the RWC of albumen for control and 200 W. The treatments of 450 W and 300 W significantly different and higher RWC value than 200 W and control. The ultrasound treatments caused stability of foam (Tables 9 and 10). Higher foam expansion indicates that more air was trapped in the foam and egg albumen reduces the surface tension and interfacial tension to a level sufficiently low to form the interfacial film that exceeds the critical thickness. As time passed, the films became progressively thinner and ruptured.^{30,51,58} Throughout the storage period, fluid is lost by lamellar water drainage, resulting in foam collapse.⁵⁸

After 6 weeks of storage, the reductions in RWC were 72.86% (control), 56.14% (200 W), 43.91% (300 W) and 42.79% (450 W). There were also significant differences in the RWC of whole eggs for control (400), and 200 W (426.5), 300 W (441) and 450 W (459) treatments. The ultrasound treatments caused stability of foam. These results clearly demonstrated that ultrasound treatments maintained both RWC of albumen and whole eggs (foaming properties: whipping capacity) due to avoiding changes in the pH during storage (Table 11, Table 12 and Table 13).

It was observed that the high power ultrasound (450 W) and higher application times present's detrimental effects on shell eggs quality during storage and therefore hold potential for their shelf-life extension and reduce economic loss from storage. Ultrasound can be viable alternative innovative techniques for maintaining functional properties (HU, YI, pH, viscosity, total solids and RWC) of shell eggs. These parameters are also used to determine albumen quality and could be an index for egg freshness quality.

Eggshell breaking strength

The eggshell protects the contents of the egg from environment impact to some extent controlling exchange of fluid and gas through the pores and also protecting microbial penetration from the environment. Eggshell quality is of considerable economic significance for commercial handling and storage. Shell quality declines, as the hens get older. The eggshell should be as strong as possible to maximise the number of eggs reaching the consumer.^{4,30,59} Any negative impact on shell strength will result in significant effect on eggs lost. The top of the eggshell has higher puncture strength than the bottom. Average breaking strength of the eggs dropped from 4.13 to 3.74 by 6 weeks storage (Table 14 and Table 15). Ultrasound treatment, the strength values were decreased and lower puncture strength (Tables 14 and 15). Statistical analyses showed that interactions of factors were not significantly important. However, main effects of ultrasound treatments on storage time and ultrasound power were statistically significant (Tables 14 and 15). Shell strength of ultrasound treated eggs depending on the storage time, power and time of ultrasound application. The puncture strength of eggshells 450 W, 300 W, 200 W was significantly lower than control eggshells (Tables 14 and 15). It is possible that ultrasound treatment had adverse effect on shell of eggs due to cavitation mechanism. Control eggs significantly exhibited the highest puncture strength than others (Tables 14 and 15). This clearly indicates the ultrasound

Table 9. Effect of ultrasonic treatment (200, 300 and 450 W) on albumen relative foaming capacity during 6 weeks of storage

Treatment	Storage time (weeks)						
	0	1	2	3	4	5	6
CNT	1075.0 ± 41.83 ^{Aa}	925.0 ± 27.39 ^{Ba}	775.0 ± 41.83 ^{Ca}	650.0 ± 31.62 ^{Da}	533.3 ± 27.3 ^{Ea}	416.7 ± 25.82 ^{Fa}	291.7 ± 31.6 ^{Ga}
200 W	1083.3 ± 38.92 ^{Aa}	1000.0 ± 39.93 ^{Bab}	945.8 ± 45.02 ^{Bcb}	891.7 ± 41.47 ^{Cb}	705.6 ± 46.4 ^{Db}	616.7 ± 39.93 ^{Fb}	475.0 ± 45.3 ^{Fb}
300 W	1092.1 ± 36.02 ^{Aa}	1054.5 ± 47.19 ^{Bbc}	975.1 ± 54.36 ^{Bb}	931.8 ± 40.30 ^{Bb}	837.5 ± 43.3 ^{Cc}	745.8 ± 49.81 ^{Dc}	612.5 ± 52.76 ^{Ec}
450 W	1129.2 ± 45.02 ^{Aa}	1079.2 ± 52.01 ^{Ac}	975.1 ± 54.36 ^{Bb}	945.5 ± 35.02 ^{Bb}	866.7 ± 44.38 ^{Cc}	770.8 ± 33.43 ^{Dc}	645.8 ± 33.43 ^{Ec}

Data are expressed as mean ± standard deviation.
^{a–g}Means in the same column with different superscript lowercase letters are significantly different ($P \leq 0.05$).
^{A–G}Means in the same row with different superscript uppercase letters are significantly different ($P \leq 0.05$).
 CNT, control.

Table 10. Effect of treatment time (2 and 5 min, at 200, 300 and 450 W) on albumen relative foaming capacity during 6 weeks of storage

Treatment	Treatment time (min)	
	2	5
CNT	669.0 ± 268.5 ^{Aa}	664.3 ± 265.1 ^{Aa}
200 W	784.1 ± 231.7 ^{Ab}	858.8 ± 195.1 ^{Bb}
300 W	878.9 ± 169.6 ^{Ac}	901.2 ± 166.2 ^{Ac}
450 W	911.9 ± 165.6 ^{Ac}	919.5 ± 166.2 ^{Ad}

Data are expressed as mean ± standard deviation.
^{a–d}Means in the same column with different superscript lowercase letters are significantly different ($P \leq 0.05$).
^{A–B}Means in the same row with different superscript uppercase letters are significantly different ($P \leq 0.05$).
 CNT, control.

Table 11. The main effects of ultrasonic power on the relative whipping capacity of whole eggs during 6 week of storage

Treatment	Whipping capacity (XX)
CNT	400.0 ± 141.4 ^A
200 W	426.5 ± 132.3 ^B
300 W	441.1 ± 130.4 ^C
450 W	459.0 ± 134.2 ^D

Data are expressed as mean ± standard deviation.
^{A–D}Means in the same column with different superscript uppercase letters are significantly different ($P \leq 0.05$).
 CNT, control.

Table 12. The main effects of storage time on the relative whipping capacity of whole eggs during 6 week of storage

Storage time (weeks)	Whipping capacity (XX)
0	673.8 ± 31.7 ^A
1	558.3 ± 38.1 ^B
2	461.0 ± 32.6 ^C
3	389.0 ± 51.8 ^D
4	350.0 ± 37.5 ^E
5	322.6 ± 31.6 ^E
6	294.0 ± 40.1 ^G

Data are expressed as mean ± standard deviation.
^{A–G}Means in the same column with different uppercase letters are significantly different ($P \leq 0.05$).

effect the shell properties and surface erosion or thickness reduction may occur.⁶⁰ These results agree with Sert *et al.*³² and Shafey *et al.*⁶⁰ ultrasound reduced shell strength.

Fourier transform near infrared analysis

NIR spectroscopy is a rapid, effective and non-destructive method for determining functional groups (C—H, N—H and O—H bonds) to identify and quantify of components in foods using specific wavelengths between 750 nm and 2500 nm.⁶¹ The results obtained from the FT-NIR analysis eggs are shown in Figure 1 and 2 wavelength range from 800 to 2500 nm, nearly the full NIR region. At the beginning of the storage of shell eggs, absorption

Table 13. The main effects of treatment time on the relative whipping capacity of whole eggs during 6 weeks of storage

Treatment time (min)	Whipping capacity (XX)
2	432.2 ± 133.8 ^A
5	441.1 ± 135.2 ^B

Data are expressed as mean ± standard deviation.
^{A–B}Means in the same column with different uppercase letters are significantly different ($P \leq 0.05$).

Table 14. The main effects of ultrasonic power on eggshell breaking strength at the top and bottom of eggs during 5 weeks of storage

Power (W)	Breaking strength (kg _f)
Top	
CNT	4.47 ± 0.42 ^A
200	4.01 ± 0.31 ^B
300	3.96 ± 0.25 ^B
450	3.833 ± 0.27 ^B
Bottom	
CNT	3.78 ± 0.29 ^A
200	3.58 ± 0.25 ^B
300	3.30 ± 0.24 ^C
450	3.02 ± 0.20 ^D

Data are expressed as mean ± standard deviation.
^{A–D}For each location (top or bottom) means in the same column with different lowercase letters are significantly different ($P \leq 0.05$).
 CNT, control.

Table 15. The main effects of storage time on eggshell breaking strength at the top and bottom of eggs during 5 weeks of storage

Storage time (weeks)	Breaking strength (kg _f)
Top	
0	4.64 ± 0.33 ^A
1	4.13 ± 0.26 ^B
2	4.01 ± 0.19 ^{BC}
3	3.93 ± 0.17 ^{BCD}
4	3.82 ± 0.20 ^{CD}
5	3.74 ± 0.15 ^D
Bottom	
0	3.59 ± 0.26 ^A
1	3.56 ± 0.35 ^A
2	3.50 ± 0.33 ^{AB}
3	3.37 ± 0.28 ^{AB}
4	3.27 ± 0.29 ^B
5	2.94 ± 0.24 ^C

Data are expressed as mean ± standard deviation.
^{A–D}For each location (top or bottom) means in the same column with different superscript uppercase letters are significantly different ($P \leq 0.05$).

spectrum showed high absorbance for water content due to freshness quality, as expected. Water absorption bands in the NIR spectrum were influenced by effects of solutes in water and were clearly observed.^{61–63} The absorbance spectrum stays relatively flat from 800 to 910 nm. The bands around at 970, 1190 and 1450 nm can be attributed to water absorption with overtone bands of the —OH and a combination band at 1940 nm (involving —OH stretching and —OH deformation) which is due to absorption by water and carbohydrate.^{62,63} The characteristic bands were identified: 1135–1200 nm (2nd overtone of the C—H stretch of —CH₂ group); 1450 nm (1st overtone of the O—H stretch); the region of 1660–1760 nm (1st overtone of the symmetric C—H stretch of CH₂ and CH₃ group); 1940 nm a combination of the O—H band and the stretching band of water^{31,62,63} (Figure 1 and 2). Increased storage time resulted in decrease of absorption bands for all treatments. The lowest peaks were observed around 970, 1190 nm for control (CNT). At the end of the storage, absorption peaks around at 970, 1190 nm dropped significantly for CNT when compared with other ultrasound treatments. Increased storage time resulted in decrease of absorption bands for all treatments. Albumen becomes thinner during storage time, which leads to a change in the transmitted spectra. The FT-NIR measurement offers the non-destructive evaluation of quality attributes such as water content.

Destructive effect of high power ultrasound on shell for 450 W treatments causes water losses. The increasing of the absorbance of —OH stretching during conservation could denote a structural change of proteins; the increase of absorbance for the wavelength of 1946 nm corresponds to water bound to protein.^{61–63} The sharp

absorption band at 1190 and 1420 nm increases in intensity due to loss of water (Fig. 1 and Fig. 2).

The yolk represents 33% of the liquid weight, and its composition consists of fat (almost all the fat in the egg) and proteins. There were clear differences between the typical average absorbance spectra corresponding to the yolk of the ultrasound treated eggs and also control at the end of the storage. The characteristic Amide I C—O stretch (1654(s)cm⁻¹), amide II NH₂ deformation (1632(sh) cm⁻¹) and amide II (1542(m) cm⁻¹) absorbance bands associated with the proteins in egg. All of these bands are clearly shown that aged sample of yolk after storage (Figure 2). The absorption peaks around at 1734 and 1714 cm⁻¹ may be due to triglyceride-derived aldehydes and acids may derive by oxidation of protein amide linkages.

CONCLUSION

We can conclude from this present study that ultrasound power between 200 W and 450 W could be used to maintain interior quality of shell eggs extends shelf life of fresh eggs. It was observed that the high power ultrasound (450 W) and higher application times present's detrimental effects on shell eggs quality during storage and therefore hold potential for their shelf-life extension and reduce economic loss from storage. There were significant increases in albumen height, HU, and yolk height of the eggs depending on the time of ultrasound power. Ultrasound can be viable alternative innovative techniques for maintaining functional properties (HU, YI, pH, viscosity, total solids and RWC) of shell eggs. These parameters are also used to determine albumen quality and could be an index for egg freshness quality. The FT-NIR measurement offers the non-destructive evaluation of quality attributes such as water content.

Further researches should also be desirable to use different treatment times and different power on liquid eggs and various perishable food products. Ultrasound applications, together with temperature treatment need to be study.

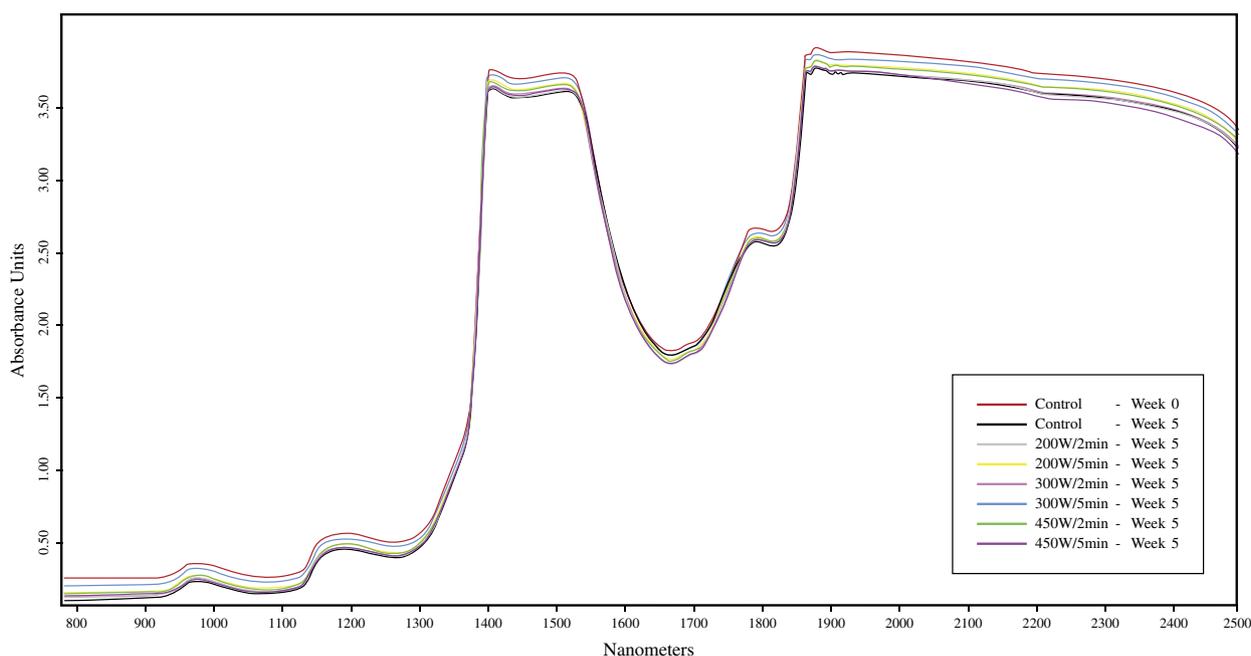


Figure 1. Effect of ultrasound treatments on average relative absorbance spectral values of eggshell albumin at the beginning (0 weeks) and at the end of storage (5 weeks).

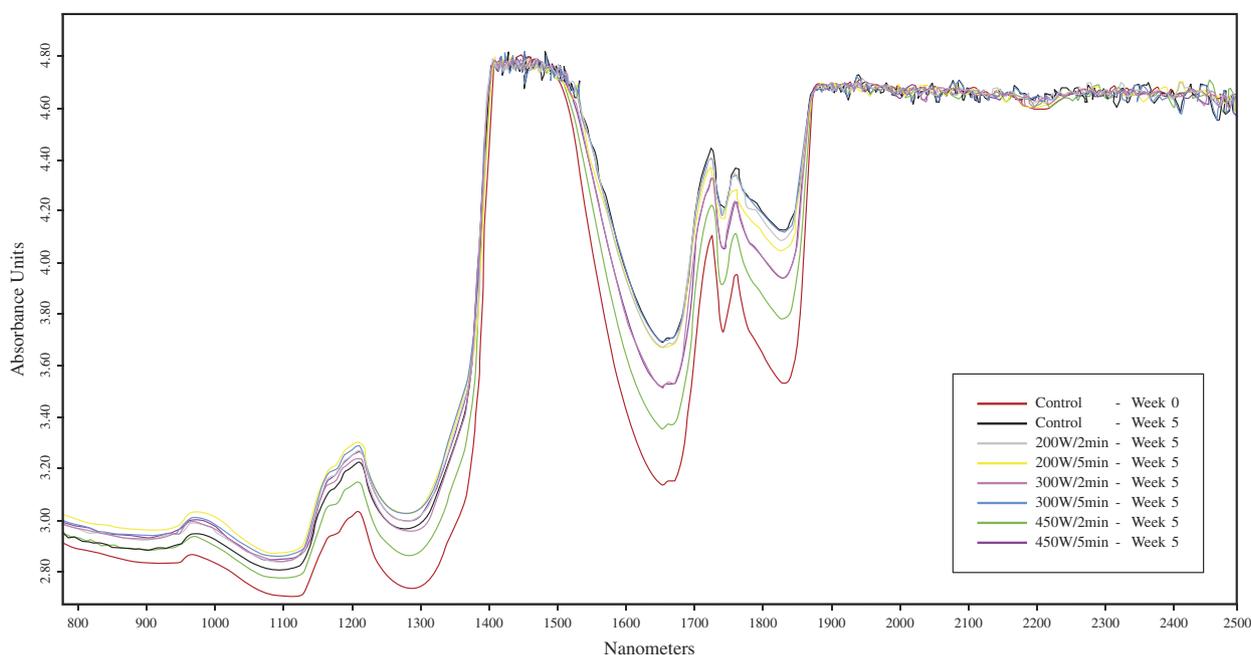


Figure 2. Effect of ultrasound treatments on average relative absorbance spectral values of eggshell yolk at the beginning (0 weeks) and at the end of storage (5 weeks).

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REFERENCES

- Hernandez-Ledesma B and Chia-Chien H, Functional proteins and peptides of hen's egg origin, in *Bioactive Food Peptides in Health and Disease*, ed. by Abdou MA, Kim M and Sato K. InTech, OALster (electronic resource), pp. 115–116 (2013).
- Anonymous, *U.S. Egg Industry Statistical Report – 2012*. Available: <http://www.ans.iastate.edu/EIC/FlockFeb2012.pdf>. [11 April 2014].
- Caner C and Cansız Ö, Effectiveness of chitosan-based coating in improving shelf-life of eggs. *J Sci Food Agric* **87**:227–232 (2007).
- Caner C and Cansız Ö, Chitosan coating minimises eggshell breakage and improves egg quality. *J Sci Food Agric* **88**:56–61 (2008).
- Strohalm J, Novotna P, Houska M, Kyhos K, Vavreinova S, Gabrovská D, *et al.*, Influence of high pressure treatment on rheological and other properties of egg white. *High Pressure Res* **19**:137–143 (2000).
- Hoppe A, Jung S, Patnaik A and Zeece MG, Effect of high pressure treatment on egg white protein digestibility and peptide products. *Innov Food Sci Emerg Technol* **17**:54–62 (2013).

- 7 Patrignani F, Vannini L, Sado Kamdem SL, Hernando I, Marco-Moles R, Guerzoni ME, *et al.*, High pressure homogenization vs heat treatment: safety and functional properties of liquid whole egg. *Food Microbiol* **36**:63–69 (2013).
- 8 Mason TJ, Paniwnyk L and Chemat F, Ultrasound as a preservation technology, in *Food Preservation Techniques*, ed. by Zeuthen P, Bøgh-Sørensen L., CRC Press, Woodhead Publishers, Cambridge, England, pp. 303–337 (2003).
- 9 Chemat F, Huma Z and Khan MK, Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrason Sonochem* **18**:813–835 (2011).
- 10 Sepulveda DRMM, Gongora-Nieto JA, Guerrero-Beltran JA and Barbosa Canovas GV, Extension of milk shelf-life by a hurdle combination of pulsed electric fields and a mild thermal treatment, in *Proceedings of the Institute of Food Technologists, Paper No: 92C-6, 2003 IFT Annual Meeting*, 12–16 July, Chicago, IL, USA (2003).
- 11 Monfort S, Gayan E, Raso J, Condon S and Alvarez I, Evaluation of pulsed electric fields technology for liquid whole egg pasteurization. *Food Microbiol* **27**:845–852 (2010).
- 12 Monfort S, Saldana G, Condon S, Raso J and Alvarez I, Inactivation of *Salmonella* spp. in liquid whole egg using pulsed electric fields, heat, and additives. *Food Microbiol* **30**:393–399 (2012).
- 13 Zhao W, Tang Y, Lu L, Chen X and Li C, Review – pulsed electric fields processing of protein-based foods. *Food Bioprocess Technol* **7**:114–125 (2014).
- 14 Debabandya M, Sabyasachi M, Saroj G and Abhijit K, Application of hurdles for extending the shelf life of fresh fruits. *Trends PostHarvest Technol* **1**:37–54 (2013).
- 15 Aday MS, Büyükcın MB, Temizkan R and Caner C, Role of ozone concentrations and exposure times in extending shelf life of strawberry. *Ozone Sci Eng* **36**:43–56 (2014).
- 16 Perry JJ, Rodriguez-Saona LE and Yousef AE, Quality of shell eggs pasteurized with heat or heat-ozone combination during extended storage. *J Food Sci* **76**:S437–S444 (2011).
- 17 Karaca H and Velioglu YS, Ozone applications in fruit and vegetable processing. *Food Rev Int* **23**:91–106 (2007).
- 18 Piyasena P, Mohareb E and McKellar RC, Inactivation of microbes using ultrasound: A review. *Int J Food Microbiol* **87**:207–216 (2003).
- 19 Ajlouni S, Sibrani H, Premier R and Tomkins B, Ultrasonication and fresh produce (Cos lettuce) preservation. *J Food Sci* **71**:62–68 (2006).
- 20 Feng H, Barbarosa-Canovas GV and Weiss J, *Ultrasound Technologies for Food and Bioprocessing*. Springer Science and Business Media, LLC, New York, NY, USA (2011).
- 21 Aday MS, Temizkan R, Büyükcın MB and Caner C, An innovative technique for extending shelf life of strawberry: Ultrasound. *LWT – Food Sci Technol* **52**:93–101 (2013).
- 22 Birmpa A, Sfika V and Vantarakis A, Ultraviolet light and ultrasound as non-thermal treatments for the inactivation of microorganisms in fresh ready-to-eat foods. *Int J Food Microbiol* **167**:96–102 (2013).
- 23 Lee DU, Heinz V and Knorr D, Effects of combination treatments of nisin and high-intensity ultrasound with high pressure on the microbial inactivation in liquid whole egg. *Innov Food Sci Emerg Technol* **4**:387–393 (2003).
- 24 Cabeza MC, Ordóñez JA, Cambero I, de la Hoz L and García ML, Effect of thermoultrasonication on *Salmonella enterica* serovar enteritidis in distilled water and intact shell eggs. *J Food Protect* **67**:1886–1891 (2004).
- 25 Huang E, Mittal GS and Griffiths MW, Inactivation of *Salmonella enteritidis* in liquid whole egg using combination treatments of pulsed electric field, high pressure and ultrasound. *Biosyst Eng* **94**(3):403–413 (2006).
- 26 Cabeza MC, Cambero MI, de la Hoz L, García ML and Ordóñez JA, Effect of the thermoultrasonic treatment on the eggshell integrity and their impact on the microbial quality. *Innov Food Sci Emerg Technol* **12**:111–117 (2011).
- 27 Hwang EF, *Liquid Whole Egg Pasteurization Using Combination Treatments of Pulsed Electric Field, High Pressure, and Ultrasound*. University of Guelph, Guelph, Ontario (2004).
- 28 Sert D, Aygun A, Torlak E and Mercan E, Effect of ultrasonic treatment on reduction of *Escherichia coli* ATCC 25922 and egg quality parameters in experimentally contaminated hens' shell eggs. *J Sci Food Agric* **93**:2973–2978 (2013).
- 29 Aygun A and Sert D, Effects of ultrasonic treatment on eggshell microbial activity, hatchability, tibia mineral content, and chick performance in Japanese quail (*Coturnix coturnix japonica*) eggs. *Poult Sci* **91**:732–738 (2012).
- 30 Yuceer M and Caner C, Antimicrobial lysozyme–chitosan coatings affect functional properties and shelf life of chicken eggs during storage. *J Sci Food Agric* **94**:153–162 (2014).
- 31 Aday MS and Caner C, Understanding the effects of various edible coatings on the storability of fresh cherry. *Pack Technol Sci* **23**:441–456 (2010).
- 32 Sert D, Aygun A and Demir MK, Effects of ultrasonic treatment and storage temperature on egg quality. *Poult Sci* **90**:869–875 (2011).
- 33 Caner C, The effect of edible eggshell coatings on egg quality and consumer perception. *J Sci Food Agric* **85**:1897–1902 (2005).
- 34 Caner C, Whey protein isolate coating and concentration effects on egg shelf life. *J Sci Food Agric* **85**:2143–2148 (2005).
- 35 Jones DR and Musgrove MT, Effects of extended storage on egg quality factors. *Poult Sci* **84**:1774–1777 (2005).
- 36 Li-Chan ECY and Nakai S, Biochemical basis for the properties of egg white. *Crit Rev Poult Biol* **2**:21–58 (1989).
- 37 Bhale S, No HK, Prinyawiwatjul W, Farr AJ, Nadarajah K and Meyers SP, Chitosan coating improves shelf life of eggs. *J Food Sci* **68**:2378–2383 (2003).
- 38 Stadelman WJ, Quality identification of shell eggs, in *Egg Science and Technology*, 4th edition, ed. by Stadelman WJ and Cotterill J. The Haworth Press, Westport, CT, and AVI Publishing, New York, pp. 39–66 (1995).
- 39 Scott TA and Silversides FG, The effect of storage and strain of hen on egg quality. *Poult Sci* **79**:1725–1729 (2000).
- 40 Biladeau AM and Keener KM, The effects of edible coatings on chicken egg quality under refrigerated storage. *Poult Sci* **88**:1266–1274 (2009).
- 41 Walsh TJ, Rızk RE and Brake J, Effects of temperature and carbon dioxide on albumen characteristics, weight loss and early embryonic mortality of long stored hatching eggs. *Poult Sci* **74**:1403–1410 (1995).
- 42 Ahn DU, Sell JL, Jo C, Chamruspollert M and Jefferey M, Effect of dietary conjugated linoleic acid the quality characteristics of chicken eggs during refrigerated storage. *Poult Sci* **78**:922–928 (1999).
- 43 Kannan S, Dev SRS, Garipey Y and Raghavan GSV, Effect of radiofrequency heating on the dielectric and physical properties of eggs. *Prog Electromagn Res B* **51**:201–220 (2013).
- 44 Silversides F and Scott T, Effect of storage and layer age on quality of eggs from two lines of hens. *Poult Sci* **80**:1240–1245 (2001).
- 45 Toussant MJ and Latschaw JD, Ovomucin content and composition in chicken eggs with different interior quality. *J Sci Food Agric* **79**:1666–1670 (1999).
- 46 Kemps BJ, Bamelis FR, Mertens K, Decuyper EM, De Baerdemaeker JG and De Ketelaere B, The assessment of viscosity measurements on the albumen of consumption eggs as an indicator for freshness. *Poult Sci* **89**:2699–2703 (2010).
- 47 Spada FP, Gutierrez EMR, Souza MC, Brazaca SGC, Lemes DEA, Fischer FS, *et al.*, Viscosity of egg white from hens of different strains fed with commercial and natural additives. *Cienc Tecnol Aliment, Campinas* **32**:47–51 (2012).
- 48 Atılgan MR and Unluturk S, Rheological properties of liquid egg products (LEPS). *Int J Food Prop* **11**:296–309 (2008).
- 49 Lomakina K and Mikova K, A study of the factors affecting the foaming properties of egg white – A review. *Czech J Food Sci* **24**:110–118 (2006).
- 50 Lucisano M, Hidalgo A, Comelli EM and Rossi M, Evolution of chemical and physical albumen characteristics during the storage of shell eggs. *J Agric Food Chem* **44**:1235–1240 (1996).
- 51 Ruth C, Veldea JV, Mathuesa W, Liedekerke PV and Moldenaers P, A rheological characterisation of liquid egg albumen, in *Inside Food Symposium*, 9–12 April, Leuven, Belgium, pp.1–6 (2013).
- 52 Heldman DR, Food preservation process design, in *Food Preservation Process Design*, ed. by Heldman DR. Heldman Associates, Mason, OH, pp. 1–18 (2011).
- 53 Gossett PW, Rizvi SSH and Baker RC, Selected rheological properties of pH adjusted or succinylated egg albumen. *J Food Sci* **48**:1395–1399 (1983).
- 54 Torricco DD, No HK, Prinyawiwatkul W, Janes M, Corredor JA and Osorio LF, Mineral oil–chitosan emulsion coatings affect quality and shelf-life of coated eggs during refrigerated and room temperature storage. *J Food Sci* **76**:S262–S268 (2011).
- 55 Jones DR, Egg functionality and quality during long-term storage. *Int J Poult Sci* **6**:157–162 (2007).

- 56 Kampf N, Martinez CG, Corradini MG and Peleg M, Effect of two gums on the development, rheological properties and stability of egg albumen foams. *Rheol Acta* **42**:259–268 (2003).
- 57 Alleoni ACC and Antunes AJ, Albumen foam stability and s-ovalbumin contents in eggs coated with whey protein concentrate. *Braz J Poult Sci* **6**:105–110 (2004).
- 58 Narsimhan G, A model for unsteady state drainage of a static foam. *J Food Eng* **14**:139–165 (1991).
- 59 Cordts C, Schmutz M and Preisinger R, New Alternatives for Improving Egg Shell Stability Through Breeding. *Lohmann Information* **26**:1–4 (2002).
- 60 Shafey TM, Hussein EOS and Al-Batshan HA, Effects of ultrasonic waves on eggshell strength and hatchability of layer-type breeder eggs. *S Afr J Anim Sci* **43**(1):56–63
- 61 Galis A-M, Dale LM, Boudry C and Théwis A, The potential use of near-infrared spectroscopy for the quality assessment of eggs and egg products. *Sci Works C Series Vet Med* **LVIII**:294–307 (2012).
- 62 Nicolai BM, Beullens K, Bobelyn E, Peirs A, Saeys W, Theron KI *et al.*, Non-destructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. *Postharvest Biol Technol* **46**:99–118 (2007).
- 63 Yukihiro O and Berry RJ, Sampling techniques in near-infrared transmission spectroscopy: mid- and near-infrared transmission spectroscopy, in *Handbook of Vibrational Spectroscopy*, ed. by Chalmers JM and Griffiths PR. Vol 2. John Wiley, Chichester, pp. 932 (2002).