An ultrasound based technique for the determination of poultry egg quality

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Abstract

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The present study investigates the possibility of the non-destructive prediction of the main quality indices of commercial eggs by calculating the ultrasound phase velocity within the egg material. The phase velocity of the ultrasound signal in the egg material was determined by analysing the recorded ultrasound signals using the Fast Fourier Transform. Three hundred commercial eggs (Boris Brown, 33 weeks age) from the first day of egg laying were purchased from a farm and divided in two groups. The first group was kept at the room temperature (22–25°C) and the second group was kept in a refrigerator (5°C). Every week, 25 eggs from both the room and the refrigerator were first submitted to the non-destructive ultrasound test at weekly basis at the room temperature. Immediately after testing, the air cell, the thick albumen heights, the Haugh unit and the yolk index of the eggs were also determined destructively for the comparison purposes. The results were analysed to find any possible correlation between the computed ultrasonic phase velocity and the destructive parameters, during a storage period of five weeks. The tests were carried out using an ultrasound beam with a frequency of 150 kHz with a sampling rate of 2.5 Gs/S on the eggs under a controlled temperature situation. Significant differences between the means of the destructive analysis on different days of the eggs storage were found using ANOVA. The results showed that the phase velocity significantly differs between the eggs stored at the room temperature and those stored in the refrigerator. It was found that the phase velocity decreased as the storage time of the eggs increased in three consecutive weeks.

Keywords: phase velocity; poultry egg; quality; ultrasound; freshness

Eggs are a non-expensive, but very nutritious food. Several chemical-physical modifications occur inside an egg during the storage period. Easily observable physical changes include an increase in the air cell, thinning of the thick albumen, and flattening of the yolk. The most evident one of these changes is the increase in the air cell mainly due to the loss of water and CO_2 through the shell and also the changes related to the ageing of the albumen and the yolk (Stadelman, Cotterill 1995). The albumen that surrounds the yolk, which is called the thick albumen, progressively liquefies and thins with time, transforming itself into thin albumen. This phenomenon is caused by the deterioration of

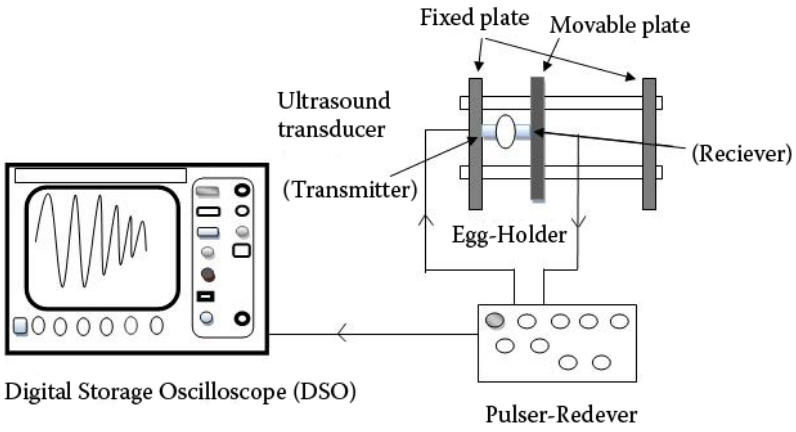

Fig. 1. Experimental arrangement setup

the gelatinous structure of the albumen that is due to the changes in the complex lysozime-ovomucine caused by the increase of pH during the storage period (Cotterill, Winter 1955; Li-Chan, Nakai 1989; Thapon, Bourgeois 1994). Another obvious change that can be observed during egg ageing is the flattening of the yolk caused by the weakening of the vitelline membrane (Fromm, Matrone 1962). These changes are used for the determination of the quality indices of the eggs. The air cell height is adopted by the European community to select eggs according to their freshness (EC 2003) while the Haugh unit, an index based on the thickness of the thick albumen and the egg mass (Haugh 1937), is adopted in the USA (USDA 1995). Another well-known quality parameter is the yolk index obtained by dividing the height by the diameter of yolk (Funk 1948). Many complex changes occur within an egg during the storage period which mostly affects the functional properties of the egg yolk and the egg albumen. These changes include: albumen thinning, increase in pH, weakening and stretching of the vitelline membrane and increasing of the yolk water content. Haugh unit values are provided for the U.S. standards for the quality of individual eggs (USDA 1995), together with the air cell measure. The air space develops by the separation of the two shell membranes immediately after the egg laying which subsequently increases in height as CO_2 and the moisture are progressively lost through the shell pores (Romanoff, Romanoff 1949).

One of the practical ways of determining the apparent quality of eggs is the candle inspection of eggs, which is a time consuming, laborious procedure, and human subjective. Basically, it is still said that the candling method is tedious and not optimal to ensure the egg quality in the future. However, eggs with tiny cracks on the shell surface are difficult to be inspected by human vision (Karoui et al. 2006). Low intensity ultrasound can be used to provide the information on the physical-chemical properties of many foods (Povey, Wilkinson 1980; Curtis et al. 1986; Javanaud 1988; Povey, McClements 1988; McClements 1994, 1995). It has been used to determine the composition, structure, and physical state of a variety of food materials. Ultrasound has advantages over many traditional analytical techniques being a rapid, non-destructive and precise measurement which can be done in a laboratory or on-line (Knorr et al. 2004). In this study, the ultrasonic method has been used as a practical method for monitoring the egg freshness. The objective of this study has been to find the difference between aged and fresh commercial eggs based on the non-destructive ultrasound technique (phase velocity) in comparison with the destructive methods (Haugh unit, air cell height, yolk height, yolk diameter, yolk index, and thick white height of the eggs) during different storage conditions.

MATERIALS AND METHODS

Sample preparation

The tests were conducted on three hundred commercial eggs having medium size and collected on the first day of egg laying by Borris Brown hens (33 week-old) in October 2008 from Kei farm near Kyoto city, Japan. On arrival in the laboratory, 50 of them were analysed and the residue were divided in two groups. The first group was kept at the room temperature (22–25°C), and the second group was kept in a refrigerator (5°C). The eggs were first submitted to the non-destructive and then to the destructive analysis. Every week, 25 eggs from the room and 25 eggs from

the refrigerator were selected randomly and allowed to equilibrate to the room temperature (24°C) before the non-destructive tests (ultrasound signal passing through the egg) were performed. The eggs were inspected individually, and the cracked eggs were discarded. The sampling continued for five-week storage period. These time steps were chosen because the quality parameters are supposed to be related to the eggs storage duration. In a parallel, ultrasound signal testing of 25 eggs stored in the room and also in the refrigerator followed non-destructively.

Ultrasonic measurement (non-destructive test)

The measurements of the ultrasound signals were carried out on intact eggs using a special probe and an egg holder which had been designed and developed for this purpose. Fig. 1 shows the ultrasonic measurement system. Two ultrasonic transducers (150 KHz, AE-901U, NF Electronic Instruments, Japan) were respectively fixed on two fixed and movable plates. These were identical transducers, one of which having been used as a transmitter and the other one as a receiver. The movable part could easily move on the four rods while the eggs were fixed between the attached probes. In this method any motion of the probe and eggs was confined.

The experimental set for the egg testing included two ultrasonic probes, one attached onto a movable plate and the other one onto a fixed plate with a transmitter-receiver system that provided the transmission and reception of the ultrasonic signals which passed through the egg and oscilloscope (LeCroy Wavesurfer 24Xs 200MHz). The repetitive pulse signal was input to the transmitter from the pulser-receiver (5055PR, Panametrics) to generate ultrasound signals. All data were stored on the digital oscilloscope at specific time intervals with the sampling rate 2.5 GHz (every week). Meanwhile, the ultrasonic signal testing was performed non-destructively on 25 eggs kept in the room and 25 eggs kept in the refrigerator conditions for 5 weeks as a control treatment. The measurements were carried out with the eggs in their vertical position around their longitudinal (vertical) axes.

Phase velocity

The velocity of the ultrasound signal within a material is one of the key acoustic parameters for a non-invasive diagnosis of the material quality. The sound velocity, determination has found applications in such areas as ultrasound based bone densitometry. Sound waves are longitudinal waves that travel away from the sound source. Each point on the source can be considered as a point source which generates a sound wave. Considering all these waves and connecting the positions of same phase on them, the resulting lines are known as the phase fronts. The phase fronts are travel with the speed of the sound in the medium, hence the name "phase velocity".

In this research, the Fast Fourier Transform (FFT) of the transmittance ultrasound signal (the ultrasound signals passed through the egg material and was recorded on the opposite side) was used to determine the phase velocity (sound velocity) within the egg material. In the following calculations, it was assumed that the eggshell thickness is the same for all eggs. In the proposed method, the ultrasound velocity within the egg material (egg liquid) was compared to its velocity within the water which replaced the egg material in the shell. The proposed equation for calculating the phase velocity is as follows:

$$\frac{1}{C_{egg}} = \left(1 - \frac{\Delta D}{D_{in}}\right)\frac{1}{C_w} + \frac{\Delta\varphi}{2\pi f \times D_{in}}$$

where:

C_{egg} – ultrasound phase velocity within the egg material (m/s)

C_W – ultrasound phase velocity within the water (after removing the egg liquid from the shell and replacing it with water, m/s)

$\Delta\varphi$ – phase difference between the signals passing through the egg liquid and water (rad)

D_{in} – inside diameter of the egg obtained by subtracting the shell thickness from the egg diameter (mm)

ΔD – difference between D (diameter of the whole egg or distance between the ultrasound probes) and D' (diameter of the egg shell moved between the ultrasound probes and filled with water)

f – ultrasound signal frequency (150 KHz)

The sound velocity of fresh water within the egg shell at 25°C (1,497 m/s) is used as the reference velocity for the comparison.

Destructive test

The following quality parameters were considered as the eggs quality indicators during storage: storage time (from laying; days), the weight loss (g),

Table 1. Mean values of main physical attributes measured at six different times of 25 eggs stored in the refrigerator (5°C and 75% humidity)

Day of storage	Weight (g)	Thick albumen height (mm)	Haugh unit	Yolk diameter (mm)	Yolk height (mm)	Yolk index	Air Cell height (mm)	Phase velocity (m/s)
0	60.7705 (1.56)	8.40^{a} (0.85)	88.912^{a} (3.2)	39.875^{a} (1.4)	17.345^{a} (1.01)	0.432^{a} (0.02)	3.52^{a} (0.2)	1573.72^{a}
7	60.0718 (1.45)	6.48^{b} (0.51)	80.131^{b} (3.8)	40.720^{ab} (0.90)	17.017^{a} (0.4)	0.421^{ab} (0.01)	4.34^{b} (0.4))	1558.32^{a}
14	59.729 (1.69)	6.176^{cb} (0.65)	76.22^{c} (5)	41.518^{bc} (1.81)	16.656^{ba}	0.406^{bc} (0.01)	4.86^{c} (0.4)	1552.17^{b}
21	59.391 (1.50)	5.987^{c} (0.25)	73.476^{dc} (2)	42.03^{c} (1.4)	16.285^{c} (0.3)	0.393^{cd} (0.01)	5.08^{c} (0.3)	1540.12^{b}
28	59.089 (1.80)	5.421^{d} (0.4)	69.642^{e} (3)	42.313^{c} (1.04)	16.033^{c} (0.45)	0.378^{d} (0.01)	5.26^{d} (0.5)	1543.18^{b}
35	58.971 (1.70)	5.031^{e} (0.35)	67.280^{e} (4)	42.87^{c} (1.3)	15.680^{d} (0.45)	0.35389^{e} (0.02)	5.60^{d} (0.4)	1544^{b}

Differences between means with the same letter within a column are not significant at $P < 0.05$; values in parentheses are standard deviations for 25 samples

air cell height (mm), thick albumen height (mm), Haugh unit, diameter and height of the yolk, and the yolk index. The air cell height was measured as the distance from the base of the shell to the contact line of the membrane to the shell, at three equidistant points and at the middle point of the same membrane. For this purpose, the egg shell was broken and the cup of shell containing the air cell was placed on paste on a horizontal flat surface to ensure that the membrane of the air cell was roughly horizontal. The air cell height was then calculated by averaging the values of these four points. The thick albumen height was calculated as the mean of three measurements taken at about 10 mm from the yolk using a tripod digital calliper. The average of this three point values is the thick albumen height. The Haugh unit was calculated by means of the Haugh Eq. (2) where the thick albumen height and egg mass are considered (Haugh 1937).

$$HU = 100 \log(H - \frac{\sqrt{G}(30W^{0.37} - 100)}{100} + 1.9)$$

where:
H – albumen height (average; mm)
G – gravitational constant (32.2)
W – weight of the egg (g)

The higher is the Haugh unit, the fresher is the egg. The albumen of fresher eggs does not spread out as much on the plate, and such eggs have a greater number of Haugh units. The spherical nature of the yolk is one of the quality characteristics of the yolk. It can be expressed as a yolk index. The yolk height and diameter were measured with a tripod digital calliper. The yolk index was determined by dividing the height of the yolk by its diameter (Funk 1948). All measurements were conducted in a temperature-controlled room (24–25°C) while

Table 2. Mean values of main physical attributes measured at six different times of 25 eggs stored in the room (24°C and 40% humidity)

Day of storage	Weight (g)	Thick albumen height (mm)	Haugh unit	Yolk diameter (mm)	Yolk height (mm)	Yolk index	Air Cell height (mm)	Phase velocity (m/s)
0	60.77 (1.597)	7.78^{a} (1.0)	87.43^{a} (8)	39.87^{a} (1.5)	17.51^{a} (0.82)	0.44^{a} (0.02)	3.58^{a} (0.47)	1571.39^{a}
7	59.34 (2.183)	5.15^{b} (0.75)	69.3^{b} (6)	42.520^{b} (0.595)	15.85^{b} (0.727)	0.37^{b} (0.01)	5.60^{b} (0.36)	1554.03^{a}
14	59.52 (1.64)	4.8^{b} (1.2)	64.74^{b} (7)	43.868^{c} (1.13)	14.53^{c} (0.55)	0.32^{c} (0.01)	6.73^{c} (0.39)	1525.06^{b}
21	58.76 (1.654)	3.50^{c} (0.65)	51.8^{c} (8)	45.412^{d} (1.39)	13.16^{d} (0.82)	0.29^{d} (0.02)	7.63^{d} (0.65)	1514.86^{b}
28	57.80 (1.817)	3.167^{c} (0.91)	48.4^{c} (6)	45.883^{d} (0.858)	13.06^{d} (0.85)	0.28^{d} (0.01)	9.26^{e} (0.62)	1518.11^{b}
35	56.44 (2.093)	2.9^{d} (0.7)	43.1^{d} (7)	46.514^{e} (0.941)	11.77^{e} (1.5)	0.25^{e} (0.02)	11.8^{f} (1.5)	1513^{b}

Differences between means with the same letter within a column are not significant at $P < 0.05$; values in parentheses are standard deviations for 25 samples

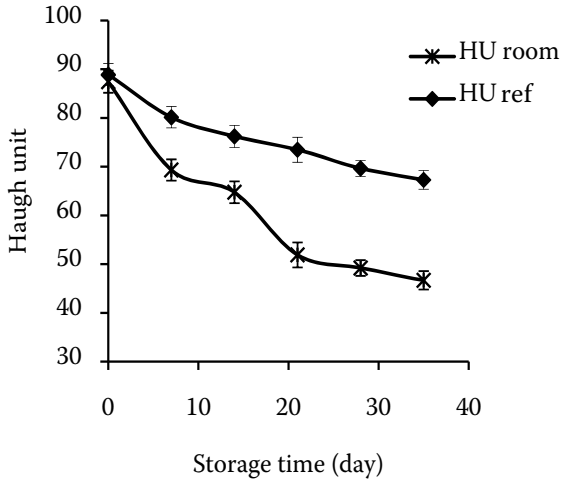

Fig. 2. Haugh unit under different storage conditions

the selected refrigerated eggs were brought to room temperature before every experiment.

RESULTS AND DISCUSSION

The initial properties and averages of the measurements results taken for each group of 25 eggs after each time interval are presented in Tables 1 and 2. The mean values and standard deviations (SD) are given for the destructive tests of all 300 eggs, measured up to 35 days of storage at 5-week intervals. The sample eggs had a mean mass of 60.77 g. The air cell height significantly increased from the 2nd day to day 35 of the storage period. In the eggs kept in the refrigerator, the air cell height was roughly doubled in the study period while it was tripled in those kept in the room temperature. However, the thick albumen height, the Haugh unit and the yolk index decreased significantly over the same period comparing the eggs kept in the room to those kept in the refrigerator.

Significant differences (at *P*-level < 0.05) between the mean values of the freshness parameters (air cell, thick albumen heights, Haugh unit, and yolk index) at different storage durations were found using ANOVA (Analysis of variance). Duncan's multiple range tests were used to find if there exists any significant differences between the variance means (SAS 9.1 for Windows).

Tables 1 and 2 show the number of the eggs tested each week together with the average of their characteristic parameters including the Haugh unit (HU), the yolk index (YI), the air cell height (Air Cell), and the egg weight.

It was observed that, as the age of the eggs increases, the ratio of the thick white to the thin white decreases. The comparison of the two tables shows that the decreasing rate of the thick albumen height at the room temperature is much higher than that in the refrigerator. This indicates that the temperature is one of the main factors influencing the egg quality during storage. The liquefaction of the thick white is largely influenced by the storage temperature. The comparison of these two tables reveals that Haugh unit quickly decreases. However, the changes in the quality of eggs kept in the refrigerator (from 88.9 to 67.2 HU) were smaller than those of the eggs kept in the room (from 87.4 to 43.1 HU), up to the end of the storage period (Fig. 2). Indeed, during storage the concentration of the free amino acid content in the egg white increases from 0.14 to 2.3 μmol under various conditions of storage (Ducay et al. 1960). During storage, some well-known physical and chemical modifications taking place are the thinning of the thick albumen (Kato et al. 1981), and mainly the increase of albumen pH caused by the loss of carbon dioxide from the egg through the pores in the shell (Hill, Hall 1980). A rapid loss of CO_2 occurs particularly with the albumen, leading to a decrease in quality until the state of gas balance is reached between the inside and outside of the egg. Albumen pH increases with the loss of CO_2 from the egg. *Carbon dioxide* migration from the egg results in an increase in albumen pH and this phenomenon is caused by the deterioration of the gelatinous structure of the albumen.

Tables 1 and 2 show how the yolk index is decreasing. It depends on the ratio of the yolk height to its diameter. Flattening of the yolk is primarily due to the

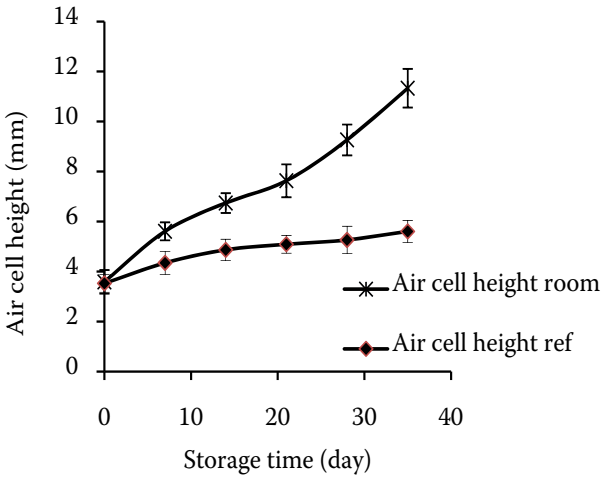

Fig. 3. Air cell size under different storage conditions

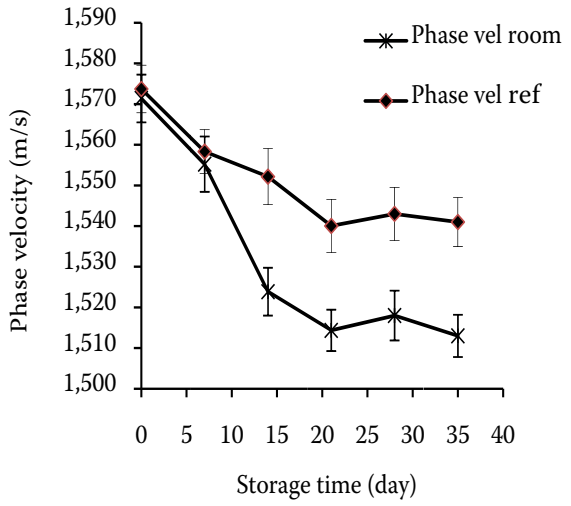

Fig. 4. Phase velocity under different storage conditions

water content increase caused by osmotic migration from the albumen through the vitelline membrane.

Fig. 3 shows that another index of egg quality is the air cell height. It can be found easily that the egg quality changes were smaller in the refrigerator (from 3.52 to 5.60 mm) than those in the room (from 3.58 to 11.8 mm). Fig. 4 shows the relations between the phase velocities at 150 kHz under various storage conditions. The phase velocities are correlated with the storage time. It seems that the phase velocity decreased similary as Haugh unit decreased during the storage time under different conditions. Fig. 5 shows this trend following 25 eggs for 5 weeks. After three weeks, some fluctuation of the data occurred. This fluctuation is not well known, but for three weeks the phase velocity had decreased, then we focused on three weeks. Fig. 4 shows the following phase velocity of eggs (25) every week using the destructive method. These experiments were carried out in two stages, first the ultrasound signals of the egg samples were recorded and subsequently the destructive parameters were measured. This treatment was done every week with 25 eggs stored in the room and with 25 of those kept in the refrigerator storage conditions. In parallel, we followed ultrasonic signals of 25 eggs without breaking eggs every week (Fig. 5). The trend of the phase velocity decrease in Fig. 4 is almost the same as in Fig. 5. These figures show that the phase velocity at the room temperature decreased to a greater extent compared to the refrigerator temperature. This trend may relate to the changes in viscosity and density of the thick white during storage (Donovan et al. 1972; Robinson, Monsey 1972; Robinson, Chokyun 1981). In other words, it can be related to liquefaction of egg changing thick white to thin white, and to yolk flattening. It seems that the sound velocity is affected not only by viscoelasticity but also by the density of the thick white.

The mean values of the Haugh unit, air cell height, and the phase velocity of the egg samples kept in the refrigerator and those kept at the room temperature are shown in Tables 1 and 2. The lower was the Haugh unit in the eggs in the refrigerator, the lower was the phase velocity in them (1,573 m/s on the first day as compared to 1,540 m/s after 21 days). Similar changes of the phase velocity are found with the eggs at the room temperature (1,571 m/s on the first day as compared to 1,514 m/s after 21 days).

CONCLUSIONS

An ultrasonic wave technique was implemented and evaluated as a possible means for non-destructive and non-invasive evaluation of the quality of commercial poultry eggs during different storage condition. The phase velocities of the ultrasonic signal propagated through the poultry egg were measured in the room and refrigerator storage conditions. At the same time, the eggs were broken and the destructive parameters were recorded. Both the Haugh unit and yolk index decreased with time over 5 weeks of storage both in the room and the refrigerator indicating the deterioration of the eggs quality. The mean value of the phase velocity change during storage was the same as the change of Haugh unit and of air cell height during the same period. Further study is needed to find the relation

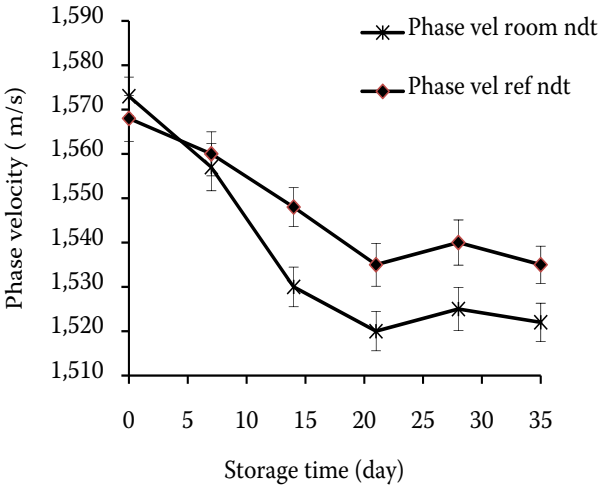

Fig. 5. Phase velocity under different storage conditions in following 25 eggs every week

between the sound velocity and viscoelasticity parameters of the egg liquid during storage. Although the phase velocity could not always correctly predict the Haugh unit and air cell height of individual eggs, it can be used to estimate the quality of the group of eggs with high thick albumen. This study shows that the ultrasound phase velocity has the potential to recognise the differences between fresh and aged eggs up to three weeks after laying.

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