Acoustic properties of egg yolk and albumen in the frequency range 20–400 MHz

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(Received 1 April 1997; accepted for publication 22 August 1997)

The acoustic propagation properties of egg yolk and albumen are characterized in the frequency range 20–400 MHz by the bioultrasonic spectroscopy system using an ultrasonic transmission comparison method. Significant differences in the attenuation, velocity, impedance, and density among yolk and thick and outer thin albumen are observed. The acoustic properties of 10% aqueous solutions of ovalbumin and bovine hemoglobin are also measured in order to investigate the contribution of proteins to the acoustic properties of albumen. The differences obtained between thick and outer thin albumen may be mainly due to their macromolecular level structural differences, as their constituents are nearly the same. © *1997 Acoustical Society of America.* [S0001-4966(97)04212-4]

PACS numbers: 43.80.Cs, 43.80.Jz, 43.80.Ev, 43.35.Bf [FD]

INTRODUCTION

Studies on biological tissue characterization using ultrasound have been conducted extensively. In general, quantitative analysis of the acoustic properties of biological tissues is difficult because of their complicated structures and various constituents, including several kinds of proteins and lipids. Poultry egg provides readily accessible biomacromolecular specimens for which the acoustic attenuation coefficient and velocity have been studied.[1–3] Choi *et al.* measured the ultrasonic absorption of albumen in the frequency range 0.2–10 MHz over the temperature range of 10 °C–50 °C.[1] Javanaud *et al.* reported the velocities of egg yolk and albumen in the frequency range 2–7 MHz and the absorption coefficients in the frequency range 2–124 MHz.[2] However, in both studies differences of the acoustic properties between thick and thin albumen were not observed. Povey and Wilkinson measured the velocities and attenuations at 1.25 MHz and reported that the attenuation of thick albumen is greater than that of thin albumen.[3] However, they did not detect differences in velocity between thick and thin albumen.

The reported data from the previous studies did not have sufficient measurement accuracy and resolution to distinguish the difference in viscoelasticity between thick and thin albumen, recognized easily by human sensibility. For a better understanding of the acoustic properties of egg yolk, and thick and thin albumen, more reliable data over a much broader frequency range are necessary.

The bioultrasonic spectroscopy system has been developed for biological tissue characterization in the VHF and UHF ranges[4] and applied to fundamental studies of bovine tissues in the frequency range 20–200 MHz.[5] The acoustic properties, viz., attenuation coefficient, velocity, impedance, and density, can be determined with high accuracy by an ultrasonic transmission comparison method using distilled water as the reference.

In this paper, egg yolk and albumen as biological macromolecular liquids are characterized in the frequency range 20–400 MHz. The acoustic properties of aqueous solutions of ovalbumin and bovine hemoglobin are also measured to provide details of the ultrasonic behavior of some biological macromolecules to help in understanding the behavior of the acoustic properties of thick and thin albumen.

I. MEASUREMENT METHOD

The measurement method and system is described in detail elsewhere.[4] The experimental configuration for characterization is shown in Fig. 1, in which a specimen is inserted between the parallel surfaces of synthetic silica (SiO_2) buffer rods having ZnO piezoelectric film transducers on their outer ends. A rf burst signal is supplied to the transmitting transducer TR(1) in order to generate ultrasonic longitudinal plane waves, which then propagate in the buffer rod. The ultrasonic attenuation coefficient, velocity, impedance, and density are determined by measuring the transducer outputs V_i $(i=1-3)$ in the ultrasonic transmission line model, as shown in Fig. 1. In the measurement method, measurement errors due to diffraction losses in the acoustic media, and to mode conversion at the buffer-rod/sample interfaces, can be corrected experimentally by performing measurements for both sample and distilled water as medium 2. The gap length between the two buffer rods is determined by measuring frequency characteristics of the interference output of V_1 and V_2 for distilled water of which the velocity is used as the reference.[4] The attenuation coefficient and velocity of the sample were measured in the transmission mode using the output V_3 of the transducer TR(2).

The velocity is obtained by the z-interference method,[4] in which the interference output of V_3 and the reference electrical signal derived from the signal generator of the measurement system is measured as a function of gap length. The acoustic impedance is obtained with the pulse echo

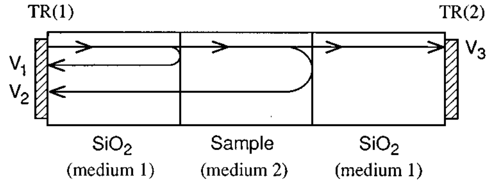

FIG. 1. Definition of transducer outputs V_i for measuring acoustic properties of liquid samples.

method by measuring V_1 for sample and for air as medium 2, in order to determine the reflection coefficient at the buffer-rod/sample interface, and by using the acoustic impedance of SiO_2 as the reference. The attenuation coefficient is obtained by measuring V_3 and by using the reflection coefficient determined in the impedance measurement with the known attenuation coefficient of distilled water. The density ρ is calculated by using the equation that $\rho = Z(f)/v(f)$, where $Z(f)$ is the acoustic impedance and $v(f)$ is the velocity. The acoustic impedance and velocity in acoustically lossy media have a frequency dependence, whereas the density is independent of frequency. The velocity and attenuation coefficient of water reported in the literature,[6,7] and the acoustic impedance of SiO_2 measured to be 13.11×10^6 Ns/m^3 were employed as the reference data.

II. EXPERIMENTS AND RESULTS

Measurements were conducted on yolk and albumen of white leghorn eggs. They were purchased at a wholesale store within less than 12 h of being laid and stored in a 5 °C temperature refrigerator until the measurement preparation procedure began. The specimens were thus measured within less than 36 h of being laid. The albumen is divided into three concentric layers, viz., outer thin albumen, thick albumen, and inner thin albumen. The yolk is composed of alternate yellow and white yolk layers. For measurements, outer thin albumen, thick albumen, and mixture of yellow and white yolk were used. After the egg shell was carefully broken on a glass plate, the thick albumen surrounding the yolk was spread and the outer thin albumen was spread beyond the thick albumen. The sample was taken from the spread material by a syringe having an inner diameter of 1 mm, and no needle attached. Yellow and white yolk became mixed during the procedure. The volume of sample required for measurements was about 1 ml, which was much less than the content of one egg.

The parameters of the ultrasonic devices used for measurements are listed in Table I.

The measurement accuracy in the present study is estimated to be better than ±0.1% for velocity and ±1% for attenuation coefficient, impedance, and density. The reproducibility is estimated to be better than ±0.02% for velocity and ±0.5% for attenuation coefficient, impedance, and density.

TABLE I. Parameters of the acoustic devices used for measuring acoustic properties of specimens (T: transmitter; R: receiver).

Device No.	No. 1 (T)	No. 1 (R)	No. 2 (T)	No. 2 (R)
Center frequency	150 MHz	150 MHz	400 MHz	400 MHz
Transducer material	ZnO	ZnO	ZnO	ZnO
Diameter of transducer	2.5 mm	2.5 mm	1.3 mm	1.3 mm
Diameter of buffer rod	8 mm	20 mm	8 mm	20 mm
Length of buffer rod	8 mm	8 mm	8 mm	8 mm

The measured attenuation coefficients of thick and outer thin albumen in the frequency range 30–400 MHz at 23.1 °C–23.4 °C are shown in Fig. 2. Two pairs of devices (see Table I), with the center frequencies of 150 and 400 MHz, were employed with the gap lengths 1200 μm for 30–150 MHz and 600 μm for 150–400 MHz, respectively. The samples for the measurements in the two frequency ranges were taken from different egg samples. The attenuation coefficients of thick albumen are greater than those of outer thin albumen in this frequency range, for example, 9.3% greater at 100 MHz. Povey and Wilkinson reported that the attenuation for thick albumen is greater than that for thin albumen at the frequency of 1.25 MHz.[3] The attenuations for both thick and outer thin albumen are proportional to the 1.4 power of frequency around 30 MHz, the 1.4–1.5 power around 100 MHz, and gradually increase with frequency up to the 1.7–1.8 power around 400 MHz.

The measured attenuation coefficients of egg yolk in the frequency range 25–400 MHz at 23.9 °C using a pair of ultrasonic devices with a 400-MHz center frequency (see Table I) are shown in Fig. 2. The gap lengths of the two ultrasonic devices were 1500, 300, and 100 μm, in the frequency ranges 25–70, 70–250, and 250–400 MHz, respec-

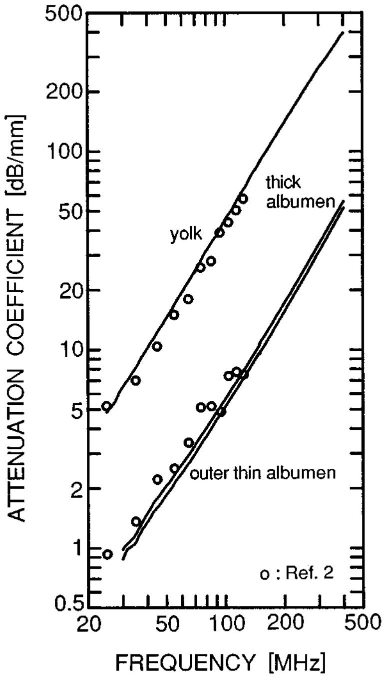

FIG. 2. Frequency dependence of attenuation coefficients of thick albumen and outer thin albumen at 23.1 °C–23.4 °C, and of yolk at 23.9 °C.

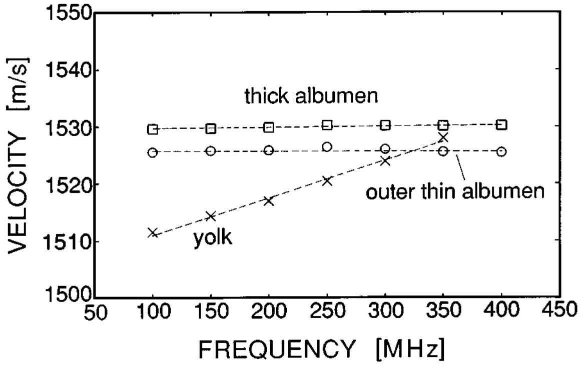

FIG. 3. Velocity dispersion of yolk, and thick and outer thin albumen at 23.4 °C–23.7 °C. Broken lines fitted by the method of least squares.

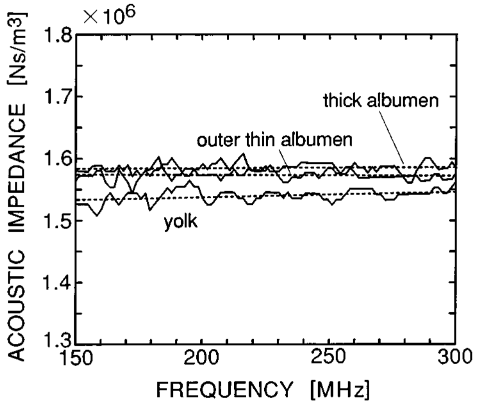

FIG. 4. Frequency dependence of acoustic impedance of yolk, and thick and outer thin albumen at 23.4 °C–23.7 °C. Broken lines fitted by the method of least squares.

tively. The attenuation coefficients of yolk are about ten times greater (in decibels) than those of albumen. The attenuation for yolk exhibits different frequency dependences than those for albumen. The attenuation for yolk is proportional to the 1.65 power of frequency in the range 25–300 MHz and dropping to the 1.5 power of frequency around 400 MHz.

The open circles in Fig. 2 show the published data measured by Javanaud *et al.* for a mixture of thin and thick albumen in the temperature range 21.5 °C–23.5 °C and yolk in the range 20 °C–25 °C, respectively.[2] The measured attenuations of yolk and albumen compare well with the data reported by Javanaud *et al.*[2]

Figure 3 shows the measured velocities for yolk in the frequency range 100–350 MHz and for thick and outer thin albumen in the frequency range 100–400 MHz at the temperatures of 23.4 °C–23.7 °C. Yolk exhibits clear velocity dispersion characteristics and thick albumen shows slight dispersion. The velocity dispersion values of 0.065 (m/s)/MHz for yolk and of 0.0019 (m/s)/MHz for thick albumen were obtained by linear approximation, as shown in Fig. 3. On the other hand, the velocity dispersion for outer thin albumen was not seen in the frequency range measured. The values of velocities for thick albumen were greater than those for outer thin albumen and the differences for the three samples ranged from 4–15 m/s.

Figure 4 shows the acoustic impedances for yolk and thick and outer thin albumen determined by measuring the reflection coefficients at the buffer-rod/sample boundary in the frequency range 150–300 MHz at the temperatures of 23.4 °C–23.7 °C. Yolk exhibits a small frequency dependence of impedance, with a rate of increase of 88 (Ns/m^3)/MHz.

Table II shows the values of the attenuation coefficient, velocity, and acoustic impedance for yolk and thick and outer thin albumen from three different eggs at 200 MHz, along with the densities calculated by dividing the measured acoustic impedances by the measured velocities. Significant differences in each acoustic parameter among yolk and thick and outer thin albumen are obtained.

The densities were also measured with a pycnometer in order to compare them with the data obtained by our ultrasonic method. The pycnometer has a volume of 10 ml and needed three eggs for each measurement. The measured results, which are average values of two different sample groups, are shown in Table II. The differences between the measured values obtained with the ultrasonic method and those with the pycnometer are 0.19%, 0.19%, and 1.8% for thick and outer thin albumen, and yolk, respectively. The measured results by the two methods agree well with each other.

III. DISCUSSION

Table III shows the percentages of water, protein, lipid, carbohydrate, and mineral contents of egg yolk and albumen.[8] As seen, egg yolk has a rich lipid content averaging 33% and a water content of 49%, and albumen is rich in water and protein with a total percentage of 99%. Thus it is tempting to ascribe the differences in the measured acoustic properties of yolk and albumen to the differences in their constituents. As the protein content of albumen is about 10% and its water content is about 88%, a 10% aqueous solution of protein may be considered a simple model of albumen. The acoustic parameters of aqueous solutions of proteins [Ovalbumin (Sigma Chemical Co., A-5503) and bovine he-

TABLE II. Measured attenuation coefficients, velocities, impedances, and densities of egg yolk and albumen at 23.4 °C–23.7 °C.

	α (dB/mm) (200 MHz)	v (m/s) (200 MHz)	Z ($\times 10^6$ Ns/m^3) (200 MHz)	ρ (kg/m^3) Ultrasonic method	ρ (kg/m^3) Pycnometer
Outer thin albumen	13.6 (13.3–14.2)[a]	1527 (1525–1529)	1.576 (1.566–1.588)	1032 (1027–1039)	1034
Thick albumen	15.7 (14.6–16.9)	1535 (1530–1540)	1.585 (1.553–1.616)	1033 (1015–1049)	1035
Yolk	129 (116–139)	1522 (1517–1526)	1.531 (1.520–1.537)	1006 (996–1013)	1024

[a] Example, mean value 13.6 dB/mm, total range of values 13.3–14.2 dB/mm.

TABLE III. Average percentages of the major groups of chemical compounds in yolk and albumen of chicken egg.[8]

Component	Average percentage	
	Yolk	Albumen
Water	48.7%	87.9%
Proteins	16.6%	10.6%
Lipids	32.6%	trace
Carbohydrates	1.0%	0.9%
Minerals	1.1%	0.6%

TABLE IV. Proteins in albumen.[9]

Protein	Molecular weight (Dalton)	Relative abundance in albumen
Ovalbumin	45 000	60%–69%
Conalbumin	76 000	9%–17%
Ovomucoid	27 000	9%–14%
Lysozyme	14 500	2%–4%

moglobin (Hb) (Sigma Chemical Co., H-2625)] were measured and the contribution of protein and water to the acoustic properties of albumen is assessed. Solutions, prepared with protein and distilled water, were stirred at room temperature and degassed under vacuum. Ten percent weight-fractional aqueous solutions of the two proteins were studied.

Figure 5 shows the measured frequency characteristics of attenuation coefficients for 10% aqueous solutions of ovalbumin and bovine hemoglobin at 23.7 °C and 23.8 °C, respectively. The attenuation coefficients of thick and outer thin albumen were also measured at 23.2 °C for comparison. The ultrasonic devices with the center frequency of 400 MHz were used for the measurements. Clearly the attenuation coefficients for all four specimens have very nearly the same frequency dependence in the frequency range 100–400 MHz, being proportional to the 1.4–1.5 power around 100 MHz and to the 1.7–1.8 power around 400 MHz. It is noted that the bovine hemoglobin and ovalbumin solutions show similar frequency characteristics of the attenuation while their molecular components and structure are different. At 200 MHz, attenuation coefficients for the 10% ovalbumin solution, the outer thin albumen, the 10% hemoglobin solution, and the thick albumen are 13.8, 14.6, 15.6, and 17.4 dB/mm, respectively.

Previously, we reported the frequency characteristics of the attenuation coefficients of bovine hemoglobin aqueous solutions in the frequency range 70–200 MHz as a function of hemoglobin concentration.[5] It was observed that as hemoglobin concentration increases, attenuation increases and the exponent on frequency decreases monotonically; around 150 MHz the exponent decreases from 1.70 to 1.52 as the concentration increases from 5% to 25%. The curves of Fig. 5 may be used to support the view that the similarity of the magnitude and frequency dependences of the attenuation coefficients of the four specimens are determined to a great extent by their protein concentrations.

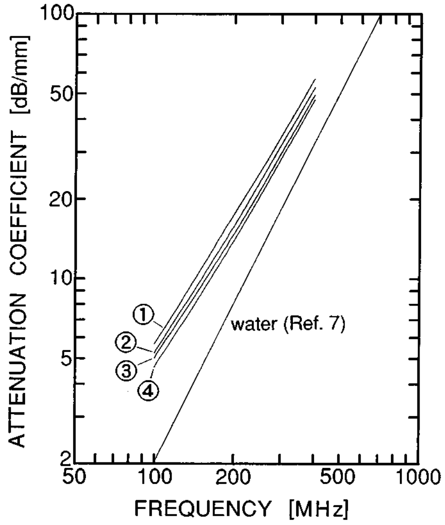

FIG. 5. Frequency dependence of attenuation coefficients of albumen and aqueous solutions of proteins. ① thick albumen (23.2 °C), ② 10% aqueous solution of bovine hemoglobin (23.8 °C), ③ outer thin albumen (23.2 °C), and ④ 10% aqueous solution of ovalbumin (23.7 °C).

Velocity dispersion was essentially not observed for the ovalbumin and bovine hemoglobin solutions in the frequency range 100–400 MHz, having values less than 0.001 (m/s)/MHz. The average values of velocities for 10% aqueous solutions of ovalbumin and hemoglobin in the frequency range were found to be 1522.2 m/s (23.7 °C) and 1520.9 m/s (23.6 °C), respectively; very close, though they represent different protein structures.

The molecular weights of ovalbumin and hemoglobin are 45 000 Dalton[9] and 64 000 Dalton,[10] respectively, while their attenuation coefficients of 10% aqueous solutions at 200 MHz are 13.8 and 15.6 dB/mm. Albumen consists largely of the proteins, ovalbumin, conalbumin, ovomucoid, and lysozyme; their molecular weights and relative abundance are shown in Table IV.[9] If it is assumed that the relative abundances of ovalbumin, conalbumin, ovomucoid, and lysozyme are 68%, 16%, 13%, and 3%, respectively, a calculated molecular weight of 46 700 Dalton for albumen can be obtained by the simple mixture law of summing each protein constituent's molecular weight multiplied by its relative abundance, yielding the value of 46 700 Dalton within 4% of the ovalbumin value of 45 000 Dalton. Table V shows the experimental results of the attenuation coefficients and velocities at 200 MHz for thick and outer thin albumen, and 10% aqueous solutions of hemoglobin and ovalbumin where it is seen that the magnitudes of both properties are greater in order of thick albumen, outer thin albumen, and 10% aqueous solution of ovalbumin. Lang and Rha[11] considered that the differences of the viscoelastic properties of thick and thin albumen are associated with their content of ovomucin which is four times greater in the thick albumen. A portion of ovomucin aggregates into filamentous super-aggregates with molecular weights of the order of 10^7 Dalton, which give the gellike character to the thick albumen. It would seem, from the measurements reported herein, that the attenuation and velocity value increases may be related to the super aggregations.

TABLE V. Measured attenuation coefficients and velocities in albumen and 10% aqueous solutions of ovalbumin and bovine hemoglobin.

	Attenuation (dB/mm) (200 MHz)	Velocity (m/s) (200 MHz)	Molecular weight (Dalton)
Thick albumen	17.4 (23.2 °C)	1535 (23.4 °C–23.7 °C)	
Outer thin albumen	14.6 (23.2 °C)	1527 (23.4 °C–23.7 °C)	
10% ovalbumin	13.8 (23.7 °C)	1522.2 (23.7 °C)	45 000[9]
10% hemoglobin	15.6 (23.8 °C)	1520.9 (23.6 °C)	64 000[10]

IV. CONCLUDING REMARKS

The acoustic properties of velocity, attenuation, impedance, and density for egg yolk and thick and outer thin albumen have been measured in the frequency range 20–400 MHz using the bioultrasonic spectroscopy system. In order to investigate the contribution of proteins to the acoustic properties of albumen, the acoustic properties of 10% aqueous solutions of albumin and bovine hemoglobin were also measured. The acoustic properties of albumen appear to be roughly dominated by their constituents of water and proteins. The differences of the acoustic properties between thick and outer thin albumen are considered to be mainly due to their structural differences at the macromolecular level as their constituents are nearly the same.

ACKNOWLEDGMENT

The authors would like to thank N. Chubachi for his helpful discussions and encouragement throughout this work.

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