Non-intrusive studies of gas contents and gas diffusion in hen eggs

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Abstract: A detailed study of the condition of eggs was performed using tunable diode lasers to monitor free gas in hen eggs. We detected oxygen and water vapor signals from 13 unfertilized eggs and studied the growth of the egg air cell over a time period of 3 weeks. We also studied the gas exchange through the egg shell, which is of particular interest for fertilized eggs. Four fertilized and five unfertilized eggs were followed over 3 weeks, the hatching period for hen eggs, and significant variations were found both in time and for the two types of eggs. Our results indicate that the techniques could be developed for automatic control of egg freshness, as well as for monitoring the hatching progress of fertilized eggs.

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1. Introduction

Hen eggs constitute an important nutrient and a very common food component in the daily life for most people [1]. With the increased awareness of food safety all over the world, food freshness has attracted an increased attention [2,3]. The suitability for consumption is a growing concern for all types of food, and recently much attention has been given to cases of contamination and best-before time tampering. In the case of hen eggs, it is not easy to assess the freshness non-intrusively [4,5]. There are some ways to evaluate the freshness of eggs: through determining the Haugh value, or the Yolk index, which relates to the shape of the egg white and the yolk, by determining the pH value of the egg white and by reflectance and fluorescence spectroscopy on the shell [6–10]. However, many of the techniques are intrusive or do not directly reflect the status of the egg interior. In contrast, we here present a method, which is fully non-intrusive, while probing the interior of the egg.

Oxygen availability affects all life. In poultry science, oxygen plays an important role in the hatching process, which can be divided into three periods; early stage, metaphase and late stage. In the different period, the embryos have different needs for oxygen [11]. Lack or overabundance of oxygen will negatively affect the growth and development of the embryos [12]. However, the knowledge in this field is limited. Since oxygen is present inside the egg due to diffusion through the shell [13–15], studies of this process are of considerable interest. The present work presents non-intrusive measurements of oxygen content in hen eggs, in order to assess the freshness. To follow the hatching process, progression the oxygen diffusion was as well monitored. We used gas in scattering media absorption spectroscopy (GASMAS) in the study [16,17]. This technique is non-invasive and critically utilizes the fact, that free gases exhibit very sharp optical absorption lines, which typically have about 10,000 times smaller linewidths in comparison with those of liquid or solids. The GASMAS technique has been used in many different areas, including food quality assessment [18,19] and medical monitoring [20–24]. The technique has been used in a preliminary study of gas in hen eggs [25]. The present study provides a considerably more detailed approach, and in particular, the diffusion processes through the egg shell have now been elucidated.
2. Materials and methods

2.1 Materials

Unfertilized eggs were bought from an Anhui province supplier. The eggs were sent by express delivery to our laboratory in Guangzhou directly after being laid, and we had an exact knowledge of the date. We stored the eggs at room temperature of 24°C and with 60 percent of relative humidity (RH). Figure 1 (a) illustrates the interior of a hen egg, and the detector position and the point of laser light entry are indicated. Figure 1(b) is a photograph of the arrangement. As illustrated in the figure the detector is placed at the egg shell above the air chamber with the light-injection fiber located at a fixed lateral position. Oxygen and water vapor absorption was detected in 13 eggs. Each egg was measured every second day for 10 minutes for oxygen and water vapor during a time period of 21 days.

In addition, five unfertilized eggs and four fertilized eggs were selected for an experimental study of gas exchange to determine the diffusion time constant of the eggs. The fertilized eggs were bought from the same egg supplier as mentioned. The fertilized eggs were kept in a hatching machine, with the temperature regulated close to 37°C and with an RH value at 70 percent, which was enabled by adding 50 ml water to the machine every day. Each unfertilized egg was put in a plastic bag, which was flushed with nitrogen for 30 minutes before the measurements for gas diffusion. In contrast, each fertilized egg was taken out of the hatching machine and put in a bag containing 30% oxygen for about 30 minutes before performing the gas diffusion measurements. These steps could ensure that the egg interior reaches a steady-state gas situation before the bags were opened, and the transition back to ambient atmospheric condition was monitored. The detection geometry was the same as described above. Signals from each fertilized egg were detected for 30 minutes every second day during its incubation period, and likewise, the unfertilized eggs were subject to the same type of measurements during 3 weeks.

2.2 Experimental set-up

A schematic diagram of the experimental system used in the study is shown in Fig. 2. One distributed feedback (DFB) laser (#LD-0760-0040-DFB-2, TOPTICA) operating at 760 nm was used as the spectroscopic light source to monitor the oxygen absorption lines. The other DFB laser (#LD-0937-0100-DFB-2, TOPTICA) operating at 937 nm, was used for water vapor detection. Each laser was connected to a laser driver (LCD201C, Thorlabs) and a temperature controller (TED200C, Thorlabs). The lasers had nominal output powers of 40
mW and 100 mW, respectively. In our Labview-controlled program, we used two analogue waves to modulate the laser outputs. A 5 Hz ramp wave was used to ensure that the laser could scan through the gas absorption line. A sinusoidal wave with a frequency of $f = 10295$ Hz and 9015 Hz, respectively, for the two gases, was used for acquiring the harmonic signals in the lock-in detection process. Those signals were sent to modulate the DFB diode lasers via an analog output (AO) channel of a data acquisition (DAQ) card (PCI6120, National Instruments). The wavelength modulation depth is optimized for maximum sensitivity, since GASMAS signals are always weak [26,27]. The laser light was transmitted through a fiber with a core diameter of 600 μm to the egg shell surface. We used a photodiode (S3204-08, HAMAMATSU) with an area of 18x18 mm² to monitor the scattered light intensity. Thereafter, the signal was boosted with a low-noise current amplifier (DLPCA-200, FEMTO) and converted into a voltage signal, which was fed to an analog input (AI) of the data acquisition (DAQ) card with a sampling rate of 400 kHz and a buffer size of 80000. Digital lock-in detection was used for retrieving signals with optimum signal-to-noise ratio [26].

Fig. 2. Experimental set up for Gas in Scattering Media Absorption Spectroscopy measurements on eggs.

3. Measurements and results

Before the measurements, we ensured that the eggs chosen for the study had no obvious anomalies. This was performed by candling, a traditional method which employs transillumination of the eggs [28,29]. Thirteen unfertilized eggs were retained for the study of oxygen and water vapor signal development, and 9 eggs were used for gas exchange experiments. In order to make sure every measurement had the same geometry, we marked the position of light injection in each egg and the detector position employed in all measurement, as show in Fig. 3 (a). From our earlier study [25], we know that the signal is not very sensitive to the detailed positions. To make sure that the recorded signal solely comes from the egg we used some wax to cover the gap between the detector and the egg surface.

GASMAS is a variety of diode-laser-based gas absorption spectroscopy [27]. Absorption measurements are governed by the Beer-Lambert law relating the transmitted intensity $I$ to the incident intensity $I_0$ according to

$$\frac{I}{I_0} = e^{-kCI} \equiv 1 - kCI$$
where $k$ is a proportionality constant specific for the studied transition, $C$ is the concentration and $l$ is the path length [27]. The linear approximation is valid for $kCl \ll l$. Then, the intensity normalized GASMAS signal is given

$$\Delta I / I = (I_0 - I) / I = kCl.$$  

We note, that for evaluation of the concentration of the substance the path length must be known. However, in a scattering medium this is not the case; only the product of concentration and the mean path length can be determined. Therefore, data obtained in GASMAS measurements are expressed as Equivalent Pathlength ($L_{eq}$) values [16,17], where the $L_{eq}$ value denotes the distance in a reference medium giving rise to the same absorptive imprint as the scattering medium. In the case of oxygen measurements, the reference medium is normally air with 21% oxygen, while for water vapor the distance in air of a particular temperature and relative humidity (RH) (both can be readily measured) is used. Since the oxygen in the unfertilized egg is 21%, the $L_{eq}$ value will basically reflect the mean optical path length through the air cell, which relates to its size. The concentration of water vapor is expressed by the empirical Arden-Buck relation [30], which allows data to easily be recalculated to other temperatures and RH values.

![Image](image.png)

Fig. 3. (a). One of the eggs in the study with ink marks, where the small circle shows the location for laser light injection into the egg and the large circle on the top of the egg where the photodetector was placed. (b). GASMAS recording of oxygen inside an unfertilized egg and a fitted high-quality line-shape recording from ambient air. The $L_{eq}$ is about 16 mm. (c). Water vapor signal from an unfertilized egg and a fitted high-quality line-shape recording from ambient air. The $L_{eq}$ is about 18 mm. Sampling number is proportional to the frequency increase of the laser, and the laser tuning range shown in Figs. 3(b) and 3(c) corresponds to about 15 GHz or 0.04 nm.

3.1 Measurements on unfertilized eggs

3.1.1 Measurements of $L_{eq}$

The oxygen and water vapor signal from thirteen eggs were measured every second day, starting on day 6 after the eggs had been laid and continued during almost 3 weeks while they were kept at a stable room temperature, resulting in 91 sets of data. Typical recorded signals for oxygen and water vapor are shown in Fig. 3(b) and (c). The average values for the oxygen and water recordings with the error bars are shown in Fig. 4. While the calibration for oxygen is straightforward, by comparing to the same type of measurement over a well-defined path length in ambient air, the situation for water vapor is more complex. Frequently, an enclosed volume of a moist material can be considered to develop water vapor of 100% RH with concentration as derived from the Arden-Buck relation [30]. The curve drawn in blue color corresponds to such an evaluation. On the contrary, if instead assuming that the egg white is a
substance without free water, and that the egg cell contains water vapor of ambient RH, we obtain the curve drawn in red color.

![Graph](image)

Fig. 4. Average $L_e$ values with error bars for oxygen and water vapor for the thirteen unfertilized eggs. The water vapor data are plotted in two different ways using two assumptions: 1. The water vapor is due to free water inside the egg, and the data plotted correspond to 100% relative humidity in the enclosed volume. 2. The water vapor inside the egg has the same relative humidity as the air outside the shell.

3.1.2 Measurements of gas exchange

In order to study the gas exchange through the shell we observed the oxygen GASMAS signal in an experimental design, where the egg was put in a plastic bag filled with nitrogen for 30 minutes. The oxygen signal was initially measured while the egg still was inside the bag. The oxygen signal was recorded repeatedly for 10 minutes. After opening the bag, the egg was exposed to the ambient atmosphere. The oxygen content in the gas-filled cell of the egg then gradually increased to the ambient air level of 21%. An experimental recording of such gas exchange is shown in Fig. 5, where the time constant for the gas exchange evaluated in an exponential fit was about 10 minutes.

![Graph](image)

Fig. 5. Gas exchange measured in an unfertilized egg. During the first 10 minutes the egg remained in the nitrogen atmosphere, which still contained some residual oxygen. The time constant obtained from the fit is about 10 min.
We performed this type of measurements on five unfertilized eggs every second day during 3 weeks. The development of the time constant is shown in Fig. 6, as averaged over the individual eggs. We observe a decreasing trend for the gas exchange time constant. If we average over the four first and the last four measurement occasions (average time separation is then 12 days) we obtain the values 11.1 (1.2) min and 6.9 (0.6) min, respectively.

![Image](image.png)

**Fig. 6.** Time evolution of the gas exchange time constant for unfertilized eggs. Average values for 5 eggs are given with error bars.

### 3.2 Measurement on fertilized eggs

The gas exchange through the shell was also measured on four fertilized eggs. The study was started by first introducing the egg from the hatching machine into a plastic bag, where it was exposed to a moderate increase of oxygen content to about 30% by volume during 30 minutes. The measurements of oxygen were then initiated with the egg remaining in the bag, like in the measurements on the unfertilized eggs. During 10 minutes, the initial signal level was recorded; then the bag was opened and exposed to ambient air. Figure 7 shows the time development of the signal returning to the level of 21% oxygen. The time constant was evaluated to 5 minutes.

![Image](image.png)

**Fig. 7.** Gas exchange measurements in a fertilized egg, initially exposed to a moderately increased air concentration of oxygen. The time constant is evaluated to 5 minutes.

Similar measurements were performed on the four fertilized eggs every second day during the 3 week incubation time. Between the measurements, the eggs were put back into the incubator environment. All eggs were subsequently observed to successfully result in a chicken after the expected time duration. Since the gas content could only be changed a little,
and not drastically for physiological reasons considering fertilized eggs, the recordings have rather low quality, and the development of the time constant over time could not be followed in a detailed way; no special tendency could be observed, though. Instead, we formed the average time constant as evaluated for the eggs, measured on 7 occasions. The average time constant for the fertilized eggs was thus found to be 6.0 (3.5) minutes, while the corresponding values for all the data shown in Fig. 6 was found to be 9.0 (2.3) minutes.

4. Discussion and outlook

We have demonstrated that GASMAS is a useful non-intrusive technique to study the contents of free gas in hen eggs. We could show that a steady size of the air chamber of unfertilized eggs with increasing storage time occurs by studying the increase of the oxygen as well as the water vapor signal. While the air cell development progresses smoothly as seen in the readily calibrated oxygen data, the situation is more complex for the water vapor data. However, the general trend is very similar for both gases, with an increase of the values over the study period by a factor 2.14 and 2.32 or 2.16, respectively, where the two water vapor values correspond to the two different types of calibrations, as discussed above. We note, that all three factors are basically the same, and reflect the continuously increasing size of the air cell. Data for oxygen display a particularly smooth age dependence, and would thus be the most precise age indicator.

Still, it should be acknowledged, that an age determination would work only for a specific type of eggs, for which the corresponding time-dependent \( L_{eq} \) curve has been established. Different types of eggs most likely have different curves, while still showing steadily increasing values. Further, an improved signal-to-noise ratio, achievable, e.g. by using a higher laser power, would clearly increase the precision attainable.

Under the assumption of 100% RH in the presence of free liquid water, a deviation between the measured equivalent path values for the two gases might be explained by differences in scattering and absorption properties of the bulk medium, which contains the gas-filled pores or cavities. It has been shown in other contexts that such deviations caused by the use of somewhat different laser wavelengths are small when the measurements are made in a geometry of transmission type, and all detected photons are known to have passed the air containing volume. Deviations were, e.g., not observed, in a study of human sinuses [31].

Deviations could also be caused by so called water activity, modifying the vapor pressure calculated by the Arden-Buck relation [32–34], effectively reducing the RH value. Water activity means, that the vapor concentration above a liquid water surface is changed if the water contains dissolved salts. While observable in precision measurements [34], the effects have been shown to be small for most wet foods, such as meat or milk [32,33]. Water activity is an important aspect in food science, since the growth of bacteria requires a high RH value [32].

It should be noted, that \( L_{eq} \) data for water vapor, evaluated assuming the presence of free water inside the egg, would have resulted in a RH of 100 percent. In Fig. 4 we show one data set evaluated under such assumption, and leading to data considerable below the oxygen ones. Since the calibration now relies on the measurement of the ambient air relative humidity for the recalculation to the case of RH = 100%, inaccuracies in the RH readings could result in a somewhat jagged curve, which is actually observed. This is in contrast to the situation for oxygen, where the curve is perfectly smooth corresponding to constant values of 21% oxygen.

In contrast, if we assume the egg white to be a homogenous substance without free water, the ambient air RH, propagating also into the air cell gas would be the situation, but now instead observed to lead to values above the ones for oxygen. Our conclusion is, that the water vapor conditions related to the egg interior is complex and possibly influenced by all the factors mentioned above.
We also note, that the scattering and absorption of the bulk medium (egg white and yolk) may be changing over the study period, in which case the GASMAS signal development could also partially reflect this aspect, apart from the increase of the air cell with time.

However, our measurements show, that for a particular type of eggs, the GASMAS technique can be used for quite good assessment of the age of the egg based on oxygen as well as water vapour monitoring, where a signal increase by a factor of about 2.2 is observed over a 3-week period.

As demonstrated, GASMAS provides a unique way to study gas diffusion through the egg shell. We repeatedly measured the time constant over a three-week period for unfertilized eggs and observed that it is decreasing substantially on a general time scale of about 10 minutes for the type of eggs studied. Of particular interest are of course the conditions for the fertilized egg during the hatching period, when the chicken embryo clearly is in demand for oxygen. Our study shows, that fertilized eggs have a faster gas diffusion than the unfertilized eggs (by about a factor of 1.5), which clearly helps the oxygenation process for the developing chicken inside the shell. Given the limited signal-to-noise ratio levels obtained in the assessment of gas exchange rates, this parameter is not a useful indicator for routine assessment of freshness for unfertilized eggs. Our study rather gives insight into the interesting time-dependent physiological changes in the permeability of the shell.

Our study indicates, that GASMAS might be developed into a valuable tool for the objective assessment of egg freshness and for studying gas contents and gas exchange during hatching. Thus, GASMAS might develop into a useful non-invasive tool in poultry science, expanding the applicability of biophotonics techniques.

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Disclosures

The authors declare no conflict of interest.

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