Quality Evaluation of Shell Eggs During Storage Using a Dielectric Technique

L. Ragni, A. Al-Shami, A. Berardinelli, G. Mikhaylenko, J. Tang

Abstract. *This article reports on investigating the possibility of non-destructively predicting basic quality parameters of shell eggs using an open-ended coaxial probe dielectric measurement technique. The studied parameters include yolk index, thick albumen height, and Haugh unit as well as air cell size as a function of storage duration at room temperature. The probe was connected to an impedance analyzer from which the dielectric spectra (dielectric constant and loss factor) were collected using a data logger and analyzed by means of partial least squares (PLS) analysis. The tests were carried out in the 10 to 1800 MHz frequency band on shell eggs stored for 1, 2, 4, 8 and 15 days at 22°C, positioning the probe in three different points on the shell eggs. For storage duration, the results showed R^2 values up to 0.985 and 0.980 in cross-validation and test-set validation, respectively, while the R^2 for the air cell height was up to 0.927 and 0.921, respectively. Yolk index, thick albumen height, and Haugh unit were also predicted with R^2 values ranging from 0.526 to 0.728 (test-set validation). No substantial differences in prediction power emerged by using the dielectric constant, loss factor spectra, or position of the probe on the shell egg. The best frequency band for prediction was from 10 to 700 MHz.*

Keywords. *Dielectric, PLS, Quality indices, Shell egg, Spectroscopy, Storage.*

Shell eggs undergo significant physical, chemical, structural, and physiological changes during storage. Easily observable physical changes include an increase in the air cell, thinning of the thick albumen (the part surrounding the yolk), and flattening of the yolk. The air cell increases as a result of loss of water and CO_2 through the shell, as well as due to physiochemical changes in the albumen and yolk (Stadelman and Cotterill, 1995). Deterioration of the gelatinous structure of the thick albumen results in albumen liquefaction and thinning (Li-Chan and Nakai, 1989). This deterioration takes place through electrostatic interaction within the complex lysozime-ovomucin associated with increased pH during storage as a consequence of the loss of CO_2 through the shell (Cotterill and Winter, 1955; Robinson and Monsey, 1972; Thapon and Bourgeois, 1994). As such, the air cell height and the Haugh unit, an index related to the thickness of the albumen and the mass of the egg (Haugh, 1937), are strongly dependent on the age of the egg. Air cell height is used in the European Community (EEC, 1989, 2003) and Haugh unit is used in the U.S. to discriminate the freshness of shell eggs. Finally, flattening of the yolk is primarily due to water increase caused by osmotic migration from the albumen through the vitelline membrane (Funk, 1948).

The key constituents contributing to the above-mentioned changes in stored eggs include proteins (10% in the albumen, on solid basis, and 17% in the yolk), lipids (34%, in the yolk), carbohydrates (1%), inorganic elements, and, above all, water (up to 89% in the albumen, and 50% in the yolk) (Stadelman and Cotteril, 1995). Ovoalbumin is the major constituent of the albumen proteins. Ovomucin and lysozime, which represent about 3.5%, are responsible for the gelatinous structure of the albumen (Johnson, 1966; Li-Chan and Nakai, 1989).

A considerable number of studies have been conducted to investigate the dielectric properties of agri-food products (Nelson, 1973; Venkatesh and Raghavan, 2004; Tang, 2005). Investigations have been carried out on eggs (Romanoff and Romanoff, 1949), grain and seeds (Nelson, 1965), fruits and vegetables (Tran et al., 1984; Nelson, 1983; Nelson et al., 1994; Berbert et al., 2002; Wang et al., 2003), juice and wine (Garcia et al., 2004), baked foods and flours (Seras et al., 1987; Zuercher et al., 1990; Kim et al., 1998), dairy products (Sone et al., 1970; Kudra et al., 1992; Green, 1997; Herve et al., 1998), fish and meat (Bengtsson and Risman, 1971; Lyng et al., 2005), and egg white solutions and thermal denatured egg albumen gels (Lu et al., 1998; Bircan and Barringer, 2002). Dielectric properties are influenced by product density, composition, structure, water activity, temperature, and frequency of the applied field (Ohlsson et al., 1974; Kent, 1977; Engelder and Buffler, 1991; Nelson, 1991, 1992; Feng et al., 2002; Nelson and Bartley, 2002; Sipahioglu and Barringer, 2003; Komarov et al., 2005).

Various techniques are used for dielectric properties measurements of food materials. Measurement devices include parallel-plate capacitors, open-ended coaxial probes, resonant structures, or waveguides that are connected to inductance, capacitance, or resistance meters or impedance, scalar,

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or vector analyzers (Nelson, 1999; Içier and Baysal, 2004). Several non-destructive techniques have been developed based on measurements of the dielectric characteristics of agri-food products. For example, measurements of the moisture content of grains based on dielectric parameters dates back to 1950s (Nelson et al., 1953; Knipper, 1959). More recent investigations were carried out to evaluate physical characteristics, such mass, density, thickness, and maturity, of fresh fruits by measuring dielectric proprieties of selected materials (Trabelsi et al., 1997; Nelson et al., 1995).

Developments of non-destructive and non-invasive methods for determining the quality characteristics of agri-food materials by means of dielectric spectroscopy have in general faced certain difficulties. The majority of the difficulties appear to be related to variability in the shape and mass of the test materials. Other factors include sensitivity and stability of the measurement methods, as well as positioning the sensors in on-line applications. To date, the most notable application of dielectric spectroscopy has been the automatic determination of moisture content (Schmilovitch et al., 1996). Despite all of above issues, evaluation of the characteristics of intact eggs by means of electrical techniques continues to be the main interest of many studies because of the non-invasive and non-destructive nature of such techniques. Budickov et al. (1965, 1968) suggested a methodology based on an electrode chamber filled with an electrolyte in which the egg was placed between the two electrodes. Significant correlations at 300 kHz were reported by those authors between electrical resistance and the yolk pH and the albumen and yolk mass. More recently, William et al. (1997) developed a system to measure total-body electric conductivity to determine the egg lean mass and the mass of albumen and water. Predicted values for the first parameter were within ±6% of actual values. Predicted values of egg lipid mass were within ±15% when using fresh egg mass in a multiple regression model. A simple methodology based on a resonant capacitor probe was used to predict several quality parameters of shell eggs, such as storage duration, air cell and thick albumen heights, and yolk index (Ragni et al., 2006). When relating a dielectric parameter to the capacitance and voltage changes induced by the egg, R^2 values of 0.850, 0.797, 0.696, and 0.674 were obtained for the above-mentioned quality indices, respectively. In the obtained linear models, the egg mass was also used as a predicting variable. Thick albumen height and Haugh unit were also examined on shell eggs by means of near-infrared spectroscopy, with encouraging results (R^2 up to 0.852) (Berardinelli et al., 2005a).

Developing a methodology to automatically and non-destructively sort shell eggs has been an interesting subject of studies in the European Community, where eggs can be stored only at room temperature for several weeks (EEC, 1989, 2003) and egg quality is currently sorted based on air cell height. Candling systems are routinely used for sorting, but they are not always capable of discriminating the freshness of eggs (Cattaneo et al., 1997), especially when eggs are characterized by low Haugh unit values. The main objective of this study was to investigate the possibility of utilizing dielectric spectroscopy as a non-invasive and non-destructive method to evaluate the quality of fresh eggs. The results of an assessment based on an open-ended coaxial probe technique that provides analysis of the dielectric constant and loss factor based on the partial least squares (PLS) statistical method are reported.

Materials and Methods

Fresh eggs were obtained from a local farm in Moscow, Idaho, on the same day laid by Plimouth Barred Rock breed hens, aged 13 months. Measurements were made after storage at room temperature (22°C) for 1, 2, 4, 8, and 15 days. These time steps were chosen because the quality parameters associated with the storage duration of eggs often show a logarithmic trend (Rossi et al., 1995; Lucisano et al., 1996; Berardinelli et al., 2005b).

Measurements of the dielectric constant (ε') and loss factor (ε'') were carried out on intact eggs in the 10 to 1800 MHz frequency band using an open-ended coaxial probe (85070B, Hewlett-Packard, Palo Alto, Cal.) connected to an impedance analyzer (4291B, Agilent, Santa Clara, Cal.). Instrumental frequency and oscillator maximum voltage resolution of the analyzer were 1 mHz and 2 μV, respectively. Two hundred points in an increment of 9 MHz were acquired in the above-mentioned frequency band. The main geometrical characteristics of the probe are shown in figure 1. Dielectric measurements were conducted at three selected locations on an egg (fig. 2): two on opposite sides (points 1 and 2), and one corresponding with the air cell (point 3). Points 1 and 2 were chosen to provide a measurement replication and to evaluate possible variation of the prediction power of the statistical models due to different but similar probe positions. Possible lack of isotropy and geometrical symmetry of egg constituents (e.g., yolk, albumen, and air cell) under the probe could influence direction and intensity of the force lines of the electric field and the consequent spectral response. Specifically, the air cell or the yolk could be closer to point 1 than to point 2, and albumen density could change from point to point of its mass.

Due to the curvature of the egg shell, measured values of ε' and ε'' are related to the dielectric characteristics of the egg and to the air-egg interface. It is appropriate to point out that in this study our main concern was accurate measurement of the overall changes of the electric field at the measurement points, as reflected in measured ε' and ε'' spectra, not the dielectric properties of each egg component.

The quality parameters considered to characterize the eggs during storage were: storage duration, air cell height, thick albumen height, Haugh unit, and yolk index. To measure the air cell height, the egg shell was cracked, and the cup of shell containing the air cell was placed on a wax mold on a horizontal flat surface to ensure that the membrane of the

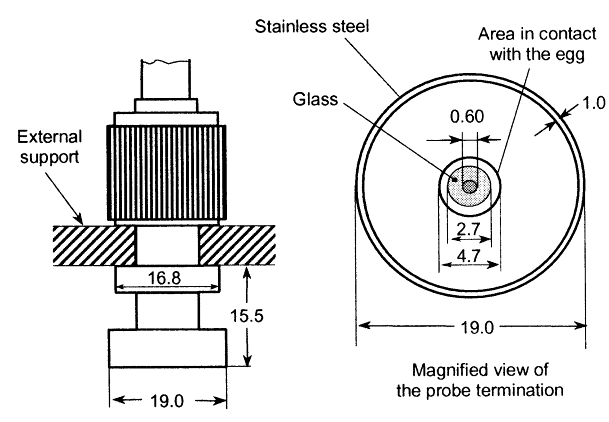

Figure 1. Schematic diagram of open-ended coaxial probe (all dimensions in mm).

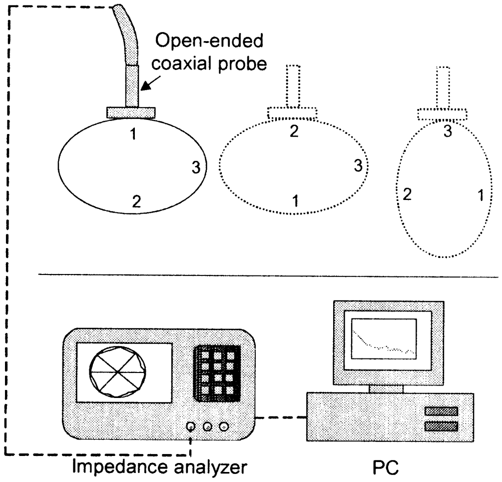

Figure 2. Measurement points on egg and experimental layout.

air cell was roughly horizontal (fig. 3a). The height of the line where the membrane was attached to the shell was measured by means of a tripod digital caliper at three equidistant points. Measurement was also made at the middle point of the membrane. The air cell height was calculated by averaging the values for these four points.

The thick albumen height was calculated as the mean of three measurements carried out at about 10 mm from the yolk using the same tripod digital caliper (fig. 3b). The Haugh unit (H_I) was calculated using the following formula (Haugh, 1937):

$$H_I = 100 \log \left[H_C - 5.67 \left(\frac{30M^{0.37} - 100}{100} \right) + 1.9 \right] \quad (1)$$

where H_C is the mean thick albumen height (mm), and M is the egg mass (g).

The yolk index was determined by dividing the height by the diameter of the yolk (Funk, 1948), as measured by digital calipers while the egg constituents were placed on a flat surface.

The mass of each egg was measured on an electronic balance (±0.001 g) before the acquisition of the dielectric spectra. Diameter and height of eggs were measured using a digital caliper (±0.01 mm). The shape index, namely the ratio between diameter and height multiplied by 100 (Romanoff and Romanoff, 1949), was calculated.

Correlations between egg quality parameters and acquired spectra were assessed using PLS analyses by exploring the predictive power of the entire frequency range and of selected frequency bands. The assessment of the frequency band that gives the best prediction for the considered quality parameters and the determination of sub-bands where the prediction remains suitable is an important step in determining the frequencies of the electric field that correlate best with egg changes during storage. Furthermore, a narrower frequency band is also useful for reducing measurement time, an important parameter for possible development of on-line applications.

PLS regression is a useful technique that combines features from principal component analysis with multiple linear regression (Abdi, 2003). The aim of PLS, especially as used in chemiometrics, is to build provisional linear models from a large set of independent variables that are characterized by high collinearity and for which multiple regression techniques cannot be applied. A frequency spectrum is a typical case of a large data set ("factor levels") where each point (e.g., the dielectric constant value for each examined frequency) represents an independent predictive variable. Although many manifesting factors may be related to a certain response, there may be a few "latent" factors that describe most of the variation in the response. Although PLS does not yield formal structured equations to predict dependent variables by means of independent variables, it is capable of extracting the sought-after latent factors.

In PLS analyses, cross-validation and test-set validation are often performed to test the reliability of the prediction models. Cross-validation uses the same set of samples to calibrate and validate the systems: one sample (one case) of the entire set is excluded (one at a time) and used for validation, while the other samples are used for calibration. Test-set validation uses two different subsets of samples: one for the calibration of the system (for example, 65% of the entire data set), and the other for model validation. In our experiment,

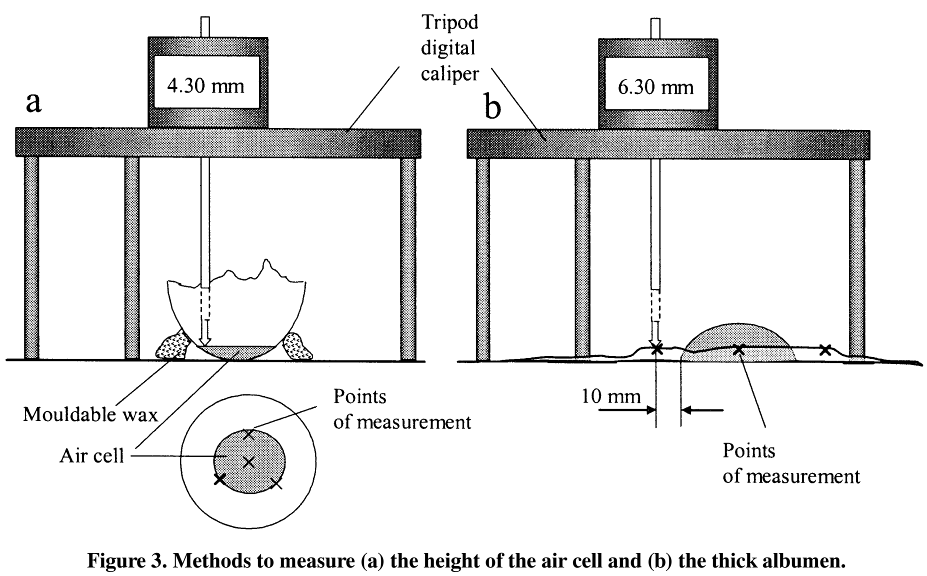

Figure 3. Methods to measure (a) the height of the air cell and (b) the thick albumen.

Table 1. Average of main physical attributes of 35 eggs measured at five different storage durations at 22°C.[a]

Day	Mass (g)	Egg Diameter (mm)	Egg Height (mm)	Shape Index (%)	Air Cell Height (mm)	Thick Albumen Height (mm)	Haugh Unit	Yolk Diameter (mm)	Yolk Height (mm)	Yolk Index
1	57.7 a (3.9)	42.5 a (1.1)	57.0 a (1.9)	74.7 a (2.6)	3.6 a (0.2)	6.2 a (0.9)	79 a (7)	42.5 a (1.1)	18.5 a (0.9)	0.44 a (0.02)
2	58.4 a (5.1)	42.4 a (1.2)	57.3 a (2.4)	74.1 a (2.9)	4.0 b (0.3)	5.6 b (1.0)	73 b (8)	42.4 a (1.3)	18.6 a (1.0)	0.44 a (0.02)
4	58.2 a (4.9)	42.4 a (1.4)	57.6 a (1.2)	73.9 a (3.1)	4.9 c (0.4)	5.0 c (0.9)	68 c (7)	42.7 a (1.3)	18.0 b (0.8)	0.42 b (0.01)
8	56.5 ab (2.4)	42.5 a (1.0)	56.9 a (1.2)	74.1 a (3.1)	6.2 d (0.4)	3.9 d (0.6)	59 d (6)	43.1 a (1.6)	17.1 c (0.9)	0.40 c (0.03)
15	55.1 b (3.5)	42.2 a (0.9)	57.1 a (2.0)	73.9 a (2.0)	7.7 e (0.5)	3.1 e (0.6)	49 e (10)	44.8 b (1.7)	14.8 d (0.6)	0.33 d (0.02)

[a] Means followed by the same letter within a column are not significantly different at $p < 0.05$. Values in parentheses are standard deviations.

cross-validation ("leave one out" method) was carried out by using 35 eggs × 5 storage durations = 175 eggs. Considering the three different probe positions, the acquired spectra (for both ε′ and ε″) were 175 × 3 = 525 (plus an extra 175 spectra obtained as the average of the spectra for points 1 and 2). The test-set validation was performed using 113 randomized spectra from the above-mentioned 175 eggs for calibration and the 62 remaining cases for validating the model. These spectra were from the average of points 1 and 2 and for the 10 to 700 MHz frequency band. Weight, dimensional, and destructive measurements of the quality parameters (air cell height, thick albumen height, and yolk index) were carried out for each of the 35 eggs at each of the five storage durations for the dielectric measurements.

PLS analysis was performed using Opus/Quant, version 5.5 (Bruker Optik, 2004). Each spectrum containing 200 data (independent variables) and the correspondent values of the quality parameters (dependent variables) were imported by the software. PLS analysis provides the coefficient of determination (R^2), the root mean square error of cross-validation (RMSECV), the root mean square error of prediction (RMSEP), and the ranks (RK), or number of used vectors, as indicators of prediction quality. With the aim of limiting the effects of the possible differences in probe-shell interface on the spectra, all spectra were preprocessed by automatically subtracting a straight line. This preprocessing treatment uses an algorithm to remove the baseline slope and offset from the spectrum by iteratively calculating the best fit straight line through a set of estimated baseline points. The RK, RMSECV, RMSEP, and R^2 values were automatically reported by the software.

Correlations between quality parameters during storage were carried out using regression analyses. Significant differences between means ($p < 0.05$) at different storage durations were explored using ANOVA or the Mann-Whitney test if significant differences emerged between variance means at the Levene test.

Results and Discussion

Experimental data of the main physical attributes (averaged for 35 eggs on each day of sampling) used to characterize changes in egg quality are shown in table 1. The air cell height increased sharply with time to more than twice the initial size after 15 day storage, but no significant weight loss was observed until after the eighth day of storage. Thick albumen height decreased sharply with storage duration at room temperature; at the end of the 15-day storage, it was reduced by almost one-half. Significant reduction in thick albumen height led to sharper reductions in Haugh unit during storage. The yolk index also changed significantly with storage duration; this was due, above all, to the decrease in yolk height.

Figure 4 provides an overview of the measured spectra for ε′ and ε″ (average values of total 175 eggs over the five storage durations) in which the influence of measurement locations is clearly apparent. It is evident that the measurement at the location adjacent to the air cell (point 3 in fig. 2) yielded much a lower dielectric constant over the whole selected frequency spectrum. Figures 5 and 6 show the spectra of the dielectric constant (ε′) and loss factor (ε″) for measurements at point 3 (corresponding to the air cell) and for different storage durations. Significant noise due to weak signals in the spectra measured at certain high frequencies (from 700 to 1800 MHz) (fig. 6) was observed. Currently, there is no effective means of isolating this noise from the true responses of the tested material to the applied electromagnetic field. Nevertheless, in the lower frequency band of less than 700 MHz, the influence of storage on measured dielectric property spectra is evident.

The ε′ spectra (figs. 4 and 5) decreased roughly from 10 to 8 with increasing frequency for the locations on the egg side and from 10 to 6 for the measurements close to the air cell. The ε″ values (figs. 4 and 6) varied between 0.2 and 0.8 and between 0.2 and 1.8 for the measurement on the egg sides and at the air cell, respectively. In previous work by Bircan and Barringer (2002), ε′ and ε″ of albumens decreased from 72 to 66 and from 15 to 10, while ε′ and ε″ of yolks decreased from 34 to 28 and from 22 to 7, respectively, as frequency increased from 300 MHz to 2450 MHz. When comparing these

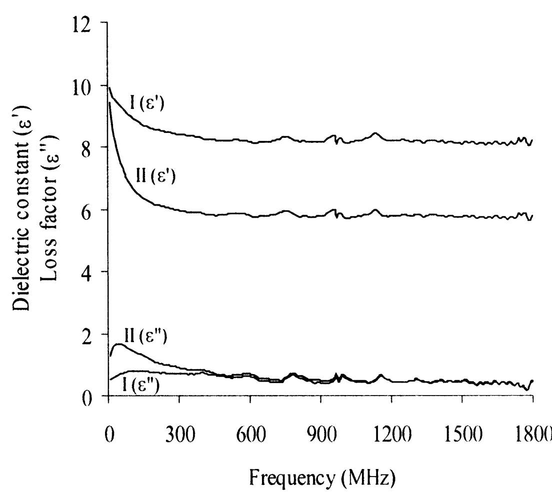

Figure 4. Dielectric constant (ε′) and loss factor (ε″) spectra for measurements at different points on shell eggs (average values for five storage durations and 35 eggs each): I = average of points 1 and 2 (opposite sides of eggs), and II = point 3 (corresponding with the air cell).

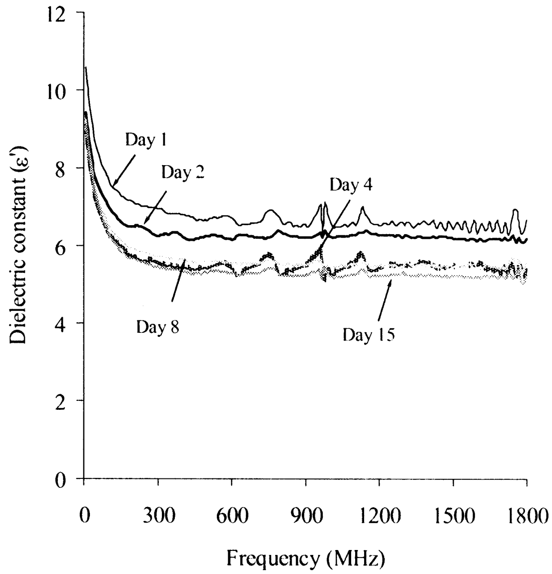

Figure 5. Dielectric constant (ε′) spectra for measurements at point 3 (corresponding with the air cell) for different storage durations (average values for 35 eggs for each duration).

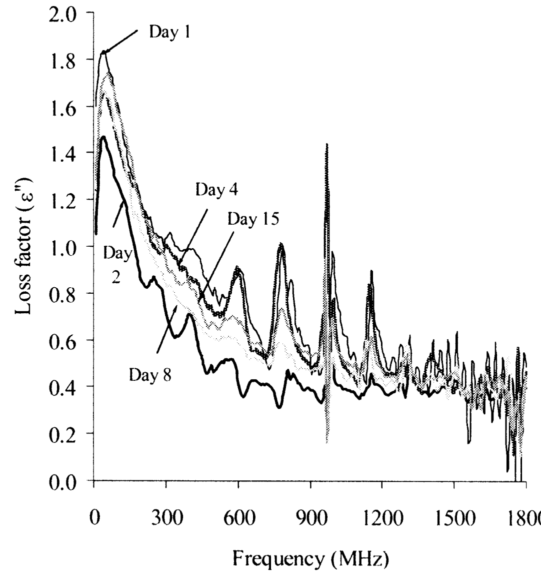

Figure 6. Loss factor (ε ″) spectra for measurements at point 3 (corresponding with the air cell) for different storage durations (average values for 35 eggs for each duration).

values with those obtained (figs. 4 to 6), it can be observed that the latter were much lower. For example, at 300 MHz, ε′ and ε ″ of albumen are one order of magnitude larger than for intact eggs. This difference reflects the strong influence of the shell and the air between the probe and the eggs constituents on the measured ε′ and ε ″ (ε′ values of the calcium carbonate and air are about 7 and 1, respectively).

Values of ε′ and ε ″ in this study are related to the complex air-egg system. Hence, it is useful to note again that the aim of this research was not the analysis of the dielectric parameters of shell eggs during storage, but rather to assess whether the information contained in the spectra can be used for predicting some key quality parameters of shell eggs.

Table 2. Values of the coefficient of determination (R^2), ranks (RK), and root mean square error of cross-validation (RMSECV) of the PLS cross-validation for predicting the quality parameters by means of the dielectric constant (ε′) spectra.

Frequency Band (MHz)	Storage Duration			Air Cell Height			Yolk Index			Thick Albumen Height			Haugh Unit		
	R^2	RK	RMSECV (days)	R^2	RK	RMSECV (mm)	R^2	RK	RMSECV	R^2	RK	RMSECV	R^2	RK	RMSECV (mm)
Measurement point 1 in figure 2															
10 - 1800	0.976	10	0.7	0.898	9	0.5	0.681	7	0.025	0.523	5	0.9	0.491	5	8
10 - 700	0.974	10	0.8	0.912	7	0.5	0.707	7	0.024	0.550	6	0.9	0.512	6	8
10 - 100	0.718	5	2.7	0.659	4	0.9	0.487	3	0.032	0.439	4	1.0	0.360	3	9
100 - 300	0.919	5	1.4	0.889	5	0.5	0.653	4	0.026	0.485	3	1.0	0.457	3	9
300 - 700	0.960	7	1.0	0.910	6	0.5	0.644	4	0.027	0.446	4	1.0	0.479	4	9
Measurement point 2 in figure 2															
10 - 1800	0.971	10	0.8	0.890	9	0.5	0.682	7	0.025	0.461	3	1.0	0.426	3	9
10 - 700	0.969	9	0.9	0.909	7	0.5	0.677	7	0.025	0.534	6	0.9	0.502	6	8
10 - 100	0.735	4	2.6	0.657	4	0.9	0.506	3	0.031	0.365	3	1.1	0.346	3	9
100 - 300	0.940	6	1.2	0.844	5	0.6	0.670	5	0.026	0.492	3	1.0	0.461	3	8
300 - 700	0.960	8	1.0	0.883	6	0.5	0.670	5	0.026	0.505	5	1.0	0.448	4	9
Average of points 1 and 2															
10 - 1800	0.976	10	0.7	0.892	9	0.5	0.693	7	0.025	0.519	5	0.9	0.482	5	8
10 - 700	0.985	10	0.6	0.927	7	0.4	0.705	7	0.024	0.557	6	0.9	0.530	6	8
10 - 100	0.807	5	2.2	0.718	4	0.8	0.593	4	0.028	0.400	3	1.1	0.358	3	9
100 - 300	0.957	6	1.1	0.881	5	0.5	0.683	5	0.025	0.497	3	0.9	0.483	3	8
300 - 700	0.969	8	0.9	0.910	5	0.5	0.650	4	0.027	0.529	5	0.9	0.461	4	9
Measurement point 3 in figure 2															
10 - 1800	0.955	10	1.0	0.887	8	0.5	0.657	7	0.026	0.499	4	1.0	0.468	4	9
10 - 700	0.964	10	1.0	0.921	8	0.4	0.686	7	0.025	0.578	6	0.9	0.499	6	8
10 - 100	0.695	4	2.8	0.672	4	0.9	0.499	4	0.032	0.428	4	1.0	0.403	4	9
100 - 300	0.889	6	1.7	0.809	5	0.7	0.671	5	0.026	0.615	4	0.8	0.426	3	9
300 - 700	0.944	7	1.2	0.907	7	0.5	0.661	5	0.026	0.552	5	0.9	0.484	5	9

Table 3. Values of the coefficient of determination (R^2), ranks (RK), and root mean square error of cross-validation (RMSECV) of the PLS cross-validation for predicting the quality parameters by means of the loss factor (ε'') spectra.

Frequency Band (MHz)	Storage Duration			Air Cell Height			Yolk Index			Thick Albumen Height			Haugh Unit		
	R^2	RK	RMSECV (days)	R^2	RK	RMSECV (mm)	R^2	RK	RMSECV	R^2	RK	RMSECV (mm)	R^2	RK	RMSECV
Measurement point 1 in figure 2															
10 - 1800	0.955	9	1.1	0.900	9	0.5	0.657	8	0.026	0.489	4	1.0	0.458	3	9
10 - 700	0.968	10	0.9	0.911	8	0.5	0.694	7	0.025	0.555	6	0.9	0.502	6	8
10 - 100	0.852	3	2.0	0.845	4	0.6	0.594	3	0.029	0.492	3	1.0	0.472	3	9
100 - 300	0.907	5	1.6	0.807	5	0.7	0.648	4	0.027	0.482	4	1.0	0.457	4	9
300 - 700	0.945	7	1.2	0.844	6	0.6	0.671	6	0.026	0.381	4	1.1	0.383	4	10
Measurement point 2 in figure 2															
10 - 1800	0.962	9	1.0	0.898	9	0.5	0.596	6	0.028	0.475	3	1.0	0.474	3	9
10 - 700	0.970	9	0.8	0.905	8	0.5	0.666	6	0.026	0.588	6	0.9	0.557	6	8
10 - 100	0.833	4	2.1	0.783	4	0.7	0.588	3	0.029	0.478	3	1.0	0.522	3	9
100 - 300	0.902	5	1.6	0.827	5	0.7	0.624	4	0.027	0.482	4	1.0	0.447	4	9
300 - 700	0.944	7	1.2	0.853	6	0.6	0.670	5	0.026	0.456	5	1.0	0.406	4	9
Average of points 1 and 2															
10 - 1800	0.968	9	0.9	0.907	9	0.5	0.684	8	0.025	0.518	5	0.9	0.493	5	8
10 - 700	0.981	10	0.7	0.918	8	0.4	0.675	7	0.026	0.578	6	0.9	0.555	6	8
10 - 100	0.882	4	1.7	0.831	4	0.6	0.638	3	0.027	0.522	3	0.9	0.548	3	8
100 - 300	0.931	5	1.4	0.839	5	0.6	0.673	4	0.026	0.508	4	0.9	0.479	4	9
300 - 700	0.965	9	0.9	0.878	6	0.5	0.656	5	0.026	0.456	6	1.0	0.412	4	9
Measurement point 3 in figure 2															
10 - 1800	0.966	10	0.9	0.915	10	0.5	0.666	9	0.026	0.557	6	0.9	0.469	4	9
10 - 700	0.969	10	0.9	0.905	9	0.5	0.649	8	0.027	0.614	6	0.8	0.536	7	8
10 - 100	0.828	3	2.1	0.776	4	0.7	0.654	3	0.026	0.512	3	0.9	0.504	3	8
100 - 300	0.884	6	1.7	0.814	4	0.7	0.598	4	0.028	0.490	4	1.0	0.401	3	9
300 - 700	0.948	8	1.2	0.853	6	0.6	0.631	5	0.027	0.408	5	1.0	0.394	5	9

The PLS analyses that correlated dielectric spectra to egg quality parameters were conducted for the complete examined frequency band (10 to 1800 MHz) and for different sub-bands to select a frequency band with the best prediction power. Tables 2 and 3 summarize the results from the analyses of dielectric constant (ε') and loss factor (ε''), respectively. As shown in table 2, the 10 to 700 MHz band provided the best overall R^2 values for all three measurement locations. Within this frequency band, three sub-bands were selected: low (10 to 100 MHz), where the most evident dependence of ε' and ε'' on the frequency was observed (figs. 5 and 6); intermediate (100 to 300 MHz), which contained the maximum curvature of ε'; and high (300 to 700 MHz), where the curve trends flattened (ε' varied less than 3%). For practical application, a narrow spectrum range is preferred to reduce computation time and increase the speed of on-line quality evaluation in a sorting system.

The values of R^2, RK, and RMSECV are indices used to assess the quality of the PLS models: the higher the R^2 value and the lower the RMSECV value, the better the fit. It should be noted, though, that very large values of RK do not always yield the best fit and may sometimes lead to over-fitting, adding noise and degrading the model (Bruker Optik, 2004). The R^2, RK, and RMSECV values in tables 2 and 3 reveal that the quality of the PLS model prediction was in general quite similar whether based on ε' or ε'' spectra. The highest prediction power was for storage duration (R^2 up to 0.985), followed by the air cell height (R^2 up to 0.927), the yolk index (R^2 up to 0.707), the thick albumen height (R^2 up to 0.615), and the Haugh unit (R^2 up to 0.557).

The storage duration parameter correlated well with the air cell height, as shown in figure 7. The primary factor causing the electromagnetic field changes, as reflected in changes of measured ε' and ε'', is the air cell height, which is continuously and significantly increasing over the storage period. This is because an aqueous liquid (the albumen), characterized by a high value of ε' and ε'', is being progressively substituted by gas, which has much lower dielectric properties. The reflected electromagnetic waves to the probe are deformed by the presence of the egg body and its local density variation (e.g., the presence of the air cell).

The correlation between air cell height and egg mass was poor (R^2 = 0.05), as well as the correlation between egg mass

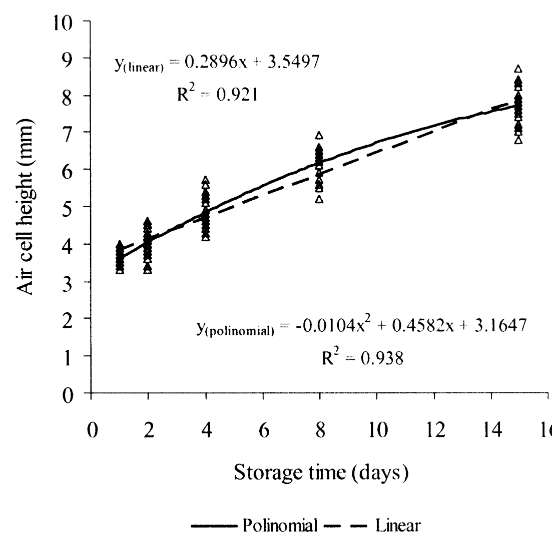

Figure 7. Correlations between storage duration (x) and air cell height (y).

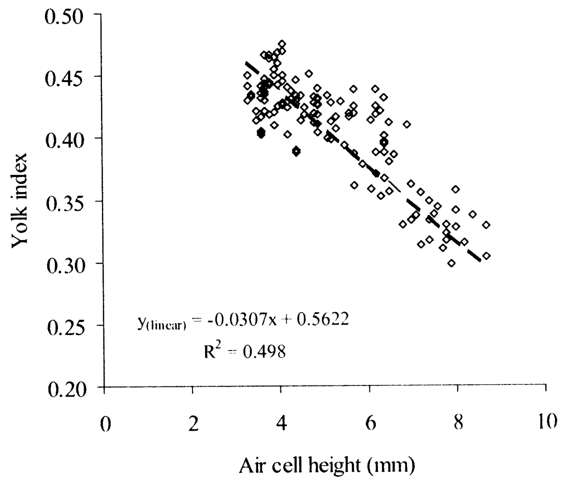

Figure 8. Correlations between air cell height (*x*) and yolk index (*y*) during 15 days of storage at 22°C.

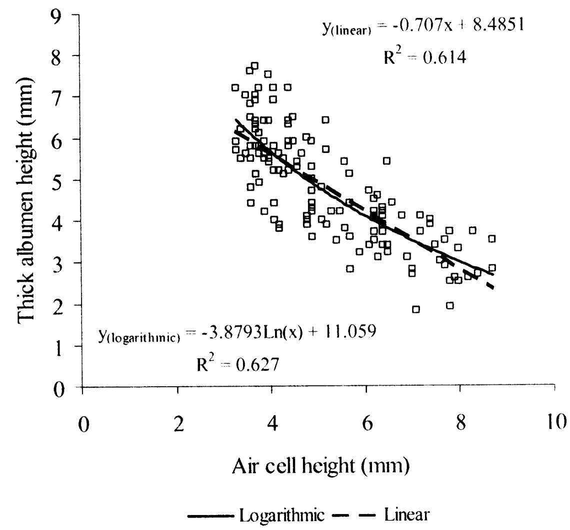

Figure 9. Correlations between air cell height (*x*) and thick albumen height (*y*) during 15 days of storage at 22°C.

and storage duration (R^2 = 0.07). This is because mass variations due to moisture loss during storage are small with respect to differences in overall egg mass. Thus, egg mass cannot be considered as a parameter responsible for the modifications in the ε′ and ε″ spectra that enabled prediction of air cell height and storage duration. Storage duration and air cell height were predicted by the PLS algorithms with high R^2 values (tables 2 and 3). As such, it can be assumed that storage duration is mainly predicted by means of the air cell height. The storage duration is necessarily measurable indirectly by means of the physical modifications of eggs.

The higher prediction power (R^2) of the algorithm for storage duration as compared to the algorithm for air cell height can be attributed to further changes that occur in eggs during storage in addition to the air cell height increase (e.g., liquefaction of albumen). That said, we have to be aware that the reasonable prediction obtained for storage duration was possible because the eggs were stored at a constant temperature (22°C). Storage temperature greatly influences air cell height and all the other quality parameters previously listed (Sauveur, 1988). Air cell height is highly correlated with storage duration in thermally constant conditions, but one cannot provide interpretations about the changes of this parameter under thermal stresses and fluctuations.

Figures 8 and 9 show correlations between air cell height and yolk index and between thick albumen height and air cell height. The R^2 values are less than 0.7, the threshold level suggested by the PLS technique (Bruker Optik, 2004) above which problems of collinearity occur.

Regarding the prediction capability of the different spectral bands, the best frequency band was often observed between 10 and 700 MHz. For prediction of storage duration and height of the air cell using ε′ and ε″ spectra, the best sub-band appeared to be at 300 to 700 MHz, while for the other quality parameters the best sub-band was at 100 to 300 MHz or 300 to 700 MHz. The variations in prediction power between the two mentioned sub-bands were often minimal. Regarding the differences due to measurement location, the highest R^2 value for storage duration, air cell height, yolk index, and Haugh unit corresponded with the average measurements at the sides of the eggs, while the yolk index appeared to be better predicted by the measurement at the location close to the air cell. The PLS parameters reported in table 4 for the test-set validation are the averages of the two measurement points on opposite sides of the egg and for the 10 to 700 MHz frequency band.

Table 4. Coefficient of determination (R^2), ranks (RK), and root mean square error of prediction (RMSEP) of the PLS test-set validation for predicting quality parameters by means of dielectric constant and loss factor spectra (spectra are from the average of points 1 and 2 and the 10 to 700 MHz frequency band).[a]

	Dielectric Constant			Loss Factor		
Quality Parameter	R^2	RK	RMSEP	R^2	RK	RMSEP
Storage duration	0.980	10	0.7	0.979	10	0.7
Air cell height	0.921	7	0.4	0.915	9	0.5
Yolk index	0.728	9	0.023	0.614	7	0.030
Thick albumen height	0.555	6	0.9	0.600	5	0.9
Haugh unit	0.502	6	8	0.526	4	9

[a] RMSEP is in days for storage duration and in millimeters for air cell height and thick albumen height.

Examination of the results in tables 2, 3, and 4 reveals that the prediction capability of the test-set validation was nearly the same as for the cross-validation. Examples of the predicted air cell height vs. observed values for cross-validation and test-set validation for ε′ and ε″ spectra are given in figures 10 and 11, respectively.

Finally, when comparing the results obtained in the present research with previous results obtained by using a parallel-plate capacitor probe in resonant conditions and with linear regression models (Ragni et al., 2006), we noted some analogies regarding the trend of the prediction power of the egg quality parameters. In fact, storage duration, air cell height, yolk index, thick albumen height, and Haugh unit were predicted with R^2 values of 0.850, 0.797, 0.696, and 0.674, respectively (Ragni et al., 2006). However, the previous study was based on broken linear regression analysis, and the egg mass was used as a predictive variable together with the dielectric parameter to obtain the statistical models.

Conclusions

Dielectric spectroscopy of shell eggs was conducted and analyzed in terms of the physicochemical changes experi-

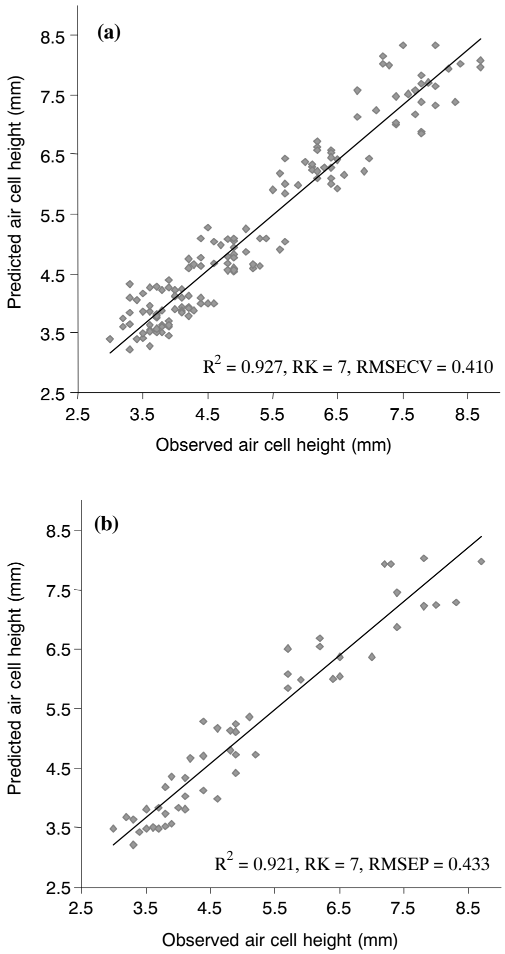

Figure 10. Observed vs. predicted values of air cell height for (a) cross-validation and (b) test-set validation using dielectric constant (ε′) spectra (spectra are from the average of points 1 and 2 and the 10 to 700 MHz frequency band).

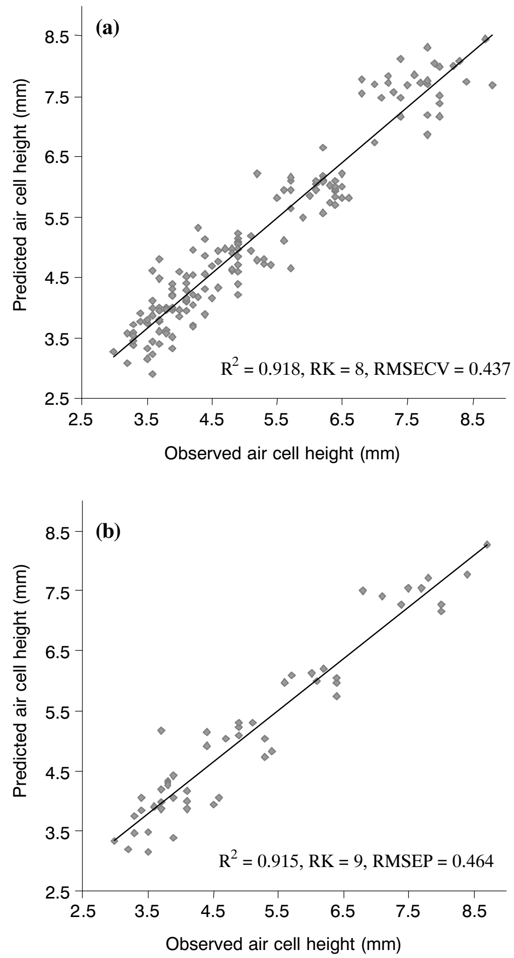

Figure 11. Observed vs. predicted values of air cell height for (a) cross-validation and (b) test-set validation using loss factor (ε″) spectra (spectra are from the average of points 1 and 2 and the 10 to 700 MHz frequency band).

enced over storage periods at room temperature. Analysis of the dielectric constant and loss factor spectra using the PLS technique in the 10 to 1800 MHz band appeared to be useful for non-destructive determination of some quality indices of shell eggs. In the 10 to 700 MHz band, storage duration (from laying) was predicted with R^2 values up to 0.985 and 0.980 for cross-validation and test-set validation, respectively; R^2 for air cell height was up to 0.927 and 0.921. Other quality parameters, such as yolk index, thick albumen height, and Haugh unit, were also assessed, with R^2 values ranging from 0.526 to 0.728 (test-set validation).

Dielectric constant and loss factor spectra measured on intact eggs provided similar prediction capability. Moderate differences were observed between measurement points on the shells of the eggs. The frequency band from 10 to 700 MHz was the best for the quality indices prediction.

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