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REVIEW ARTICLE

Romdhane Karoui · Bart Kemps · Flip Bamelis · Bart De Ketelaere · Eddy Decuypere · Josse De Baerdemaeker

Methods to evaluate egg freshness in research and industry: A review

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Abstract The modern poultry industry is not satisfied with the traditional system of the handling and processing of eggs which is based on candling and visual inspection of the eggs. Currently, the operator of the conveyer does not have the opportunity to inspect 120 000 eggs per hour and to estimate the freshness, weight, bacterial infection, presence of technical spoilage, eggshell defects without elimination of subjectivity, fatigability and destruction. That is why the problem of automatization of egg quality control is rather difficult. In order to assure a high and consistent egg quality, an attractive and alternative strategy for determining the state of egg freshness can be achieved by sensors technologies. These techniques (e.g., near-infrared, mid-infrared, fluorescence spectroscopies, etc.) appear to be very promising for non-destructively determining egg freshness because they are relatively not expensive. Such methods cannot eliminate the need for more detailed physico-chemical analyses, but they may help to screen samples that require further examination.

Keywords Egg quality · Egg freshness · Non-destructive techniques

Introduction

Freshness makes a major contribution to the quality of egg and egg products. One of the most important objective in the food industry is that of achieving a uniform quality both of raw materials and of the final product. One of the main concerns of the egg industry is the systematic determination of egg freshness, because consumers may perceive variability in freshness as lack of quality. Egg white and egg yolk are extensively utilised as ingredients because of their unique functional properties, such as gelling and foaming. Foams are used in the food industry for the manufacture of bread, cakes, crackers, ice creams, etc. Hen egg yolk has good emulsifying properties. The foaming and emulsifying properties of albumen and yolk respectively are affected by protein concentration, pH, ionic strength. The changes that occur in egg during storage are many and complex and affect the functional properties of egg yolk and egg albumen. These changes includes: thinning of albumen, increase of pH, weakening and stretching of the vitelline membrane and increase in water content of the yolk.

Freshness can be explained to some extent by some objective sensory, (bio)-chemical, microbial and physical parameters and can therefore be defined as an objective attribute [1]. Knowledge of the various descriptors of properties that are encountered in egg immediately after lay must be known as well as the changes in properties that take place over time. This information can be gained by performing controlled storage experiments that extend from the time after lay, loss in freshness and spoilage can thus be monitored; once dynamics and the rate of various changes that occur have been measured, the next step is to try to develop a model in order to determine age of commercial shell eggs in terms of equivalent egg age or predicted the remaining shelf life of an unknown sample. To achieve this aim, it is useful to combine several measurements obtained by different methodologies and correlate them with sensory assessments, which are used to evaluate egg freshness. The aim of this article is to review the potential of both destructive and non-destructive techniques for the evaluation of egg freshness. Another goal is the development and application of spectroscopic techniques as research tools for egg products and sensors for on-line measurements.

R. Karoui (✉) · B. Kemps · F. Bamelis · B. De Ketelaere · E. Decuypere · J. De Baerdemaeker
Egg Quality and Incubation Research Group,
K.U. Leuven Kasteelpark Arenberg 30,
3001 Leuven, Belgium
e-mail: Romdhane.Karoui@biw.kuleuven.be
Tel.: +32(0)16321470
Fax: +32(0)16328590

Destructives egg freshness determination

Sensory evaluation

Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyse and interpret

characteristics of food as perceived by the senses of sight, smell, taste, touch and hearing. Sensory tests can be divided into three groups: discriminative test, which indicates whether there is a difference between samples; descriptive tests and affective tests [2]. Descriptive and discriminative tests are objective analytical tests in which a trained panel is used. Affective tests are subjective consumer tests that are based on a measure of preference or acceptance. The choice of method depends on the purpose of the application of the sensory evaluation and whether it is used in product development, quality control, consumer studies or research.

Characteristic sensory changes occur in the appearance, odour, taste and texture of egg during aging. Only few papers have underlined the effect of storage and temperature on the sensory attributes of eggs. Campo et al. [3] have used panellists to express their acceptability of the white, yolk and whole raw egg on the visual appreciation, in a 0–100 scale. In this research, the eggs were stored at different temperatures (e.g., 4, 18 and 32 °C) and at different days of storage (e.g., 0, 7, 14 and 21 days).

For visual appreciation yolk acceptability, the researchers reported that temperature was the main factor with influence on the evaluation of each parameter. Storage time has also an effect but appeared less significant than temperature. Indeed, panellists were able to appreciate clear differences between, on the one hand, fresh egg yolks (68.9±7.3) and those stored during 21 days at 18 °C (45.6±8.5) and, on the other hand, between fresh eggs yolks and those stored during 7 days at 32 °C. The appreciation of the yolk visual aspect of eggs stored during 3 weeks at 18 °C was significantly better ($P<0.5$) than those of eggs kept 7 days at 32 °C [3]. However, panellists were unable to differentiate the new-laid eggs from the eggs stored at 4°C for any length of time, which maintained its good yolk appearance. Concerning albumen acceptability, the panellists were able to differentiate between new-laid eggs and those stored at 18 and 32 °C whatever the storage time (7, 14 or 21 days).

In a second step, the panellists were asked to evaluate texture, white flavour intensity, white favour acceptability, yolk aroma, yolk flavour intensity and yolk favour acceptability in a 0–100 scale. Regarding yolk texture, no difference were found between treatments in yolk aroma and yolk flavour intensity attributes, while yolk favour acceptability attribute depend from the storage temperature and storage time [3]; indeed, a significant difference were found between new-laid egg from those stored during 18 days at whatever the storage temperature (4 or 18 °C).

Regarding albumen texture, significant difference were found ($P<0.5$) between eggs stored for 21 days at 4 °C which had a high albumen consistency, and those stored for 14 days at 18 °C. Acceptability of white flavour was better judged in new-laid eggs and those stored at 4 °C for 7 days than eggs stored 21 days at 18 °C [3]. This difference has been attributed to the degradation of suphur-containing volatiles which are the main factor influencing on the acceptability of albumen [4].

From the above study, the researchers concluded that storage temperature had a higher effect on the acceptability of eggs by a trained panel than the duration of its storage especially for egg visual appreciation. However, the texture of such eggs found to be dependent by both temperature and time. Indeed, eggs stored under refrigeration can maintain their sensory quality almost invariably up to 3 weeks from date of lay, while those stored at 18 °C lose a part of their sensory quality after 2 weeks.

Physico-chemical methods

The characteristic of fresh eggs change during aging, being influenced by both storage temperature and environmental conditions (concentrations of O_2 and CO_2, relative humidity). The albumen has a major influence on overall interior egg quality. Thinning of the albumen is a sign of quality loss. When a fresh egg is carefully broken out onto a smooth flat surface, the round yolk is in a central position surrounded by thick albumen. When a stale egg is broken out, the yolk is flattened and often displaced to one side and the surrounding thick albumen has become thinner, resulting in a large area of albumen collapsed and flattened to produce a wide arc of liquid. The most widely method used for the determination of albumen quality is the Haugh Units [5] which is based on determining both the weight on the intact weight and the albumen height of a broken egg. The Haugh Units has been reviewed extensively by Williams [6], although many authors have criticised it [7–10] and have shown that the adjustment of egg weight implied by the Haugh Units is incorrect except possibly in the sample of eggs determined by Haugh [5]. Indeed, Williams [6] reported that the strain and age have an effect on the height of albumen: the albumen height decreases when the age of hen increases, even as the egg weight and the total amount of albumen increase [11, 12].

Another indices used to evaluate egg freshness is air cell height which is affected by egg weight and storage relative humidity [13, 14]. Air cell height, the only quantitative egg freshness parameter considered by the European Union regulation depends on the egg weight. Theoretically, a grade A egg at packaging has to keep the characteristics of its grade (air cell height <6 mm) up to expiring date. However, the strong dependence of the air cell height from environmental relative humidity and temperature makes it difficulty to guaranty the quality without a strict control of these two variables throughout the whole egg marketing cycle.

The pH has been used to determine the egg freshness. Hence, the pH of albumen from a newly laid egg is between 7.6 and 8.5 [15]. During the storage of shell eggs, the pH of albumen increases at a temperature-dependent rate to a maximum value of about 9.7 [16]. The rise in the albumen pH is caused by a loss of carbon dioxide from the egg through the pores in the shell. Indeed, the pH of albumen is dependant on the equilibrium between dissolved carbon dioxide, bicarbonates ions, carbonate ions and proteins.

Albumen refraction index, which consist to measure the liquid concentration of albumen (index of refraction) by utilizing the refraction phenomenon of light at the boundary plane between the lane of a small prism exposed at a

part of the detection section of the refractometer and the liquid to be measured, has also been used as an indicator of egg freshness by Stanescu et al. [17]. However, this technique is too laborious and need a long time compared to the measurements determined by pH and Haugh Units. Recently, new chemical indices that increase both in albumen and yolk during the storage of eggs (e.g., uridine and pyroglutamic acid) have been considered as descriptors of shell egg freshness [14]. Furosine, ε-*N*-(2-furoylmethl-L-lysine), produced by acid hydrolysis of the Amadori compounds, is a promising shell egg freshness index when determined in albumen [18, 19]. This index shows high repeatability and low natural variability in fresh eggs and moreover, is independent from egg weight, hen age and storage relative humidity. Recently, Hidalgo et al. [20] have confirmed the possibility of expressing shell egg freshness as equivalent egg age, using furosine as a reference index.

Non-destructives egg freshness determination

Nowadays, the modern poultry industry is not satisfied with the traditional system of the handling and processing of eggs which is based on candling. That is why the problem of automatization of egg quality control is rather difficult. To reply to this request, non-destructive methods for determining egg freshness have been proposed. Results published in the last years showed that spectroscopic techniques in combination with multivariate statistical methods have broad application in the determination of egg freshness.

Infrared spectroscopy

Infrared radiation (IR) or the term infrared alone refers to energy in the region of the electromagnetic radiation spectrum at wavelengths longer than those of visible light, but shorter than those of radio waves. The applications of this technique for animal nutrition, agricultural and food sciences have increased considerably in the last decade [21, 22].

Near infrared (NIR) spectroscopy is widely used for the determination of organic constituents in feeds, foods, pharmaceutical products and related materials. The technique is advantageous for many applications because it can provide rapid, non-destructive and multi-parametric measurements. The technique is also suitable for at-line and on-/in-line process control. The NIR is based on the absorption of electromagnetic radiation at wavelength in the range 800–2500 nm. NIR spectra of food absorption correspond mainly to overtones and combinations of vibrational modes involving C–H, N–H and O–H chemical bonds. At present time, there are two technological ways to use NIR technology: Near-Infrared Reflectance (NIRR) and Near-Infrared Transmittance (NIRT). NIRR normally requires sample grinding to obtain a uniform surface for measurement of reflectance, while NIRT requires little or no sample preparation. Consequently, NIRT is a faster and more reproducible technique than NIRR. But NIRT is less sensitive than NIRR. One of the strength of NIRR technology is that it allows several constituents to be measured concurrently [23].

In the sector of egg, the application of NIR for the determination of egg freshness is rather limited. Only a few studies have been published about the potential of this technique to determine the egg freshness. The first publication on measuring eggs by NIR addressed an early stage of Norri's work [24]. However, these measurements of freshness covered only a few hours of egg storage. The feasibility of evaluating freshness of intact eggs in the course of 28 days in storage was recently proved by Schmilovitch et al. [1].

The spectral region of wavelengths below 1100 nm is often called near-NIR. Usually, diode array spectrophotometers cover the spectrum range below 1100 nm. Schmilovitch et al. [1] have used near-NIRT to determine the freshness of eggs. The results obtained from the Partial Least Square (PLS) showed that the variables, days after hatching, air chamber size, weight loss and pH could be predicted by near-NIRT with a correlation coefficient varying between 0.9 and 0.92. However, these high correlation coefficients refer to group means rather than to individual egg. Bamelis [25] has used visible (VIS) in the range of 300–750 nm, and NIR (in the range of 750–2500 nm) spectral analysis, to monitor the freshness of eggs during storage; this author showed a large variation between the spectra of individual eggs in a batch. This variation depends on both internal egg characteristics and shell characteristics. In addition, a difference between time-dependent and time-independent variability of the transmission spectra has been found because shell conductance, shell thickness and shape index are correlated significantly with the time-independent variability [25].

Recently, Kemps et al. [26] assessed the potential of VIS-NIRT spectroscopy to determine the internal quality of eggs. A total of 600 eggs were monitored during storage at 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 days. On the same eggs and for the different storage time, Haugh Units and pH were also measured. Using PLS, the correlation coefficients for the prediction of the Haugh Units and pH were 0.82 and 0.86, respectively. The obtained results show better prediction of pH than Haugh Units. This could be due as explained here above by the low exactitude to determine the Haugh Units compared to the measurement of the pH.

The superposition of many different overtone and combination bands in the NIR region causes a very low structural selectivity for NIR spectra compared to the mid infrared (MIR) where many fundamentals can usually be observed in isolated positions. Many overlapping signals is the NIR give rise to a spectrum with broad peaks, making it difficult to interpret compared to the conventional MIR spectrum [27]. Starting from these explanations, it appears very difficult to study the secondary structure of egg proteins using NIR.

MIR represents the spectrum of the absorption of all the chemical bonds having an infrared activity between 4000 and 400 cm^{-1}. The acyl-chain is mainly responsible for the absorption observed between 3000 and 2800 cm^{-1},

whereas the peptidic bound C–NH is mainly responsible of the absorption occurring between 1700 and 1500 cm^{-1} [28]. Most of the absorption bands in the MIR region, but not in the NIR region, have been identified and attributed to chemical groups [27]. The triacylglycerols ester linkage C–O (~1175 cm^{-1}), C=O (~1750 cm^{-1}) group and acyl chain C–H (3000–2800 cm^{-1}) stretch wavenumbers are commonly used to determine fat [29]. The infrared bands appearing in the 3000–2800 cm^{-1} region are particularly useful because they are sensitive to the conformation and the packing of the phospholipid acyl chains [27, 28, 30, 31]. For example, the phase transition of phospholipids (sol-to-gel state transition) can be followed by the MIR spectroscopy [29]. Indeed, increasing temperature results in a shift of the bands associated with C–H (~2850, 2880, 2935 and 2960 cm^{-1}) and carbonyl stretching mode of the phospholipids [29].

The development of Fourier transform infrared (FTIR) spectroscopy in recent years also affords the possibility of obtaining unique information about protein structure and protein–protein and protein–lipid interactions without introducing perturbing probe molecules [32]. The Amide I and II bands (1700–1500 cm^{-1}) are known to be sensitive to the conformation adopted by the protein backbone. The secondary structures of proteins can be deduced from their FTIR spectra since there are good correlations between the Amide I band (1700–1600 cm^{-1}) and the levels of α-helix, β-sheet and unordered structure in proteins [31].

Although the peptide bonds are essentially responsible for the absorbance of proteins in the 1700–1500 cm^{-1} region, the side chains of several amino acids (glutamic acid, aspartic acid, glutamine, asparagine, lysine, arginine and tyrosine) can contribute to the signal in the Amide II region [33]. The carboxylate groups of the side chains of aspartic and glutamic acids absorb between 1580 and 1520 cm^{-1}. However, as the whole fresh egg contained a considerable amount of water, the water is absorbed in this region and may affect the interpretation of the spectra. Water is a very strong infrared absorber with prominent bands centred at 3360 cm^{-1} (H–O stretching band), at 2130 cm^{-1} (water association band) and at 1640 cm^{-1} (the H–O–H bending vibration) [34]. Infrared spectroscopy can be used with proteins in aqueous solution. Precise subtractions of the H_2O band are possible because of the frequency precision achievable with FTIR. The subtraction of a large H_2O band from a large absorbance spectrum of protein in H_2O to get a small spectrum of protein was difficult considering older dispersive infrared spectrometers [35]. In addition, the development of the attenuated total reflectance (ATR) device allows the sampling problems encountered when collecting spectra from opaque and viscous samples to be overcome. It can be concluded that the MIR could be a suitable technique for monitoring the changes that occurred during the storage of eggs.

Despite the advantage of MIR to study the secondary structure of proteins, this technique was seldom used to study the egg freshness. To our knowledge, only Narushin et al. [36] have used diffuse infrared spectroscopy (5000–640 cm^{-1}) to predict egg shell quality (e.g., shell thickness, shell weight, shell fracture force, maximal deformation and shell stiffness). Among these parameters, shell thickness was the best predicted parameter by MIR since the correlation coefficient was 0.52. Accuracy of the prediction of the shell maximum deformation (r=0.35–0.42), the shell weight to surface area ratio (r=0.40–0.45), and the shell stiffness (r=0.24–0.29) was slightly improved but the difference between the correlation coefficients were not significant.

Front-face fluorescence spectroscopy

Absorption of light by molecule causes the excitation of an electron moving from a ground state to an excited state. After the electron has been excited, it rapidly relaxes from the higher vibrational states to the lowest vibrational state of the excited electronic state. After reaching the lowest vibrational state of the excited electronic state, the excited state may decay to the ground state by the emission of a photon (fluorescence). Due to energy losses, the emitted fluorescence photon always carries less energy than the absorbed photon [37, 38].

Fluorescence spectroscopy offers several inherent advantages for the characterization of molecular interactions and reactions. First, it is 100–1000 times more sensitive than other spectrophotometric techniques [39]. Second, fluorescent compounds are extremely sensitive to their environment. For example, tryptophan residues that are buried in the hydrophobic interior of a protein have different fluorescent properties than residues that are on a hydrophilic surface [40]. This environmental sensitivity enables to characterize conformational changes such as those attributable to the thermal, solvent or surface denaturation of proteins, as well as the interactions of proteins with other food components. Third, most fluorescence methods are relatively rapid.

If absorbance is less than 0.1, the intensity of the emitted light is proportional to fluorophore concentration and excitation and emission spectra are accurately recorded by classical right-angle fluorescence device. When the absorbance of the sample exceeds 0.1, emission and excitation spectra are both decreased and excitation spectra are distorted [40]. To avoid these problems, a dilution of samples is currently performed so that their total absorbance would be less than 0.1. However, the results obtained on diluted solutions of food samples cannot be extrapolated to native concentrated samples as the organisation of the food matrix is lost [38]. To avoid these problems, the method of front-face fluorescence spectroscopy can be used.

Fluorescence probes or fluorophores represent the most important area of fluorescence spectroscopy. Intrinsic fluorophores include the aromatic amino-acids—tryptophan, tyrosine and phenylalanine in proteins, vitamin A and B_2, NADH derivatives of pyridoxal and chlorophyll, some nucleotide, and numerous other compounds that can be found at low or very low concentration in food [41, 42]. The fluorescent properties of aromatic amino acids of proteins [40, 43, 44] can be used to study protein structure or

protein-hydrophobic molecule interactions [44]. The major proteins of egg contain at least one tryptophan residue, the fluorescence of which allows the monitoring of the structural modifications of proteins during aging.

As mentioned above, fluorescent molecules are extremely sensitive to their environment. For example, the emission of tryptophan is highly sensitive to its local environment, and is thus often used as a reporter group for protein conformational changes [43]. Spectral shifts have been observed as a result of several phenomena, such as tertiary structure change, binding of ligands and protein–protein association. In addition, the emission maxima of proteins reflect the average exposure of their tryptophan residues to the aqueous phase [43]. The fluorescence properties of tryptophan, along with its chromophore moiety-indol ring, have been studied extensively due to its use as a standard optical probe for protein structure and dynamics [45].

Vitamin A in egg yolk can be a good fluorescent probe for the determination of protein–lipid interaction during the storage of eggs. Indeed, in cheese, it has been reported that the excitation spectra of vitamin A recorded between 250 and 350 nm with the emission wavelength set at 410 nm [46] provide information on development of protein–fat globule interactions during milk coagulation. In addition, the shape of the vitamin A excitation spectrum is correlated with the physical state of the triglycerides in the fat globule [47].

Front-face fluorescence spectroscopy has been used extensively in the field of dairy products. However, in the literature, only preliminary studies explored the application of front-face fluorescence for the determination of egg freshness [48]. This may be explained by the fact that eggs are complex products containing numerous fluorescent compounds, which makes it difficult to derive molecular information from their spectra. Posudin [48] has assessed the potential of front-face fluorescence spectroscopy to determine the freshness of egg. This author has used ultraviolet radiation for the quality evaluation of eggs with different level of pigmentation. The emission spectra of different eggs showed two maxima located at 635 and 672 nm after excitation at 405, 510, 540 and 557 nm. These excitation wavelengths are related to the pigments of porphyrin nature and porphyrin derivatives of florin and oxoflorin. The obtained results showed that the intensity at 672 nm depends on the egg freshness. Indeed, an egg shell emits vivid red autofluorescence by ultraviolet radiation, because of the presence of porphyrin on it. The autofluorescence of a fresh egg is stronger than that of an old one because the intensity of autofluorescence depends on the amount of porphyrin on the shell surface. From these results, it was concluded that fluorescence spectroscopy could be a promising approach for quantitative estimation of porphyrin in eggs and thus to determine the egg freshness.

Other techniques

Besides the above-mentioned techniques, other non-destructive measurements have been investigated to determine the egg freshness. Völgyi [49] has used microwave sensors to determine the freshness of eggs and reported that the microwave attenuation (water content) and the bistatic radar cross section (dimensions) of eggs change during 30 days of storage. Their future plan will be the development of a microwave device for the automatic selection of old eggs. Using this equipment the quality control of eggs will be very quick in contrast to the traditional method.

Dutta et al. [50] have used an electronic nose-based system, which employs an array of four inexpensive commercial tin-oxide odour sensors to analyse the state of the egg freshness stored over a period of 20–40 days. These authors have applied multivariate statistical analysis to define regions of clustering in the multi-sensor space according to the state of the freshness of eggs. Their results suggest that it is possible to predict egg freshness into one of three states with up to 95% accuracy. However, no explanation about the chemical compounds that are involved in determining the egg freshness was given.

The aging process of eggs implies many modifications of high complexity, which take place simultaneously or successively. As the whole fresh egg contains a considerable amount of water; water plays an essential role in determining the structure of proteins. It is important to study the dynamic properties of water and its interactions with other components in the egg matrix. NMR relaxation and diffusions measurements have been shown to give detailed information on the state of water and to provide insight into the dimensions and geometries of diffusive domains. Recently, Dutta et al. [50] have investigated changes in molecular mobility of water in eggs stored at different conditions varying in combination of temperature (e.g., 5, 20 and 25 °C), atmosphere (air, CO_2) and lighting (dark, artificial light) at a constant relative humidity of 60% by using nuclear magnetic resonance (NMR). During the first week of storage, an exponential decrease in the transversal relaxation time has been observed; afterwards, this parameter decreased linearly, especially at high temperatures [50]. The observed modifications have been attributed to the changes in the physico-chemical environments due to the structural rearrangements of protein matrix, contributing to the change of water mobility during aging. These modifications were found to be due to the increasing liquefaction of albumen during storage [50–52].

Conclusion

During the past few years, spectroscopic methods have gained importance in the evaluation of food quality parameters. The advantages of spectroscopic methods are their ability to provide rapid analysis and simultaneous evaluation of several parameters, and their potential for on-line or at-line. Although, fluorescence and infrared spectroscopies are techniques whose theory and methodology have been extensively exploited for studies of both chemistry and biochemistry, the utility of these spectroscopies for molecular studies has not been yet fully recognized in food science and especially in the field of egg products.

Fluorescence and infrared spectroscopies have the same potential to address molecular problems in food science as in biochemical science field, because the scientific questions that need to be answered are closely related.

The future aim of our group will be to assess the potential of these two spectroscopic techniques (e.g., infrared and fluorescence) for the evaluation of egg freshness. Then a mathematical model for predicting the freshness, egg age or remaining shelf life of an unknown egg sample will be built. Such a model could complement both sensory and physico-chemical analyses for egg freshness evaluation in the near future. However, more research is needed; this should include controlled storage experiments of different strains of hen eggs to obtain valid parameters for use in mathematical models.

The development of spectroscopic and microscopic methods should also increase our understanding of the determinants of food-texture and may allow to devise a structure engineering of egg products.

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