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"Pullum Ovum". Highlighting the Nutrient Content through Innovative Determination Methods

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Abstract: Introduction. The benefits or risks of egg consumption have been a challenge for researchers in identifying the most effective methods for evaluating micronutrients and, implicitly, the nutritional properties of eggs. The purpose of this work was to highlight the comparative nutritional properties of eggs from hens with similar productive yields through innovative methods, such as FT-IR spectra. Methods. This paper aimed to determine the omega 3 fatty acid (O3FA) content in eggs. In this study were used eggs from four different breeds of laying hens: Lohmann Brown, Isa Brown, Australorp and a hybrid breed. The eggs were collected daily, and their evaluation was carried out at an interval of 24 h after collection. For the calibration of the measurements, the Omacor product whose omega 3 content was evaluated by the National Agency of Medicines and Medical Devices from Romania was used as a reference standard. Results. The study supports that there is a significant difference in the polyunsaturated FA and saturated FA content between eggs of various hen breeds. Conclusions. The composition of egg contents differs in terms of micronutrients both at the level of the egg white, yolk and shell, depending on the breed of the laying hen. This experiment can support the idea that eggs with a lower atherogenic potential can be recommended based on the chicken breed.

Keywords: eggs; omega 3; polyunsaturated fatty acids; IR spectroscopy

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1. Introduction

The nutritional value of food plays a pivotal role in ensuring the optimal supply of macro- and micronutrients. [1]. The role of nutrition in the occurrence of some diseases has been demonstrated in various pathologies, just as the importance of lifestyle and diet has been demonstrated especially in the management of chronic [2–5], autoimmune [6–8] or acute diseases [9,10]. The SARS-CoV-2 pandemic also demonstrated the importance of nutritional status correlated with the survival rate or the occurrence of complications [11] but also the importance of hygienic and healthy dietary practices in supporting antiviral immunity [12]. Also, the iatrogenicity of the treatment of chronic conditions such as dyslipidemia or hyperuricemia can be prevented by adopting a lifestyle that allows the reduction in or even elimination of drug treatment [13–15].

The current evidence of these correlations between dietary habits and the occurrence of certain diseases or their complications did not allow the prevention of these mechanisms,

not only for reasons related to the insufficient dissemination of information or the fact that there were studies with different or sometimes even divergent conclusions on certain themes or pathologies. Very often, aspects related to the direct beneficiary, the patient, are omitted from the analysis or intervention. Thus, for example, in the case of a patient who has suffered a stroke, the therapist should take into account not only the nutritional needs but also the patient's mastication and swallowing disorders. If in such cases the impact of these aspects is easily noticeable, there is also the situation of patients with oral breathing, in which developmental disorders may occur [16].

In patients with oral breathing, regardless of its etiology, obstructive or functional, developmental disorders may occur that have nothing to do with eating style, even if constitutional changes in the asthenic longilin type, poorly represented adipose tissue or anemia would suggest this [17]. The benefits or risks of egg consumption have been a challenge for researchers in identifying the most effective methods for evaluating micronutrients and, implicitly, the nutritional properties of eggs. The combination of standardized methods determines the increase in the accuracy of measurements, and the use of procedures that combine physical and chemical methods can only increase the accuracy and, implicitly, the value of the results obtained. The absorption in the IR is due to the interaction between the electric dipoles of a molecule and the incident electromagnetic radiation; through physical methods, molecules can be identified based on the chemical structure and the vibrations of some chemical bonds within the molecules [18]. Taking these aspects into account, it was seen that IR spectroscopy is not only a particularly useful method but also relevant in terms of the accuracy of the information obtained. This accuracy has been proven even in the evaluation of screening for cervical cancer, comparing the results with those obtained through classical histological methods [19,20].

This work aimed in particular to determine the omega 3 content in eggs, with a specific emphasis on quantifying the polyunsaturated FAs. Probably as a result of this general concern, people who want to supplement their diet with polyunsaturated fatty acids have at their disposal a various range of food supplements that, according to the packaging, contain these substances. According to the legislation, food supplements do not have therapeutic or diagnostic properties, and the composition of food supplements is not standardized as in the case of medicine. For this reason, the search for the standard regarding the calibration of measurements to identify the components of polyunsaturated fatty acids directed our research towards a product approved by the National Agency of Medicines and Medical Devices in Romania so that the research carried out on egg consumption is reported from this point of view to a structure whose effectiveness has been proven both from a curative and preventive point of view. From the point of view of the wavelength range of the electromagnetic radiation spectrum, the field of interest belongs, according to the classification, to the mid-IR range, 4000–350 cm^{-1} [18]. The assignment of FTIR bands to biological tissues and the spectral interpretation of chemical bonds allowed the method to be used for both the quantitative and qualitative determination of various study materials [21].

The existence of different breeds of laying hens may suggest the existence of different nutritional properties of eggs obtained by administering the same standardized feed and that these differences are due to the breed.

The purpose of this work was to highlight the comparative nutritional properties of eggs from hens with similar productive yields through innovative methods.

2. Materials and Methods

Four laying hen breeds, Australop Isa Brown, Lohmann and Blue Moravia (standardized hybrid breed), were selected for this study. All chickens, regardless of race, were about 68 weeks old at the beginning of the study.

For all chickens, the living conditions of the hens were identical, specific to the geographical area in central Romania during the spring–summer months (April–June), with them being raised outdoors but without the possibility of having access to feed other than

standardized feed for laying hens provided by a local authorized producer. The standardized bird feed, AN FEED F1008.02/20/F/AF, contained a mixture of cereals, protein straws, fatty mass, calcium carbonate and a vitamin–mineral premix. The average amount of feed allocated to one hen was approximately 180 g.

In order to prevent contamination or illness, the possibility of contact with other domestic or wild birds was excluded, and the feeding of the birds and the sanitization of the space was performed by a single person who always wore appropriate equipment. Eggs were collected daily, and their evaluation was carried out at an interval of 24 h after collection. Eggs were collected during the daytime period of the day by checking the nests every 4 h, for a period of 12 weeks, and the eggs to be determined were those that came 12 weeks after the introduction of standardized foods that were mentioned exclusively throughout the study. Eggs subject to physical determinations were re-collected according to the measurements. The four chicken breeds in the study had a similar egg production rate, with the average production being about 320–360 eggs per year. The identification of the species from which the egg was obtained was based on the different colors of the shell. Each collected sample was measured only once, with the method used being standardized. For this purpose, in the initial phase, the hens were carefully monitored during egg laying. To obtain FT-IR spectra, pellets were made using 200 mg of potassium bromide to which 20 mg of eggshell or 25 μmL of yolk or white was added. Given that the study focused in particular on the omega 3 component and polyunsaturated fatty acids, in order to calibrate the measurements, it was necessary to identify an agreed and attested benchmark for the benefits it can bring through its intake. For this purpose, we have used as a benchmark the Omacor preparation whose omega 3 content was evaluated by the National Agency of Medicines and Medical Devices in Romania, which approved the product as a medicine. Recording the FT-IR spectra of the product approved by the National Agency of Medicines and Medical Devices in Romania allowed the identification of the wavelengths of interest for the identification of omega 3 fatty acids that can be attributed triglyceride- and cholesterol-reducing properties. Considering the fact that excessive consumption of eggs can also represent a risk for the increase in cholesterol and triglycerides due to the content of fatty acids and cholesterol, the determinations carried out also sought to identify these risks. The measurements were carried out in the laboratory of nuclear magnetic resonance and sensor physics of the Technical University of Cluj Napoca, using fresh eggs harvested within the first 24 h after obtaining them. A Jasco 6200 single-beam spectrometer was used for FT-IR spectroscopy measurements. In the first step, a support suitable for pressed KBr pellets was used to record the background. Thus, the signal from potassium bromide, but especially from water vapor, carbon monoxide, carbon dioxide, nitrogen and dust particles in the atmosphere is eliminated. A total of 64 scans (accumulations of the IR signal) were used. For the actual measurement, the support was extracted from the spectrometer, and 25 μL of yolk and egg white were pipetted onto the potassium bromide pellet. For recording the FT-IR spectrum of eggshell, 20 mg of eggshell was mortared together with potassium bromide, and only a 200 mg potassium bromide pellet was used as a reference. The FT-IR signal was recorded in about 1 min and 20 s using all 64 scans to increase the signal-to-noise ratio by 8 times. The FT-IR signal recorded in the measurement window of Jasco software version 2.01.02 (Jasco Corporation, Tokyo, Japan) was automatically transmitted to its processing window. Baseline correction procedures and a smoothing procedure with a factor of 11 were performed for each recorded FT-IR signal. The FT-IR spectrum thus obtained was saved in a text file. For graphical representation, the text file was imported into the Origin software (Origin Pro 2022b (64 bit) 9.9.5.167 Academic licence) without further procedures. Analysis of specific domains from low wavenumbers of 400–1000 cm^{-1}, which were associated with saturated fatty acids, and high wavenumbers of 2800–3100 cm^{-1}, which were associated with polyunsaturated fatty acids, was performed. The statistical interpretation was carried out with the help of principal component analysis, or PCA, which allowed a reduction in the size of the large data set and variables to a smaller number but which contained the

most important information from our data set. This decrease did not reduce the accuracy of the data. The data were previously checked to confirm their distributions [22].

3. Results

An evaluation of the nutritive properties of the eggs was performed through FT-IR spectrometry regarding the content of omega 3 polyunsaturated fatty acids.

Figure 1 shows the individual variations of the FT-IR spectra measured at the level of the yolk, white and shell for the eggs from the five hens of the Australorp breed.

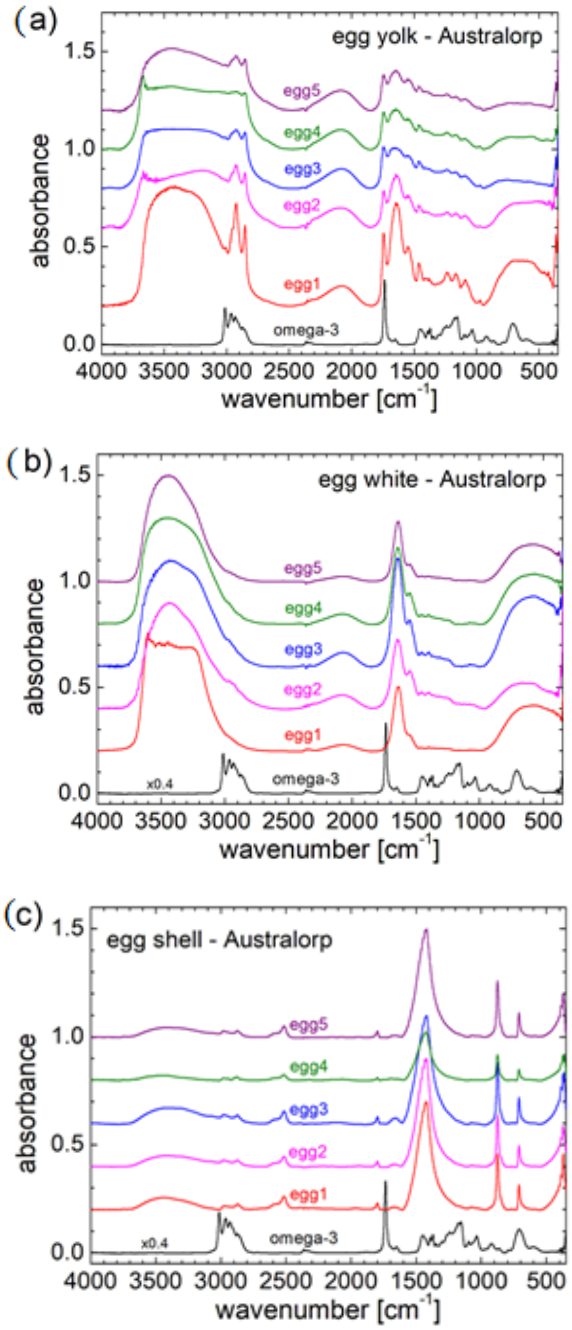


Figure 1. FT-IR spectra measured for 5 Australorp hens' (**a**) yolk, (**b**) white and (**c**) eggshell compared to the FT-IR spectrum of the omega 3 standard.

A comparison with the standard shows that the specific domains from the high wavenumbers of 2800–3100 cm^{-1}, which have been associated with polyunsaturated fatty acids, lipids, phospholipids and cholesterol, are found predominantly in the yolk in a

small amount in the shell and are almost absent in the egg white. At the same time, the specific domains from small wavenumbers of 400–1000 cm^{-1} have a greater presence in the shell and white compared to the yolk of Australorp hens. Within the domains from small wavenumbers, there is a more important share at the level of the eggshell of the chemical structures containing deoxyribonucleic acid, corresponding to a wavelength of 771 cm^{-1}, and of the structures representing the carotenoid content, corresponding to the wavelength of 827 cm^{-1}.

At the same time, corresponding to the range of wavelengths between 1396 and 1403 cm^{-1}, a higher proportion of the presence of protein methyl groups can be observed at the level of the eggshell, a situation not found in the case of the white or the yolk. Corresponding to the wavelength of 1540 cm^{-1} is Amide I, and corresponding to the wavelength of 1600 cm^{-1} is Amide II, with the presence of these protein structures being identified both in the yolk and in the egg white. However, the signal recorded through FT-IR spectroscopy for the wavelength of 1600 cm^{-1} is much better represented at the level of the egg white.

Figure 2 shows the individual variations in the FT-IR spectra measured at the level of the yolk, white and shell for the eggs from the five hybrid breed hens related to high polyunsaturated fatty acids purified with therapeutic properties from the preparation approved by the National Agency of Medicines and Medical Devices in Romania.

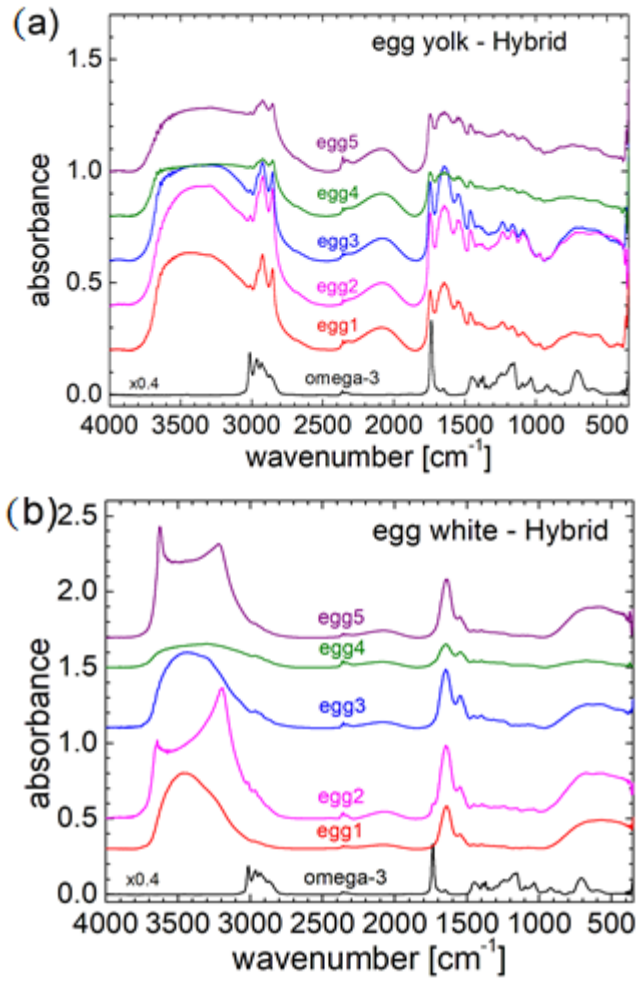


Figure 2. *Cont.*

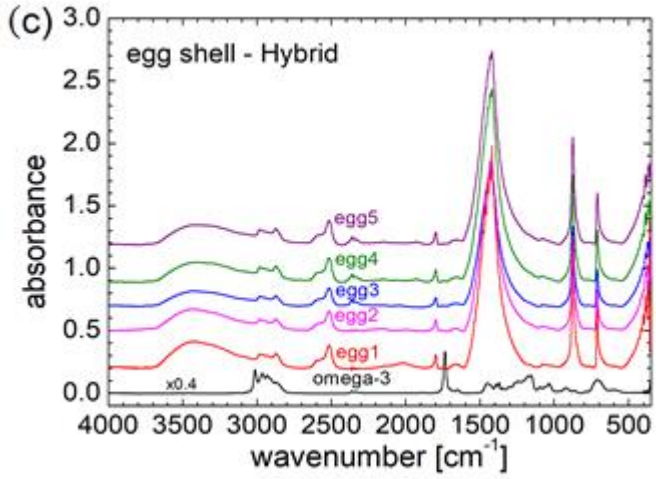


Figure 2. FT-IR spectra measured for 5 Hybrid hens' (**a**) yolk, (**b**) white and (**c**) eggshell compared to the FT-IR spectrum of the omega 3 standard.

A comparison with the standard shows that the specific domains from the high wavenumbers of 2800–3100 cm^{-1} that have been associated with polyunsaturated fatty acids, lipids, phospholipids and cholesterol are found predominantly in the yolk; in a small, relatively constant amount at the level of the shell; and to be almost absent at the level of the albumen. At the same time, the specific domains from small wavenumbers of 400–1000 cm^{-1} have a greater presence at the level of the shell compared to the yolk and white of the hybrid hens' eggs. Within the domains from small wavenumbers, there is a more important share at the level of the eggshell of the chemical structures containing deoxyribonucleic acid, corresponding to the wavelength of 771 cm^{-1}, and of the structures representing the carotenoid content, corresponding to the wavelength of 827 cm^{-1}.

At the same time, corresponding to the range of wavelengths between 1396 and 1403 cm^{-1}, a higher weight of the presence of protein methyl groups can be observed at the level of the eggshell, a situation not found in the case of the egg white, and in the case of the yolk, the weight of these structures is much lower. Corresponding to the wavelength of 1540 cm^{-1} is Amide I, and corresponding to the wavelength of 1600 cm^{-1} is Amide II, with the presence of these protein structures being identified both in the yolk and in the egg white. The signal recorded through FT-IR spectroscopy for the wavelength of 1600 cm^{-1} is approximately equally well represented at the level of the two structures.

Figure 3 shows the individual variations in the FT-IR spectra measured at the level of the yolk, white and shell for the eggs from the five Isa Brown hens.

A comparison with the standard shows that the specific domains from the high wavenumbers of 2800–3100 cm^{-1} that have been associated with polyunsaturated fatty acids, lipids, phospholipids and cholesterol are found predominantly in the yolk; in a small, relatively constant amount at the level of the shell, especially the specific domains from the high wavenumbers of 2800–3000 cm^{-1}; and to be almost absent at the level of the albumen. At the same time, the specific domains from small wavenumbers of 400–1000 cm^{-1} have a greater presence at the level of the shell compared to the egg white and yolk of Isa Brown hens, and, in the case of the latter, the signal is almost absent. Within the domains from small wavenumbers, there is a more important share at the level of the eggshell of the chemical structures containing deoxyribonucleic acid, corresponding to the wavelength of 771 cm^{-1}, and of the structures representing the carotenoid content, corresponding to the wavelength of 827 cm^{-1}.

At the same time, corresponding to the range of wavelengths between 1396 and 1403 cm^{-1}, a higher proportion of the presence of protein methyl groups can be observed at the level of the eggshell, a situation not found in the case of the egg white and the yolk. Corresponding to the wavelength of 1540 cm^{-1} is Amide I, and corresponding to the wavelength of 1600 cm^{-1} is Amide II, with the presence of these protein structures being

identified both in the yolk and in the egg white. The signal recorded by FT-IR spectroscopy for the wavelength 1600 cm^{-1} is much better represented at the egg white level.

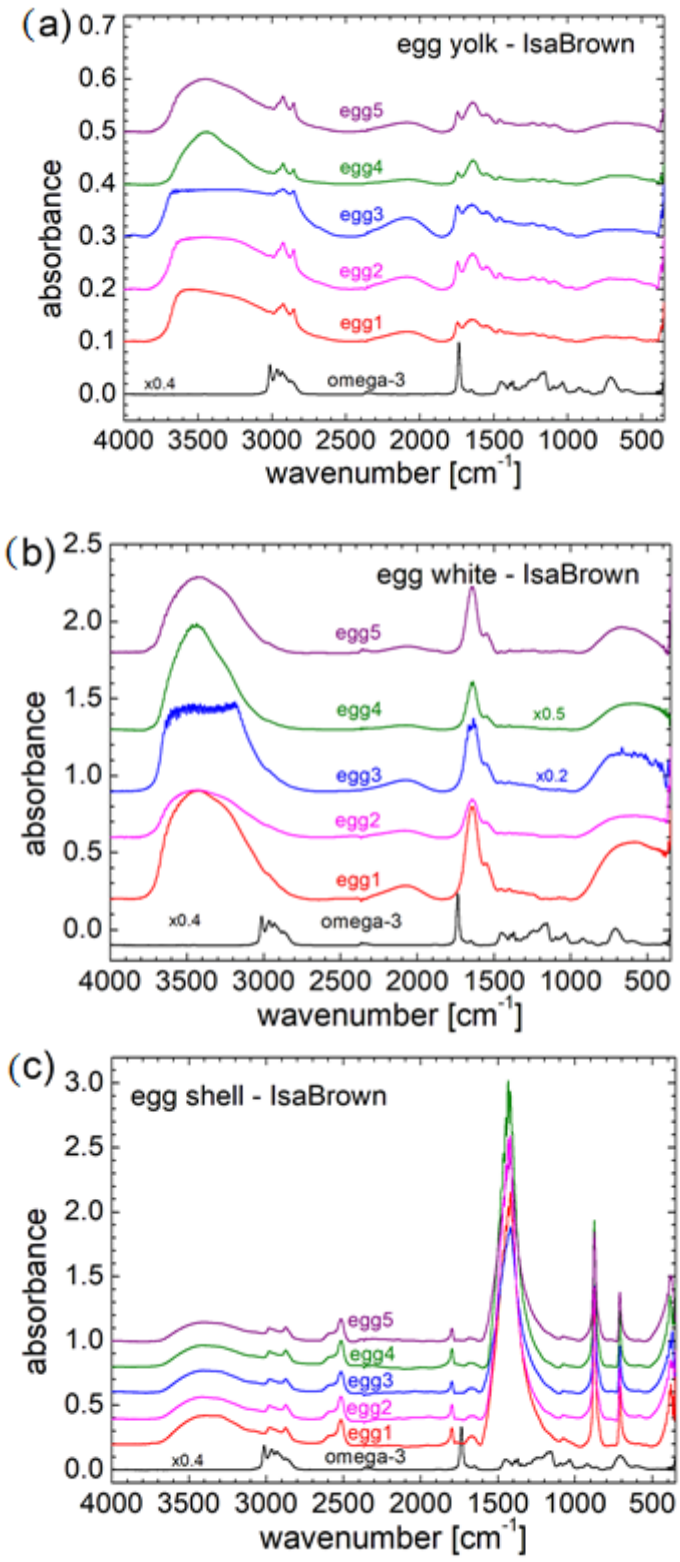


Figure 3. FT-IR spectra measured for 5 hens of the Isa Brown species: (**a**) yolk, (**b**) white and (**c**) eggshell compared to the FT-IR spectrum of the omega 3 standard.

Figure 4 shows the individual variations in the FT-IR spectra measured at the level of the egg yolk, white and shell for the eggs from the five Lohmann Brown hens.

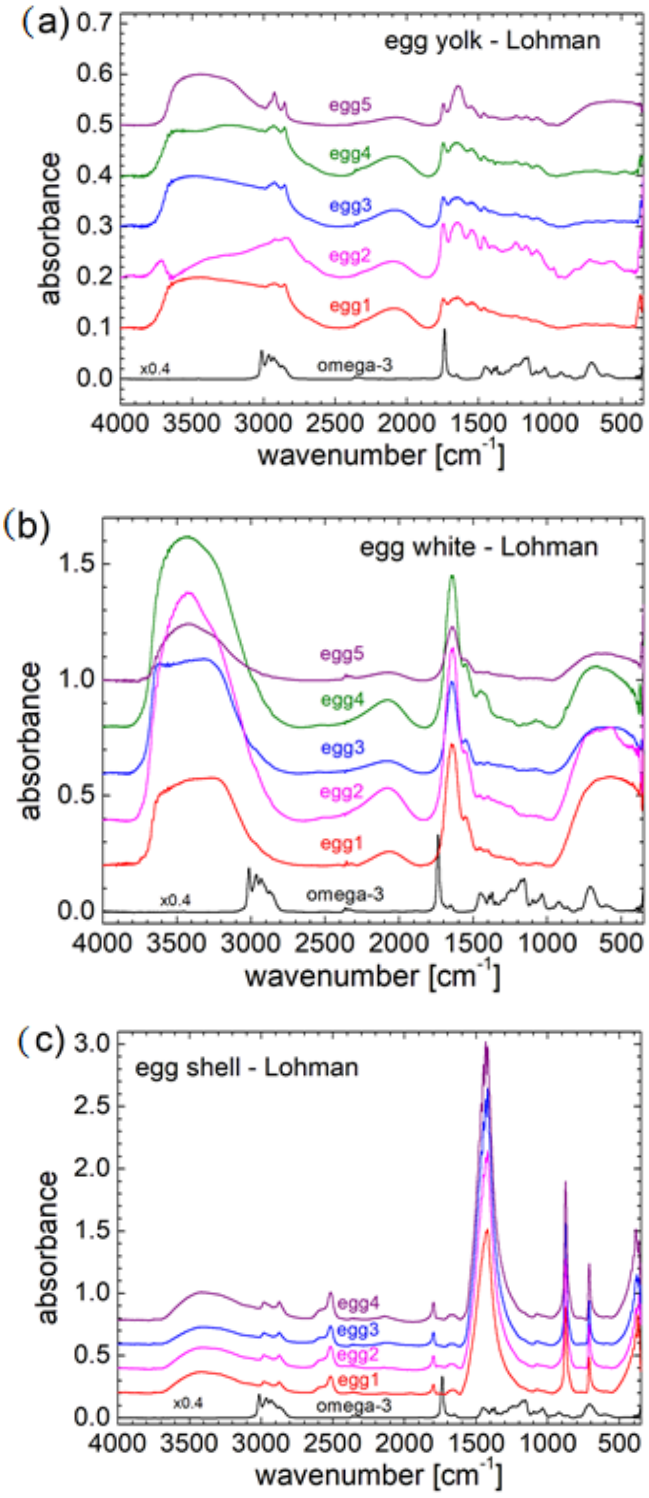


Figure 4. FT-IR spectra measured for 5 hens of the Lohmann Brown species: (**a**) yolk, (**b**) white and (**c**) eggshell compared to the FT-IR spectrum of the omega 3 standard.

A comparison with the standard shows that the specific domains from the high wavenumbers of 2800–3100 cm^{-1} that have been associated with polyunsaturated fatty acids, lipids, phospholipids and cholesterol are found predominantly in the yolk; in a small, relatively constant amount at the level of the shell, especially the specific domains from the high wavenumbers of 2800–3000 cm^{-1}; and to be almost absent at the level of the albumen. At the same time, the specific domains from small wavenumbers of 400–1000 cm^{-1} have a

greater presence at the level of the shell compared to the egg white and yolk of Lohmann Brown hens, and, in the case of the latter, the signal is almost absent. Within the domains from small wavenumbers, there is a more important share at the level of the eggshell of the chemical structures containing deoxyribonucleic acid, corresponding to the wavelength of 771 cm^{-1}, and of the structures representing the carotenoid content, corresponding to the wavelength of 827 cm^{-1}.

At the same time, corresponding to the range of wavelengths between 1396 and 1403 cm^{-1}, a higher weight of the presence of protein methyl groups can be observed at the level of the eggshell, a situation encountered in a very small weight in the case of the egg white and the yolk. Corresponding to the wavelength of 1540 cm^{-1} is Amide I, and corresponding to the wavelength of 1600 cm^{-1} is Amide II, with the presence of these protein structures being identified both in the yolk and in the egg white. The signal recorded through FT-IR spectroscopy for the wavelength of 1600 cm^{-1} is much better represented at the egg white level. The comparative evaluation of the content in the monitored micronutrients required the determination of the absorbances measured as integral areas for each species of chickens (Australorp, hybrid, Isa Brown and Lohmann Brown) and reported to the corresponding wavelength range. This comparative analysis, corresponding to the lipid structures, was carried out on the egg yolk and is represented in Table 1.

Table 1. Absorbents measured as integral areas for chicken species of lines corresponding to polyunsaturated fatty acids centered at wavelengths of 3010, 2960, 2940, 2910 and 2855 cm^{-1}.

Race	Hen	PolyN				
		3010 cm^{-1}	**2960 cm^{-1}**	**2940 cm^{-1}**	**2910 cm^{-1}**	**2855 cm^{-1}**
Australorp	G1	10.4	7.2	12.7	4.3	2.8
	G2	11.3	5.2	7.7	3.2	3.4
	G3	9.4	9.3	11.1	4.3	14.8
	G4	9.6	10.5	6.5	2.2	11.8
	G5	8.3	8.7	7.7	7.3	2.1
	Average	9.8	8.18	9.14	4.26	6.98
Hybrid	G1	3.8	3.7	6.9	8.4	8.3
	G2	3.0	2.7	7.7	7.5	8.7
	G3	3.7	4.9	10	5.7	8.7
	G4	5.6	6.7	8.9	6.6	8.7
	G5	5.9	6.4	8.5	5.7	3.8
	Average	4.4	4.88	8.4	6.78	7.64
Isa Brown	G1	4.9	4.1	5.6	7.1	2.3
	G2	2.8	2.9	6.1	6.7	6.0
	G3	3.4	2.9	6.1	6.7	2.2
	G4	4.9	4.8	8.1	7.8	2.4
	G5	3.8	3.5	6.1	7.4	5.9
	Average	3.96	3.64	6.4	7.14	3.76
Lohmann Brown	G1	7.0	6.3	6.4	3.8	1.5
	G2	7.4	3.9	3.5	3.6	10.6
	G3	4.4	5.3	4.5	12.9	1.0
	G4	5.9	8.2	8.4	5.3	14.1
	G5	6.7	4.1	4.2	11.6	12.4
	Average	6.28	5.56	5.4	7.44	7.92

Considering the fact that the measurements within each breed showed individual variability, a mean value corresponding to each wavelength analyzed within each species was calculated. This calculation is useful for a more efficient comparative evaluation of the content of a micronutrient relative to the breeds of laying hens.

The comparative evaluation of the content of saturated fatty acids and nucleic acids required the determination of absorbances measured as integral areas for each species of chicken (Australorp, hybrid, Isa Brown and Lohmann Brown) and reported to the corresponding wavelength range. This comparative analysis was carried out on egg yolk and is represented in Table 2.

Table 2. The absorbances measured as integral areas for each species of chickens (Australorp, hybrid, Isa Brown and Lohmann Brown) of the lines corresponding to saturated fatty acids and nucleic acids centered at the wavelengths of 974, 912, 827, 771, 736, 680 and 576 cm^{-1}; measurements made on egg yolk.

Race	Hen	FAs						
		974 cm^{-1}	**912 cm^{-1}**	**827 cm^{-1}**	**771 cm^{-1}**	**736 cm^{-1}**	**680 cm^{-1}**	**576 cm^{-1}**
Australorp	G1	0.8	2.9	7.5	8.9	10.3	12.5	19.8
	G2	0.8	0	5.1	6.6	9.7	16.5	16.9
	G3	1.5	0.4	24.6	5.0	8.4	16.8	27.5
	G4	1.8	0.8	11.3	10.7	11.0	15.8	19.7
	G5	2.9	1.2	11.3	1.3	3.2	26.3	22.3
	Average	1.56	1.06	11.96	6.5	8.52	17.58	21.24
Hybrid	G1	4.1	0.7	20.8	12.2	11.6	6.0	26.5
	G2	2.1	0.4	9.9	22.4	18.3	15.1	15.6
	G3	1.9	0.2	9.7	10.1	16.1	10.6	4.2
	G4	6.2	10.5	15.3	8.9	8.3	14.3	12.3
	G5	6.7	6.6	13.7	12.0	12.6	14.8	18.1
	Average	4.2	3.68	13.88	13.12	13.38	12.16	15.34
Isa Brown	G1	3.0	0.6	7.1	8.9	22.4	14.7	24.3
	G2	1.9	1.5	10.8	7.1	18.8	19.3	20.5
	G3	2.6	0.5	6.8	13.3	10.5	21	23.4
	G4	0.9	0.1	3.3	11.5	15	15.6	18.6
	G5	1.4	1.8	11.2	6.7	20.5	16.6	11.8
	Average	1.96	0.9	7.84	9.5	17.44	17.44	19.72
Lohmann Brown	G1	4.2	0.8	6.2	8.2	11.1	11.9	18
	G2	5.2	0.6	6.9	17.1	1.0	17.8	27.1
	G3	2.4	0.1	1.6	6.1	4.2	7.8	4.2
	G4	1.0	1.0	1.2	19.3	15.4	12.9	13.9
	G5	0.2	0.4	6.5	11.0	17.1	27.5	13.2
	Average	2.6	0.58	4.48	12.34	9.76	15.58	15.28

Considering the fact that the measurements within each breed showed, also in this case, individual variability, an average value was calculated corresponding to each analyzed wavelength within each species. This calculation is useful for a more efficient comparative evaluation of the content of a micronutrient relative to the breeds of laying hens.

The content of polyunsaturated fatty acids (PolyN) but also in saturated fatty acids (FAs) is very important in terms of the ratio between the two structures. Thus, a PolyN/Fas ratio is favorable the higher the subunit value (Figure 5).

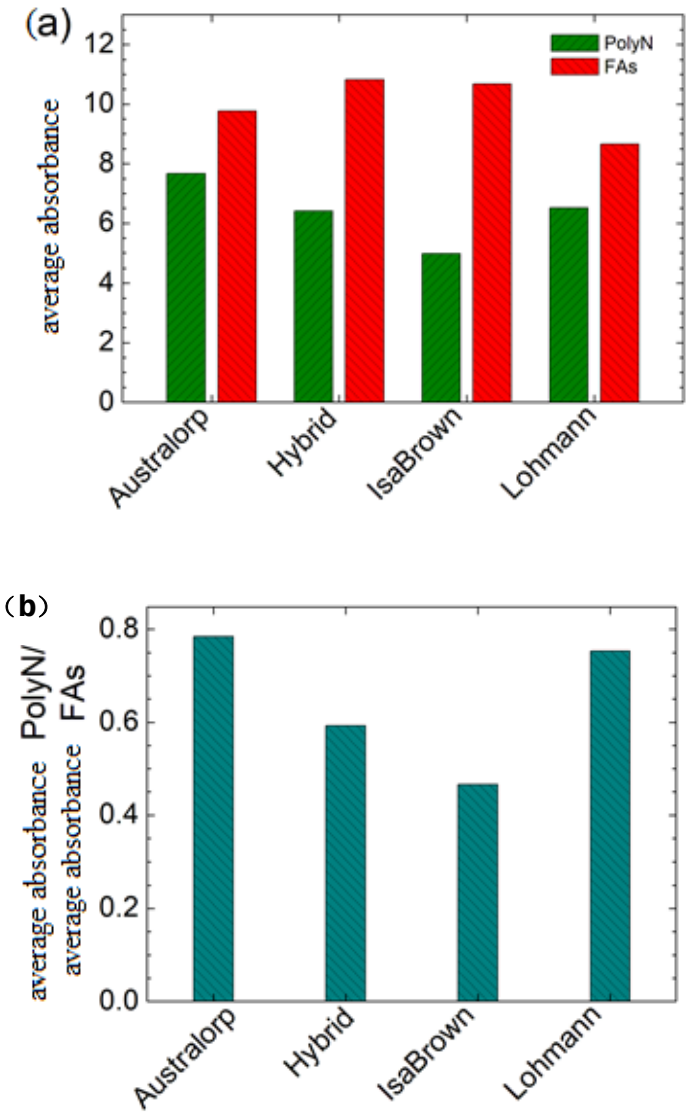


Figure 5. (**a**) Average absorbance calculated for all Australorp, hybrid, Isa Brown and Lohmann Brown chickens for polyunsaturated fatty acids (green) and saturated fatty acids domains. (**b**) The ratio of the average absorbents for the domains of polyunsaturated and saturated fatty acids for the same species of chickens.

Figure 6 shows the PCA for the evaluation of the variations in the values recorded in eggs from each species of Australorp (black), hybrid (blue), Isa Brown (brown) and Lohmann Brown (red) hens.

In Figure 6, it can be seen that the PCA carried out in order to evaluate the variations in the values recorded in the eggs from the same breed in order to identify the breed that has the lowest individual variability highlighted the advantages of using hens from the Australorp and Isa Brown breeds.

The analysis of PC1 and PC2 highlighted the fact that the structures corresponding to the average absorbance values measured as the integral area for the lines in the FT-IR spectrum centered at 3010 cm^{-1} and 827 cm^{-1}, respectively, i.e., highly purified omega 3 fatty acids and carotenoids can represent the structures whose evaluation accuracy is increased.

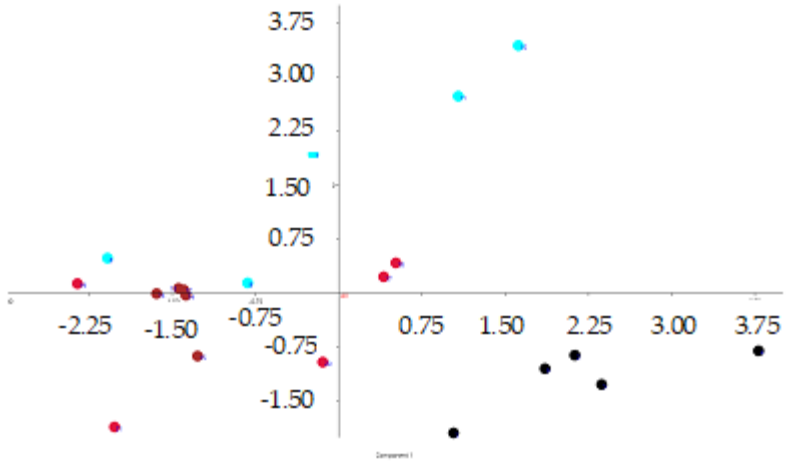


Figure 6. PCA for the evaluation of the variations in the values recorded in eggs from each species of Australorp (black), hybrid (blue), Isa Brown (brown) and Lohmann Brown (red) hens.

4. Discussion

At the level of the yolk, the highest concentration of omega 3 polyunsaturated fatty acids with a high degree of purification, corresponding to the average absorbance values measured as the integral area for the lines in the FT-IR spectrum centered at 3010 cm^{-1}, and the highest concentration of lipids corresponding to the values of the average absorbance measured as the integral area for the lines in the FT-IR spectrum centered at 2960 cm^{-1} and 2940 cm^{-1} are found in eggs from the Australorp hen breed. A 2018 study showed that FTIR spectroscopy can be used as a non-destructive method to classify eggs in terms of hen culture method and to monitor their freshness [23], while another study showed that FT-IR spectroscopy combined with multivariate models can be used as an effective method to detect both the surface and internal constituents of eggs [24]. The highest concentration of cholesterol corresponding to the average absorbance values measured as the integral area for the lines in the FT-IR spectrum centered at 2855 cm^{-1} is found in the egg yolk belonging to the Lohmann Brown breed, and the lowest is found in the egg yolk belonging to the Isa Brown breed. The average absorbance values measured as the integral area for the lines in the FT-IR spectrum centered at 2910 cm^{-1} corresponding to the oscillations determined based on the chemical bonds of phospholipids and cholesterol show the lowest concentration in the egg yolk belonging to the Australorp breed, and the highest is in the egg yolk belonging to the Lohmann Brown breed.

Regarding the nucleic acid content corresponding to the average absorbance values measured as the integral area for the lines in the FT-IR spectrum centered at 974 cm^{-1}, the fatty acid content calculated by determining the average absorbance values measured as the integral area for the lines in the FT-IR spectrum centered at 912 cm^{-1}, the carotenoid content calculated by determining the average absorbance values measured as the integral area for the lines in the FT-IR spectrum centered at 827 cm^{-1} and the deoxyribonucleic acid content calculated by determining the average absorbance values measured as the integral area for the lines in the spectrum FT-IR centered at 771 cm^{-1}, the highest concentrations were recorded in the yolk of eggs belonging to the hybrid breed.

The determination of the average absorbance values measured as the integral area for the lines in the FT-IR spectrum centered at 736 cm^{-1} showed that the egg yolk from the Isa Brown breed had the highest concentration of guanine, and the egg yolk from the breed Australorp had the highest concentration of chemical structures containing the phenolic group calculated by determining the average absorbance values measured as the integral area for the lines in the FT-IR spectrum centered at 680 cm^{-1} and the highest concentration of chemical structures containing the methyl group and the phenols calculated by determining the average absorbance values measured as the integral area for the lines in the FT-IR spectrum centered at 576 cm^{-1}.

Further research will include a closer examination of the predictions through formalization, which would allow the understanding of this process by the general public. Thus, the formal model allows the identification of all the stages of this research, and it will be possible to make the presentation of these data much easier [25–27].

The use of standardized feed in this study, as opposed to other studies in this field, was not intended to demonstrate how certain physico-chemical properties of some nutrients can influence the quality or nutritional properties of the egg.

The study showed that if the breed of laying hens was not considered and the nutrient recipe was extrapolated to another breed, it would be possible to suspect an error in the recipe or working method, raising the suspicion that the method is not reproducible.

Evaluating the nutritional properties of the egg can involve many aspects, but addressing the benefits weighed against the risks that egg consumption can have is a real challenge. Even if an overdose of vitamin A, a lipophilic organic compound that is also contained in the egg, can affect the normal development of the fetus, this happens very rarely [28–30]. The fear related to the consumption of eggs is that of the atherogenic risk, which some authors state is correlated with the number of eggs consumed [31,32]. However, we consider that the evaluation of the ratio of saturated and polyunsaturated fatty acids can represent an additional indicator regarding the different atherogenic risks depending on the breed of laying hens.

The study confirmed that there is a different proportion of the ratio of polyunsaturated fatty acids to saturated fatty acids, and this may represent a criterion by which eggs with a lower atherogenic potential than those of other breeds of laying hens can be recommended. Such a finding might, on the one hand, suggest marking on the egg packaging the breed of origin, and, on the other hand, improve selection for the optimization of the egg quality of the breeds that have this sanogenic potential appropriately labeled [33,34]. Complementing the existing markings [35] by introducing codes to identify the breed of the laying hen would lead producers to be concerned with breeding hens that provide superior egg quality. The concern of farmers regarding the increase in egg quality by changing the feed of hens [32,36] correlated with the information regarding the breed of laying hens that come closest to the proposed objectives regarding the nutritional quality of the egg could facilitate the achievement of their objectives.

If the human diet mainly includes the consumption of the egg white and the yolk, and, from a nutritional point of view, it is considered that the yolk has the greatest value [37], the analysis regarding the content of polyunsaturated fatty acids in the shell, egg white and yolk compared to the polyunsaturated fatty acids with a high degree of purification from the omega 3 standard used and accredited by the Romanian National Agency for Medicines and Medical Devices as having therapeutic properties showed that chemical structures similar to those in the standard are found in different proportions in all three structures of the egg. From this point of view, the hypothesis was confirmed that the evaluation of the three structures is important because the modification of the food of the laying hens can enrich the content of omega 3 polyunsaturated fatty acids, but it is very important to establish the percentage of these modifications correlated with the element at which the modification occurs—the peel, white or yolk. Thus, studies based on chicken feed can improve feed formulations in terms of evaluating the effectiveness of such interventions. The degree of variability, sometimes high within a breed of hens, under conditions of compliance with identical living conditions and food, could be attributed to the nyctemeral rhythm, since the eggs whose properties were determined were collected in the last 24 h before carrying out measurements but without marking the time of day when the collection was made.

Research results have shown that the inclusion of essential nutrients in laying hen diets can be a strategy to improve the quality of eggs [38], and the factors that can influence the extent to which the respective nutrients will be found in the egg require research.

5. Conclusions

After comparing the nutritional properties of the eggs of different breeds of chickens, we highlighted that the compositions of the eggs differ in terms of micronutrients in the egg white, yolk and shell depending on the breed of laying hen.

There is variability in the composition of the eggs in the same breed of laying hens, but a specific trend of each breed could be observed depending on the chemical structure identified through FT-IR spectroscopy.

The study showed that genetic determinism specific to the breed of a laying hen can influence the composition of the egg. Thus, the way in which a nutrient influences the nutritional properties of the egg must not be extrapolated to all breeds of laying hens, as well as the risks that excessive consumption of eggs represents. This feature of the egg composition proven to be correlated with the hen breed could allow their selection according to the objective pursued for optimizing the quality of the egg based on the nutrients used in feeding the chickens, thus optimizing the way of fulfilling the objective of other studies.

The results of this study contribute to understanding the extent to which the qualities of eggs and the effects of supplementation can be influenced by the particular characteristics of the breed of the laying hen.

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