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Use of RGB channels in multivariate analyses to characterize commercial and conventional eggs in laying hen farming

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Abstract

The present study aimed to apply multivariate analysis based on data extracted from digital images to characterize eggs from laying hens raised in conventional and free-range systems, seeking an objective approach to egg differentiation in poultry farming. Twelve samples were analyzed, subdivided into shell, white, and yolk from commercially purchased eggs and eggs from local farms for personal consumption, differentiated by shell color (white, red, or brown and blue). The images were obtained using a standardized light chamber at a height of 30 cm with white walls and processed using the ImageJ software with the help of the Color Inspector 3D plugin. Thus, the intensities of the RGB (Red, Green, and Blue) channels were extracted for the shell, yolk, and white. The data were submitted to Principal Component Analysis (PCA) in the statistical environment R. PCA explained 97.4% of the total variability in the first two dimensions, with PC1 strongly associated with the R channel. Free-range egg yolks showed higher intensities in the R and G channels (averages of up to 156.8 and 79.6, respectively), significantly higher than those of commercial eggs ($p < .01$), reflecting a diet rich in carotenoids. White shells clustered close to the blue vector (mean: 215.3), while red and blue shells exhibited greater variation in the R and G channels ($p < .05$), indicating genetic and dietary influence. The whites presented smaller variations ($p > .05$), with translucent coloration and low saturation. It can therefore be concluded that multivariate analysis based on RGB data is an objective and effective tool for describing and differentiating eggs according to the breeding system, with potential application in visual quality control and traceability of origin.

Keywords: digital image; color analysis; non-destructive analysis; quality control; ImageJ.

Practical application: Multivariate RGB analysis offers an objective method for differentiating eggs by production system.

1 INTRODUCTION

Egg production in Brazil is one of the most representative activities in the national poultry industry, generating substantial production volumes and contributing significantly to the country's food sector and economic security (Brasil, 2023). According to the Municipal Livestock Survey, in 2023 Brazil reached the milestone of 39.2 billion eggs produced, with the Southeast and Northeast acting as the primary growth centers (Instituto Brasileiro de Geografia e Estatística [IBGE], 2023).

Alongside intensive production, there has been an increase in demand for eggs from chickens raised in alternative systems, such as free-range and commercial farm systems, which are valued by consumers who associate these products with better sensory attributes and greater social and environmental responsibility (Lusk, 2019; Rondoni et al., 2020). However, objective differentiation between commercial and free-range eggs is still a technical challenge, especially regarding visual aspects, such as shell color, and their internal components: white and yolk (Hidalgo et al., 2008; Liao et al., 2023).

The use of digital imaging technologies in agribusiness has promoted significant innovations in product quality control and is widely used for objective measurement of physical and visual characteristics (Brosnan & Sun, 2004). The use of RGB (Red, Green, Blue) color channels, extracted from digital photographs, allows for the quantitative analysis of chromatic parameters of eggshells, yolks, and whites with precision and reproducibility (León et al., 2006; Milovanovic et al., 2021). These channels are effective in assessing color intensity and uniformity, overcoming the limitations of subjective visual inspections made with the naked eye (Wu & Sun, 2013). In addition, image-based methods offer advantages such as low cost, rapid data collection, and the possibility of industrial-scale automation (Atwa et al., 2024; Brosnan & Sun, 2004).

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To properly interpret the data extracted from the RGB channels, statistical techniques such as multivariate analysis have been employed, allowing for the simultaneous treatment of multiple variables that describe the same object (Jolliffe & Cadima, 2016). Among these techniques, Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) stand out, enabling the distinction between groups of eggs based on their color patterns (Campmajó et al., 2019; Dibakoane et al., 2023). The application of these tools has made it possible not only to classify eggs by type of farming, but also to identify variations associated with feed, bird age, and environmental conditions (Odabaşi et al., 2007; Samiullah et al., 2015; Van den Brand et al., 2004). Thus, multivariate analysis based on RGB data contributes to the traceability and valorization of alternative products in the consumer market (Campmajó et al., 2019; Dibakoane et al., 2023).

In view of the above, this study aimed to apply multivariate analysis to data extracted from the RGB channels of digital images, characterizing and comparing eggs from laying hens raised in conventional and free-range systems. The aim is to offer an objective, accessible, and scientifically validated approach to differentiating eggs in poultry farming, strengthening the link between visual quality and traceability of origin.

1.1 Relevance of the work

The topic is highly relevant as it demonstrates how objective digital image analysis can improve poultry farming practices. By applying multivariate analysis of RGB values from egg components, researchers can differentiate eggs from conventional and free-range systems with precision. This approach reduces subjectivity in visual inspection, supports traceability of origin, and enhances consumer confidence in product labeling. It also provides valuable insights into the influence of diet and genetics on egg quality, particularly yolk coloration linked to carotenoids. This method has practical applications in quality control, certification, and automated classification, rendering it a promising tool for modern agribusiness.

2 MATERIALS AND METHODS

The experiment was conducted using egg samples from two different laying hen farming systems: the conventional (commercial farm) and the alternative system (free-range), purchased from commercial establishments and independent local producers, respectively. The eggs were classified according to shell color into three groups: white, red (brown), and blue. All samples were kept at room temperature until the time of analysis.

For image acquisition, a light chamber was developed with white interior walls and a standard height of 30 cm to ensure uniform lighting and minimize external interference. The light source consisted of a lamp with a neutral white LED bulb (4,000–4,500K), positioned laterally and directed toward the center of the sample. The images were captured with a high-resolution smartphone (minimum 12 MP), attached to a fixed support at a constant distance from the sample. Each egg was photographed separately across three components: intact shell, yolk, and egg white, the latter two obtained after controlled breaking of the egg on a transparent Petri dish.

The images obtained were processed using the ImageJ software (version 1.54p), widely used for morphometric and spectral analyses in agro-industrial studies (Schindelin et al., 2012; Schneider et al., 2012). Initially, the area of interest was isolated manually using the free selection tool. Then, the external regions were removed using the Edit > Clear Outside command to avoid background interference in the chromatic analyses. The RGB (Red, Green, Blue) color channel analyses were performed based on the pixel intensity distribution histogram, on a scale of 0 to 255, using the Analyze > Histogram command, as recommended by León et al. (2006).

In addition, the Color Inspector 3D plugin, available in the official ImageJ library, was also used, allowing for a three-dimensional visualization of the chromatic distribution in the RGB channels and contributing to the spatial understanding of the spectral data.

The data extracted from the RGB color channels (intensity values for red, green, and blue) referring to the images of the eggshells, yolks, and egg whites were organized in a spreadsheet (Microsoft Excel) and subsequently imported into the statistical environment R (version 4.3.2; R Core Team, 2023), using the readxl package for data reading and the FactoMineR (Lê et al., 2008) and factoextra (Kassambara & Mundt, 2020) packages for multivariate analysis.

Principal Component Analysis (PCA) was applied to reduce the dimensionality of the data as well as identifying patterns of variation between different types of eggs, as suggested by Jolliffe and Cadima (2016). This approach allows for a set of correlated variables (RGB values) to be transformed into a new set of uncorrelated variables called principal components, facilitating the visualization of groupings and trends.

3 RESULTS

Chromatic analysis of eggshells revealed significant variations between types of farming and coloration. The red shell from free-range chickens showed high intensity in the red and green channels (averages of 210.6 and 172.6, respectively) and a lower contribution from the blue channel (113.7), giving the surface an earthy, orange hue (Figure 1A). In turn, the blue shell exhibited a whitish hue with a subtle bluish tint, associated with higher averages in the red (221.95) and green (216.01) channels, while the blue, although lower (190.69), presented greater variability (Figure 1B). The white shell, typical of commercial Leghorn chickens, exhibited a highly luminous and uniform color, with averages of 231.5 (R), 224.7 (G), and 215.3 (B), indicating a balance between the three channels (Figure 2A). The red shell of commercial eggs exhibited an intense brown hue, with a predominance in the R (182.3) and G (110.7) channels, while the B channel (47.5) presented low intensity, characteristic of eggs with protoporphyrin IX deposition (Figure 2B).

The analyses of egg whites showed translucent coloring with slight variations between the farming systems. The red-shelled free-range egg white presented a faint yellowish hue, with averages

of 137.9 (R), 124.2 (G), and 87.3 (B) (Figure 3A), while free-range egg whites from blue-shelled eggs showed slightly lower values, mainly in the blue channel (41.1), which visually accentuated the cream tone (Figure 3B). Commercial egg whites, both white and red shell, had averages ranging from 132.3 to 148.9 in the red channel and from 111.9 to 138.5 in the green channel. The blue channel had the lowest averages, contributing to the yellowish and uniform visual perception of the samples (Figures 4A and 4B).

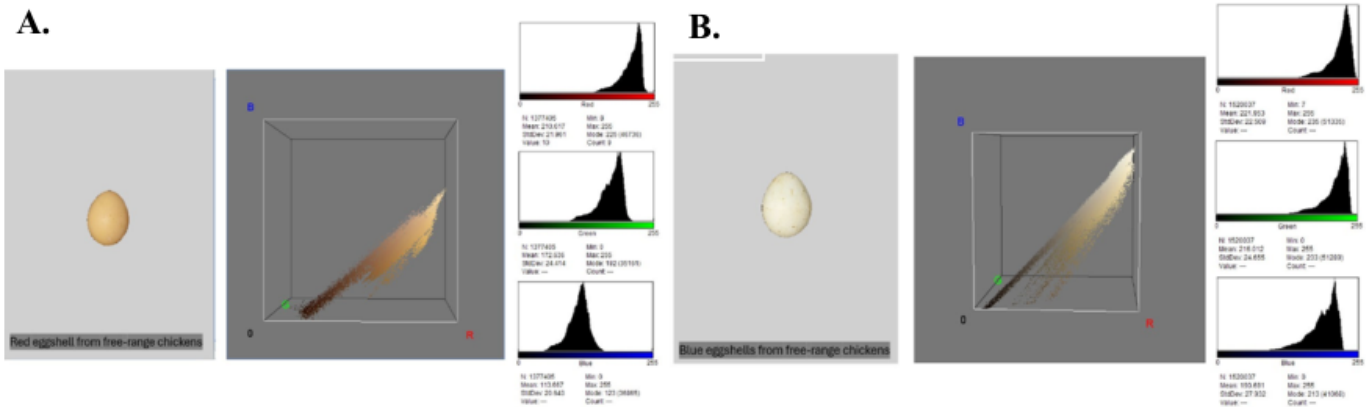


Figure 1. (A) Chromatic analysis of a red eggshell from a chicken raised in a free-range system, using a digital image processed in ImageJ software with the Color Inspector 3D plugin. (B) Chromatic analysis of a blue eggshell from a chicken raised in a free-range system, using a digital image processed in ImageJ software with the Color Inspector 3D plugin.

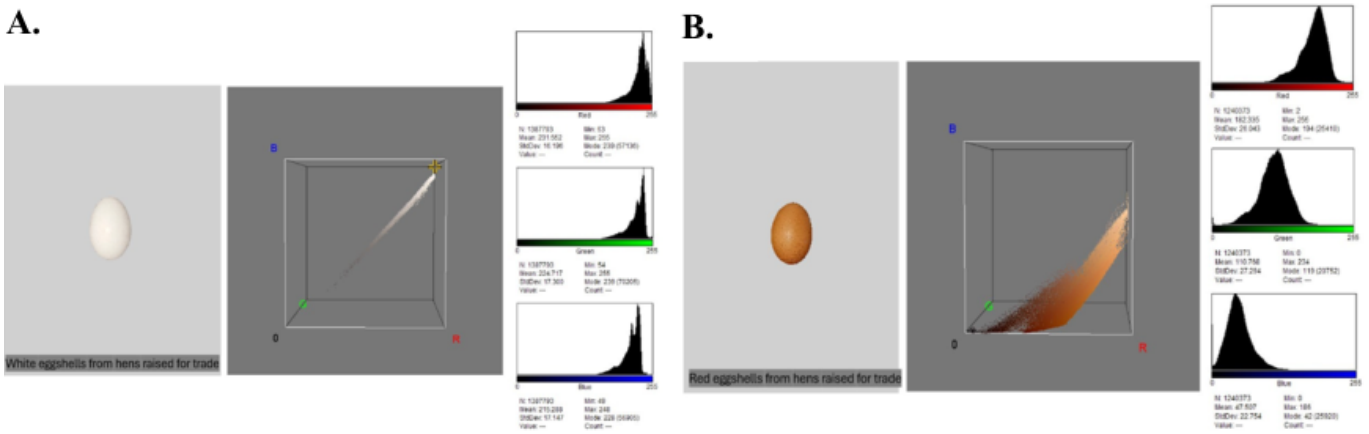


Figure 2. (A) Chromatic analysis of a white-shelled egg from commercial laying hens, using a digital image processed in ImageJ software with the Color Inspector 3D plugin. (B) Chromatic analysis of a red-shelled egg from commercial laying hens, using a digital image processed in ImageJ software with the Color Inspector 3D plugin.

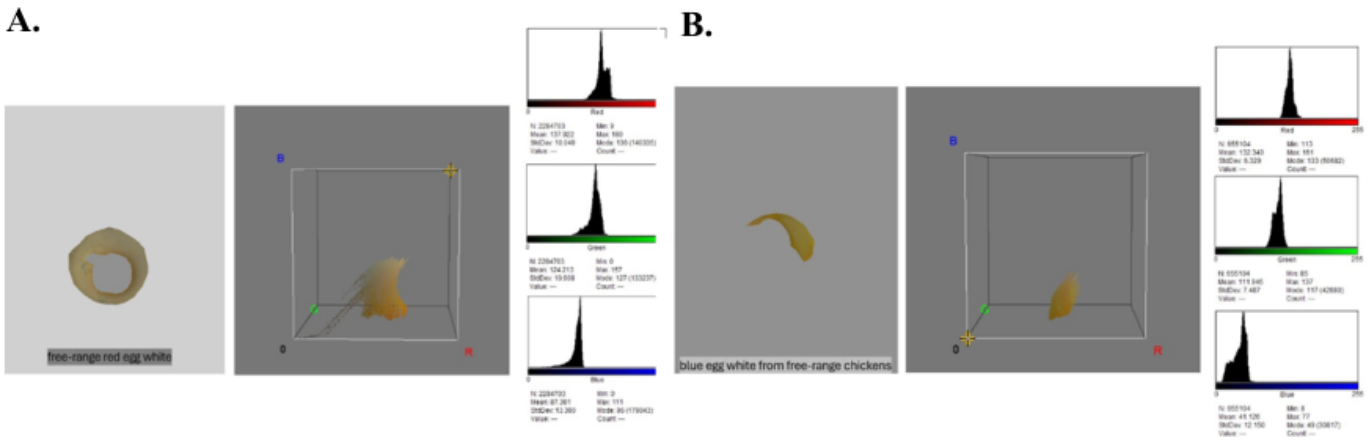


Figure 3. (A) Chromatic analysis of egg white from free-range chickens with red shells, using a digital image processed in ImageJ software with the Color Inspector 3D plugin. (B) Chromatic analysis of free-range chicken egg whites with blue shells, using a digital image processed in ImageJ software with the Color Inspector 3D plugin.

The yolks were the components with the greatest chromatic variation among the groups analyzed. The yolks of free-range eggs were intensely orange in color, with averages of 156.8 (R) and 79.6 (G), and very low values in the blue channel (10.6), reflecting high saturation and a strong influence of the natural diet rich in carotenoids (Figure 5A). The yolk from free-range chickens with blue shells presented similar values, with averages of 154.5 (R), 76.3 (G), and 9.4 (B), maintaining the pattern of intense pigmentation (Figure 5B). In contrast, commercial egg yolks, although also orange in color, displayed reduced values in the R and G channels, with averages between 163.4 and 170.0 (R) and between 75.7 and 90.7 (G), and minimum values in blue (7.0 to 11.8), reflecting the standardized use of pigments in diets (Figures 6A and 6B).

Principal Component Analysis (PCA) provided a clear visualization of the differences between egg components (shell, white, and yolk) and between breeding systems. Dimension 1 (PC1) accounted for 85.8% of the total variability and was strongly associated with the red channel, while Dimension 2 (PC2), with 11.6%, was associated with the blue channel, totaling 97.4% of the explanation of variability (Figure 7). The yolks of free-range eggs were clustered within distinct regions of the scatter plot, reflecting greater saturation in the R and G channels. Commercial yolks, on the other hand, were less dispersed, indicating chromatic uniformity due to feed control.

The shells also formed distinct clusters in the multivariate analysis. White shells were concentrated near the blue vector due to their high brightness and low saturation, while red and blue shells, particularly those from free-range eggs, exhibited greater variation in the red and green channels. These differences reflect the genetic and dietary diversity present in the alternative system, as well as suggesting potential for visual discrimination between eggs based on their origin and shell coloration.

The egg whites, in turn, exhibited lower variability between groups, with stable and translucent coloring, presenting themselves as the least discriminating component among those analyzed. However, the results indicate that RGB analysis can identify subtle patterns related to the quality, freshness, and uniformity of egg whites, reinforcing the applicability of this approach in studies of traceability, automated classification, and quality control in the poultry industry.

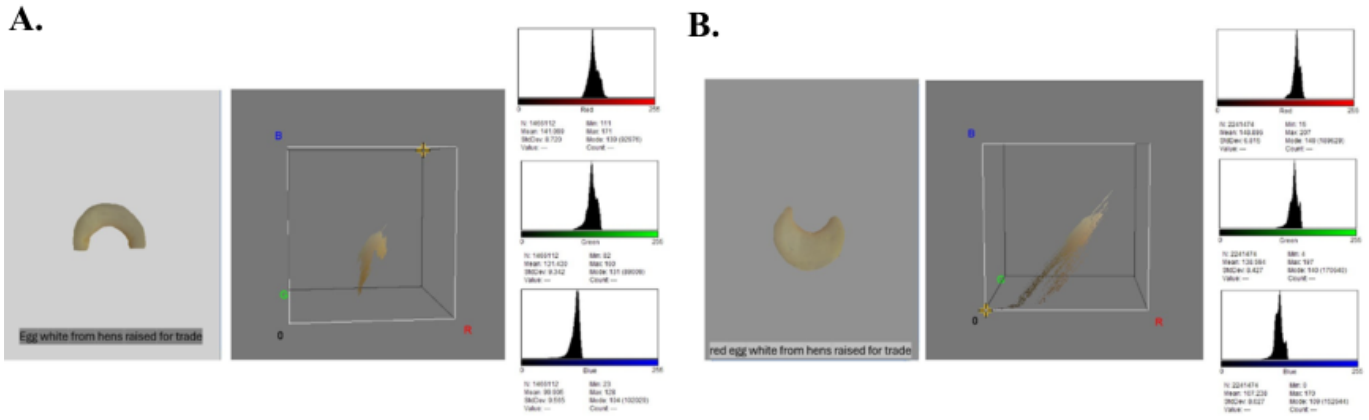


Figure 4. (A) Chromatic analysis of egg whites sourced from commercial chickens (farm-raised), using a digital image processed in ImageJ software with the Color Inspector 3D plugin. (B) Chromatic analysis of egg white from commercial red-shelled laying hens, using a digital image processed in ImageJ software with the Color Inspector 3D plugin.

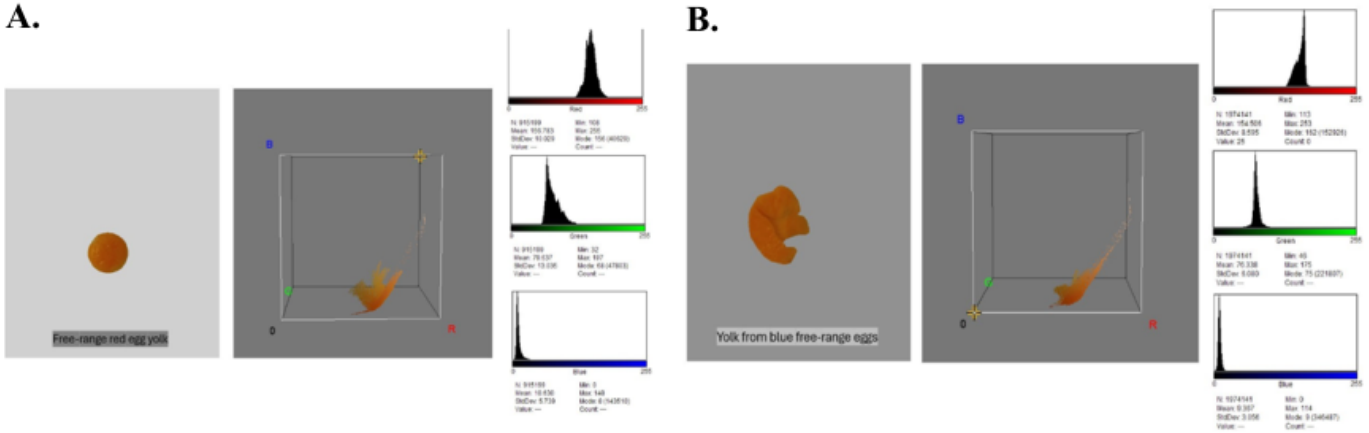


Figure 5. (A) Chromatic analysis of egg yolks from free-range chickens with red shells, using a digital image processed in ImageJ software with the Color Inspector 3D plugin. (B) Chromatic analysis of free-range chicken egg yolks with blue shells, using a digital image processed in ImageJ software with the Color Inspector 3D plugin.

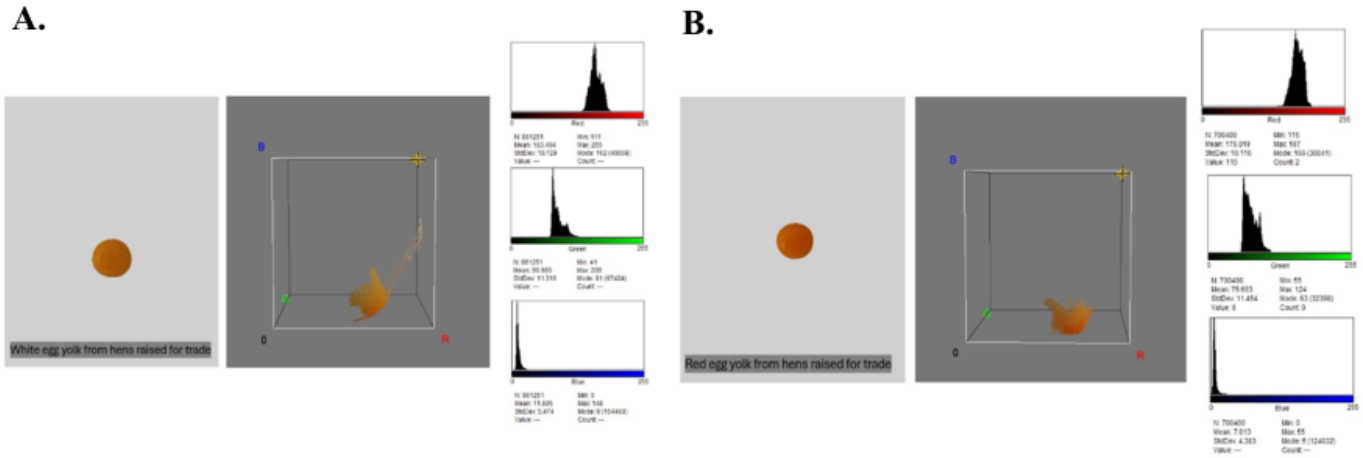


Figure 6. (A) Chromatic analysis of egg yolk coloration from commercial white-shelled laying hens, using a digital image processed in ImageJ software with the Color Inspector 3D plugin. (B) Chromatic analysis of egg yolk from commercial chickens with red shells, using a digital image processed in ImageJ software with the Color Inspector 3D plugin.

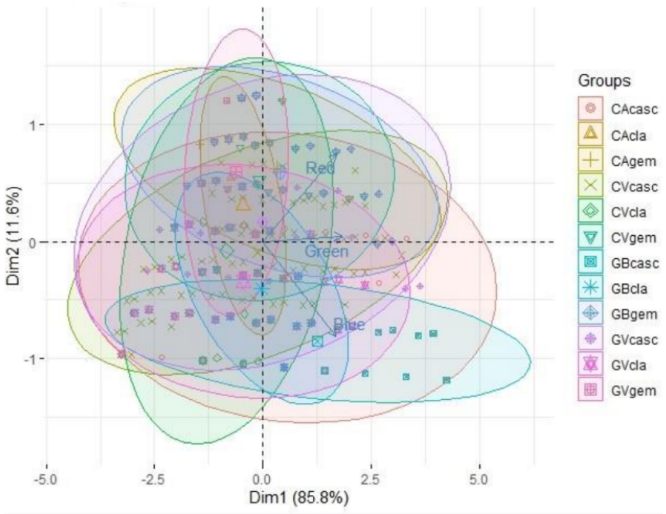


Figure 7. Principal component analysis of the variables evaluated in relation to the groups evaluated: (CAcasc), (CAcla), (CAgem), (CVcasc), (CVcla), (CVgem), (GBcasc), (GBcla), (GBgem), (GVcasc), (GVcla), (GVgem).

4 DISCUSSION

The data obtained through RGB digital analysis revealed that eggshell coloration is strongly related to the breeding system and genetics of the chickens. The predominance of red and green channels in red shells and the greater contribution of the blue channel in white and bluish shells confirm previous observations by Samiullah et al. (2015) and Wang et al. (2013), which point to the influence of protoporphyrin deposition and other natural pigments in the visible spectrum of the shell. Although these differences are visually apparent, digital techniques allow for standardization and reproducibility, favoring industrial applications and traceability (Milovanovic et al., 2021; Wu & Sun, 2013).

The white shell, observed in commercial Leghorn eggs, exhibited high uniformity and brightness, a characteristic associated with the genetic line of commercial Leghorn hens (Samiullah et al., 2015). This uniformity was confirmed by the low standard deviations in the three RGB channels, especially in blue. Red and blue shells, which are frequently associated with free-range chickens, displayed greater variation in the R and G channels, evidencing genetic diversity and dietary influence on coloration (Gautron et al., 2021; Odabaşi et al., 2007; Samiullah et al., 2015).

Although the egg whites exhibit lower color variability than shells and yolks, they still displayed subtle differences associated with the farming system. Egg whites from alternative systems (free-range) exhibited a subtle cream hue, possibly related to differences in diet, since egg quality traits vary among production systems (Hidalgo et al., 2008; Yenice et al., 2016). On the other hand, commercial egg whites revealed a more translucent and uniform color, reflecting greater control over feed and storage conditions (Oliveira et al., 2024; Van den Brand et al., 2004).

These color variations in egg whites are also related to the freshness and integrity of the albumin, whose protein structure directly interferes with light refraction (Eisen et al., 1962; Silversides & Scott, 2001). Non-destructive optical methods, including image-based color analysis, have been proposed for monitoring albumen freshness (Atwa et al., 2024; Gao et al., 2025). Therefore, even though the egg white is the least discriminating component in PCA, its objective evaluation through RGB analysis can assist in monitoring the internal quality of eggs.

The yolks stood out as the component with the greatest variation between farming systems. Free-range egg yolks depicted greater intensity in the red and green channels, reflecting a diet rich in natural carotenoids, such as lutein and zeaxanthin, from corn and forage (Karadas et al., 2006; Ortiz et al., 2021). The high chromatic saturation observed in these eggs is consistent with studies that associate yolk color with the farming system and type of feed (Hidalgo et al., 2008; Machado et al., 2024).

In contrast, commercial egg yolks exhibited lower chromatic dispersion, with more standardized coloring and less intense values in the RGB channels. This uniformity is attributed to the use of synthetic pigments in the laying hens' diet, such as canthaxanthin, paprika extracts, or marigold, in order to meet consumer visual requirements (Karunajeewa et al., 1984; Maia

et al., 2022; Matache et al., 2024). Other studies have previously highlighted that standardized yolk coloration is preferred by the market and directly associated with the perception of quality and stability (Berkhoff et al., 2020; Rondoni et al., 2020).

Multivariate analysis via PCA confirmed the discriminatory capacity of RGB data extracted from digital images. The separation between shell, white, and yolk, as well as between types of farming systems (free-range and commercial), reinforces the robustness of the technique employed. Studies such as those by Campmajó et al. (2019) and Dibakoane et al. (2023) also demonstrated the applicability of multivariate analysis in differentiating foods through visual attributes, consolidating the use of statistical tools associated with digital imaging in the poultry sector.

Furthermore, the possibility of applying these analyses in real time, at a low cost and without destroying the samples, renders this approach extremely useful for traceability, standardization, and quality control in the egg production chain. The combination of digital imaging, RGB channels, and multivariate statistics offers a reliable and accessible method for differentiating between farming systems, thereby aligning with modern consumer demands for safe, natural and traceable food products (Atwa et al., 2024; Lusk, 2019; Rondoni et al., 2020).

5 CONCLUSION

Principal component analysis (PCA) demonstrated high discriminatory capacity between egg components (shell, white, and yolk), farming systems (free-range and commercial), and shell color, with 97.4% of the variability explained by the first two dimensions.

The yolks exhibited the greatest color variation between groups, with free-range chicken yolks associated with more intense red and green values, evidencing the direct influence of a natural diet rich in carotenoids, while commercial yolks showed more standardized coloring due to the use of pigments in the feed.

White, red, and blue eggshells formed distinct groups in PCA, with white shells related to the blue channel (low saturation), while red and blue shells, especially from free-range birds, showed greater dispersion and intensity in the red and green channels, reflecting genetic and dietary variability.

Egg whites, regardless of system or shell color, presented less variability in the RGB channels, with homogeneous coloring and low saturation, representing the least discriminating component in the multivariate set, yet remaining useful for the validation of freshness and integrity standards.

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