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ANALYSIS OF CAROTENOID CONTENT AT VARIOUS PLANT SOURCES USED TO INTENSIFY MEAT COLOR IN CHICKEN BROILER

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Abstract

The increasing demands for the `yellow chicken` have led poultry farmers to adopt different technological strategies, including the administration of carotene-rich cereals, in quantities that ensure the desired coloration. In this context, we set out to determine the carotenoid content of the main color source used in poultry farming (six maize hybrids) and several plant species (marigold, red pepper, turmeric and sea buckthorn); all samples were analyzed by spectrophotometric methods. In maize hybrids, the total carotenoid content was only 0.29-0.74 mg/g, while in the tested plants it was 0.81-8.31 mg/g. The dosage of β-carotene (responsible for the red-orange color) showed that the highest content was in turmeric (0.84-96.63% higher than the other samples), a situation also valid for lycopene (yellow-orange-red color) at which the turmeric was ahead of the other samples by 21.15-99.72%. The results obtained indicate that, unlike the classic corn dye, the tested plants can ensure a much more intense coloring of the meat of the chicken broiler.

Key words*: lycopene, plant sources, pigments, β-carotene, yellow chicken.*

INTRODUCTION

Skin and muscle pigmentation in broiler chickens is a quality parameter with a major impact on the acceptability of the product on the market, being closely correlated with consumer preferences and the commercial value of the meat. The yellow colour of the skin and adipose tissue is mainly determined by the accumulation of dietary carotenoids in tissue lipids, since birds cannot synthesize these isoprenoid compounds and depend exclusively on their dietary intake (Pérez-Vendrell et al., 2001; Surai et al., 2002; Rosati et al., 2009).

In poultry nutrition, maize (*Zea mays* L.) is the traditional source of xanthophylls (especially lutein and zeaxanthin), being used both as a major energy ingredient and as a natural pigmenting agent. However, the total carotenoid content of commercial maize hybrids shows considerable variability, influenced by genetic, pedoclimatic and technological factors (Breithaupt et al., 2007; Popa et al., 2025). Relatively low levels of carotenoids can limit the intensity of pigmentation, especially in intensive growth systems, where the length of the production cycle is short.

In this context, interest in alternative natural sources rich in carotenoids has increased significantly, both for technological reasons and due to consumer pressure to reduce the use of synthetic dyes. Plant species such as marigold (*Calendula officinalis* L.), red pepper (*Capsicum annuum* L.), turmeric (*Curcuma longa* L.) and sea buckthorn (*Hippophae rhamnoides* L.) are recognized for their high carotenoid content, including β-carotene and lycopene, as well as for the presence of other bioactive compounds with antioxidant and immunomodulatory potential (Rao & Rao, 2007; Surai et al., 2014).

β-carotene, a precursor to vitamin A, contributes to both pigmentation and support immune function and protection against oxidative stress, while lycopene is associated with high antioxidant capacity due to its multi-conjugated double bond structure (Rao & Rao, 2007; Tudorache et al., 2023).

In poultry farming, supplementing diets with natural sources rich in carotenoids has been correlated not only with increased skin coloration, but also with improved health and performance parameters (Custură et al., 2024; Moise et al., 2024; Bittencourt et al., 2025).

In view of these considerations, the objective of the present study was to compare the total carotenoid content and specific fractions (β-carotene and lycopene) in medicinal plants and

maize, by spectrophotometric determinations, to identify effective natural alternatives for the intensification of meat pigmentation in chicken broiler.

MATERIALS AND METHODS

The study aimed to evaluate the colour potential of some medicinal plants, compared to that of some corn hybrids. In this regard, four medicinal plants recognized for their high pigment content were analyzed (sea buckthorn - *Hippophae rhamnoides*; red pepper - *Capsicum annuum*; turmeric - *Curcuma longa* and marigolds - *Calendula officinalis*) (Figure 1), as well as six corn hybrids - *Zea mays* L.: (FABULO CX5039; P9415; P9398; P9944; ZPSC388 and P9590 (Figure 2).



Figure 1. Analyzed medicinal plants: A - *Capsicum annum,* B - *Hippophae rhamnoides,* C - *Curcuma Longa*, D - *Calendula officinalis* (own source)

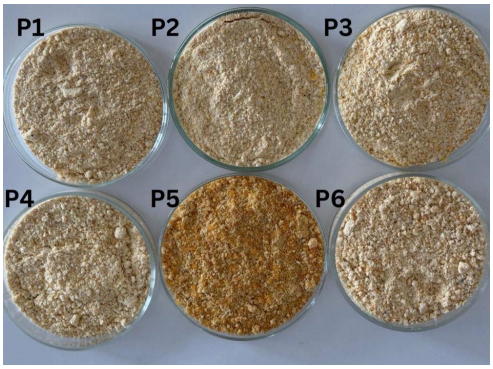

Figure 2. Corn hybrids analyzed:
P1- *P9398*, P2- *FABULO CX5039*, P3- *P9415,* P4- *P9944*, P5- *P9590*, P6- *ZPSC388* (own source)

The plant material was represented by powders obtained from parts specific to each plant. The samples (medicinal plants + maize hybrids) were dried and finely ground to the same grain to eliminate the influence of particle size on the extraction and spectrophotometric determination of carotenoids.

Solvent and ultrasonic extraction of biologically active compounds

The sample to be analyzed (1 g powder) was mixed, one at a time, with 10 mL hexane + acetone (3:1), then the mixture was homogenized at room temperature for 5-6 minutes (Figure 3).

The samples were kept in the ultrasonic bath for 45 minutes at 40 kHz and 40°C. Each sample was then centrifuged at 5000 rpm at 4°C for 10 minutes.

The resulting supernatants were analyzed from the point of view of phytochemical composition.



Figure 3. Solvent and ultrasonic extraction of compounds (own source)

Determination of the total content of carotenoids, β-carotene, lycopene

The quantitative determination of the total carotenoid content, including the compounds β-carotene and lycopene, was carried out by spectrophotometric methods, using a procedure adapted according to the literature (Butnariu, 2016). This method involves dissolving a defined amount of the extract obtained - i.e. 2 mL of a solution with a concentration of 10 mg/mL - in the solvent used in the extraction step (Figure 4), followed by measuring the spectrophotometric absorption at wavelengths specific to each compound of interest.

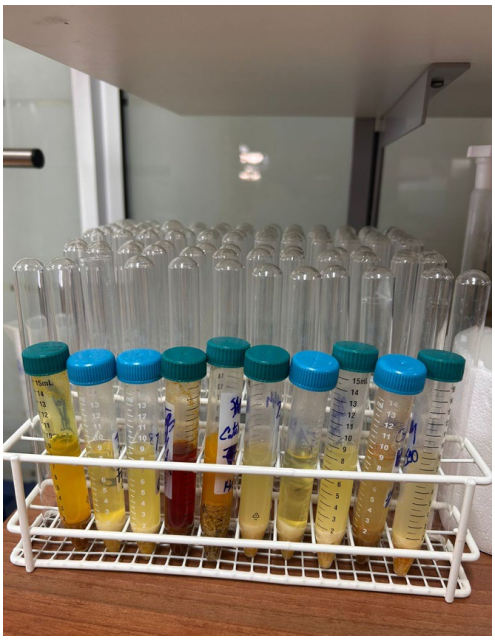

Figure 4. Quantitative determination of carotenoids, β-carotene and lycopene (own source)

More specifically, the absorption measurements were carried out at:
- 450 nm for the determination of total carotenoids;
- 470 nm for β-carotene quantification;
- 503 nm for lycopene content estimation.

With the help of the absorption values recorded at these wavelengths and based on the relationship proposed by Butnariu M. (2016) the concentration of carotenoid compounds in the analyzed samples was calculated, according to the following formula:

$$Carotenoid\ (mg/g\ s.u.) = \frac{A \times V \times DF}{m \times l \times \varepsilon}$$

where:

A = represents the absorption of the sample at the appropriate wavelength;

V = is the volume of solvent used to solubilize the concentrated extract (mL);

DF = is the dilution factor applied to the sample, where further dilution was required;

m = indicates the mass of the concentrated extract (g);

l = is the length of the optical path of the vessel used in spectrophotometry (1 cm in the case of quartz vessel);

ε = is the extinction coefficient specific to each compound analyzed:
- 2500 for total carotenoids,
- 2590 for β-carotene,
- 3450 for lycopene.

RESULTS AND DISCUSSIONS

Total carotenoid content

The comparative analysis of the total carotenes content highlights a clear demarcation between the metabolic profile of medicinal plants and maize hybrids (*Zea mays* L.). The results indicate a massive accumulation of carotenoids in the species *Hippophae rhamnoides* (sea buckthorn) and *Capsicum annum* (pepper), where concentrations of 8.312 mg/g and 7.827 mg/g, respectively, far exceed the biosynthetic potential of maize hybrids. This underlines the evolutionary specialization of medicinal plants in the storage of secondary metabolites with antioxidant role in chromoplasts. In contrast, although maize hybrids show significantly lower carotenoid values (with a maximum of 0.748 mg/g in sample P2), the significant variation observed within this group (over 150% between sample P2 and P5) suggests a different genetic potential despite the limitations imposed by the starchy matrix of the endosperm.

From the perspective of the total concentration of carotenoids, the comparison of the average of maize hybrids (0.546 mg/g) to the values recorded in medicinal plants highlights a major physiological contrast. Thus, the cereal matrix represents a massive accumulation deficit, the total carotenoid content being 93.35% lower compared to that of sea buckthorn (a reduction of 15.03 times) and 92.94% lower than that of red pepper (14.15 times lower). Even compared to turmeric, corn registers a 90.95% lower concentration of carotenoids (a decrease factor of 11.04 times).

A difference is also observed in the case of marigolds, which registers an average value of 0.81 mg/g, about 48.35% higher than the average for corn hybrids (0.546 mg/g).

The total carotenoid content determined in the six maize hybrids analysed showed significant difference, with values ranging from 0.296 to 0.748 mg/g (Figure 5).

This heterogeneity of the data can be attributed mainly to the genetic background of the hybrids, and it has been documented that the accumulation of these compounds in the endosperm is regulated by the differentiated expression of genes involved in the biosynthetic pathway (Yan et al., 2010; Gheorghe et al., 2021).

The values obtained converge with the previously reported data for conventional maize genotypes, confirming the influence of genetic and pedoclimatic factors on the metabolic profile (Chen et al., 2024). The higher levels identified in certain hybrids (P2) indicate outstanding antioxidant potential and additive nutritional value, essential in the context of biofortification and protection against oxidative stress (Rodriguez-Amaya et al., 2001; Bouis et al., 2010; Custură et al., 2019).

As for medicinal plants, there were marked differences, with concentrations ranging from 0.811 to 8.310 mg/g. The analysis highlighted Sample B (sea buckthorn) as the richest source (8.310 mg/g), while the minimum level was detected in Sample D (marigolds - 0.811 mg/g). The massive accumulation of carotenoids in sea buckthorn is consistent with the literature, which establishes this species as the primary source of b-carotene and zeaxanthin (Britton, 1995; Rodriguez-Amaya, 2010).

It should be noted that the minimum value recorded in the group of medicinal plants (0.811 mg/g) exceeds the maximum threshold determined in maize hybrids (0.748 mg/g). This value difference emphasizes the metabolic specialization of medicinal plants in the storage of secondary metabolites.

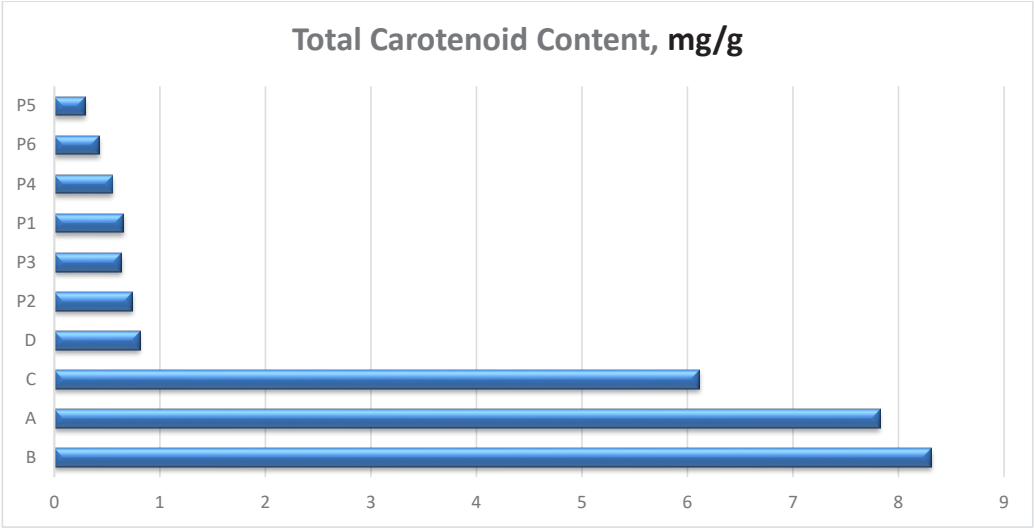

Figure 5. Variation in total carotenoid content by plant source

β-carotene content

As regards the fraction of b-carotene analysed, the disparity between the cereal matrix and medicinal species remains pronounced. The average of corn hybrids (0.410 mg/g) registers a critical deficit compared to red pepper, being 92.14% lower (a decrease factor of 12.73 times). Similar downward trends were identified in relation to sea buckthorn (-91.65%, factor 11.98) and turmeric (-90.83%, factor 10.90).

The β-carotene content determined in the six maize hybrids analyzed ranged from 0.207 to 0.598 mg/g produced, showing moderate variability between genotypes. The maximum level (0.598 mg/100 g) was recorded in sample P2, while the minimum concentration (0.207 mg/g) was observed in sample P5 (Figure 6). The values obtained fall within the ranges reported in the literature for conventional maize, where β-carotene is present in relatively small quantities compared to lutein, the predominant carotenoid in the endosperm (Ortiz-Monasterio et al., 2007; Baranowska et al., 2025). Inter-genotypic variability is associated with differences in the expression of genes involved in the carotenoid biosynthetic pathway, especially crtRB1 and lcyE, which influence the accumulation of β-carotene in the grain (Yan et al., 2010; Gupta et al., 2022)

In the case of the four medicinal plants analysed, the β-carotene content was considerably higher, ranging from 0.671 to 5.892 mg/100 g product.

The maximum level was found in red pepper (sample A) and indicates an increased capacity for carotenoid accumulation in photosynthetic tissues, a phenomenon explained by the structural and photoprotective role of these compounds in chloroplasts (Britton, 1995; Fraser & Bramley, 2004).
Compared to maize, medicinal plants showed concentrations up to about 10 times higher, which highlights major differences between the cereal matrix and green vegetative tissues in terms of biosynthesis and carotenoid storage.
From a nutritional point of view, β-carotene is the main precursor of vitamin A, and the high levels identified in plant species can contribute significantly to dietary intake of provitamin A (Stahl & Sies, 2003; Lungu & Moise, 2025; Kurilich, 1999). The differences observed between the two categories of samples underline the decisive influence of the type of plant matrix on the total and specific carotenoid content.

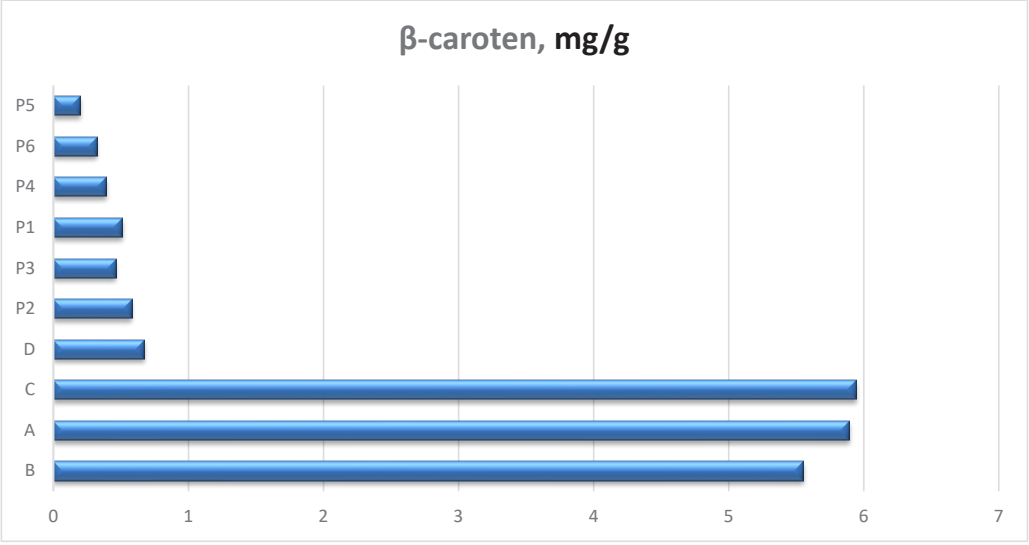

Figure 6. Variation in β-carotene content by plant source

Lycopene content
The assessment of lycopene content reflects the most pronounced discrepancy between medicinal plants and maize hybrids, highlighting the limited storage capacity of this carotenoid in the endosperm of *Zea mays* L. compared to the tissues of medicinal plants. The average of corn hybrids (0.066 mg/g) represents a drastic decrease compared to red pepper (4.681 mg/g), being 98.53% lower, which corresponds to a reduction factor of 67.84 times. A similar segregation trend is observed in relation to sea buckthorn (3.687 mg/g), where the average concentration in cereals is 53.49 times lower.
Although in the case of total carotenoid content the differences were more balanced, in terms of lycopene, even medicinal plants have moderate accumulation, such as turmeric and marigolds. Corn hybrids register deficiencies of 86.17% compared to turmeric and 85.53% compared to marigolds, being 7.23 and 6.91 times poorer in this bioactive compound, respectively.
The lycopene content determined in the six maize hybrids analyzed ranged from 0.014 to 0.125 mg/g produced, highlighting small but detectable quantitative differences between genotypes. The maximum value (0.125 mg/g) was recorded in sample P2, while the minimum concentration (0.014 mg/g) was observed in sample P5 (Figure 7). The value range obtained confirms that lycopene is a minor carotenoid in the cereal matrix of maize, compared to lycopene and β-carotene, which are the predominant compounds in the endosperm (Ortiz-Monasterio et al., 2007; Boileau, 2003).
Inter-genotypic variability may be correlated with differences in gene expression involved in the biosynthetic pathway of carotenoids,

especially the lycopene enzymes β-cyclase and ε-cyclase, which cause the conversion of lycopene to xanthophylls and cyclic carotenoids (Fraser & Bramley, 2004). In corn, the reduced accumulation of lycopene is explained by its rapid conversion into derived compounds, which limits its free concentration in grains (Yan et al., 2010).

The concentrations determined in the present study are within the limits previously reported for conventional maize, where lycopene is described as present in low or even undetectable amounts in certain genotypes (Rodriguez-Amaya et al., 2001; Cvetkovic et al., 2013). Although the levels identified are low, the contribution of lycopene to the total antioxidant capacity of grains cannot be neglected, given its high efficiency in neutralizing singlet oxygen and free radicals (Stahl & Sies, 2003; Hidayat et al., 2023; Buckler, 2008).

The lycopene content determined in the four analyzed medicinal plants showed considerable variations, the values being between 0.476 and 4.681 mg/g product. The highest concentration (4.681 mg/g) was recorded in sample A, while the minimum level (0.476 mg/g) was determined in sample D. The quantitative differences observed can be attributed both to the biochemical peculiarities specific to the raw materials and to the technological processing (drying, grinding, exposure to light), factors known to influence the stability of carotenoids.

According to the literature, lycopene is present in high concentrations in tomato and red pepper products, being one of the main compounds responsible for the intense red colour (Shi et al., 2000). Differences between plant species may reflect differences in the initial content of the raw material, but also the oxidative degradation of lycopene during processing and storage (Giovannucci et al., 1999; Rodriguez-Amaya et al., 2001; Martinez-Valvere et al., 2002).

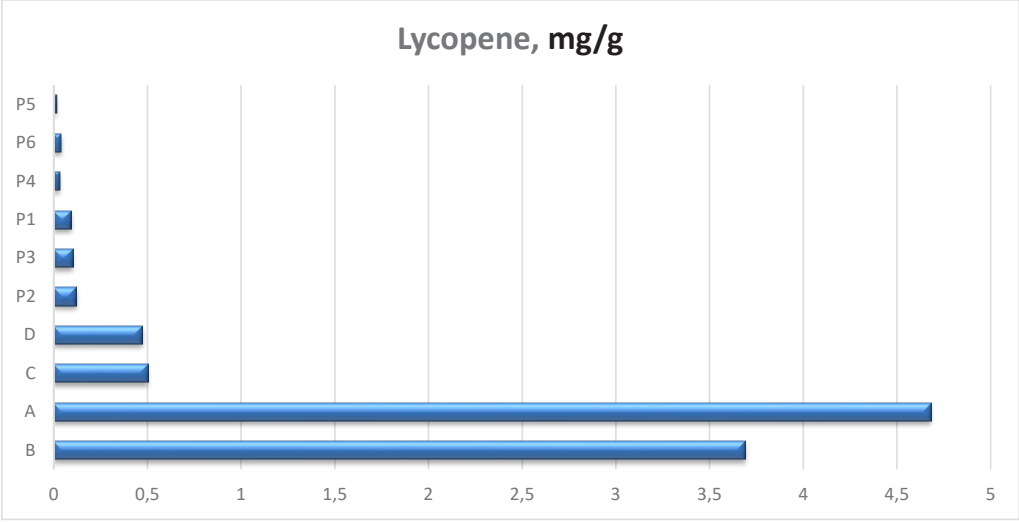

Figure 7. Variation in lycopene content by plant source

CONCLUSIONS

The results of the study highlight major differences in the pigmentation capacity of the analyzed raw materials, with direct implications on the correct formulation of feed rations for the chicken broiler

From the perspective of the hierarchy of pigmentation sources, medicinal plants, especially red pepper and sea buckthorn emerge as high-density feed additives.

The massive lycopene deficiency of maize compared to red pepper (67.84 times lower) suggests that the exclusive use of maize in the diet of the chicken broiler may be insufficient to achieve intense pigmentation, requiring supplementation with concentrated sources.

Red pepper and sea buckthorn are recommended for intensifying orange/red shades due to the extreme density of total carotenoids.

Lycopene, identified in higher concentrations in medicinal plants than corn (deficit of over 85% even for marigolds), is essential for the yellow base of pigmentation, highlighting the importance of these plants as chromatic "boosters".

Among the corn hybrids analyzed, the P2 sample represents the highest coloristic potential, having a β-carotene and lycopene content clearly higher than the other hybrids (P5 showed a pigment content 9.6 times lower than P2). The use of the P2 hybrid in feed recipes can reduce the dependence on synthetic pigments, providing a natural alternative for achieving a high colour score.

The conclusion of the study is that the inclusion of sea buckthorn powder or red pepper in compound feeds for the chicken broiler can represent an effective and sustainable strategy in obtaining high quality carcasses, but also to improve the health of the birds.

Although maize remains the basic energy source in poultry farming, the genetic variability identified between hybrids requires a rigorous selection of them based on pigment content criteria.

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