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Comparative Metabolomic Profiling of Eggs from 3 Diverse Chicken Breeds Using GC-MS Analysis

Affo Dermane [*], Kodjo Eloh [†,1], Koffi Kibalou Palanga [‡], Diane Tchakinguena Adjito, [†] Oumbortime N'nanle [§], Damintoti Simplice Karou, [§] Tchilabalo Abozou Kpanzou [#], and Pierluigi Caboni [†]

[*]*Laboratory of Chemistry, Faculty of Health Sciences, University of Lomé, Lomé, Togo;* [†]*Laboratory of Organic Chemistry and Environmental Sciences, Department of Chemistry, University of Kara, Kara, Togo;* [‡]*Laboratory of Applied Agronomic and Biological Sciences, High Institute of Agricultural Professions, University of Kara, Kara, Togo;* [§]*Regional Center of Excellence in Poultry Science, University of Lome, Lome, Togo; and* [#]*Laboratory of Mathematical Modelling and Decision Statistical Analysis, Department of Mathematics, University of Kara, Kara, Togo*

ABSTRACT Eggs, as a crucial source of essential nutrients for consumers, possess a high nutritional value owing to their rich composition of vital components essential for human health. While previous research has extensively investigated genetic factors influencing egg quality, there has been a limited focus on exploring the impact of specific strains, particularly within the African context, on the polar metabolite profile of eggs. In this extensive study, we conducted an untargeted analysis of the chemical composition of both albumen and yolk from 3 distinct strains of hens—Blue Holland, Sasso, and Wassache—raised under identical feeding conditions. Utilizing gas chromatography coupled with mass spectrometry (**GC-MS**), we meticulously examined amino acids, carbohydrates, fatty acids, and other small polar metabolites. In total, 38 and 44 metabolites were identified in the whites and yolk, respectively, of the 3 studied strains. The application of chemometric analysis revealed notable differences in metabolite profiles with 8 relevant metabolites in each egg part. These metabolites include amino acids (N-α-Acetyl-L-lysine, lysine, L-valine, L-Tryptophan), fatty acids (oleic acid, linoleic acid, palmitic acid and stearic acid), and carbohydrates (d-glucose, maltose, lactose). These findings shed light on strain-specific metabolic nuances within eggs, emphasizing potential nutritional implications. The ensuing discussion delves into the diverse metabolic pathways influenced by the identified metabolites, offering insights that contribute to a broader understanding of egg composition and its significance in tailoring nutritional strategies for diverse populations.

Key words: egg composition, metabolomics, gas chromatography-mass spectrometry, hen strain, nutritional implication

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INTRODUCTION

Eggs, esteemed for their widespread global consumption, stand out as a nutritional powerhouse, boasting essential minerals, vitamins, fatty acids, amino acids, and pigments (Blanton et al., 2006). About 75% of the total weight of the edible portion of eggs is attributed to water, with lipids and proteins being the primary contributors to its nutrient value. In addition to these, minor quantities of carbohydrates in the form of simple sugars, including glucose, sucrose, fructose, lactose, maltose, and galactose and minerals are also found. The whole egg is comprised of approximately 9 to 11% eggshell, 60-63% albumen, and 28 to29% yolk. Proteins are predominantly present in both yolk and white, while lipids are primarily concentrated in the yolk. The eggshell is mainly composed of minerals. In addition, eggs encompass various other components. Although present in minor quantities, these components gain significance in bioscience and biotechnological applications (Mine, 2008; Dong & Zhang, 2021).

Acknowledged as a comprehensive nutrient source for human dietary needs, eggs are agricultural products sourced from diverse farms and are integral components of culinary traditions worldwide, featured both as standalone delicacies and key ingredients in a myriad of dishes across diverse cultural landscapes (Zaheer, 2015). In

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[1]Correspondence author: kodjoeloh@outlook.com

2017, global hen egg production surpassed 80 million tons, and this figure has shown a yearly increase (Faostat, 2019). Despite the continuous growth in food production, a staggering 820 million individuals worldwide still face inadequate access to sufficient nutrition for a healthy and active life (Boliko, 2019). Addressing the challenge of hunger, eggs, readily available from hens, have the potential to contribute to the provision of livestock-derived foods on a global scale.

Three strains of hens are usually found in West African poultries. The Wassache hen that is the result of a crossbreeding between 2 hen strains, namely the Kokochiè and the Rhode Island Red. The term 'Wassache' means 'satisfaction hen' in Bambara (Mali) (Davenport C.B., Inheritance in poultry, 1906, Carnegie Institution of Washington; Washington, DC.). Unlike traditional hens, the Wassache hen represents a remarkable genetic improvement. Adult females can lay between 160 and 170 eggs per year, averaging 4.5 kg, compared to local strains that lay between 60 and 80 eggs. The North Holland hen, formerly known as the Blue Holland, is a domestic hen strain. It is among the 108 hen strains recognized by the British Poultry Standard (Allonby & Wilson, 2018). The ideal weight is 3 to 4 kg for the rooster and 2.5 to 3 kg for the hen. And the Sasso hen that is characterized by a superior taste, robustness, and slower, natural growth. In 1978, Sasso was formally created within the Poultry Selection of Sarthe and Southwest (**S.A.S.S.O.**). The Sasso strain stands out for its ability to easily adapt to free-range farming, slow growth, and hardiness. At adulthood, it averages around 3 kg between 12 and 14 wk (Kassa et al., 2021).

In the realm of nutritional science, the exploration of food metabolomics has emerged as a pivotal avenue for unraveling the intricate biochemical compositions that underlie the nutritional value of dietary components (Chaudhary et al., 2021). Metabolomics, a comprehensive analysis of small molecules present in biological samples, has provided unprecedented insights into the diverse metabolic pathways inherent in various food items. Among these, eggs stand out as a quintessential source of essential nutrients, offering a rich amalgamation of proteins, lipids, vitamins, and minerals crucial for human health (Wang et al., 2023). The importance of genetic factors in determining egg composition has been the interest of various research highlighting significant breed-specific differences in egg metabolite profiles. For example, in a recent study conducted by Goto et al., 2019, an analysis of 81 eggs from 5 distinct chicken breeds demonstrated notable variations. Utilizing One-way ANOVA, the researchers observed significant breed-related effects across 10 egg traits, 20 yolk amino acid traits, and 15 albumen amino acid traits, highlighting the impact of genetic differences on egg composition. Furthermore, in a study by Nishimura et al. (2021) focusing on 2 Japanese breeds, notable impacts of genotype were observed. Specifically, the researchers found significant effects of genotype on 10 egg traits, 8 yolk amino acids, and 11 albumen amino acids contents, indicating the influence of genetic factors on egg composition. The understanding of egg composition has traditionally centered on genetic factors and the influence of specific hen strains on the metabolite profile of eggs was reported by few works (Goto et al., 2019; Yang et al., 2023). In the context of the African continent particularly, the terrain has remained relatively unexplored.

Gas chromatography-mass spectrometry (**GC-MS**) is a powerful analytical technique widely employed in metabolomics research for its ability to provide detailed insights into the composition of complex biological samples. Gas chromatography-mass spectrometry excels in the separation and identification of volatile and semi-volatile compounds, making it particularly suitable for the analysis of small molecules such as amino acids, fatty acids, and carbohydrates found in eggs (Dunn et al., 2011). The high throughput and sensitivity of GC-MS allow for precise quantification and differentiation of metabolites, contributing to a comprehensive understanding of the metabolomic landscape.

If hen strain and variety factors were assessed as source of variability for eggs yolk and white macroelements and microelements, carbohydrates, moisture, ashes, protein, fat (polyunsaturated and saturated), sugars, cholesterol, and α-tocopherol, multivariate data analysis coupled with metabolomics is scarce. This research undertakes a thorough examination of the polar metabolite profiles found in eggs originating from 3 specific hens strains—Blue Holland, Sasso, and Wassache—highlighting the importance of distinctive metabolic nuances inherent to each strain in influencing the nutritional composition of eggs. Utilizing GC-MS, we explored the intricate interactions among amino acids, carbohydrates, and fatty acids present in both the albumen and yolk. This investigation adds valuable insights to the expansive realm of food metabolomics, unraveling the complex metabolic signatures inherent in eggs. It sets the stage for customized nutritional strategies designed to meet the diverse needs of various populations. This exploration aims to deepen our comprehension of the dynamic interplay between hen strains and the metabolomic composition of eggs, thereby contributing to the formulation of well-informed dietary recommendations.

MATERIAL AND METHODS

Materials and Reagents

Methanol, chloroform, hexane, pyridine, methoxamine hydrochloride, potassium chloride, N-methyl-N-(trimethylsilyl)trifluoroacetamide (**MSTFA**), and standards for amino acids, fatty acids, and carbohydrates were obtained from Merck (Sigma-Aldrich, Milan, Italy). The gas chromatograph (**GC**) used in this study was an Agilent Technologies 78,90B system, and the mass spectrometer (**MS**) was an Agilent Technologies 59,77B system (Agilent Technologies Inc., Santa Clara, CA).

Eggs sourced from 3 distinct hen strains, cultivated within the experimental poultry unit of the Regional Center of Excellence in Poultry Science (**CERSA**) at the University of Lomé, Togo, were utilized for this study. In

this experimental phase, 4 eggs were selected from each strain. Subsequently, both the albumen and yolk of each egg were meticulously separated by hand and subjected to lyophilization. The lyophilized components were stored at −20°C, awaiting the extraction process on the designated day.

Sample Extraction for Metabolomic Analysis

The Bligh and Dyer extraction method (Bligh & Dyer, 1959) was employed to effectuate the separation of small metabolites from lipids in the egg whites and yolks of diverse hen strains under investigation. In the metabolite extraction procedure, 20 mg of lyophilized egg white and yolk samples were meticulously transferred into Eppendorf tubes, followed by the addition of 250 μL of methanol and 125 μL of chloroform. The samples underwent vigorous vortexing at 15 min intervals, totaling 4 cycles. Subsequently, 380 μL of chloroform and 90 μL of a 0.2 M aqueous solution of potassium chloride were introduced, followed by a 30 s vortexing. The resultant suspension underwent centrifugation at 13,572 × g for 10 min at 4°C. Postcentrifugation, the aqueous phase (upper layer) was meticulously transferred into a glass vial and subsequently subjected to lyophilization.

To enhance the suitability of polar and volatile metabolites for GC-MS analysis, the desiccated extracts underwent derivatization using MSTFA and methoxamine. In this process, 50 μL of pyridine containing 10 mg/ml of methoxamine hydrochloride were carefully introduced to each desiccated extract. Following a 17 h incubation period, 100 μL of MSTFA were added and allowed to react for 1 h. Subsequently, each sample underwent dilution with 600 μL of hexane before GC-MS analysis.

GC-MS Analysis of Metabolites

One microliter of each derivatized sample was injected in splitless mode into a gas chromatograph coupled with a mass spectrometer. The injector temperature was set at 200°C, and the gas flow through the column was 1 mL/min. The fused silica capillary column used was a DB5-MS column with a thickness of 0.25 μm (30 m × 0.25 mm id.; J&W Scientific Inc., Folsom, CA). The temperature program in the oven was set as follows: 3 min of isothermal heating at 50°C, then increased to 250°C at 3°C/min and held at 250°C for 25 min. The transfer line and ion source temperatures were 280 and 180°C, respectively. Ions were generated with an electron beam energy of 70 eV in electron impact ionization and recorded at 1.6 scans/s over a mass range of 50 to 550 m/z.

Metabolite identification was carried out by comparing their mass spectra with those in the NIST08 mass spectral database. Other online databases were used as needed to determine the structure of certain molecules, or their retention indices were compared to those reported in the literature. When available, authentic standards were used to confirm the structure of the metabolites.

Deconvolution and Integration of Chromatograms

The XCMS online platform (Gowda et al., 2014) was used to identify different peaks in the chromatograms along with their corresponding ions. Each ion (mass) was treated as a variable, resulting in a total of over 3000 ion variables. The abundance of each ion was identified by the platform to create the data matrix. One matrix was obtained for the albumen, and another for the yolk for each egg. Each matrix, therefore, comprised over 3000 variables (ions) and 12 observations (eggs) (12 columns and over 3000 rows). These matrices underwent multivariate analysis.

Multivariate Statistical Analysis and Data Visualization

For each matrix, the ion intensities of metabolites were adjusted to sum to 1,000. Each feature in each matrix was standardized by subtracting the mean and dividing by the standard deviation across all samples. In the case of asymmetric distribution, a logarithmic transformation was applied, and symmetry improvement was assessed using asymmetry tests available in the SIMCA18 software (Umetrics, Sweden). Partial least squares discriminant analysis (**PLS-DA**) was conducted using the same software. The quality of PLS-DA models and the optimal number of principal components were determined based on cumulative R2Y (indicating classification ability) and Q2Y (assessing predictive ability during cross-validation) parameters, following methods integrated into the SIMCA-18 program. A permutation test comprising 500 permutations was employed to assess the validity of the PLS discriminant model and mitigate the risk of overfitting. Important indicators extracted from the PLS-DA models included Variable Influence on Projection (VIP values had to be >1.5) scores and coefficients that highlighted the influence of metabolites, considering all validated components, in the separation of hen strains and the comparison of metabolite levels within each hen strain (Eriksson et al., 2013).

To validate the discriminant metabolites identified through multivariate analysis, univariate statistical analysis was conducted. The analysis was carried out using Minitab 21.4.1 software. An ANOVA one-way analysis was conducted on the GC-MS areas of discriminant metabolites among the various hen strains under study. The analysis provided information on means, p-values, pooled standard deviations, and the significance of differences ($P < 0.05$).

RESULTS

Overview of Egg Metabolomes

The low molecular weight metabolites from yolk and white egg samples of the three strains were studied using GC−MS, and the analysis of both types of chromatograms (Figures 1 and 2, for yolk and white, respectively) allowed

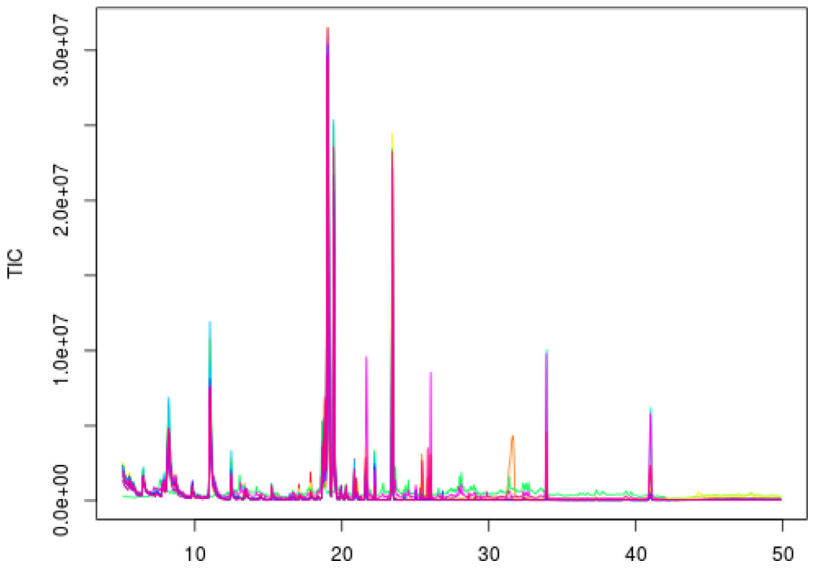

Figure 1. Retention time corrected total ion GC-MS chromatograms of overlaid chromatograms of the studied strains egg yolks.

the identification of amino acid molecules, carbohydrates, and short-chain fatty acids. Globally, a total of 38 metabolites were identified in the egg whites of the diverse hen strains under investigation. These included 7 fatty acid and derivatives, 20 carbohydrates and derivatives, 3 amino acids and other metabolites as outlined in the Table 1. In the yolk, a total of 44 metabolites were identified from eggs of various hen strains, including 16 amino acids, 8 fatty acids, 13 carbohydrates and other metabolites as listed in Table 2.

Multivariate Analysis

Initially, for an overview of the sample distribution, detection of outliers, divergent features, and common trends, Principal Component Analysis (**PCA**) of the GC-MS data was conducted. The results of these analyses, performed considering the 3 egg groups demonstrate that eggs from the 3 strains, Blue Holland, Sasso, and Wassache, were not enough distinguishable by an unsupervised approach from a metabolomic perspective, therefore we applied a supervised PLS-DA. Partial least squares discriminant analysis of chromatographic areas has facilitated the identification of metabolites that discriminate among the three egg groups, both in the yolk and the White. The PLS-DA score plots are depicted in Figures 3 and 4 for white and yolk, respectively. The previous multivariate statistical analyses have identified 8 discriminant metabolites, based on the VIP values (>1.5), in the whites of different strains, including 1 amino acid and 7 carbohydrates, as illustrated in Figure 5.

In the egg yolks, the study has also identified 8 discriminant metabolites among yolks from different

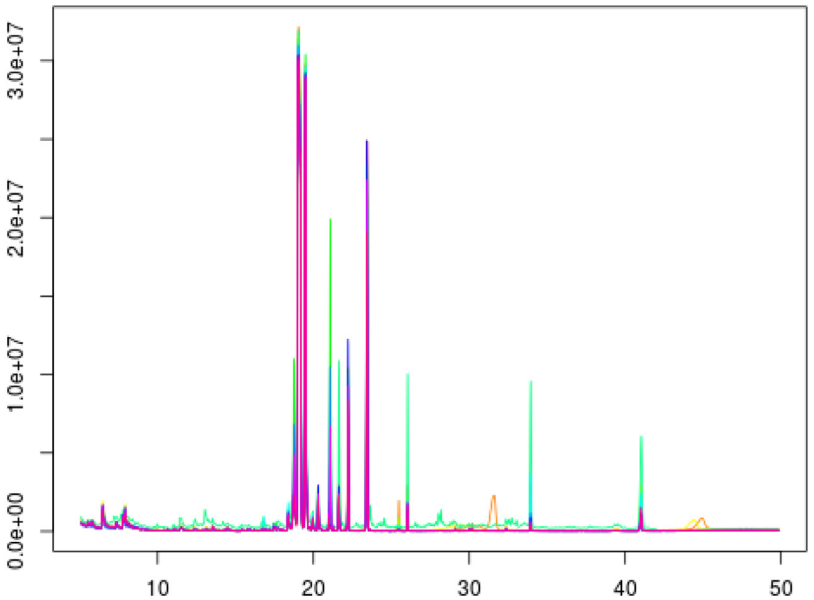

Figure 2. Retention time corrected total ion GC-MS chromatograms of overlaid chromatograms of the studied strains egg whites.

Table 1. Key Metabolites identified in the white of eggs from different hen strains listed in chromatography retention time order with their features highest variables important for the projection (**VIP**) .

N°	Retention time (min)	Compounds	VIP
1	5.28	N-α-Acetyl-L-Lysine	0.77
2	6.48	Methyl aminoacetate	1.38
3	8.08	δ -gluconolactone	1.44
4	11.64	Phenylalanine	0.76
5	12.05	β-Arabinopyranose	1.78
6	12.43	β-Lyxopyranose	1.04
7	13.13	D-Ribofuranose	1.11
8	13.93	Ribitol	1.21
9	14.23	D- (+)-Arabitol	0.96
10	14.35	L-Fucitol	1.41
11	14.48	*Trans*-4-hydroxy-L- proline	1.70
12	15.37	D- (-)-Lyxofuranose	0.75
13	15.85	D-Xylofuranose	0.60
14	16.20	D-Pinitol	1.63
15	16.72	Citric acid	1.45
16	16.79	Myristic acid	0.83
17	16.94	α-D-Galactopyranose	0.94
18	17.48	1,5-Anhydrohexitol	1.44
19	17.72	d-Galactose	0.42
20	18.36	D- (-)-Fructose	1.37
21	19.06	D-Glucose	1.83
22	19.47	D-Talose	1.61
23	19.78	D-Mannitol	0.91
24	19.96	D-Sorbitol	1.58
25	20.30	Maltose	1.64
26	20.87	D- (+)-Talofuranose	1.25
27	20.98	D-Lactose	1.43
28	21.28	D-Allofuranose	1.29
29	21.62	Palmitic Acid	1.24
30	22.09	*scyllo*-Inositol	1.40
31	23.32	*N*-Acetyl-D-glucosamine	1.52
32	23.43	*myo*-Inositol	1.32
33	25.26	Linoleic acid	0.67
34	25.32	Oleic acid	0.26
35	26.03	Stearic acid	1.30
36	29.99	Dulcitol	0.96
37	33.92	1-Monopalmitin	1.36
38	41.02	1-Monostearin	0.96

Table 2. Key Metabolites identified in the egg yolk of different hen strains listed in chromatographic retention time order with their variables important for the projection (**VIP**).

N°	Retention time (min)	Compouds	VIP
1	5.53	L-Threonine	1.45
2	5.79	Glycine methyl ester	0.87
3	6.49	*N*-(4-aminobutyl)acetamide	1.68
4	7.21	L-5-Hydroxylysine	1.26
5	8.23	L-Aspartic acid	1.94
6	8.71	L-5-Oxoproline	1.06
7	9.84	L-Valine	1.30
8	10.65	4-Aminobutanoic acid	0.89
9	10.83	L-Ornithine	1.38
10	11.04	L-Glutamic acid	1.54
11	11.64	Phenylalanine	1.18
12	12.48	Asparagine	1.60
13	13.40	L-Lysine	1.61
14	14.24	Ribitol	1.40
15	14.48	*trans* 4-hydroxy-L-proline	1.95
16	15.22	L-Glutamine	1.22
17	15.60	Azelaic acid	1.02
18	16.42	DL-Ornithine	1.37
19	16.58	Citrulline	1.44
20	17.07	*N*-α-Acetyl-L-Lysine	1.42
21	17.20	D-Pinitol	1.58
22	17.87	Tyrosine	1.45
23	18.35	D- (-) -Fructose	1.26
24	18.71	D- (+) -Talose	1.26
25	18.83	D-Galactose	1.35
26	19.06	D-Glucose	1.81
27	19.44	D-Allose	1.66
28	19.94	D-Sorbitol	1.64
29	20.28	Lactulose	1.25
30	20.86	Pantothenic acid	1.23
31	21.62	Palmitic Acid	1.38
32	22.08	*scyllo*-Inositol	1.39
33	23.32	*N*-Acetyl-D-glucosamine	1.37
34	23.43	*myo*-Inositol	0.73
35	25.26	Linoleic acid	1.30
36	25.35	Oleic Acid	1.42
37	25.83	L-Tryptophan	0.78
38	26.04	Stearic acid	1.28
39	26.84	Glyceryl phosphate	1.44
40	28.54	Arachidonic acid	1.10
41	31.70	Cholesterol	0.84
42	33.90	1-Monopalmitin	0.84
43	37.29	Sucrose	1.36
44	41.02	1-Monostearin	0.58

strains, including 4 amino acids and 4 carbohydrates as illustrated in Figure 6. Univariate statistical analysis was instrumental in confirming the discriminant metabolites. All the 16 metabolites exhibited significant differences in composition in at least one strain compared to the others (Table 3).

DISCUSSION

Overview of Egg Metabolomes

The GC-MS analysis enabled the identification of 38 metabolites in the egg whites and 44 metabolites in the yolks of various hen strains. In a metabolomic study investigating the freshness of hen eggs, Rivera-Vélez and Villarino identified 31 markers in both the egg white and yolk, primarily consisting of amino acids and carbohydrates (Zhang et al., 2020). Apart from the analysis of the growth performance, egg production, and survival rate of hen strains, very few studies have delved into the exploration of their egg metabolome differences (Cavanna et al., 2018). In their study, (Liu et al., 2022) utilized LC-MS to detect 43 different metabolites in egg whites and 16 in yolks. However, Goto et al., 2019 employed a more sensitive GC-MS/MS method, which enabled the identification of 138 yolk and 132 albumen metabolites in 2 Japanese chicken breeds (Rhode Island Red and Australorp). Beyond the commonly studied amino acids, carbohydrates, and fatty acids, which are often the focus of targeted studies, this untargeted research with an available GC-MS serves to identify characteristic metabolites in the eggs of 3 hen strains—Sasso, Bleu de Hollande, and Wassache. These findings may be of particular interest to consumers or patients experiencing nutrient deficiencies.

Multivariate Analysis

The overall differences in the metabolite profiles among egg yolks and whites of different strains were assessed.

Figure 3. Partial least square discriminant analysis (PLS-DA) score plot of the 3 strains eggs whites GC-MS data ((R2Y(cum) = 0.99 and (Q2 (cum) = 0.82). Circles represent Holland blue, triangles represent Wassachiè while squares represent Sasso strain.

Principal component analysis (**PCA**) allowed to identify clusters in the 4 replicate samples of strains indicating potential metabolomic differences (Leng et al., 2021; Sebzalli & Wang, 2001). To obtain a better understanding of hens strains egg yolks and whites metabolome differences, the metabolomic data were subjected to PLS-DA (Arendse et al., 2018; Kang et al., 2022). The PLS-DA score plots of the 3 strains egg yolks and whites were shown in Figures 3 and 4, respectively. The 3 groups of samples were significantly separated, suggesting that the PLS-DA clearly distinguishes Blue Holland, Sasso, and Wassache egg yolks and whites metabolite profile. The R2X(cum)-value of the PLS-DA analysis of white was 0.71, meaning that 71% of the variation in the egg white metabolome dataset could be modeled. In addition, an excellent goodness-of-fit of 99 % (R2Y(cum) = 0.99) and goodness-of-prediction of 82 % predictability (Q2 (cum) = 0.82) for the discrimination of white metabolite profiles were determined. Yolk metabolome differences between Blue Holland, Sasso, and Wassache eggs were also analyzed by PLS-DA, and the R2Y(cum)-value and Q2(cum)-value were 0.99 and 0.72, respectively. This PLS-DA result showed that the metabolomes of egg yolks were significantly different and that the model could clearly evaluate differences in metabolome between the studied strains egg whites and yolks.

As revealed by the Variable Importance in the Projection (VIP) analysis generated by SIMCA-18 software, the egg whites from Blue Holland exhibited higher concentrations of *β*-arabinopyranose, *trans*-4-hydroxy-L-proline, D-glucose and *N*-acetyl-D-glucosamine when compared to the Sasso and Wassache strains eggs (Figure 5). According to the findings of Mariette et al. (2021), *β*-arabinopyranose assumes a pivotal role in providing energy and serves as an essential foundational substance. Trans-4-hydroxy-L-proline with high levels in Holland Blue eggs holds significant importance as a crucial amino acid widely applied in medicinal and industrial sectors. Its value extends to serving as a valuable chiral building block in the organic synthesis of pharmaceuticals (Zhang et al., 2021). Concerning variations in the D-glucose, a carbohydrate composition of egg samples, it was observed that this phenomenon might be linked to the genetic characteristics inherent in the studied hen strains (Silberstein et al., 2003). Additionally, *N*-acetyl-D-glucosamine, found in elevated levels in Blue Holland eggs, is recognized as a widely accepted treatment for osteoarthritis, the most prevalent joint disease and a primary cause of physical disability among elderly individuals (Kubomura et al., 2017).

D-Sorbitol exhibited higher concentrations in the egg white of Sasso compared to both other species. D-Sorbitol

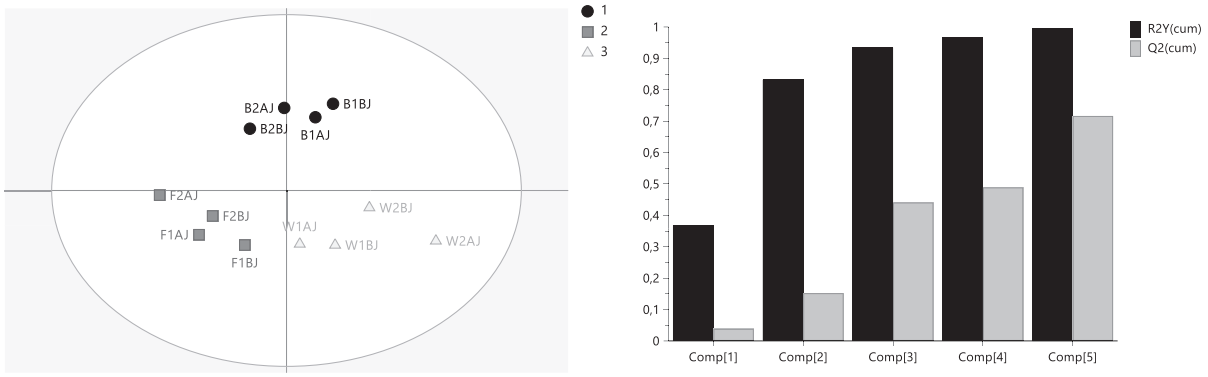

Figure 4. Partial least square discriminant analysis (PLS-DA) score plot of the 3 strains eggs yolk GC-MS data (R2Y(cum)= 0.99 and Q2 (cum) = 0.72). Circles represent Holland blue, triangles represent Wassachiè while squares represent Sasso strain.

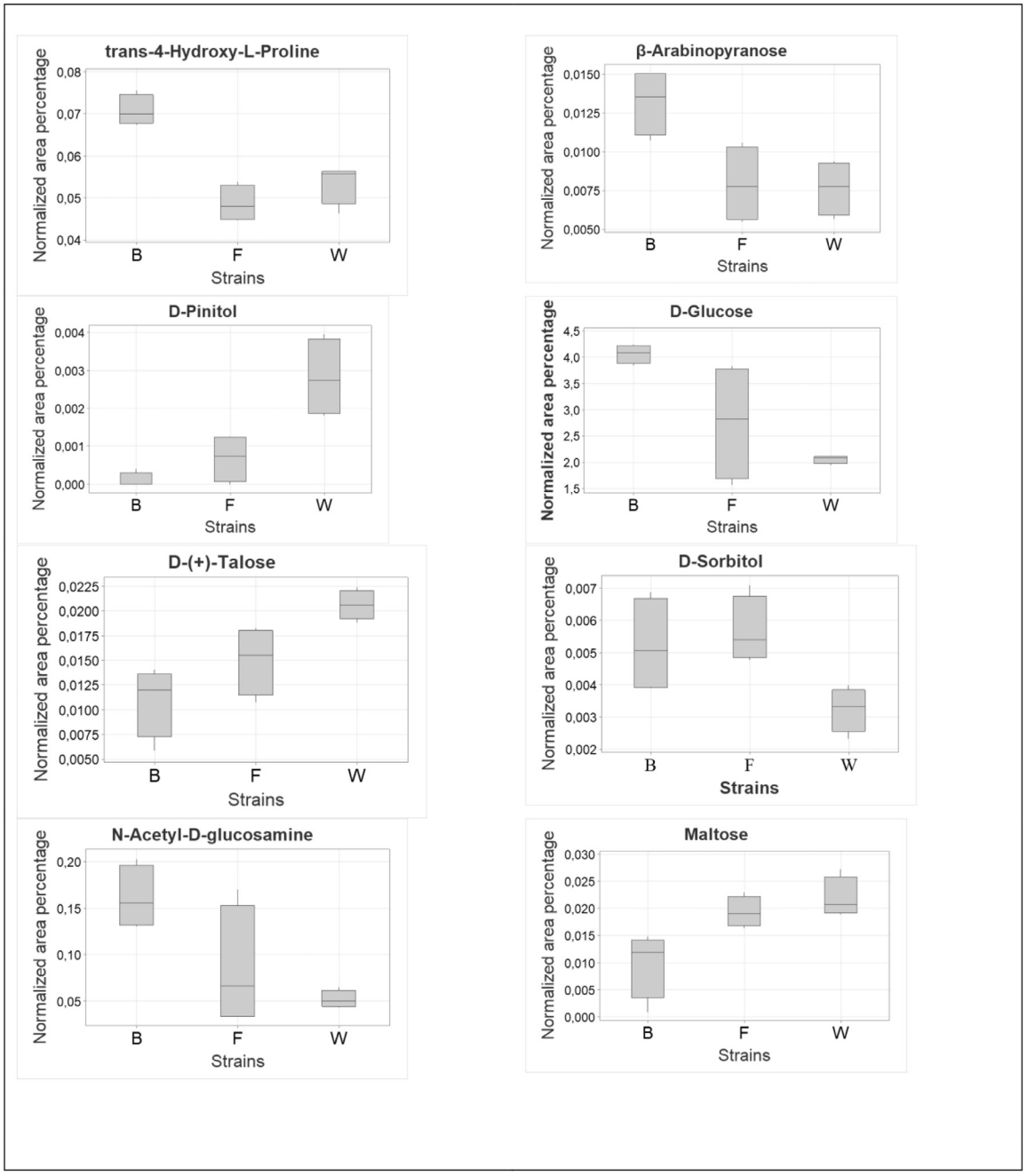

Figure 5. Boxplots of discriminant metabolites in the white of eggs from different strains. "B" referred to Holland blue strain, "F" to Sasso and "W" to Wassachiè.

is an important constituent of living cells, used as a sweetener in the food industry, and for its other physico-chemical and organoleptic properties (Ibrahim, 2016).

Wassache showed increased levels of carbohydrates D-pinitol, D-(+)-talose and maltose. D-Pinitol is frequently reported as an antidiabetic agent making Wassache eggs potentially beneficial for diabetic patients (Pandi et al., 2022). More, D-talose is an important and rare sugar involved in the metabolism of *Daphnia magna* (a species of small crustaceans), its alteration could be attributed to specific genetic characteristics of the studying strains. Maltose, a highly nutritious disaccharide for yeast, used in beer brewing and bread fermentation, was also found in higher quantities in the egg whites of Blue Holland compared to Sasso and Wassache.

The concentrations of *trans*-4-hydroxyproline, L-lysine, D-pinitol and D-sorbitol in the egg yolk of Blue Holland exceeded those in the egg yolks of Sasso and Wassache (Figure 6). *trans*-4-hydroxy-proline possesses antitumor, immunosuppressive, anti-HIV, and anti-inflammatory properties and its versatility has led to a global increase in its use (Gong et al., 2023). Lysine being one of the 9 essential amino acids, its high quantity in the yolks of Blue Holland eggs may enhance the nutritional significance of these eggs. A recent study found that lysine was among the differing amino acids observed in 4 distinct commercial sources of eggs

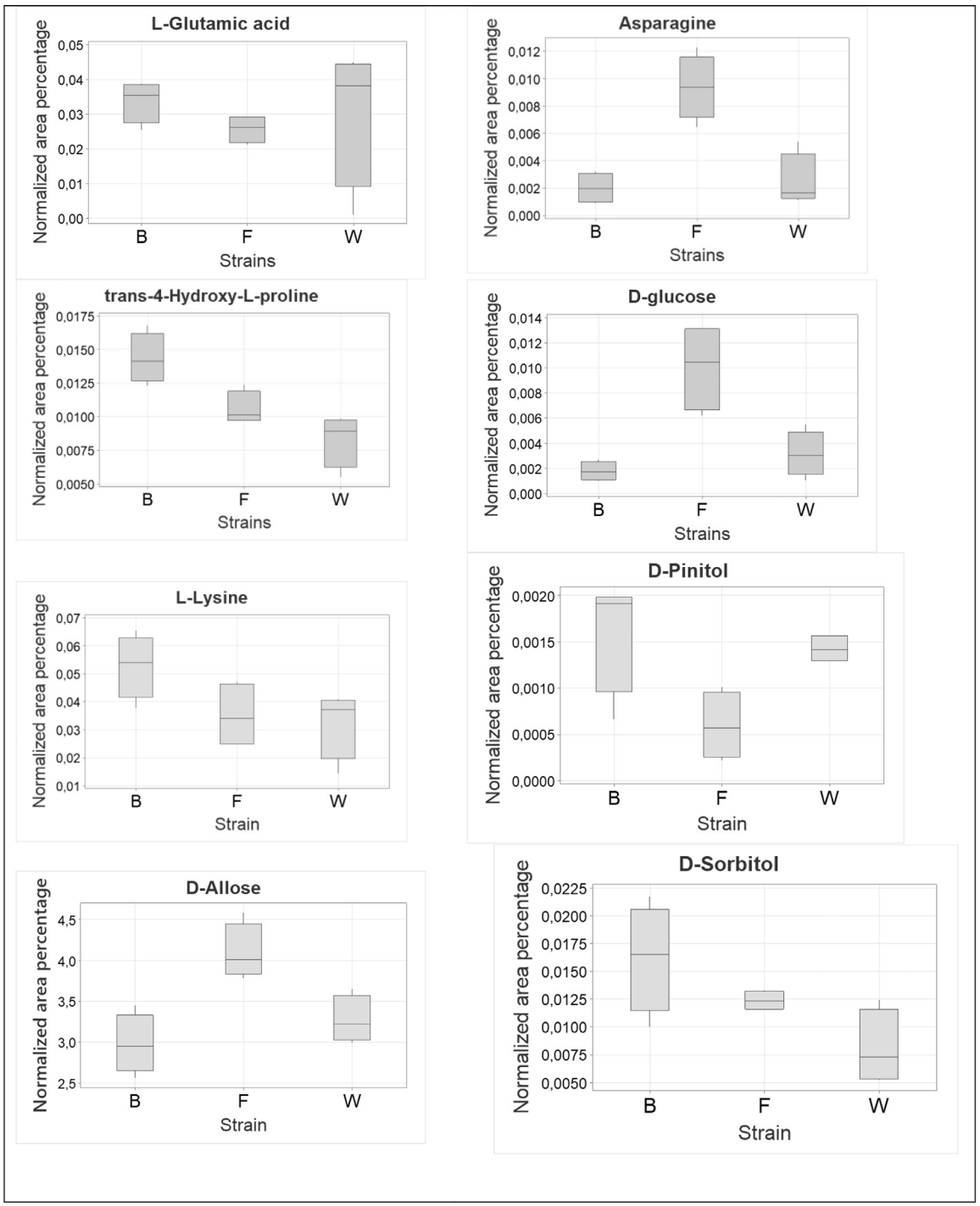

Figure 6. Boxplots of discriminant metabolites in the egg yolk from different hen strains. "B" referred to Holland blue strain, "F" to Sasso and "W" to Wassachiè.

available in a retail market (Attia et al., 2020). The presence of D-pinitol and D-sorbitol may be associated with carbohydrate composition, influenced by specific metabolic regulatory mechanisms (Liu et al., 2018).

Asparagine, a crucial component in ammonia biosynthesis vital for neuron function according to Lomelino et al. (2017), and D-glucose and D-Allose, subject to race-specific metabolic regulation, were more abundant in the egg yolk of Sasso compared to the egg yolks of Blue Holland and Wassache. Finally, L-glutamic acid, a vital component in protein composition, acting as an excitatory neurotransmitter within the central nervous system had statistically higher levels in the egg yolk of Wassache compared to Blue Holland and Sasso.

From the obtained results, it was evident that each egg yolk from the 3 strains exhibited significantly higher amino acid content than its respective egg white aligning with reported works (Mine, 2008; Li et al., 2021). The Blue Holland egg strain displayed a greater number of discriminant metabolites compared to the Sasso and Wassache strains. The Wassache egg strain exhibited a lower number of discriminant metabolites compared to

Table 3. ANOVA one-way analysis of normalized GC-MS chromatographic areas of metabolites discriminating the different hen strains.

Discriminant metabolite	Feature normalized area mean[1]			*Pooled StDev*	*P*-value
	B	F	W		
Egg white					
trans-4-Hydroxy-L-Proline	0.07078^{a}	0.04860^{b}	0.05357^{b}	0.00429	0.000
β-Arabinopyranose	0.01322^{a}	0.00790^{b}	0.00765^{b}	0.00217	0.009
D-Pinitol	0.00010^{a}	0.00067^{b}	0.00280^{c}	0.00072	0.001
d-Glucose	4.05930^{a}	2.76300^{b}	2.05720^{b}	0.65863	0.006
D-(+)-Talose	0.01098^{a}	0.01502^{ab}	0.02060^{b}	0.00299	0.005
D-Sorbitol	0.00522^{ab}	0.00565^{a}	0.00324^{b}	0.00112	0.031
N-Acetyl-D-glucosamine	0.16110^{a}	0.08410^{ab}	0.05206^{b}	0.04286	0.016
Maltose	0.00988^{a}	0.01937^{ab}	0.02188^{b}	0.00446	0.010
Egg yolk					
L-glutamic acid	0.03384^{a}	0.02577^{a}	0.04564^{b}	0.00521	0.001
Asparagine	0.00201^{a}	0.00938^{b}	0.00247^{a}	0.00188	0.001
Trans-4-hydroxy-L-proline	0.01431^{a}	0.01056^{b}	0.00830^{b}	0.00172	0.003
D-Glucose	0.00180^{a}	0.01007^{b}	0.00315^{a}	0.00233	0.002
L-Lysine	0.05781^{a}	0.03515^{b}	0.03248^{b}	0.01029	0.013
D-Pinitol	0.00161^{a}	0.00059^{b}	0.00142^{ab}	0.00043	0.020
D-Allose	2.98000^{a}	4.09900^{b}	3.27400^{b}	0.33216	0.003
D-Sorbitol	0.01616^{a}	0.01239^{ab}	0.00808^{b}	0.00342	0.027

[1]Means that share the same letters are not significantly different ($P < 0.05$)

the Blue Holland and Sasso strains. These observations highlight that the discriminant metabolites identified in the egg whites and yolks of various strains may be influenced by genetic, metabolic, or other inherent characteristics specific to each hen strain, irrespective of dietary factors.

Previous research indicates that both the housing environment and laying hen strain influence performance and egg quality (Sharma et al., 2022). Additionally, in a study conducted by Goto et al. (2021), specific amino acids effects in hen eggs were investigated, revealing that genetic factors can not only modify amino acid contents but also affect sensor values of bitterness. This suggests that selecting the combination of breed and feed may allow the production of designer eggs enriched in amino acids and enhanced in taste. These previous recent studies demonstrated the crucial importance of evaluating the essential metabolites of eggs from the most commonly consumed chicken breeds and strains in their production environment. The eggs from the chicken strains examined herein (Blue Holland, Sasso, and Wassache) are among the most produced in Africa. Previous studies on eggs from these strains have primarily focused on parameters such as egg weight, yolk height, yolk width, albumen height, total protein, total cholesterol, uric acids, triglyceride level, Haugh unit, and yolk index (Assefa et al., 2023; Dzungwe et al., 2024). This study provides, for the first time, insights into their differential metabolomes in a tropical context.

In conclusion, in the present work, we evaluated for the first time the chemical composition of different hen strains egg white and yolk. Different metabolites such as amino acids and carbohydrates were clearly identified as discriminant compounds among the studied strains. Thus, the selection of a particular hen strain can have implications for the nutritional composition, taste, and texture of eggs, emphasizing the significance of the strain in shaping egg characteristics and their impact on consumer health. Further studies with a higher scale are needed to confirm our understanding.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Affo Dermane: Conceptualization, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Kodjo Eloh:** Conceptualization, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Koffi Kibalou Palanga:** Visualization, Writing – original draft, Writing – review & editing. **Diane Tchakinguena Adjito:** Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Oumbortime N'nanle:** Conceptualization, Writing – review & editing. **Damintoti Simplice Karou:** Conceptualization, Writing – review & editing. **Tchilabalo Abozou Kpanzou:** Formal analysis, Visualization, Writing – review & editing. **Pierluigi Caboni:** Conceptualization, Visualization, Writing – review & editing.

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DISCLOSURES

The authors declare no conflicts of interest.

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