Review

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Metabolomics for quality assessment of poultry meat and eggs

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Abstract

The poultry industry is experiencing rapid growth worldwide. This accelerated growth has led to multiple food fraud incidents across the food supply chain, which consequently created a demand for precise determination of quality poultry production. This increase in demand for precise poultry production quality has necessitated advanced solutions. Metabolomics has emerged as a viable solution by offering detailed differentiation of biochemical indicators throughout the poultry supply chain. Additionally, this study provides a means to address risk factors affecting the poultry industry without compromising animal welfare, which is a critical concern. This review focuses on important issues related to poultry product quality assessment. Food adulteration has escalated in recent years as it is driven by the increasing focus on consuming high-quality and nutritious food. However, there is no specific guideline for such determinations, especially when appearance, texture, and taste can be manipulated by substituting for food components. Metabolomics can pave the way for a deeper understanding of existing and novel biochemical indicators responsible for determining the quality of poultry meat and eggs. This approach holds the potential to enhance the overall quality of poultry meat and egg products while also preventing food fraud.

Keywords: Poultry; metabolomics; welfare; application; authentication.

Introduction

According to the Food and Agriculture Organization of the United Nations (2014), poultry cover a wide variety of domestic birds that humans rear for their meat, eggs, or feathers. These birds are typically classified under the superorder Galloanserae (fowl) and the order Galliformes. Commonly, there are two main categories of poultry: domestic poultry and commercial poultry. Commercial poultry can be further classified, based on breeding terms, into two types: meat-type poultry and egg-type poultry, which are raised for meat and egg consumption, respectively (de Carvalho *et al.*, 2018). The U.S. Department of Agriculture (Food Safety and Inspection Service in Health and Safety, 2011) has refined the classification of commercial poultry (Table 1). Selecting the appropriate type of poultry is crucial for ensuring the outcome of any dish.

There has been a notable increase in poultry consumption and production worldwide (Del Bosque *et al.*, 2021). Valceschini (2006) attributed this rapid growth, which has been consistent since the 1990s, to poultry's nutritional properties, lack of religious restrictions, and affordability in comparison to other meat products. Baéza *et al.* (2022) presented data from different countries and highlighted a 17% increase in poultry meat consumption in Europe over recent decades. The European Commission (2021) forecasted a significant increase in the production and consumption rates of poultry meat by 2030. This growth is anticipated to lead to an increase in poultry exports that is driven by rising global demand. According to the European Commission findings (2021), the increase in meat exports and consumption has been substantial, and measures are needed to improve net poultry production to meet this growing demand.

In Malaysia, the poultry industry plays a pivotal role as a primary source of meat protein for consumers, during which it is experiencing rapid growth each year. According to the Federation of Livestock Farmers' Associations of Malaysia (2019), the ex-farm value of livestock products in 2020 exceeded MYR 24 billion (US\$5.8 billion), in which poultry

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Class of poultry	Age (weeks)	Specification
Rock Cornish game hen or Cornish game hen	<5	Immature chicken Both sexes Two pounds or less ready-to-cook carcass
Broiler or fryer chicken	<10	Both sexes Tender meat with soft, pliable, and smooth-textured skin Flexible breastbone cartilage
Roaster or roasting chicken	8–12	Both sexes Five pounds or more ready-to-cook carcass Tender meat with soft, pliable, and smooth-textured skin Less flexible breastbone cartilage compared to broiler or fryer chicken
Fryer roaster turkey	12	Immature turkey Both sexes Tender meat with soft, pliable, and smooth-textured skin Flexible breastbone cartilage
Capon	<16	A surgical neutered male chicken Tender meat with soft, pliable, and smooth-textured skin

Source: The U.S. Department of Agriculture (Food Safety and Inspection Service in Health and Safety, 2011).

meat accounted for over MYR 12 billion (US\$2.8 billion). This figure has been rising steadily over the past decades. The swift expansion of the poultry industry has triggered several transformations, affecting farming techniques, feed types, and the quality of end products. Modern poultry farming utilizes diverse rearing methods, including battery cage method, free-range method, indoor raising method, organic farming method, and deep litter method. Simultaneously, alternative poultry production has gained prominence as a current trend shows that consumers increasingly favour organically and naturally raised meat and eggs (Ricke, 2021). Consumers play a pivotal role in shaping the poultry industry as their demand serves as the primary driving force behind its growth. Poultry meat has become a favoured choice among consumers due to its high content of proteins and minerals as well as its affordable price point (Valceschini, 2006; Adamski et al., 2017) According to a survey conducted by Adamski et al. (2017), 46% of consumers consume meat two to three times a week, followed by 35% who consume it weekly. Additionally, 10% of consumers opt for poultry meat once every 2 weeks, whereas a mere 4% of consumers consume meat less than once a month. Remarkably, 84% of consumers favour chicken meat over other meat options, further emphasizing its popularity. This escalating demand has stimulated the emergence of various companies in the poultry industry, each striving to meet the poultry product purchase specifications.

Consequently, there is a surge in research and innovation aimed at ensuring and enhancing poultry product quality, thus intensifying competition among producers. Many new brands and claims are currently being introduced into the industry, encompassing both the meat itself and its by-products like eggs. Assessing meat quality is a multifaceted task and requires consideration of various factors. This is because consumers particularly prefer poultry meat and eggs that not only offer a good level of nutritional content but also ensure that specific sensory attributes are taken care of.

Traditional methods are still the predominant methods used for assessing meat quality and they can be categorized into subjective methods, which involve sensory evaluation based on human senses, and objective methods, where scientific experiments are conducted using instruments to assess physical, chemical, and microbiological properties (Kamruzzaman *et al.*, 2015; Shi *et al.*, 2021; Wu *et al.*, 2022a). Subjective methods depend on inspectors' experience and are difficult to quantify, whereas objective methods provide quantifiable results through scientific experiments. However, these current conventional laboratory methods in assessing meat quality are destructive, time-consuming, and unable to meet the demand from modern poultry meat production companies (Shi *et al.*, 2021). Rapid and automated methods are preferred to address these challenges in quality assessment, and these rapid technologies have been extensively reviewed elsewhere (Shi *et al.*, 2021; Wu *et al.*, 2022a).

The knowledge gaps in understanding the components that affect meat quality could limit progress in the development of rapid and accurate quality assessment methods. Metabolomics, a systematic study of small molecular compounds with molecular weight of less than 1500 Da that are present in biological systems (Wen et al., 2020), has gained attention in the areas of food quality and safety. It allows comprehensive profiling of metabolites in food that influence its taste, aroma, nutrition content, quality (both quality and contamination), and authenticity. Metabolomic studies utilize high-throughput analytical instruments such as mass spectrometry (including liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS)) and spectroscopies (infrared spectroscopy and nuclear magnetic resonance spectroscopy). These technologies have been extensively reviewed by Wu et al. (2022b), who discussed their principles and applications in food safety and quality assessment.

Metabolomic technology offers significant advantages to the food industry, including in the poultry sector, as it can be applied throughout the supply chain, from breed identification to the final product. This approach provides vital insights into food quality, processing stages, safety, nutritional content, and sensory attributes. Metabolomic analysis is essential for understanding the transformation of raw materials into end products, considering the chemical and sensory changes occurring during various treatments and processes in food production (Utpott *et al.*, 2022). It has been demonstrated to be excellent for analysing complex food compositions, including biological samples such as poultry meat and eggs (Wu *et al.*, 2022b). Moreover, the importance of metabolomic analysis in identifying essential biomarkers across diverse food matrices for food authentication has been extensively discussed by Mialon *et al.* (2023). This review will explore and discuss the parameters used in assessing the quality of poultry meat and eggs, factors affecting meat and egg qualities, and various applications of metabolomics in determining the quality of poultry meat and eggs.

Poultry Product Quality and Parameters

According to the Food and Agriculture Organization (FAO), meat quality is determined by an objective measure, that is, its nutritional value, and by the consumer's subjective evaluation of eating quality, including colour, flavour, juiciness, and tenderness. The FAO also highlights the significance of pale, soft, and exudative meat and dark, firm, and dry meat in evaluating meat quality, underscoring the importance of proper meat care post-slaughter, during transportation and storage. Meat quality is the key factor considered by consumers when making purchasing decisions, thus prompting the poultry industry to focus on producing high-quality meat. Poultry meat is renowned for its abundance of high-quality protein and minimal fat content. Its nutritional composition is influenced by factors such as poultry feed composition and consumption (Petracci and Baéza, 2007; Shaviklo, 2023). In addition to feed, the rearing environment also affects the metabolic profile of poultry products (Choi et al., 2023). In essence, the nutritional quality of poultry, in terms of high protein content, low fat and cholesterol contents, and the presence of other micronutrients, is equally important as the appearance and flavour of the poultry product itself in determining meat quality. Furthermore, the increasing health consciousness among consumers and concerns about the welfare of poultry animals should also be considered when the poultry industry is progressing. Acknowledging these aspects is crucial for the advancement of the poultry industry.

The World's Poultry Science Journal has previously introduced a procedure aimed at harmonizing meat quality traits and their methodologies to improve the overall reference value (Petracci and Baéza, 2011). This effort primarily focuses on physical traits, including pH value, R-value, colour, waterholding capacity, texture, and sarcomere length, as well as chemical traits such as moisture content, total lipid content, protein content, ash content, fatty acid composition, cholesterol, susceptibility to oxidation, amino acid content, collagen content, and pigments. Notably, sensory evaluation has yet to be incorporated as part of the quality determination process. To establish standardization, specific quality parameters, commonly used in scientific research and the food industry, are employed to assess poultry quality. Quality checks represent pivotal steps in the food supply chain. Table 2 provides a summary of selected quality parameters of poultry and their products, as utilized by various researchers.

The quality of an egg, on the other hand, is determined by external factors such as colour, cleanliness, and size before measuring the egg's weight when held in one's hand. These factors evaluate the quality of the egg based on the appearance of the egg after cracking (colour, viscosity), and eventually through sensory evaluation. As discussed by Berkhoff et al. (2020), consumer decisions when purchasing eggs are primarily influenced by price and egg size, with additional consideration of the egg yolk colour, which is preferable to a darker yellow shade. The nutritional quality of eggs, on the other hand, encompasses the types and levels of protein, lipids, minerals, and carbohydrates. The ratio of these compounds varies according to the egg components, which are the shell, egg white, and egg yolk (as thoroughly reviewed by Gautron et al., 2022). The composition of eggs is dependent mainly on the genetic strain of the laying hens and is also moderately affected by feed composition and consumption (Gautron et al., 2022). Although consumers nowadays tending to favour free-range chicken eggs due to their belief in the eggs' superior nutritional quality, they still base their preferences on the valuable physical characteristics of the eggs. Coutts and Wilson, in the book of 'Optimum Egg Ouality—A Practical Approach' (Coutts et al., 2007), further defined egg quality by both internal quality (viscosity of egg white, size, colour, and firmness of egg yolk and size of air cell) and external quality (shell thickness, height, texture, cleanliness, and width), as shown in Figure 1.

Egg quality is generally determined by egg grade, which is correlated with egg weight. In Malaysia, there are six grades of eggs, with grade AA (70 g) representing the highest quality, followed by grade A (65–69.9 g), grade B (60–64.9 g), grade C (55–55.99 g), grade D (50–54.9 g) and the lowest quality, grade E (<50 g). Prices of eggs also vary according to these grades. Currently, egg quality is primarily determined by external factors. Other factors, particularly nutrient content, are yet to be considered as key indicators of egg quality.

Table 2. Quality parameters measured in poultry and its products

Poultry	Quality parameter	Reference
Poultry products (chicken nugget, chicken fillet, chicken burger, chicken meatball, and chicken kabab)	Proximate composition, pH, water holding capacity, total volatile base, microbial quality	Hussain et al., 2016
Chicken	Proximate composition, pH, colour, shear force, microbial quality, and sensory characteristic	da Silva <i>et al.</i> , 2017
Turkey	pH, colour, water-holding capacity	de Carvalho <i>et al.</i> , 2018
Chicken	pH, colour, water holding capacity, tenderness, fibrousness, and sensory characteristic	Escobedo Del Bosque et al., 2020
Quail	pH, colour, drip loss, thawing loss, cooking loss, shear force, sarcomere length, and myofibrillar fragmentation index	Carvalho dos Santos <i>et al.</i> , 2020
Turkey (meat to sausage products)	pH, colour, thawing loss, cooking loss, shear force, antioxidant capacity, water-holding capacity	Kluth <i>et al.</i> , 2021

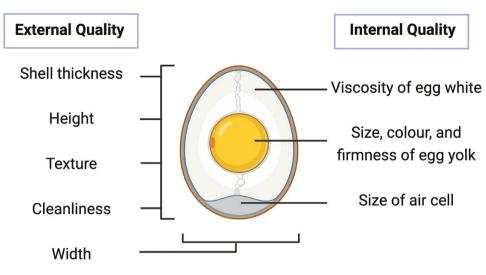


Figure 1. Factors affecting egg quality. Created with BioRender.com

Specific parameters, in addition to weight measurements, are used to determine the overall egg grade. For instance, the thickness of the eggshell is considered a crucial factor in improving overall egg quality. The yolk is protected from being released into the oviduct after it is laid. Conventionally, eggshell thickness is measured using gauge metres and spherical probes. However, the development of terahertz waves has allowed for a more precise and non-destructive method for measuring eggshell thickness (Khaliduzzaman *et al.*, 2020).

Eggshell strength is also a vital parameter for determining egg quality. It can be measured through fracture observation and egg force readers to gauge the egg's resistance to damage from external factors. This evaluation is especially important because eggs travel along the food supply chain before reaching consumers, and stronger eggshells help maintain egg quality. The strength of the vitelline membrane, a multilayered membrane that maintains the shape of the yolk and prevents it from mixing with the egg white, is another critical measurement parameter. Additionally, the Haught unit, which is a measurement unit for albumen height, is a widely used standard parameter for evaluating egg quality, particularly with regard to internal factors as it is directly proportional to egg internal quality.

On the other hand, factors such as cholesterol content, nutritional composition, and sensory acceptability also influence egg quality. Furgasa *et al.* (2019) stated that egg quality is determined by a combination of internal and external parameters, leading to the classification of eggs into three grades (AA, A, and B). However, further research is needed to determine its quality. Table 3 shows a summary of the selected quality parameters of eggs, as reported by various researchers.

The quality of poultry meat and eggs can be measured in various ways. Nevertheless, appearance and texture still play vital roles in consumers' acceptance and purchase intent (Escobedo del Bosque *et al.*, 2021; Cardona *et al.*, 2023). The texture quality of a poultry product is correlated with its nutritional composition. Quality is subjective between different individuals, based on their knowledge and preferences. Modern poultry meat and egg production, however, require minimal errors in the production system to produce uniform quality products. Thus, a more precise assessment method is required to not only produce quality poultry meat and eggs but also overcome fraudulent issues, particularly in adulteration and breed authentication.

Factors Affecting Poultry Product Quality

Feed

Feed consumption plays a major role in determining poultry product quality. Cereal grains are commonly used in poultry feed due to their nutrition content and impact on poultry growth. Specific nutrition has been proven to enhance poultry growth and meat quality, encompassing factors such as flavour, texture, and appearance. For example, studies have shown that male broilers fed probiotics exhibited higher breast muscle weight (Cramer et al., 2018). Probiotics that were administered at high ambient temperatures were shown to improve egg quality (Fathi et al., 2018). Feed intake with vitamin E supplementation has been found to be beneficial for preventing lipid oxidation (Kennedy et al., 2005). Additionally, a high-fat diet with an exogenous emulsifier was found to enhance overall performance and nutrient utilisation in broilers by facilitating digestion (Saleh et al., 2020). Jiang et al. (2019) further highlighted the significance of DL-methionine and DL-methionyl-DL-methionine supplementation at a minimum of 0.30% to improve meat quality and antioxidant activity in pigeon meat. DL-Methionine, a sulphur-containing amino acid, balances the amino acid content in poultry, thus promoting protein synthesis, growth, and meat elasticity. Another study demonstrated that antioxidant activity and meat quality could be improved with synbiotic supplements containing both prebiotics and probiotics (Li et al., 2019). These supplements not only improved meat quality but also enhanced overall poultry health and growth.

Environment

The environment indirectly influences the quality of poultry and its products, with factors ranging from production area temperature to overall production systems. Research by Ranjan *et al.* (2019) indicates that environmental stress during the growing period adversely affects meat quality. In poultry production, indoor rearing systems are most prevalent, but alternative methods such as free-range production have recently gained traction. According to El-Deek and El-Sabrout

Table 3. Quality parameters measured in poultry eggs

Eggs	Quality parameter	Reference
Eggs of Sri Lankan village chicken, commercial chicken, duck, quail and turkey	External quality (egg weight, length, weight, shape index, shell thickness) Internal quality (albumen height, yolk height, yolk width, yolk colour, yolk index and haugh unit) Functional properties (gelling strength, viscosity, Colour L^* , a^* , and b^* values for light- ness, red and green, and yellow and blue, respectively)	Wijedasa <i>et al.</i> , 2020
Eggs of Laying Hens strain (Hisex Brown, Hy-Line Brown, Isa Label and Lohmann Brown)	External quality (egg weight, egg diameter, egg length, specific gravity, shell weight, shell percentage, and shell thickness) Internal quality (albumen height, Haugh units, yolk weight, yolk percentage, albumen weight, and albumen percentage)	de Almeida <i>et al.</i> , 2021
Eggs of hens	External quality (egg weight, shape index, eggshell surface area, eggshell strength, and egg- shell thickness) Internal quality (yolk weight, weight of thick and thin albumen, thick albumen height, yolk colour, Haugh units, specific density of thick albumen and yolk) Lysozyme and fatty acid profile	Kowalska <i>et al.</i> , 2020
Eggs of brown and white laying hens	External quality (egg weight, egg shape index, eggshell weight, eggshell thickness, eggshell strength, eggshell colour) Internal quality (yolk weight, yolk index, albumen weight, albumen index, Haugh units)	Kraus <i>et al.</i> , 2020
Eggs of village and commercial chicken	External quality (blood spots, meat spots, shell cracks, egg weight, shell percentage, shell colour, and shape index) Internal quality (yolk percentage, albumen percentage, albumen pH, yolk pH, Haugh units, yolk colour, albumen protein percentage, and thick and thin albumen viscosity) Fatty acid content	Lordelo <i>et al.</i> , 2020
Eggs of Japanese quail	External quality (egg weight, egg shape index, shell thickness, shell weight and shell per- centage) Internal quality (albumen height, albumen weight, albumen index, albumen percentage, yolk height, yolk weight, yolk index, yolk percentage, and Haugh units)	El-Attrouny and Iraqi, 2021
Eggs of quail	External quality (egg length and width, egg weight, egg shape index, egg surface area) Internal quality (percentage of hatching weight and hatchability)	Kostaman and Sopiyana, 2021

(2019), a housing system can be classified into three types: intensive (indoor) systems, which consist of deep litter and battery (cage), semi-intensive systems, and extensive (outdoor) systems. Free-range production systems, in particular, have produced notably higher quality poultry than other systems. El-Deek and El-Sabrout (2019) found that this system, which provides more space for chickens to carry out physical activities, helps the chickens grow healthier legs and produce higher quality meat. This is supported by a separate study, which showed that both conventional and free-range production systems affect breast muscle yield, with the free-range method producing meat with superior appearance and lower fat content (Davoodi and Ehsani, 2020).

Climate changes, including global warming, exert significant impacts on the quality of poultry and its products by altering the structure of meat and eggs. It has been reported that heat stress can disrupt the whole production cycle of birds. High temperature was shown to reduce reactive oxygen species that are crucial for follicle maturity during ovulation, leading to infertility in birds (Nawab *et al.*, 2018). Heat stress is one of the primary concerns of climate change that significantly impacts poultry growth, productivity, laying performance, meat quality, and eggs (Abdel-Moneim *et al.*, 2021). Table 4 further summarizes the impacts of heat stress on chickens.

The heat stress is determined by the air relative humidity and the air temperature. Heat stress often affects poultry behaviour, which results in low-quality meat and eggs. For instance, heat stress increases panting in poultry, thereby elevating the blood pH and creating more stress and hormonal changes in poultry. Fouad *et al.* (2016) reported that heat stress reduces muscle pH, which in turn reduces the quality and appearance of meat. This effect can be mitigated through proper diet, as demonstrated by Attia *et al.* (2017), where supplements such as vitamins C and E improved the overall effect of chronic heat stress.

Age

Age has a significant effect on the quality of meat and eggs, with many quality traits in poultry meat affected by age. Li et al. (2020) revealed that older chicken meat had a higher pH value and was darker and redder. A higher pH value increases the water-holding capacity of the meat, resulting in increased juiciness and taste. Juiciness, along with appearance and texture, serves as a key indicator of meat quality. Prolonged rearing duration contributes to superior meat quality through enhanced muscle content and growth efficiency (Poltowicz and Doktor, 2012). Poultry age also plays a crucial role in egg quality. Uyanga et al. (2020) reported that a breeder's age significantly affects specific egg quality traits such as yolk weight, yolk percentage, and albumen content. In goose production, it has been reported that the meat fibre content is influenced by goose age, with 90–120 d being recommended as the ideal market age, as fibre production accelerates between 70 d and 90 d (Weng et al., 2021). Fibre degeneration significantly increases at 28 d and continues until 46 d in both sexes of broiler chickens, indicating age-related changes (Radaelli et al., 2017).

Breed

In Asian countries, jungle fowls are considered native breeds and have experienced increased demand in recent years.

Table 4. Impact of heat stress on chickens

Impact	Response
Behavioural	Higher respiration rate Panting Appetite loss Altered metabolism More time drinking
Physiological	Dehydration Acid–base imbalance Oxidative stress Immune response Increased heterophil and lymphocyte (H/L) ratio Impaired intestinal integrity and permeability
Growth	Reduced feed intake Reduced live weight gain Reduced feed efficiency Reduced meat quality Reduced liveability Poor fertility Mortality
Laying performance	Egg production Egg quality

Source: Abdel-Moneim et al. (2021)

Although native poultry is generally trusted for its quality and nutrition, this is not always the case. For instance, a comparative study conducted in Thailand between native and broiler chickens revealed that native chickens exhibited higher meat quality (including high water-holding capacity, high protein content, low fat content, and firm texture), whereas broiler chickens showed better productive performance that can be enhanced through crossbreeding (Jaturasitha et al., 2002). Other than meat quality, significant differences in egg quality traits exist between breeds, such as eggshell weight and colour, volk weight, and higher ribitol content in the albumen (Goto et al., 2019). Consequently, hens with different genetic traits would lay eggs with varying nutrient content. Crossbreeding enables the production of specific meat and egg traits with favourable metabolite content and improved taste to meet consumer demands. While genetic diversity plays a vital role, specific or irreplaceable genes also make significant contributions to the poultry industry in producing better-quality poultry products. This was proven by Mori et al. (2020), where varying breeds resulted in noticeable differences in eggshell colour, indicating that selecting breeds and optimizing the environment help in producing higher-quality eggs. Research on native breeds in India-namely, Aseel and Kadaknath-revealed superior egg yolk, albumen, and shell weight in the Aseel breed compared to the Kadaknath breed. Kadaknath, however, produced a higher number of eggs at 44 weeks of age (Haunshi et al., 2013).

Slaughtering method

Technically, slaughtering involves the humane killing of livestock under specific procedures to ensure their swift and painless death. Slaughtering holds a prominent position in various religious and is supported by research findings. There are two religious slaughtering methods: (1) kosher, which requires all blood residues to be removed through salting, and meat is prohibited for consumption if an error occurs during the process; and (2) halal, where naturally remaining blood in meat is acceptable post-slaught (Zurek *et al.*, 2021). According to Ahmed et al. (2018), the amount of blood removed during slaughter determines the method's effectiveness and enhances the quality of the meat as an end-product. As halal slaughtering increases the amount of blood removed from broiler meat, the quality of meat produced through this process is better and the meat has a better shelf-life (Hafiz et al., 2015). Studies by Awad and Ali (2011) have shown that the Islamic hanging method during slaughtering yields superior quality in broiler chicken meat when compared to the traditional Islamic method and the commercial electrical stunning method. It has also been reported that halal-slaughtered chicken contains lower amounts of iron, reduced lipid oxidation, and lower total bacterial count during storage (Ahmed et al., 2018; Sohaib et al., 2020). Hakim et al. (2020) noted that halal slaughtering results in less residual blood, reducing the bacterial count and consequently increasing the shelf-life of chicken meat.

Detection of Defects in Poultry Products with Relation to Animal Welfare

Animal welfare is a paramount concern for those involved in poultry rearing, slaughtering, transporting, and processing. Consequently, defect detection plays a pivotal role in the industry. However, traditional methods for defect detection do not provide comprehensive information, especially concerning poultry welfare. According to Falkovskaya and Gowen (2020), hyperspectral imaging offers numerous advantages over traditional methods by providing accurate results in bacterial contamination detection, physical defect detection, product quality assessment, and sensory quantification to meet consumer standards without compromising animal well-being.

Ensuring animal welfare in rearing environments is crucial for producing healthy and high-quality poultry products. Adequate feed and water supply, a safe environment with minimal to no injury risk, and a comfortable living space that reduces stress and promotes natural growth are essential aspects of rearing conditions. Computer learning systems to monitor poultry (both bioprocess and bio-response), particularly in rearing environments, are becoming valuable tools in animal welfare. These systems fall into two categories: conventional-based systems and deep learning-based systems (Okinda et al., 2020). Okinda et al. (2020) further explained that a machine learning-based monitoring system processes depth images of chickens, by preprocessing steps such as resizing, colour-space transformation, contrast enhancement, normalization, and denoising. This is followed by region of interest (ROI) segmentation through background subtraction, threshold-based methods, ellipse modelling, watershed, and point distribution modelling. The process then proceeds to feature extraction (morphological, locomotor, and optical flow measures), followed by modelling with machine learning algorithms, and classification or regression for monitoring bioprocess or bio-response. On the other hand, deep learning-based monitoring systems acquire depth images, extract features using a classifier, and then perform classification or regression for monitoring bioprocess or bio-response (Okinda et al., 2020).

Extensive research on computer vision technology, both hardware and software, is underway to enhance and implement these techniques in poultry farms with the aim of improving poultry performance (Abd Aziz *et al.*, 2021).

Wilhelmsson *et al.* (2019) emphasized the significance of organic broiler welfare, focusing on birds' natural behaviours. Their findings revealed a decline in the welfare of fast-growing birds after 6 weeks of rearing, but a similar situation was observed for slow-growing birds at a later stage. Additionally, due to the indirect effect of the COVID-19 outbreak on the poultry industry, limited farm workers were available daily to maintain animal welfare, thus further impacting poultry well-being (Hafez and Attia, 2020).

Metabolomics

The aim of 'omics' technology is to identify all genes (genomics), transcripts (transcriptomics), proteins (proteomics), and metabolites (metabolomics) present in biological samples (Weckwerth, 2003). Additional 'omics'-related tools include foodomics, lipidomics, nutrigenomics, metagenomics, and toxicogenomics. Metabolomics, specifically, encompasses all metabolites (carbohydrates, amino acids, lipids, fatty acids, vitamins, and others) weighing less than 1500 Da and are endogenously or exogenously present in a biological system (Johnson, 2020; Wen et al., 2020). Various metabolomics studies have been conducted across different research fields, including toxicology (Bonvallot et al., 2018), plants (Barrett et al., 2021), nutrition (Gonzalez-Granda et al., 2021), and microbiology (Pavlidis et al., 2021), as summarized in Table 4. Metabolomics studies can be categorized into targeted and untargeted approaches. Targeted metabolomics, which is generally applied in functional metabolomics studies, measures specific metabolites in samples and can be developed into quantitative or semi-quantitative analysis using reference metabolites (Guo et al., 2021a). Untargeted metabolomics, on the other hand, is used to profile a wider range of metabolites, which enhances the likelihood of detecting new or unknown and variant metabolites (Cao et al., 2020; Johnson, 2020). Cao et al. (2020) suggested that while targeted metabolomic approaches offer quantification advantages, they lack coverage of all metabolites. Untargeted metabolomic approaches can mitigate this limitation in detecting novel compounds. Untargeted metabolomics, as defined by Roberts et al. (2012), involves the comprehensive analysis of all compounds, including unknown ones, in a sample. This analysis is performed in conjunction with multivariate analysis (MVA), such as principal component analysis (PCA) and partial least square (PLS) to latent structure. Worley and Powers (2013) noted that the most abundantly used MVA in metabolomic is PCA followed by PLS, as PCA only provides information when variation within a group is minimal compared to variation between groups. However, they also recommended employing other multivariate analyses such as hierarchical clustering analysis and nearest-neighbour clustering, based on the nature of the research (targeted or untargeted) and objectives (Worley and Powers, 2013). The choice of multivariate analysis relies on the specific research context. Metabolomics analysis can provide detailed information on the nutritional content of poultry meat and eggs, which can aid in product quality improvement. Emwas et al. (2015) highlighted the specificity of the metabolomics approach in detecting disease biomarkers. Metabolomics explores various chemical compounds, including carbohydrates, lipids, amino acids, and organic acids, within a biological system (Escudero et al., 2017). Alterations in biological systems impact the chemicals present in the systems, and these changes can be specifically determined via a metabolomics approach.

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The metabolomics approach has been widely used in many industries as a tool of authentication because fraud cases are becoming more common. Metabolomics allows the identification of changes in biological systems that are affected by age, medication, climate, stress, pathology, and temperature more efficiently than other 'omics' technologies due to their quantitative and comprehensive nature (Yuan et al., 2008). Drastic technological development and the fast-paced world have created many issues regarding the originality and safety of many products used by consumers all over the world. This consequently increases the importance of food authentication to ensure that safe and quality foods are consumed. Therefore, the development of a proper authentication system is crucial, especially in the food industry to protect consumers. Metabolomics has attracted increased interest in the area of food authentication (Cubero-Leon et al., 2014) while continuously expanding to other industries (Rocchetti and O'Callaghan, 2021). It is becoming a powerful tool for analysing meat quality and authenticity, which indirectly and directly helps improve poultry welfare and product quality. In the future, sensory evaluation might be integrated into the metabolomics experimental design to further enhance the comprehensiveness of food quality assessment (Zhang et al., 2021a). As the poultry industry is rapidly growing, fraud cases involving the substitution of low-quality poultry meat and eggs are becoming more common. The metabolomics approach opens a new pathway for distinguishing poultry meat and eggs by offering highly detailed information with greater accuracy, starting from day 1 of chicken rearing until the products reach consumers.

Metabolomics Applications in Poultry Meat Quality Assessment

The metabolomics approach can be used for quality measurement of poultry from its raw form to its processed form. The metabolites present in these products can vary and change (either change in structure/concentration or release of new metabolites) due to many factors, such as stress, storage, pre- and post-slaughtering processes, and geographical origin. The metabolomics approach can also be used to determine meat quality prior to processing, the effects of stress conditions, and the welfare status of animals (Muroya et al., 2020). According to Wen et al. (2020), storage days of chilled chicken meat caused changes in the metabolite content (amino acids, amines, nucleosides, nucleotides, carbohydrates, and organic acids) and their stability, which consequently affected the overall quality of the meat. The freshness of chilled chicken meat depends on several important metabolites such as indole-3-carboxaldehyde, urine monophosphate, s-phenlymercapturic acid, gluconic acid, tyramine, and serylphenylalanine (Zhang et al., 2020). In addition, Zhang et al. (2021b) reported that a metabolomic approach can also be used to detect spoilage bacteria such as Brochothrix, Pseudomonas, and Serratia, which release undesired metabolites through different pathways such as histidine metabolism, glycine-serine metabolism, betaine and methionine metabolism, urea cycle, glutathione metabolism, purine metabolism, carnitine metabolism, and pyrimidine metabolism.

The metabolites present in poultry and their products can also be determined by the feed given to the poultry. The metabolomics approach can help prevent the development of infection in poultry at a very early stage, as reported by Wu *et al.* (2021), where infection caused by pathogen *Cryptosporidium baileyi*, which causes respiratory disease in chickens, was determined by identifying metabolite biomarkers in the blood serum using ultrapure liquid chromatography-mass spectrometry (UPLC-MS) techniques. This innovative technique not only helps in early infection detection but also establishes a novel approach for producing high-quality poultry meat while minimizing waste. Furthermore, the metabolomics approach can also mitigate environmental effects on meat quality. For example, copper toxicity in broiler chickens was found to impact 62 different metabolites, primarily those associated with mitochondrial metabolism in the kidney (Liao *et al.*, 2021).

A metabolomics study conducted by Cônsolo *et al.* (2020) successfully detected different metabolites in chicken breast with and without myopathy disease. Myopathy is a disease that affects the muscles that control voluntary action. According to Boerboom *et al.* (2018), myopathy is a concern for the broiler industry, affecting the quality of meat produced. Shukla *et al.* (2018) mentioned that stress in poultry causes a drastic effect on the poultry industry, and this can be ascertained by the presence of biochemical indicators (corticosterone and adrenal gland ascorbic acid) that can be detected by the metabolomics approach. Furthermore, pre-slaughter stress can also change the metabolomics in poultry, where higher values of muscle glycogen and muscle peroxidation are often observed in poultry (Santonicola *et al.*, 2017).

Metabolomics has been widely used in the poultry industry, from the determination of nutritional composition to the prevention of poultry-related diseases. This will consequently increase the quality of poultry meat and eggs. According to Beauclercq et al. (2016), the metabolomics approach in the determination of glycogen level in meat muscle can be insightful in determining the ultimate pH value of meat, which is an indicator of meat quality. In addition, the fatty acid composition in chicken meat due to the feeding regime can also be identified via lipid metabolome analysis. The determination of metabolomics content clearly differentiated between healthy and diseased (Eimeria acervuline infection) chickens, and showed altered fatty acid metabolism, nucleotide, β-oxidation, and microbial-related products due to malabsorption of the chicken (Aggrey et al., 2019). In addition, Wu et al. (2021) reported that a significant number of metabolites are involved in lipid metabolism as a result of Cryptosporidium baileyi infection during the very early age of chicken, which can be used as biomarkers for early detection. Therefore, the metabolomics approach will not only help improve the product quality in terms of nutritional content and sensorial characteristics but also help to prevent poultry quality deterioration due to external factors.

Abbas et al. (2020) confirmed that the untargeted determination (liquid chromatographymetabolomics ionization-tandem electrospray mass spectrometry, LC-ESI-MS/MS) in poultry can distinguish two different slaughtering methods, namely the non-Zabiha method (detaching spinal cord) and the Zabiha method (without detaching spinal cord). This research generated a clear separation of Zabiha and non-Zabiha characteristics in the orthogonal partial least squares discriminant analysis (OPLS-DA) plot, which was further confirmed with a set of blind samples. This statistical analysis tool provides a controlled data interpretation, which is ideal for metabolome studies. In addition to the specification of the research, such identification

sets a great reference for authentication for different types of slaughtering methods, which are in demand for halal classification in the food industry.

According to Akhtar et al. (2021), studies on H-NMRbased metabolomic identification would be very beneficial for overcoming food adulteration problems, as up to 37 metabolites were identified in their research, which contributes to the nutritional and sensory value of the sample. However, not all the discovered metabolites significantly distinguish the meat sample. More in-depth research on the detection of food adulteration should be encouraged, particularly by focusing on a single type of meat sample, which will have a greater impact on defining and differentiating the research samples. Consideration should be given to the causes of food adulteration when this conclusion is reached. On the other hand, research by Rangel-Huerta et al. (2022) reported that precooked chicken fillets have improved with vacuum-packed packaging, but other methods did not show much effect on metabolite profiling. However, more precise research should be conducted on pre-cooked meat products to obtain better differentiation, which can be used as an identification tool. In addition to raw poultry food products, metabolome identification can also be applied to processed foods. Ivanne et al. (2016) emphasized the importance of metabolomics analysis in determining the response of chickens to various external factors such as diet (feed and supplements), infections, and treatments. The study concluded that by using a metabolomics approach quality poultry can be attained.

The metabolomics approach is particularly valuable for determining additives and veterinary drugs in poultry meat. This approach is crucial because certain additives have been found to have negative effects, and concerns arise when these effects also impact consumers, and not just meat quality. In short, while additives and veterinary drugs are primarily used to enhance the quality of meat's end product, they should not jeopardize consumer health. For instance, a study has reported that 77.5% of chicken samples from Lebanese farms contained antibiotic residues, with 53.75% of these samples containing multiple drug residues, including antibiotics such as quinolones, amoxicillin, and tetracyclines (Jammoul and Darra, 2019). Metabolomics can be applied to generate data that are extremely helpful in establishing maximum residue limits for veterinary drugs in poultry products to improve food safety guidelines. Another metabolomics study detected 11 quinolones in 60 chicken meat samples obtained from local markets, with recovery rates ranging from 70.4% to 98.4%, including the detection of enrofloxacin and ciprofloxacin (Lu et al., 2019). A highly efficient method for detecting antibiotics in chicken breast meat was developed, in which six antibiotics were successfully extracted from four different classes with recovery rates between 75.68% and 101.3% (Doyuk and Dost, 2023). Additionally, a study demonstrated a reliable method for detecting veterinary drug residues, identifying 169 residues in chicken eggs in China (Luo et al., 2019). The overall application of metabolomics in assessing poultry meat quality is further summarized in Table 5.

Metabolomic Applications in Poultry Eggs Quality Assessment

The nutritional value of eggs, which is oftentimes overlooked throughout the manufacturing process, is important for consumers. It is therefore imperative to identify and examine the Table 5. Applications of metabolomics in assessing the poultry meat quality

Metabolomics application	Description	Example	Reference
of metabolic specific metabolic biomarkers with desirable of meat quality training	Metabolomics can help identify specific metabolites associated with desirable or undesirable meat quality traits, such as flavour, tenderness, or oxidative stability	Non-targeted metabolomics was used to resolve the taste differences in muscle tissue of four major livestock species (chicken, duck, pork, and beef) by LC-MS/MS and e-tongue analysis	Wang <i>et al.</i> , 2022b
		Studies on metabolites 'fingerprinting' to enhance meat quality using FTIR, GC-MS, and UHPLC-TOF-MS showed neck slaughtering chicken meat has a higher number of health-promoting metabolites such as <i>n</i> -3-polyunsaturated fatty acids (PUFA), triglyceride (TG), cytadine, and uridine, compared to neck pocking chicken meat	Shikh Zahari <i>et al.</i> , 2021
		The untargeted metabolomics technique was used to identify unique metab- olites and pathways that demonstrate the impact of selective breeding on the quality of chicken meat	Shi <i>et al.</i> , 2022
		A substantial difference was found between storage times of 0 d and longer times when using a metabolomics approach of LC-MS/MS to measure the quality of chilled chicken meat	Wen <i>et al.</i> , 2020
		544 biomarkers and multiple metabolic pathways affecting the chicken meat flavour in the breast muscle of Beijing You chicken were identified by HPLC- QTRAP-MS approach	Ge et al., 2023
Assessing Metabolomics can be used to nutritional measure the concentrations of various nutrients in poultry meat, including amino acids, fatty acids, vitamins, and min- erals, providing insights into its nutritional value	Mass spectrometry-based metabolomics revealed that metabolic path- ways of amino acids and purines, creatine, betaine, L-anserine, inosine 5'-monophosphate, 33 hypoxanthine, inosine, and phospholipid are all in- volved in chicken meat's taste, meat quality, and composition improvements by medium-chain monoglyceride supplementation	Liu <i>et al.</i> , 2021	
		The addition of dietary mixed edible oils (MEO) improved feed conversion, increased beneficial fatty acid levels, and changed muscle metabolites of breast muscle in chickens when investigated using untargeted LC-MS	Cui <i>et al.</i> , 2020
trol and au- verifying the auther	Metabolomics can assist in verifying the authenticity and quality of poultry meat prod-	LC-ESI-MS/MS results revealed distinct metabolic differences between the Zabiha (neck cut without spinal detachment) and non-Zabiha (complete neck detachment) groups	Abbas <i>et al.</i> , 2020
	ucts by analysing specific me- tabolite profiles and comparing	Metabolites in serum and skeletal muscle tissues of different chicken breeds from commercial farms were profiled using GC-MS/MS	Tan <i>et al.</i> , 2021
them with established reference standards (halal authentication, breed authentication, organic authentication, and adulter- ation)	Metabolites in serum and skeletal muscle tissues of different chicken breeds from commercial farms were profiled using ¹ H-NMR	Tan <i>et al.</i> , 2023	
Evaluating Metabolomics can provide a non-invasive approach to assess the impact of stressors on poultry welfare by monitoring changes in metabolite levels associated with stress response pathways	non-invasive approach to as- sess the impact of stressors on	Chicken breast tissues with myopathies were able to be discriminated by profiling metabolites in normal breast, wooden breast, and white striping myopathies of broiler chicken that are correlated with hypoxia and oxidative stress using proton NMR	Cônsolo <i>et al.</i> , 2020
	associated with stress response	Analysis of metabolic pathways revealed that heat stress has an impact on the glycerolipid, glycerophospholipid, linoleic acid, and alpha-linolenic acid pathways. These results reveal new important information on how heat stress affects the lipidomics of the liver of indigenous broiler chickens (Huaixiang chickens)	Guo <i>et al.</i> , 2021b
		Serum metabolomics in chickens infected with <i>Cryptosporidium baileyi</i> using UPLC-MS reveal 138 differing serum metabolites between mock and <i>C. baileyi</i> -infected chickens, with pathways related to energy, lipid metabolism, and some important immunity pathways being enriched	Wu <i>et al.</i> , 2021
Detection of foodborne contaminants	Metabolomics can be employed to detect and quantify the presence of potentially harmful substances or contaminants, such as antibiotics, pesticides, and/or mycotoxins, in poultry meat	Antibiotic residues in poultry meat samples from Germany, Poland, and Lithu- ania were evaluated using targeted UHPLC-MS/MS. However, low enrofloxacin and doxycycline concentrations were established in tested samples	Bartkiene <i>et al.</i> , 2020
		Method strategy is applied to capillary electrophoresis (CE) coupled to tandem mass spectrometry (MS/MS) for the multi-residue detection of quinolone in chicken meat	Lara and García- Campaña, 2022
		The selective and sensitive HPLC-MS/MS method was developed to provide a reliable tool for monitoring and regulating the presence of antimicrobial residues in poultry litter	Yévenes <i>et al.</i> , 2021
		This study developed a precise method using ultra-high performance liquid chromatography-quadrupole/orbitrap HRMS to simultaneously detect 23 mycotoxins in broiler tissues	Yang <i>et al.</i> , 2021
		Pesticide levels were studied in chicken samples from Tanzanian farms using GC-MS. Kidney and liver samples had higher concentrations, especially in Dar es Salaam sites. Many concentrations exceeded safe limits, posing risks to both livestock and public health	Mahugija <i>et al.</i> , 2018

Table 5. Continued

Metabolomics application	Description	Example	Reference
Under- standing processing effects	Metabolomics can shed light on the impact of different processing techniques (e.g. cooking, marinating) on the	This study investigated the effect of different heating temperatures on the metabolite changes in chicken meat using LC-MS, where increases in levels of endogenous and degraded metabolites in the chicken stock were detected, thus encouraging a lower temperature cooking	Ohta <i>et al.</i> , (2022)
	metabolic profiles of poultry meat, influencing its flavour, texture, and overall quality	This study investigated changes in metabolomes related to the meat quality properties of pre-cooked chicken fillets that underwent different food preservation methods using LC-HRMS. Findings showed that vacuum packaging was the best method for preserving meat quality	Rangel-Huerta et al., 2022

metabolite content of the eggs to ensure that their quality is preserved. As myriad factors may influence the nutritional profile of the eggs, metabolomic analyses can help in elucidating their nutritional changes. Currently, poultry eggs are undergoing a drastic transformation and metabolomics can be used to measure the quality of those eggs. Goto *et al.* (2019) used GC-MS/MS to determine thoroughly that different types of breeds, namely Rhode Island Red and Australorp, and different feed, which are fermented feed and mixed feed, influence the content of metabolites (identifying 3 metabolites in yolk and 12 metabolites in albumen) in chicken eggs. On the other hand, specific acids were identified in the egg yolks of hens, which were used as the model matrix of animal origin and can be used to differentiate origin, breed, and farming system (Hajjar *et al.*, 2021).

Application of metabolomics can be used to differentiate different types of eggs, especially village chicken egg and the conventional egg. A metabolomics study has shown better nutrient quality in organic eggs (both egg yolk and egg white) than in conventional eggs (Xia et al., 2022). However, more research is ongoing to improve the quality of conventional eggs. As a result of metabolomics studies, it is possible to identify changes occurring in egg yolk during embryonic development that have led to the discovery of different metabolites. On the other hand, Wang et al. (2023) demonstrated that metabolomics studies can be used wisely in cooked egg studies as well, where metabolites in egg yolk were observed to change in boiled eggs, resulting in an increase in specific nutritional contents (fat-soluble vitamins, riboflavin, and biotin). In addition to preserving egg quality, the metabolomics approach also aids in the identification of microbial contamination. Eggs can be easily contaminated with microorganisms such as Salmonella, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, and Campylobacter spp. Microbial contamination can alter the metabolic profiles of eggs, which involve metabolic pathways such as amino acid and lipid metabolisms. Therefore, metabolomics can help in assessing changes in the food system due to microbial contamination. A study discovered several potential spoilage markers that are present in eggs, especially stored eggs, and a combination of these identified markers helps increase the accuracy of spoiled egg detection (Chang et al., 2021). However, until recently, relatively limited research has applied the metabolomics approach to detect microbial contamination in eggs. As the metabolomics approach offers a rapid and highly sensitive method, further exploring the utilization of metabolomics in addressing egg contamination is highly encouraged.

A recent study emphasizes the importance of ontinuous monitoring of antibiotic residues in poultry eggs in China, as 18.13% of samples tested positive for antibiotics (Wang et al., 2022a). It is important to consider that rapid methods and early detection of additives and drugs are vital in the fast-growing poultry egg industry. In addition, another study developed a rapid, efficient, and highly sensitive method for detecting antibiotic contamination by simultaneously determining levamisole, mebendazole, and the two metabolite residues of mebendazole in various types of poultry eggs (hen, duck, and goose) using a metabolomics approach (Chen et al., 2022). The metabolomics approach is also advantageous for rapidly analysing multiple compounds in a single run within a short time. A previous study reported that 244 chemical compounds that contaminated eggs, including B-agonist, insecticides, and steroidal hormones, in real samples can be analysed in 30 min (Zhang et al., 2022). Moreover, 11 quinolones were previously detected in 110 egg samples obtained from the local market using a metabolomics approach with a recovery rate between 66.9% and 99.0% (Lu et al., 2019). On the other hand, a study aimed to develop an effective method for analysing 64 antibiotics from nine different classes in chicken eggs. Five antibiotics were successfully detected in commercial eggs, further suggesting the possibility of multiclass antibiotic detection in eggs (Wang et al., 2017). Therefore, the metabolomics approach proves valuable for identifying various additives and veterinary drugs in poultry eggs. In the long term, this method may pave the way for the use of new additives and veterinary drugs with fewer side effects in the poultry industry.

In addition to detecting adulteration and distinguishing different types of eggs, metabolomics studies also contribute to the production of high-quality eggs. Despite eggs being simple and nutrient-rich food that can be easily cooked or prepared, there is a need to focus on enhancing their nutritional content. This area of focus aligns with the concept introduced by Goto *et al.* (2019), known as 'designer eggs', which are nutritionally modified or enriched beyond their natural states and their quality can be assessed using a metabolomics approach. Consequently, designer eggs, such as omega-3-enriched eggs, low-cholesterol eggs, and eggs enriched with vitamins and minerals, are already available on the market, with further advancements expected in future research.

In addition to nutritional content, egg quality is also determined by other factors, such as colour and freshness. Egg freshness is a critical aspect of quality and safety for egg consumption, and an untargeted metabolomics approach has been utilized to identify various compounds associated with freshness (Cavanna *et al.*, 2018). Microbial contamination poses a common challenge in egg production and has a significant implication for the entire poultry egg industry. To maintain egg quality in a robust manner, it is vital to maintain each egg to be free from contamination to ensure that it is safe and of high quality for consumers, and metabolomics has proven to be valuable in enhancing the assessment of egg quality.

Conclusions

Metabolomics has contributed significantly to the food industry by improving quality assessment, reducing fraud cases, and safeguarding consumers. These technologies allow the profiling of metabolites that have potential as biomarkers for food quality assessment. Metabolomics allows systematic and comprehensive profiling of existing metabolites in poultry samples, which significantly enriches databases in poultry science compared to non-metabolomics studies. Whereas recent metabolomics studies of poultry meat and eggs have focused on ensuring superior product quality, its applications should also be extended to monitoring quality changes during storage and transportation, which can impact the end product. Modern consumers' demand for high-quality, safe, delicious, and nutritious meat products with fewer additives and veterinary drugs and metabolite analysis can help ensure that these demand specifications are met by the poultry industry. Additionally, improving food quality through an effective assessment system such as the metabolomics approach will also help improve consumer health and reduce health risks in the population. In conclusion, metabolomics pave ways towards improving the analytical assessment of the quality and authenticity of poultry meat and egg products systematically, thoroughly, and has high accuracy and specificity.

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Author Contributions

Conceptualization, Jinap Selamat, Nuzul Noorahya Jambari, and Suganya Murugesu; writing original draft preparation, M.S. Yogeswari; review and editing, Jinap Selamat, Nuzul Noorahya Jambari, Alfi Khatib, Mohd Hishammfariz Mohd Amin, and Suganya Murugesu; funding acquisition, Jinap Selamat and Nuzul Noorahya Jambari. All the authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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