THESES OF THE PHD DISSERTATION

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Gödöllő

2026



Hungarian University of Agriculture and Life Sciences

INVESTIGATION OF GREEN DISCOLORATION IN FATTENED GOOSE LIVER FOR FOOD QUALITY IMPROVEMENT

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Gödöllő

2026

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1. BACKGROUND AND OBJECTIVES

The production of fattened goose and duck liver has a long-standing tradition, with its origins dating back several millennia. The practice of force-feeding waterfowl was already known in Ancient Egypt, where it was used to promote fat deposition in birds. References to foie gras as a delicacy can be traced back to the 4th century BC, while the earliest European written records concerning goose husbandry date from the 8th century AD.

Goose breeding and production have a particularly rich history in Hungary, where goose husbandry became established during the 13th century and has remained an important agricultural activity ever since.

Foie gras has consistently been regarded as a premium food product and continues to occupy a distinguished position on the menus of fine dining establishments worldwide. This highly valued gastronomic specialty is produced through a specialized force-feeding process, during which birds receive large quantities of carbohydrate-rich feed. Consequently, the amount of feed consumed substantially exceeds what geese would voluntarily ingest under ad libitum feeding conditions.

Hungary is one of the leading producers of fattened waterfowl liver, with an annual production volume exceeding 3,500 tonnes. Although the industry has undergone considerable technological development and numerous automation solutions have been introduced, goose production remains highly labor-intensive.

In recent years, several external factors—including prolonged drought periods, recurring outbreaks of avian influenza, the COVID-19 pandemic, and the Russia–Ukraine conflict—have contributed to increased production costs throughout the sector. These developments have led to a substantial rise in the market price of foie gras.

Given its high economic value and importance as an export commodity, it is essential to investigate all factors that may adversely affect product quality. Equally important is the maximization of shelf life. In industrial practice, livers are typically vacuum packaged and subsequently frozen. However, a green surface discoloration is frequently observed during storage under these conditions. Understandably, this phenomenon often generates negative consumer perceptions, as many consumers associate the discoloration with microbial spoilage, ultimately leading to product rejection.

Within the framework of my doctoral research, the objectives of the study were formulated based on the occurrence of this undesirable green surface discoloration. The specific aims were to determine:

- whether individual slaughterhouse processing steps—particularly pre-slaughter watering, stunning procedures, exsanguination following neck vessel severance, and the applied evisceration technology—affect the development of greening on the surface of fattened goose liver;

- whether the green discoloration is associated with microbial activity and whether differences can be detected in the microbial communities of affected and unaffected livers, with special emphasis on hydrogen sulfide-producing microorganisms;
- whether bile production, as an emulsifying digestive secretion synthesized by the liver, is involved in the development of the discoloration;
- whether differences exist in the amino acid composition of greened and non-greened livers, particularly regarding sulfur-containing amino acids such as cysteine and methionine;
- whether concentrations of macroelements, microelements, and trace elements differ between affected and unaffected livers; and
- how vacuum packaging, the most commonly applied packaging technology for foie gras, influences the occurrence of green surface discoloration.

2. MATERIALS AND METHODS

2.1. Experimental Background

The present research involved both laboratory investigations and experiments conducted under industrial processing conditions. Practical trials were carried out at one of Hungary's largest poultry processing facilities, operated by Pannon Fine Food Ltd. (Orosháza, Hungary). A total of three slaughtering experiments were performed at the facility. During these industrial-scale trials, the effects of stunning, pre-slaughter watering, and different evisceration technologies on the quality characteristics of fattened goose liver were investigated.

The livers were transported to the laboratory on the day of slaughter in vacuum packaging under refrigerated conditions (0–3°C), placed on ice during transportation. In all experiments, the samples were subjected to frozen storage at –18°C for five weeks (35 days). Following the initial measurements conducted on Day 0, analyses were performed at weekly intervals, resulting in six measurement points throughout the storage period.

All experiments were carried out using Grey Landes geese (*Anser anser*). From hatching onward, birds were reared in closed housing systems where temperature, stocking density, ventilation, lighting, and relative humidity were adjusted according to age-specific husbandry requirements. During the first eight weeks of rearing (Days 1–56), feed and water were provided *ad libitum*.

The force-feeding period lasted from Day 57 to Day 70. Birds were fed every seven hours, beginning with 160 g portions and gradually increasing the ration size over seven days until reaching 440 g per feeding. From Day 64 until the conclusion of force-feeding on Day 70, all geese received a constant 440 g feed ration at each feeding. Water remained available *ad libitum* throughout the force-feeding period.

At the end of the fattening phase, the live body weight of each goose was individually recorded, ranging between 6.4 and 7.1 kg. Subsequently, birds were placed into plastic transport crates (three birds per crate) and transported to the slaughterhouse in a properly ventilated vehicle.

2.2. Experiment I – Effects of Pre-Slaughter Watering and Different Evisceration Technologies

The objective of the first experiment was to evaluate the effects of pre-slaughter watering and different evisceration procedures—namely cold evisceration and warm evisceration—on the quality characteristics of fattened goose liver.

Seventy-two geese were randomly selected at the slaughterhouse and allocated to four experimental groups (18 birds per group):

Group	Pre-slaughter watering	Evisceration method
1	No	Cold evisceration
2	No	Warm evisceration
3	Yes	Cold evisceration
4	Yes	Warm evisceration

Watering was performed using a 5-mm silicone tube connected to a 50-mL syringe. Each bird received 200 mL of potable tap water administered directly into the crop. Following watering, birds were allowed to rest for two hours before shackling. No stunning procedure was applied prior to slaughter.

After shackling, the cervical blood vessels were severed and blood loss during exsanguination was collected and quantified individually. Subsequent processing steps included scalding (66°C), defeathering, paraffin waxing (53°C), cooling (16°C), paraffin removal, and foot removal.

Carcasses assigned to cold evisceration (Groups 1 and 3) were stored in a chilling chamber at –2°C for eight hours. At the end of the chilling period, the core temperature reached approximately 6°C. Following chilling, carcasses were eviscerated, livers were removed, and liver weight was recorded individually.

For warm evisceration (Groups 2 and 4), liver removal was performed immediately after cooling (16°C), followed by storage on ice for eight hours.

Prior to individual vacuum packaging, liver hemoglobin content was determined using Drabkin's reagent and spectrophotometric analysis at 540 nm (Hitachi U-2900, Hitachi Ltd., Tokyo, Japan). Liver pH was measured using a Testo 206 pH2 instrument (Testo GmbH, Vienna, Austria).

Following vacuum packaging in polyethylene (PE) bags and prior to freezing, objective colour measurements were performed at three predefined locations on each liver using a portable chromameter (Minolta CR-400, Konica Minolta Inc., Tokyo, Japan). Colour parameters were evaluated in the CIELab colour space, where L* represents lightness, a* indicates the red–green axis, and b* represents the yellow–blue axis. Total colour difference (ΔE*) values were also calculated.

After freezing, colour measurements were repeated weekly. At the end of the storage period, microbiological analyses were performed focusing on hydrogen sulfide-producing spoilage organisms, particularly members of the genus *Pseudomonas*. Identification was conducted using MALDI-TOF mass spectrometry (Bruker Corp., Billerica, MA, USA).

Statistical analyses were performed using IBM SPSS Statistics Version 26 (IBM Corp., Armonk, NY, USA). Two-way ANOVA was applied to evaluate pH and hemoglobin content, while one-way ANOVA was used for exsanguination-related variables. Colour measurements

were analysed using two-way MANOVA. Normality and homogeneity of variance assumptions were verified using the Shapiro–Wilk and Levene tests, respectively. Statistical significance was established at $P \leq 0.05$.

2.3. Experiment II – Targeted Investigation of the Effects of Evisceration Technology

Based on the findings of Experiment I, the second experiment focused specifically on the development of green surface discoloration. The primary objective was to investigate potential causes of greening in vacuum-packaged fattened goose livers stored under frozen conditions, with particular emphasis on the possible involvement of sulfhemoglobin formation and bile pigments.

Following arrival at the processing facility, 60 geese were randomly selected and allocated to two groups:

Group	Number of birds	Evisceration method
1	30	Cold evisceration
2	30	Warm evisceration

Birds were slaughtered without stunning following shackling and bleeding. Standard processing procedures included scalding, defeathering, paraffin waxing, water chilling, paraffin removal, and foot removal.

Birds assigned to warm evisceration were processed immediately after chilling, whereas carcasses designated for cold evisceration were held at –2°C for eight hours before liver removal.

After vacuum packaging in PE bags and freezing, colour measurements were performed at weekly intervals throughout the five-week storage period. Additional analyses conducted at the end of storage included:

- lipid oxidation assessment using the thiobarbituric acid (TBA) assay;
- qualitative bile pigment detection using the Gmelin test;
- elemental analysis by ICP-OES following microwave-assisted digestion;
- amino acid profiling with special emphasis on sulfur-containing amino acids;
- detection of hydrogen sulfide through a lead nitrate test.

Statistical evaluation was conducted using paired-sample t-tests for colour measurements, TBA values, elemental composition, and amino acid profiles. Statistical significance was accepted at $P \leq 0.05$.

2.4. Experiment III – Effect of Stunning on the Development of Green Discoloration

The third experiment examined the effect of pre-slaughter stunning. Stunning is routinely applied in poultry processing to improve animal welfare by eliminating voluntary movement before slaughter. Following stunning, birds remain calm during shackling and bleeding, thereby facilitating handling procedures.

The objective of this experiment was to determine whether stunning influences the efficiency of blood removal and consequently affects the occurrence of green discoloration during frozen storage.

Sixty fattened geese were randomly assigned to two experimental groups:

Group	Number of birds	Stunning treatment
1	30	Electrically stunned before slaughter
2	30	Slaughtered without stunning

Birds in Group 1 were subjected to electrical stunning at 110 V before slaughter. Subsequent processing steps included slaughter, bleeding, scalding, defeathering, paraffin waxing, water chilling, paraffin removal, and foot removal.

Following processing, carcasses from both groups were chilled at –2°C for eight hours before liver removal. Hemoglobin content was measured before vacuum packaging. Colour measurements were performed immediately after packaging and repeated weekly during frozen storage.

At the end of the storage trial, additional investigations included:

- solvent extraction and spectrophotometric characterization of green pigments using acetone and distilled water;
- evaluation of pigment solubility;
- assessment of packaging atmosphere effects on greening through modified atmosphere packaging (MAP) containing 80% CO_2 and 20% O_2.

Statistical analyses were performed using paired-sample t-tests for hemoglobin concentration and colour parameters. Statistical significance was established at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Experiment I – Effects of Pre-Slaughter Watering and Different Evisceration Technologies

No significant differences were observed between watered and non-watered birds regarding either the volume of blood removed during exsanguination ($P = 0.765$) or the rate of bleeding ($P = 0.426$). On average, more than 50% of the total blood volume was removed during bleeding, corresponding to approximately 4.2% of live body weight.

Pre-slaughter watering significantly reduced the hemoglobin content of fattened goose livers ($P < 0.001$). The most plausible explanation is that the administered water diluted the circulating blood, thereby reducing its viscosity and facilitating blood circulation. In contrast, the applied evisceration method had no significant effect on liver hemoglobin concentration ($P = 0.656$).

Watering did not significantly influence any of the measured colour parameters, including lightness (L*, $P = 0.715$), redness (a*, $P = 0.979$), or yellowness (b*, $P = 0.964$). However, the evisceration technology exerted a significant effect on all colour attributes ($P < 0.001$). Statistically significant colour changes were first detected on Day 21 of storage, suggesting that the development of discoloration occurred between Days 14 and 21. Significant differences in total colour change (ΔE*) between cold- and warm-eviscerated groups were observed from Day 21 onward ($P < 0.001$). ΔE* values exceeding 5 indicated colour differences that were readily perceptible to the human eye.

Neither watering ($P = 0.225$) nor evisceration method ($P = 0.606$) significantly affected liver pH. Although pre-slaughter watering is traditionally believed to calm animals before slaughter, the present results did not support this assumption, as no pH differences were observed between watered and non-watered birds. Furthermore, the absence of pH changes associated with cold evisceration suggests that no substantial formation of acidic or alkaline compounds occurred during the chilling period.

Microbiological analyses revealed the presence of *Escherichia coli*, *Brochothrix thermosphacta*, *Carnobacterium maltaromaticum*, and *Aeromonas bestiarum*. *E. coli* was detected exclusively in warm-eviscerated samples, whereas the occurrence of the other bacterial species was independent of the evisceration method. Notably, only a single sample tested positive for a member of the genus *Pseudomonas*. These findings indicate that microbial contamination was unlikely to be the primary cause of green discoloration.

3.2. Experiment II – Targeted Investigation of the Effects of Evisceration Technology

The combination of cold evisceration and frozen storage for 35 days significantly affected all measured CIELab colour parameters, including L* ($P \leq 0.05$), a* ($P < 0.001$), and b* ($P \leq 0.05$). Cold-eviscerated livers became darker and developed visible green discoloration on their surfaces, whereas no statistically significant colour changes were observed in warm-eviscerated samples relative to their initial values.

Consistent with the findings of Experiment I, significant colour differences emerged on Day 21 of storage, indicating that the onset of greening occurred between Days 14 and 21. Total colour difference (ΔE*) values differed significantly between treatment groups from Day 21 onward ($P < 0.001$), with values exceeding 5 indicating substantial and visually obvious colour deviations.

No significant differences were observed in lipid oxidation between greened and non-greened liver samples ($P = 0.326$). The mean thiobarbituric acid (TBA) value was 1.552 mg MDA/kg liver in greened samples and 1.492 mg MDA/kg liver in unaffected samples. These results suggest that lipid oxidation is unlikely to be responsible for the observed discoloration.

The Gmelin test yielded a positive reaction for the bile control sample but produced negative results for all liver extracts. Consequently, neither bilirubin nor biliverdin—the principal pigments responsible for bile coloration—were detected in the discoloured liver tissues. This finding indicates that bile contamination is unlikely to be the source of the green surface discoloration.

Elemental analysis identified significant differences between greened and non-greened samples only for potassium (K), iron (Fe), and manganese (Mn) concentrations ($P \leq 0.05$). Although iron may theoretically contribute to discoloration phenomena, the observed differences were insufficient to explain the development of the pronounced green colour. Likewise, while potassium and manganese concentrations differed significantly, no evidence currently exists linking these elements directly to the greening process.

Analysis of sulfur-containing amino acids revealed no significant differences between greened and non-greened livers. Methionine concentrations were 2.78% and 2.95% in greened and unaffected samples, respectively ($P = 0.274$). Similarly, cysteine concentrations were 1.21% and 1.00%, respectively ($P = 0.187$). These findings suggest that sulfur-containing amino acids are unlikely to serve as sulfur donors in the sulfation of hemoglobin.

In contrast, the lead nitrate test produced positive reactions exclusively in greened samples, confirming the presence of hydrogen sulfide. Since hydrogen sulfide is a prerequisite for sulfhemoglobin formation, its detection strongly supports the hypothesis that sulfhemoglobin plays a central role in the development of green discoloration.

3.3. Experiment III – Effect of Stunning on the Development of Green Discoloration

Statistical analysis demonstrated that stunning had no significant effect on any colour parameter, including lightness (L*, $P = 0.347$), redness (a*, $P = 0.421$), or yellowness (b*, $P = 0.324$). In both experimental groups, decreases in colour values were first observed on Day 21 of storage, indicating that discoloration developed between Days 14 and 21 regardless of stunning treatment.

No significant differences were detected in total colour difference (ΔE*) at any point during the five-week storage period ($P = 0.325$). Calculated ΔE* values ranged between 1 and 2, corresponding to colour differences that were only barely perceptible to the human eye.

Similarly, stunning prior to neck vessel severance had no significant influence on liver hemoglobin content (P = 0.426). Although previous studies have suggested that properly executed stunning may facilitate more efficient bleeding, the present results did not support this assumption. Comparable hemoglobin concentrations were measured in livers obtained from stunned and non-stunned birds, indicating that blood removal efficiency was essentially identical between treatments.

Spectrophotometric analyses conducted after acetone extraction revealed similar absorption patterns in both greened and non-greened samples, although slight differences in absorption maxima were observed. Comparable results were obtained following extraction with distilled water; however, absorbance values differed from those obtained using acetone, and no distinct absorption peaks were detected.

The investigation of oxidative and reductive environments yielded particularly noteworthy results. Immediately after thawing, the previously pronounced green discoloration visibly diminished. This observation suggested a direct relationship between freezing and the appearance of greening. Following packaging under modified atmosphere conditions (80% CO_2 and 20% O_2), exposure to oxygen further reduced the discoloration, and the livers progressively regained their original colour.

After four hours of storage under oxygen-containing modified atmosphere packaging, the green discoloration had completely disappeared. These findings clearly demonstrate that oxygen exerts an inhibitory effect on the development and persistence of green surface discoloration in fattened goose liver.

4. CONCLUSIONS AND RECOMMENDATIONS

Based on the findings of the first experiment, it can be concluded that pre-slaughter watering may be beneficial in terms of reducing the blood content of the liver; however, its practical implementation on an industrial scale would be difficult, and the presumed calming effect could not be verified. Furthermore, the results obtained from livers processed using different evisceration technologies demonstrated that although cold evisceration protects the liver from mechanical damage, it also contributes to the development of undesirable green surface discoloration.

Microbiological analyses indicated that the hydrogen sulfide required for sulfhemoglobin formation—the pigment most commonly associated with green discoloration—was not the result of post-processing microbial contamination. Consequently, the source of hydrogen sulfide is likely endogenous rather than microbiological in origin.

The results of the second experiment further reinforced the conclusions drawn from the first study. The application of cold evisceration clearly promoted the development of greening. The absence of bilirubin in greened liver samples, as demonstrated by the Gmelin test, indicated that the discoloration was not associated with bile contamination but was more likely related to blood-derived pigments.

The elemental analyses suggested that macroelements, microelements, and trace elements do not play a decisive role in the development of the discoloration. Likewise, amino acid profiling revealed that sulfur-containing amino acids, specifically cysteine and methionine, do not appear to function as sulfur donors during hemoglobin sulfation and therefore are unlikely to participate directly in the formation of sulfhemoglobin.

The positive hydrogen sulfide reactions observed in greened samples strongly support the hypothesis that sulfhemoglobin formation represents the primary mechanism responsible for the appearance of green discoloration. Since hydrogen sulfide is an essential prerequisite for sulfhemoglobin formation, its detection in affected samples provides substantial evidence for this proposed pathway.

The results of the third experiment demonstrated that pre-slaughter stunning should primarily be regarded as an animal welfare measure rather than a means of improving bleeding efficiency. No statistically significant differences were observed in liver hemoglobin content between stunned and non-stunned birds, indicating comparable blood removal efficiency in both groups. Moreover, stunning had no measurable effect on the incidence or severity of green surface discoloration.

Investigation of oxidative and reductive conditions revealed that the presence of oxygen inhibits the development and persistence of green discoloration on the surface of fattened goose liver. This finding represents one of the most practically relevant outcomes of the study.

Based on the knowledge and practical experience gained during the slaughtering experiments conducted throughout the doctoral research project, it can be concluded that the undesirable green surface discoloration can be prevented. However, achieving this objective requires

modifications to certain stages of the currently applied processing technology as well as reconsideration of the packaging system used for product storage.

Although cold evisceration is a relatively gentle processing method that minimizes mechanical damage to the liver, the pre-chilling period may facilitate the diffusion of sulfur-containing compounds produced within the intestinal tract throughout the body. According to the findings of the present study, these sulfur-containing compounds may contribute to the development of green discoloration in fattened goose liver.

Consequently, replacing cold evisceration with warm evisceration may be advisable. Immediate liver removal without prior chilling could reduce the distribution of sulfur-donor compounds generated during digestive processes and thereby decrease the likelihood of subsequent sulfhemoglobin formation and greening.

In addition to modifications in processing technology, changes in packaging practices should also be considered. Evaluation of the experimental findings together with available scientific literature strongly suggests that green discoloration is closely associated with the oxygen tension of the surrounding environment.

Therefore, the use of modified atmosphere packaging (MAP) containing oxygen is recommended for fattened goose liver products. Such packaging systems may effectively prevent the formation of green discoloration. Although the presence of oxygen may reduce shelf life and increase susceptibility to lipid oxidation and rancidity, preventing visible discoloration would provide substantial commercial benefits for producers and significantly reduce consumer complaints related to product appearance.

5. NOVEL SCIENTIFIC FINDINGS

1. I demonstrated that pre-slaughter watering performed two hours before neck vessel severance, involving the administration of 200 mL of tap water directly into the crop, did not significantly affect either the rate of bleeding or the total volume of blood removed during exsanguination. However, it significantly reduced both the hemoglobin concentration and pH of the liver in fattened Grey Landes geese. Furthermore, neither cold nor warm evisceration significantly influenced liver pH.

2. I established that cold evisceration, the industrially applied practice of removing internal organs following pre-chilling, resulted in green surface discoloration in polyethylene vacuum-packaged fattened goose livers stored at –18°C for 35 days. In contrast, no significant colour changes were observed in livers removed immediately after slaughter without prior chilling (warm evisceration).

3. I demonstrated that the appearance of green discoloration is not immediate but develops progressively during storage, typically becoming evident between Days 14 and 21 of frozen storage.

4. I determined that electrical water-bath stunning applied before neck vessel severance (110 V) did not significantly affect liver hemoglobin concentration in fattened Grey Landes geese.

5. I established that the green discoloration observed in cold-eviscerated, vacuum-packaged fattened goose livers stored at –18°C for 35 days is not attributable to lipid oxidation mechanisms. This conclusion was supported by the absence of significant differences in TBA values between greened and non-greened liver samples.

6. I demonstrated that green-discoloured fattened goose livers stored at –18°C for 35 days regained their original colour within four hours after thawing and subsequent packaging under a modified atmosphere containing 80% CO_2 and 20% O_2.

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