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MONITORING FOOD QUALITY ALTERATIONS INDUCED BY STRESS FACTORS USING NEAR INFRARED SPECTROSCOPY, ELECTRONIC TONGUE, AND ELECTRONIC NOSE

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1. INTRODUCTION

Quality assurance of food products is undoubtedly one of the most relevant topics related to food security. It is also directly related to the satisfaction of consumer expectations and demands, who focus on certain intrinsic and extrinsic characteristics of foods such as color, shape, size, freedom from defects, texture, sweetness, acidity, aroma, flavor, shelf life, and nutritional value (Margeta *et al.*, 2019). Quality assurance, and therefore consumer acceptance, is linked to the control, study, and implementation of practices that ensure optimal products throughout the entire food production process, from the initial production of raw materials to the final product. Agricultural products are often perishable and subject to varying quality, which introduces uncertainty and requires active involvement in primary production to ensure food safety (Tadesse, 2024). Furthermore, once raw materials are processed into food products, their quality may continue to change depending on storage, transportation, and treatment conditions prior to consumption (Dunno *et al.*, 2016).

The production and consumption of fresh eggs is important due to their nutritional contribution to the human diet. Furthermore, enriched eggs, which offer greater nutritional benefits, can be produced by varying the diet of laying hens, including relevant nutrients that will be assimilated and transferred to the egg. However, such dietary changes can alter the organoleptic characteristics of the eggs, which in many cases can have a negative effect and decrease consumer acceptability. It is advisable to conduct sensory evaluations of the organoleptic profile of eggs, which have traditionally been performed through human evaluation panels (Hayat *et al.*, 2010; Mian K. *et al.*, 2017). Although valuable, this approach has limitations due to the subjectivity and variability inherent in human perception. Given the growing interest in the consumption of enriched eggs through feed modification, this research used advanced sensory technologies (Aouadi *et al.*, 2020), including electronic tongue (e-tongue) which detects soluble compounds in liquids and the electronic nose (e-nose) which identifies volatile compounds in gases and aromas, for a more objective evaluation of the sensory of enriched eggs from laying hens feed with an industrial brewery by-product enriched with organic zinc on the diet.

Probiotics are important food supplements that promote a positive balance of beneficial microbiota in the gastrointestinal tract, bringing potential advantages for overall health. This beneficial supplements are predominantly sourced from bacterial groups such as Lactobacillus, Bifidobacterium and Enterococcus, as

well as yeast strains such as Saccharomyces boulardii (Menezes *et al.*, 2018; Sanders *et al.*, 2018). Therefore, the study of stress factors that may compromise the viability of these probiotics, such as the temperature of the probiotic beverage preparation water and its concentration, is relevant.

Microgreens are also important agricultural products which have gained popularity being recognized as functional foods with notable nutritional benefits and high acceptance in modern gastronomy (Paraschivu *et al.*, 2022). Therefore, the proper cultivation of these little plants is relevant to achieve a high quality product. Environmental factors influence plant development and, consequently, their morphological characteristics and biochemical components. It is important to adequately establish the influence of these factors using techniques that allow for adequate monitoring and comprehensive evaluation.

In this regard, optical methods like Near Infrared Spectroscopy (NIRS) have gained popularity for their non-destructive, environmentally friendly, fast, and real-time monitoring capabilities. NIRS measures the interaction between light and matter to determine food quality features. It is particularly useful because the NIR spectrum corresponds to overtones and combinations of chemical bonds such as C-H, O-H, and N-H, which relate to food structure and its properties (Burns and Ciurczak, 2008; Ozaki, Genkawa and Futami, 2017). NIRS shows potential in characterizing probiotic drinks prepared at different water temperature and concentration conditions, and predicting their viability; on the other hand, NIRS shows some potential for characterization and agronomical and biochemical parameter prediction of plant products like pea microgreens grown under different temperature and photoperiod conditions. The study aimed to demonstrate NIRS's capability in providing real-time, non-invasive monitoring for the considered analyzed matrixes (probiotic supplements and pea microgreens) which have been subjected to stressing conditions.

Despite their advantages, these technologies (e-tongue, e-nose and NIRS) require expertise in measurement interpretation and in adjusting mathematical and statistical models to fit new conditions and food matrices. To enhance their effectiveness, they are often combined with chemometric approaches such as Principal Component Analysis (PCA), Discriminant Analysis, and Partial Least Squares Regression (PLSR). These techniques help to extract relevant patterns from complex data, classify food samples, and predict various quality parameters.

2. OBJECTIVES

The primary aim of this thesis is to determine the applicability and effectiveness of rapid correlative methods: NIRS, e-tongue, e-nose, for assessing alterations in food quality caused by significant stress factors, offering advantages over conventional quality evaluation techniques.

The first research aim was to evaluate the applicability of e-tongue and e-nose to detect the possible alteration of the organoleptic properties of eggs produced by hens, with diets containing different levels of an organic zinc-enriched by-product.

1. Develop models for e-tongue to discriminate, classify, and predict eggs based on the level of zinc-enriched by-product in the diet.

2. Develop models for e-nose to discriminate, classify, and predict eggs based on the level of zinc-enriched by-product in the diet and storage time.

The second aim of our study was to determine the applicability of NIRS to detect changes in probiotic drinks prepared with varying concentrations of probiotic powder and different water temperatures prior to consumption.

1. Develop models for characterization of three commercial probiotic food supplement powders containing lactic acid bacteria (LAB) subjected to probiotic concentration and water temperature conditioning factors.

2. Develop models for viability prediction of lactic acid bacteria (LAB) from three commercial probiotic food supplement powders subjected to probiotic concentration and water temperature conditioning factors.

The third research aim was to determine the applicability of NIRS for detecting changes induced by different environmental conditions during the growth of pea microgreens.

1. Develop models to characterize pea microgreens and predict key agronomical and physicochemical properties under varying temperature and photoperiod conditions.

2. Develop and assess models for two different sample types: Microgreens fresh-cut samples pea samples and Aqueous microgreens extracts samples pea samples.

3. MATERIALS AND METHODS

3.1. Materials and methods for egg sensory evaluation

This study evaluate the sensory qualities of eggs derived from Lohmann Brown Classic hens subjected to various dietary treatments. The dietary treatments included a control feed (0% Zincoppyeast) and Zincoppyeast supplemented feeds: ZP 2.5% (2.5% Zincoppyeast), and ZP 5.0% (5.0% Zincoppyeast). The evaluation was designed to assess the impact of these dietary variations on the sensory properties by human sensory analysis, e-tongue and e-nose and additionally the effect of storage time by e-nose.

Eggs from the three groups feeding groups were collected for evaluation on day 30 (batch 1) and day 60 (batch 2) of the experimental period, totaling 90 samples per batch for human sensory analysis, 18 for e-tongue analysis, and 90 for e-nose.

For human sensory analysis eggs were evaluated, for five trained panelists, on three presentations: fresh raw (Albumin color, Yolk color, Yolk shape, Albumin density), boiled (Albumin color, Yolk color, Egg odor, Unusual odor, Albumin flavor, Unusual taste, Albumin flexibility, Yolk creaminess) and fried eggs (Yolk color, Egg odor, Sweet aroma, Unusual odor, Egg taste, Sweet taste, Unusual taste, Texture) in a scale from 0 to 9. Evaluation was established according to three feeding groups ZP 0%, ZP 2.5%, ZP 5% for two the batches. Results from the sensory evaluation were statistically analyzed using SPSS software (version 20.0). Differences between treatment groups were evaluated using one-way ANOVA, and Tukey's Honestly Significant Difference (HSD) post hoc tests ($p < 0.05$).

An Alpha Astree electronic tongue from AlphaMOS was used to evaluate the soluble compounds present in of the fresh egg samples by seven potentiometric sensors. To prepare each sample, 2 g of homogenized egg mixture was transferred to individual 100 mL volumetric flasks and made up to the mark with distilled water. Six parallel samples were taken from each of the three experimental groups, giving a total of 18 eggs samples for the two batches tested. These samples were then subjected to analysis using e-tongue. Chemometrics was applied to the resulting data from e-tongue analysis, by Euclidean distances, PCA and LDA for classification between feeding groups for each of the two batches. The validation type for the LDA models was three-fold cross validation (by repeat) by data splitting: 2/3 for training and 1/3

validation.

The evaluation of the aroma profiles of egg samples was carried out using the ultrafast chromatograph Alpha MOS Heracles NEO electronic nose. One type of evaluation was made in fresh eggs for Batch 1 and Batch 2 with preheating temperature of 50 °C and 80 °C during e-nose chromatograph analysis. Models were performed according to each preheating temperature by applying PCA-LDA for classification between feeding groups. A second type of evaluation consisted on the classification of eggs according to storage time, were the number of days considered for storage were 0, 30, 60 days. In this case, two preheating for the creation of the classification models were also considered. The models were validated by three-fold cross validation (by repeat) were data was splitted in 2/3 for training and 1/3 for validation.

Kovats index (related to each peak detection) were identified. These indices were checked against the AroChemBase database to recognize the volatiles associated with the odors. The methodology for sensory analysis of the enriched eggs experiment is shown in Figure 1.

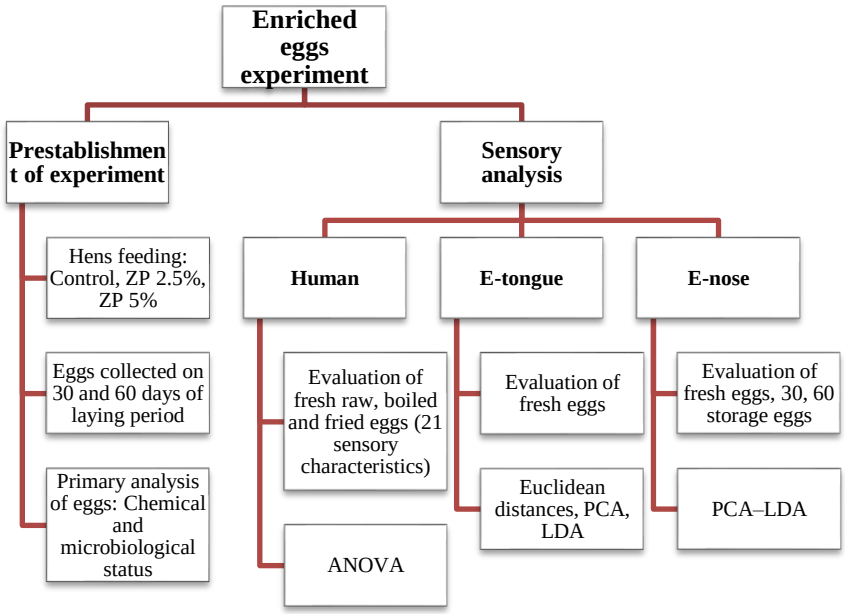

Figure 1. Methodological scheme for sensory and analytical assessment of enriched eggs

3.2. Materials and methods for probiotics evaluation

It was evaluated three commercial probiotic food supplements in a powder format named as probiotic N (Istanbul, Turkey), P, and A (Budapest, Hungary), in this research. These probiotics which contained lactic acid bacteria (LAB) were prepared as beverages by adding water at different temperatures of 25 ºC (control), 60 ºC and 90 ºC, and considering three different probiotic concentrations (C1: 3 g/125 mL, C2: 2.5 g/125 mL, and C3: 2 g/125 mL). Once water (at different temperatures was poured onto the probiotics), the mixture liquid was let to cool down before measurements. A total of three repetitions of each preparation were made, resulting in 81 samples for the three probiotic products (three probiotics × three concentrations × three temperatures × three repetitions). For the determination of the viability of probiotics under the stress factors under study, microbiological analysis was performed, which consisted in the culture of lactobacillus spp. in a low-selective medium MRS agar by pour plating. The plates were incubated at 37 °C for 72 hours, after which the number of colonies (log CFU/g) was counted. A statistical analysis on the Viable counts (Log CFU/g) of the samples was performed using ANOVA and Tukey's test ($p < 0.05$) to evaluate the differences between the groups.

A Benchtop MetriNIR spectrophotometer was used to collect the transflectance spectra of the probiotic samples. Samples were placed in a self-made, thermo-regulated circular cuvette at 25 °C, with a metallic wall (inner diameter: 5 cm; outer diameter: 8.5 cm) and a 0.4 mm thick crystal layer, which also included a white reflector. The 81 samples were scanned in three parallel and three consecutive scans. A total of 729 scans were obtained: 243 for each probiotic product. The recorded spectra was analyzed in the 950 to 1650 nm range.

PCA-LDA models were built for classification according to probiotic type, concentration, and temperature groups separately. Classification models were performed by three-fold cross-validation. In each step of the cross-validation, the data of one repeat of the samples was left out. Two-thirds of the data were used for model building, while the remaining third was used for external validation. Single and combined spectra pretreatments were evaluated to determine the best possible PCA-LDA models, resulting in a total of 41 evaluated spectra pretreatments. Single spectra pretreatments were: Savitzky–Golay (SG) smoothing filter, second-order polynomial (13, 17, or 21 points), first derivative, second derivatives, multiplicative scatter correction (MSC), standard normal variate (SNV), and de-trending (deTr). Following the acquisition of PCA-LDA results, the most suitable models were identified based on the spectral

pretreatments that yielded the highest CV accuracy percentage. For the PLSR regression model used to predict viability, two-thirds of the data were used to build the PLSR models (for calibration and cross-validation). The final model was tested to ascertain its robustness by using the remaining one-third of the data for prediction. The methodology for probiotics experiment is shown in Figure 2.

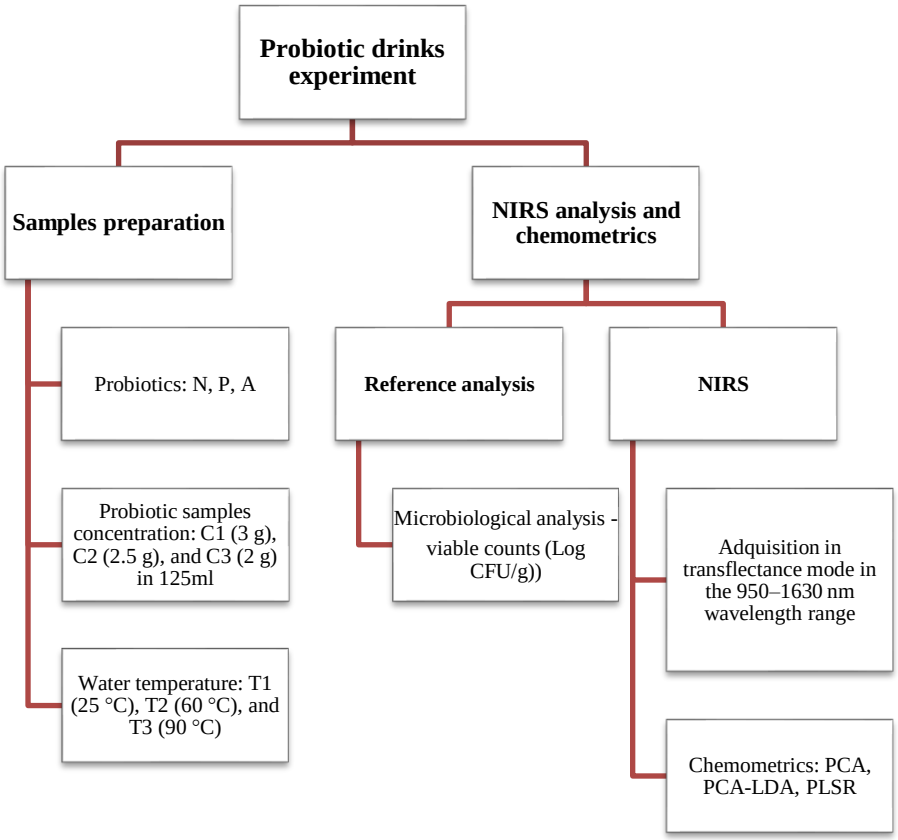

Figure 2. Methodological scheme for probiotic drinks assessment

3.3. Materials and methods for microgreens evaluation

For the purpose of this study, a critical phase of the project involved the development of custom-designed climate chambers, achieved through a stepwise approach. Initially, individual control components were developed separately and subsequently integrated into a unified system. The developed set up for microgreens growth permitted to regulate the temperature and photoperiod with photon flux density (PPFD) of 75.7 µmol/m²/s ± 4.96. Additionally, sensors coupled inside the chambers for real-time humidity monitoring and ventilation control. Pea microgreens (Debrecen sötétzöld) were planted in soil (organic

horticultural substrate). Twelve different environmental conditions (temperature-photoperiod) were considered for microgreens growth: temperature (15, 20, 25 ºC), photoperiod (0 hours of light, 6 hours of light, 12 hours of light, 18 hours of light), relative humidity around 70-80%. At temperatures of 20 and 25 ºC, higher temperatures promoted faster emergence and growth, plants were harvested at 7, 11 and 14 days after sowing. Meanwhile, at a temperature of 15 ºC, emergency and growth were slower, so plants were harvested at 11, 14 and 18 days.

NIRS analysis was conducted independently on two different types of prepared samples: microgreens fresh-cut samples (measured in reflectance in a benchtop XDS Rapid Content Analyzer) and Aqueous microgreens extracts samples (measured in transmittance in a benchtop XDS Rapid Liquid Analyzer). For NIRS analysis of fresh cut samples, which consisted on microgreens cut in homogeneous pieces around (2.5cm) (without roots), samples were placed in a circular cuvette of 0.4 mm layer thickness, meanwhile, for NIRS analysis of aqueous extracts, consisting on the same microgreens but subsequently blended with distilled water (in a proportion 1:5) and filtered, samples were place in a 1 mm pathlength quartz cuvette.

The total number of scans for each type of the two matrices consisted of 324 spectra (3 temperatures x 4 photoperiods x 3 harvesting days x 3 repeats x 3 scans). The spectral analysis was focused on the 1150 to 1850 nm which showed better results compared to other NIR ranges tested. The spectral pretreatments consisted on Savitzky-Golay smoothing (2nd polynomial, and 45 window width) and standard normal variate. Next, Principal component analysis (PCA) data exploration by harvest day, temperature and photoperiod was performed on the full data for pattern recognition. Followed by PCA-LDA analysis, for classification of microgreens, performed according to temperature, photoperiod, temperature-photoperiod, harvesting day. Supervised three-fold cross-validation was applied (leaving out one from three repeats for CV in each iteration).

PLSR was carried out for height, weight, Lab color components, pH, conductivity, °Brix, chlorophyll A, B, total carotene. Two from three repeats were used for model calibration and cross-validation, and the last repeat for prediction. Additionally, PLSR models were performed for total water soluble polyphenolic compounds (TPC) and antioxidant capacity (TAC). Leave-one-out cross-validation was used for the PLSR models in this case. The experimental design is summarized in Figure 3.

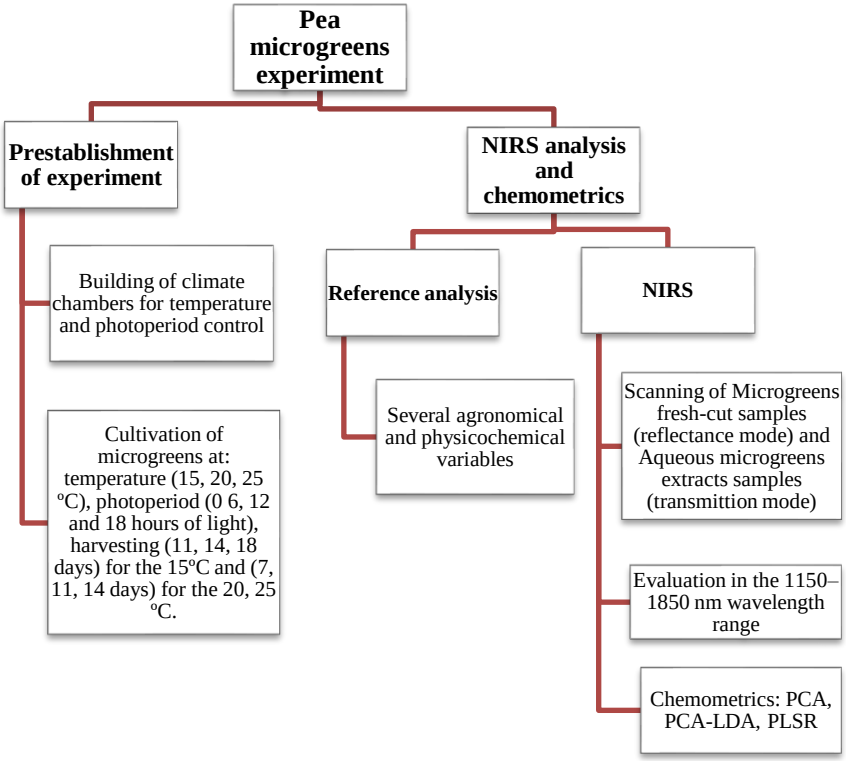

Figure 3. Methodological scheme for pea microgreens assessment

4. RESULTS

4.1. Results of sensory evaluation of eggs

The sensory evaluation of eggs by a trained human panel assessed differences across three feeding groups (control, ZP 2.5%, and ZP 5.0%) using eggs prepared raw, boiled, and fried from two production batches. Statistical analyses (ANOVA and Tukey tests) revealed that most sensory attributes did not show significant differences between the groups, especially in boiled and fried eggs, which were generally described as representative of fresh eggs. Notably, raw eggs from Batch 1 in the ZP-supplemented groups demonstrated more intense sensory characteristics, such as greater yolk color intensity, yolk convexity, and protein density, compared to the control. Fried eggs from the same batch also showed higher values for yolk color, odor, and sweet flavor in the ZP-fed groups. In contrast, boiled eggs from Batch 1 exhibited slight differences, with the ZP 2.5% group displaying slightly more intense yolk color and white flavor. However, results from Batch 2 were less pronounced, suggesting that hens may have adapted to the supplemented diet over time, leading to more uniform egg characteristics. Overall, the findings suggest that while some sensory differences emerged, especially in the first batch, these were not consistent or strongly apparent across all samples.

The LDA models from e-tongue analysis showed major overlapping with some degree of separation within each group of eggs (Control, ZP 2.5% and ZP 5.0%) for the two Batches tested. The average calibration accuracy for batch 1 was 95.92%, with a cross-validation accuracy of 64.81%. Additionally, batch 2 exhibited an average calibration accuracy of 100% and a cross-validation accuracy of 56.23%. This suggests that the models captures some useful information from real data, but there is evidence of overfitting. The analysis of a matrix of random numbers showed a calibration accuracy of 80.07% and cross-validation accuracy of 29.95%. Differences between the CV values of real data (64.81%) and random data (29.95%) demonstrates that the real data contains relevant information for classification.

The e-nose analysis provided valuable results to establish sensory profile of eggs from different feeding groups and storage durations. Linear discriminant analysis based on principal component analysis (PCA–LDA) revealed high discrimination between batches and feeding groups, particularly between Control and 5.0% ZP. In the classification of fresh eggs according to the feeding groups in both batches at 50 °C, the average calibration accuracy was 98.00%, and cross-validation accuracy 68.49%. At 80 °C, the calibration and cross-

validation accuracy was 82.65%and 62.22%, respectively. The three-fold cross-validation results showed some misclassification between adjacent egg groups. However, the Control and ZP 5.0% groups showed a clearer tendency toward separation. A significant gap was identified between the calibration and cross-validation results obtained from the PCA-LDA models from e-nose. Analysis on simulated data (random numbers) showed a calibration accuracy of 98.77% and a cross-validation accuracy of 39.64%. Difference between the CV accuracy of real data (68.49%) and simulated data (39.64%) shows that the real data contains relevant information for classification. Moreover, PCA-LDA models from the analysis according to eggs stored at 0, 30 and 60 days showed complete separation of samples according to storage time, with 100% correct classification in calibration and cross-validation.

Important sensors involved in the differentiation to both dietary treatment and storage effects were linked to different volatile compounds including acetaldehyde, methyl acetate, 2-methylpropanal, between others. Overall, by e-nose, it was possible to determine differences attributed to feeding regimes and storage conditions on the volatile profiles of eggs.

4.2. Results of probiotic samples evaluation

During the assessment of probiotic supplements under varying conditions, microbiological analysis showed that microorganism viability is strongly influenced by temperature, were high temperatures (60 °C and 90 °C) decreases the viability of probiotics.

The PCA-LDA analysis on pretreated spectra with SG 2-17-0 performed the best on the three probiotics (N, A, and P) at 25°C correctly separating the groups, with 100% of the groups being correctly classified for calibration and 99.18% for cross-validation. Probiotic N showed the most distinct separation compared to probiotics A and P which were more closely related. Additionally, classification by concentration showed clear separation between levels at 90°C, with minor overlap between adjacent concentrations. The models reached 100% accuracy in calibration and over 90% in cross-validation. Probiotic A had the highest cross-validation accuracy (95.06%), followed by probiotic P (93.52%) and probiotic N (90.12%). The best pretreatments were DeTr + MSC for probiotic N, SG 2-21-0 + DeTr for probiotic A, and SG 2-17-0 + SG 2-17-2 for probiotic P. At lower temperatures, classification depends more on the probiotic type. NIR spectroscopy with PCA-LDA appears effective for identifying probiotic concentrations in solutions. Classification based on temperature also

resulted in high accuracy. Probiotic A performed the best, with 100% accuracy in both classification and cross-validation. Probiotics P and N also showed high classification accuracy, both above 90%, with minor misclassifications between adjacent temperatures. The best pretreatments were de 2-13-0 + SG 2-21-1 for probiotic N, SG 2-17-0 + MSC for probiotic A, and DeTr for probiotic P. NIR spectroscopy showed effectiveness for distinguishing probiotic solutions based on temperature. Regarding PLSR, the most accurate predictive model for CFU counts used SG 2-21-0 and SG 2-13-2 pretreatments, resulting in an R^2Pr of 0.82 and an RMSEP of 0.64 Log CFU/g. Wavelengths between 1300–1600 nm were important for predicting probiotic viability, with relevant molecular interactions involving water and organic compounds, including OH and NH stretching, notably at 1458 nm, 1484 nm, and 1140 nm.

4.3. Results of pea microgreens samples evaluation

The evaluation of agronomic and phytochemical characteristics of pea microgreens grown under different environmental conditions of photoperiod and temperature showed in a major extent the classical behavior reported in literature. Higher temperatures, especially 25 °C, and longer light exposure significantly improved plant growth and pigment accumulation. For instance, microgreens under 25 °C with 18-hour light showed enhanced plants height, weight, deeper green coloration, and higher chlorophyll and carotenoid levels. In contrast, plants grown in complete darkness were taller but lacked pigmentation due to etiolation.

°Brix values were highest at lower temperatures and longer light exposure, indicating greater sugar retention. pH remained relatively stable, and no specific trends were found, while electrical conductivity varied without a consistent pattern.

Color analysis confirmed that increased light reduced L* and b* values which improved green intensity. Color and pigment content was directly correlated with photoperiod length, emphasizing the importance of light for photosynthetic and nutritional quality.

The parameters were evaluated through different days, in general height, weight, and pigments consistently increase over the analyzed period. In the case, of the other parameter the behavior was more variable and specific photoperiod-temperature dependent.

Overall, optimal conditions for producing pea microgreens related for the majority of parameters tested are 20–25 °C with 12–18 hours of light per day. Except for TAC and TPC which increase at lower temperatures 15°C.

Classification of Pea Microgreen Samples via PCA-LDA

The data collected was assessed by performing classification models on aqueous microgreens extracts samples and classification models on fresh-cut microgreens samples. Microgreens grew at 12 different conditions of temperature-photoperiod (treatments). Each treatment was classified according to harvesting days. The models from aqueous microgreens extracts samples achieved better results compared to microgreens fresh-cut samples in most treatments (classified by harvesting day), reporting CV between 81.45% to 100% for treatments from 15 ºC and 25 ºC, meanwhile for 20 ºC, it was between 66.67% and 82.26%.
In a different approach, by selecting a specific day, samples were classified according to photoperiod-temperature treatment. Once again, aqueous microgreens extracts samples achieved higher CV classification accuracies (between 56.47% to 87.72%) compared to microgreens fresh-cut samples (between 48.39% and 59.72%). Next, samples were classified by selecting a specific day and classification according to temperature. The PCA-LDA models showed higher accuracy at day 11 for aqueous microgreens extracts samples with a CV of 85.58%, meanwhile microgreens fresh-cut samples achieved 81.79%. In the case of photoperiod, the best classification was at day 7 for aqueous microgreens extracts samples with a CV of 85.45%, meanwhile for microgreens fresh-cut samples was 67.83% at day 14. The last approach and consistent with the previous results, one more time aqueous microgreen extract samples had better discrimination were all the samples from the dataset was selected at the time and the classification models were according to harvesting day, treatment, temperature and photoperiod with CV accuracies of 95.59, 68.34, 88.87 and 66.89%, respectively.

Partial Least Squares Regression (PLSR) models on Pea Microgreen Samples

Models for physical traits (weight and height) performed better in fresh-cut samples (R^2pr = 0.78 and 0.70) than in extracts.

Color components L* and b* showed similar R^2pr values for microgreens fresh-cut samples (R^2pr = 0.73 for L* and 0.70 for b*) and aqueous microgreens extracts samples (R^2pr = 0.71 and 0.65). While a* showed poor predictability.

For pigments, PLSR models showed consistent values, with R^2pr = 0.71, 0.62, and 0.73 for chlorophyll A, B, and carotene in microgreens fresh-cut samples; and R^2pr = 0.68, 0.65, and 0.69, respectively, in aqueous microgreens extracts samples.

°Brix models showed fair accuracy (R^2pr ~0.70), but pH and

conductivity models performed poorly due to limited variability.

TAC and TPC were predicted more accurately in aqueous extracts (R^2CV = 0.73 and 0.71), meanwhile models for microgreens fresh-cut samples behaved poorly.

PLSR models using the full spectral range (1150–1850 nm) showed acceptable performance (R^2 > 0.6), but often required many latent variables (LV), increasing overfitting risk. Focusing on significant wavelength ranges reduced the number of LV, particularly for fresh-cut microgreens, without significantly compromising accuracy.

Similar important wavelength profiles was revealed in several PLSR models, principally for height, weight, pigments (chlorophyll A, B, and total carotene), and °Brix in pea microgreens. This similarity is likely attributed from their shared physiological and biochemical bases, mainly water content, carbohydrates, and proteins, which are determinant for plant growth and biomass accumulation. Notably, for solid microgreens, the most relevant wavelengths for height and weight were around 1196, 1286, 1392, 1417, 1446, 1480, 1508, 1543, 1600, 1704, and 1838 nm. In contrast, aqueous extract samples showed key wavelengths at 1337, 1368, 1396, 1409, 1433, 1460, 1484, 1530, 1590, 1640, 1685, 1706, 1746, and 1793 nm.

5. CONCLUSIONS AND RECOMMENDATIONS

Human sensory analysis, e-tongue, and e-nose were used to assess sensory variations of eggs enriched through dietary supplementation, in laying hens, with ZP (0%, 2.5% and 5.0%). Human panelists did not report consistent sensory differences across treatments or egg types (raw, boiled, fried), with all samples being perceived as fresh. However, e-tongue analysis revealed a certain degree for discrimination between the feeding groups, especially between the Control and ZP 5.0% groups and showing greater misclassification between neighboring groups (Control-ZP 2.5%) and (ZP 2.5%- ZP 5%). These results were consistent in both the eggs belonging to Batch 1 and those of Batch 2, which refer to eggs collected on day 30 and day 60 of the laying period, respectively.

For the e-nose evaluation, important contributing sensors related to the Kovats index and specific volatile compounds established the results in the classification models. Sensory variations were detected in the e-nose evaluations, marking the same pattern in the classification models (similar to e-tongue), which showed greater misclassification between adjacent groups, while lower misclassification was evident between the control and ZP 5.0% groups. These results were true for both Batch 1 and Batch 2. And they were true for both the 50°C and 80°C preheating temperature classification models. In the case of E-nose, an additional analysis was carried out in which fresh eggs were stored for a specific time (0, 30, and 60 days) at 10-14°C. Evaluation highlighted stronger differentiation based on storage duration (with full classification between groups) rather than feeding treatment (which, as noted, presented misclassification). These findings suggest that electronic sensing is more effective for detecting storage-related changes than diet-induced differences.

Regarding probiotics, three commercial probiotic food supplement powders N, A and P containing lactic acid bacteria (*LAB*) were evaluated. To prepare the probiotic drinks were considered three concentrations (3 g/125 mL, 2.5 g/125 mL, 2 g/125 mL) and temperatures of water (25 °C, 60 °C, 90 °C) as stressing factors. Overall, applying chemometrics to the NIR spectra resulted in PCA-LDA classification models with high accuracy in both calibration and cross-validation. Temperature has an important impact on sample classification. The most effective models were obtained at 90 °C, showing high accuracy in both recognition and prediction. However, prediction performance declined at lower temperatures, with a notable drop at 25 °C for two of the three probiotics. Moreover, at higher temperature levels, both calibration and cross-validation

accuracies were consistently high, approaching optimal performance. Additionally, the partial least squares regression (PLSR) model showed potential of NIRS for predicting colony-forming units (log CFU/g) of the probiotic samples.

Pea microgreens were grown at 15, 20, and 25 °C under photoperiods of 0, 6, 12, and 18 hours, harvested at different intervals from 7 to 18 days depending on the temperature; and NIRS scanning and chemometric evaluation was performed on two different sample preparations in reflectance mode for fresh-cut samples and in transmittance mode for aqueous extracts. The classification patterns of pea microgreens were generally better in aqueous extracts than in fresh-cut samples. Global classification models (analysis on the entire data) confirmed higher accuracy in aqueous samples, particularly when grouping by harvesting day and temperature. Similarly, to determine if specific datasets such as a treatment (temperature-photoperiod) could be adequately classified by harvesting day, individual models were created for a specific temperature or photoperiod, or if microgreens from a specific day could be classified according to treatments, temperatures, or photoperiod. Similarly, models generally performed better on aqueous extracts than on fresh-cut samples.

PLSR models showed low to moderate accuracies on the various parameters analyzed. Generally, models had similar although slightly better predictions in fresh-cut samples for physical parameters (height, weight), color components, pigments, °Brix, while in aqueous extracts was better predicted TPC and TAC. It was evidenced a narrow range or lack of structured variations of the data belonging to a* color component, pH and conductivity parameters, consequently showing poor predictability. PLSR models using the full spectral range (1150–1850 nm) often required many latent variables (LV), increasing overfitting risk. In some cases, the LV was reduced significantly without substantially compromising accuracy by selecting significant wavelength ranges, particularly for fresh-cut microgreens. Height, weight, pigments (chlorophyll A, B, and total carotene), and °Brix in pea microgreens showed similarities on the important wavelength profiles which may be attributed to shared physiological and biochemical interactions that determine these parameters.

Imperfect classification was present in both the enriched egg (by LDA for e-tongue and PCA-LDA for e-nose) and microgreens (by PCA-DA) experiments due to the nature of the samples analyzed and limitations encountered during the chemometric analysis, thus some models could incur in certain degree of

overfitting. To this end, through parallel analysis on simulated data, the superiority of the classification models was established when using real data, which establishes that the data contains important information for the classification.

Although certain valuable results were found in this research, future studies could explore the applicability of these correlative methods (NIRS, e-tongue, and electronic nose) on a larger scale, given that this study was conducted with a limited number of samples. Likewise, whether this technology is transferable to other food matrices subject to the stress factors considered in this study, for example, whether Zincoppyeast or another analogous element can cause changes in the sensory characteristics of eggs (or even on meat) of different poultry species and be detected by (e-senses). Additionally, future probiotics experiments using NIRS are encouraged to evaluate how temperature and concentration as conditioning factors may affect the viability of other probiotic strains beyond lactic acid bacteria (LAB). Or in the case of microgreen whether NIRS can detect differences in other species (besides pea) when subjected to different temperature and photoperiod conditions. It may also be worthwhile to consider the use of alternative chemometric approaches (e.g., PLS-DA, ANN, k-NN, SVM) to achieve more accurate classification and prediction models.

6. NEW SCIENTIFIC RESULTS

For the purpose of these new scientific findings, the term benchtop MetriNIR spectrophotometer refers to the MetriNIR (MetriNIR, Research Development and Service Co., Budapest, Hungary), whereas the term benchtop NIR XDS spectrophotometer refers to the NIR XDS spectrometer (Metrohm, Herisau, Switzerland), with two separate attachable modules: Rapid Solid Analyzer (RCA) and Rapid Liquid Analyzer (RLA). The term e-tongue refers to the Alpha Astree potentiometric electronic tongue (AlphaM.O.S, Toulouse, France) equipped with seven sensors specifically developed for food application (called by the manufacturer: BB, HA, ZZ, GA CA, JE, JB), an Ag/AgCl in 3M KCl reference electrode and a 16-position autosampler. E-nose refers to the Alpha MOS Heracles NEO electronic nose (e-nose), which functions as an ultrafast gas chromatograph analyzer featuring dual columns (MXT-5 and MXT-1701) and performs evaluation of odor intensity associated with volatile substances through the Kovats index.

New scientific findings focusing on eggs evaluation

Sensory attributes of enriched eggs produced by hens fed with feed with added brewer's yeast and wet yeast biomass enriched with organic zinc, polyphenols, and vitamins (ZP) at concentrations of ZP 0% (Control), ZP 2.5%, and ZP 5.0% as feeding regimes were analyzed. Batch 1 and batch 2 correspond to the eggs collected for evaluation on day 30 and day 60 of the experimental period, respectively.

Human sensory analysis

1) This study shows that eggs enriched with Zincopyeast (ZP) at 2.5% and 5.0% did not consistently differ in sensory attributes from non-supplemented eggs (control group) across two production batches in case of boiled (albumin color, yolk color, egg odor, unusual odor, albumin flavor, unusual taste, albumin flexibility, and yolk creaminess) and fried eggs (yolk color, egg odor, sweet aroma, strange odor, egg taste, sweet taste, strange taste, and texture). While some statistically significant differences were observed between feeding groups in certain sensory characteristics, these differences were not consistently replicated between the two batches. Therefore, ZP supplementation at the tested levels does not appear to alter the overall sensory profile of boiled or fried eggs.

Characterization of eggs by e-tongue

2) The ability of an electronic tongue (e-tongue) to effectively distinguish egg samples based on feeding regimes with different levels of Zincoppyeast (ZP) supplementation was proven. ZP 2.5%, and ZP 5% were correctly distinguished from the Control showing a 64.81% accuracy in cross-validation for fresh eggs collected at day 30 of the laying period. The largest differences were observed between the groups Control and ZP 5.0% samples.

Characterization of eggs by e-nose

3) The effectiveness of electronic nose (e-nose) to classify enriched eggs according to storage time was proven. Eggs from 0, 30, and 60 days of storage were correctly classified with 100% accuracy in cross-validation. Moreover, the use of e-nose prove to be valuable distinguishing fresh eggs samples based on different feeding-(ZP) supplementation regimens. ZP 2.5%, and ZP 5% were correctly distinguished from the Control with 76.5% accuracy in cross-validation.

4) The e-nose analysis revealed that specific volatile compounds played a critical role in distinguishing storage durations. Among these, methyl acetate and 2-methylpropanal (sensor 528.86), acetaldehyde (469.52 and 430.57), 2,4,5-trimethyl-3-oxazoline and 2-butanone, 3-mercapto (818.98), as well as 2-hexanol and hexanal (803.41) were the primary contributors to the observed separations of eggs stored at 0, 30 and 60 days. Moreover, the major volatile compounds responsible for the separation of the feeding regimes in fresh eggs included, 2-butanol and n-butanol (602.94), homofuraneol and methyl 3-pyridinecarboxylate (1140.88), methyl acetate and 2-methylpropanal (528.86), as well as 2-propanone and propanal (494.47).

New scientific findings focusing on microgreens evaluation

Prediction of probiotics viability by NIRS

5) It was proven that viability of the probiotic samples, influenced by concentration and temperature stress factors, can be predicted through NIR spectrophotometry coupled with PLSR modeling. The models achieved a R^2Pr of 0.82 and RMSEP of 0.64 Log CFU/g.

New scientific findings focusing on microgreens evaluation

Pea microgreens grown under different environment stress conditions of temperature (15, 20, 25 ºC), and photoperiod (0, 6, 12, 18 hours of light) and harvested at 7, 11 and 14 and 18 days were scanned in two modes: reflectance for Microgreens fresh-cut samples and in transmission for Aqueous microgreens extracts samples (1:5 plant - distilled water) and analyzed in the 1150–1850 nm range and applied spectral pretreatment SG (p=2, n=45, m=0) + SNV. Classification PCA-LDA models and partial least squares regression (PLSR) models were developed to test prediction capacity for 13 agronomical and physicochemical variables.

Classification of pea microgreens by NIRS

6) The near-infrared spectroscopy (NIRS), combined with PCA-LDA analysis, enabled effective classification of pea microgreens according to harvesting day, temperature, photoperiod, and combined treatment in both fresh-cut and aqueous extract samples. In fresh-cut samples, cross-validation accuracy were 6.98% for harvesting day, 75.74% for temperature, 71.05% for photoperiod, and 58.54% for treatment. In contrast, aqueous extract samples yielded higher classification rates of 95.59%, 88.87%, 66.89%, and 68.34% for the same parameters, respectively. These results indicate better class separability in aqueous extracts, likely due to the homogenized nature of the samples and enhanced spectral response under transmission mode, reflecting the compositional changes induced by these environmental stressors.

Prediction of pea microgreens for physical characteristics, pigments and bioactive compounds by NIRS

7) The temperature and photoperiod combinations successfully reproduced known growth patterns in pea microgreens. Under these combined stress conditions, NIRS predicted height and weight in fresh-cut samples with R^2 values of 0.78 and 0.70, respectively. Aqueous extract samples yielded lower values of 0.64 and 0.65, despite the theoretically more favorable optical properties of homogeneous solutions in transmission mode, there might be some structural and morphological characteristics retained in fresh-cut samples measured in diffuse reflectance mode such as tissue density, stem thickness, and leaf arrangement that better correlate with physical traits like height and weight. NIRS shows potential as a non-destructive method for estimating biomass traits under environmental stress.

8) It was proven that pea microgreens pigments are influenced for temperature and photoperiod. 20C_18L and 25C_18L treatments showed higher pigments accumulation, denoting that especially photoperiod is the most limiting factor in this regard when 0L treatments presented chlorophyll values close to 0. The PLSR pigments prediction models had R^2pr of 0.71 for chlorophyll A, 0.62 for chlorophyll B and 0.73 for total carotenes in microgreens fresh-cut samples, comparable to 0.68, 0.65, and 0.69, respectively for aqueous microgreens extracts samples. These results proves the moderate potential of NIRS to measure pigments (chlorophyl A, B, total carotenes) of pea microgreens, subjected to temperature-photoperiod stress factors, in both microgreens fresh-cut samples and aqueous microgreens extracts samples.

9) °Brix evaluation showed that lower temperatures (15 °C) favor sucrose accumulation compared to higher temperatures (20 °C and 25 °C); furthermore, microgreens with 18 hours of light had higher °Brix values compared to other treatments. The results indicates that the lower temperature and higher photoperiods in this study promotes °Brix accumulation in pea microgreens. The PLSR prediction of °Brix for microgreens fresh-cut samples showed R^2pr of 0.70 and for aqueous microgreens extracts samples R^2pr of 0.68, but pH and conductivity had low predictive accuracy (below 0.34) for both (aqueous microgreens extracts samples and microgreens fresh-cut samples). It is proven that NIRS provides modest accuracy for prediction of chemical properties of pea microgreens subjected to temperature-photoperiod stress factors, however it is capable of measuring °Brix in some extent, in both microgreens fresh-cut samples and aqueous microgreens extracts samples.

10) In the bioactive compound analysis in pea microgreens, it was proven that lower temperatures (15 °C) and longer photoperiods enhance phenolic compounds accumulation and antioxidant capacity, with 15C_18L being the most effective (particularly on day 14). Moreover, the results show proof of the moderate potential of NIRS for measuring TAC and TPC of pea microgreens subjected to temperature-photoperiod stress factors, especially for aqueous microgreens extracts samples. the PLS regression for TAC and TPC for aqueous microgreens extracts samples achieved R^2CV of 0.73 and 0.71 in aqueous microgreens extracts samples, compared to 0.35 and 0.56 in microgreens fresh-cut samples, respectively.

11) The study proves the effectiveness of Near Infrared Spectroscopy (NIRS) for simultaneous prediction of correlated agronomic and physicochemical variables in pea microgreens. Height, weight, pigments (chlorophyll A, B, and total carotene), and °Brix PLSR models for pea microgreens showed similar spectral profiles. The notable wavelengths for weight and height, which had a broad spectral profile and can be compared with the other variables, included important wavelengths at 1196, 1286, 1392, 1417, 1446, 1480, 1508, 1543, 1600, 1704, and 1838 nm in microgreens fresh-cut samples, while the prominent wavelengths for aqueous microgreens extracts samples were 1337, 1368, 1396, 1409, 1433, 1460, 1484, 1530, 1590, 1640, 1685, 1706, and 1746 nm. These wavelengths, pinpointed through PLSR models, underline the capability of NIRS to detect shared spectral markers across diverse variables, advancing its application in quality assessment and predictive modeling of plant characteristics.

LIST OF PUBLICATIONS IN THE FIELD OF STUDIES

Journal articles

David Tjandra Nugraha, John-Lewis Zinia Zaukuu, **Juan Pablo Aguinaga Bósquez**, Zsanett Bodor, Flora Vitalis and Zoltan Kovacs. Near-Infrared Spectroscopy and Aquaphotomics for Monitoring Mung Bean (Vigna radiata) Sprout Growth and Validation of Ascorbic Acid Content. SENSORS (1424-8220): 21 (2) Paper 611. 20 p. (2021). **Q1. IF (3.576).** https://doi.org/10.3390/s21020611.

Flora Vitalis, David Tjandra Nugraha, Balkis Aouadi, **Juan Pablo Aguinaga Bósquez**, Zsanett Bodor, John-Lewis Zinia Zaukuu, Tamás Kocsis, Viktória Zsom-Muha, Zoltan Gillay and Zoltan Kovacs. Detection of Monilia Contamination in Plum and Plum Juice with NIR Spectroscopy and Electronic Tongue. CHEMOSENSORS (2227-9040): 9 (12) Paper 355. (2021). **Q2. IF (3.398).** https://doi.org/10.3390/chemosensors9120355

Juan Pablo Aguinaga Bósquez, Zoltan Kovacs, Zoltán Gillay, György Bázár, Csaba Palkó, Hajnalka Hingyi, Éva Csavajda, Márta Üveges, Zsuzsanna Jókainé Szatura, Iuliana Diana Barbulescu, Mihaela Begea and Tamás Tóth. Evaluating the Effect of a Brewery By-Product as Feed Supplementation on the Quality of Eggs by Means of a Human Panel and E-Tongue and E-Nose Analysis. CHEMOSENSORS (2227-9040): 9 (8) Paper 213. 20 p. (2021). **Q2. IF (3.398).** https://doi.org/10.3390/chemosensors9080213

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