PhD THESIS

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MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

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Production biology and complex evaluation of lettuce (*Lactuca sativa* L.) varieties by testing LED lighting programs

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1. BACKGROUND AND OBJECTIVES

Lettuce (*Lactuca sativa* L.) is a widely grown and world-wide known vegetable plant, which is mostly consumed raw, fresh or pre-washed and packaged as a convenience product.

Agriculture is under increased pressure, so cultivation solutions where production can be maximized over a small area of floor space have come to the fore again. Such a solution is the use of closed cultivation systems with artificial lighting. Depending on plant species and varieties, the spread and reliable use of the system requires a high level of research support, with an increasing focus on research into plant-specific LED-based light recipes.

There is relatively little, difficult to compare information on plant physiology responses to LED light programs and phytonutrient changes. This is mainly due to the fact that the varieties and parameters set in each experiment are very varied (light programs, nutrient supply, temperature, humidity, CO_2content, planting arrangement, etc.). Thus, it is important to clarify in research the importance of each parameter of light sources (spectrum range, light intensity, photoperiod) and their characterization in the design and presentation of a plant experiment.

As food, the lettuce must comply with food safety standards, where the determination of its nitrate content is a key element. Although it is a popular product and has an expanding market share, the classification of different lettuce types have not been seen in public consciousness. Consumers' opinions, shopping habits, motivation and preferences for lettuces should therefore be explored. Decision support methods should provide them with information and assistance in understanding and selecting the optimal product/products.

My scientific objectives are:

1. My aim is to define the parameters that are primarily important for plants and to evaluate the LED lighting systems based on these parameters.
2. My goal is to test different LED light environments on batavia lettuces (*Lactuca sativa* L.) via their vegetative development, level of phytonutrients and macronutrients, as well as the colour parameters and coordinates.
3. My aim is to develop a new rapid analytical method for determining the nitrate content of different lettuce types (batavia, butterhead). My goal is to be able to carry out the measurement quickly, chemical-free and to routinely.
4. My aim is to test consumer acceptance of lettuce consumption product combinations among a special consumer group. My aim is also to determine the ideal product combination, their utility levels and importance values for the target group under consideration.
5. My aim is to test the applicability of the combination of the eye camera test method and value-based segmentation for leaf vegetables.
6. My goal is to evaluate the lettuce types in a complex way, taking into account phytonutrient values, by combining statistical methods.

2. MATERIAL AND METHODS

The experiments were conducted in Gödöllő, in a laboratory (with computer-controlled and monitored environment) designed by Institute of Mechanical Engineering and Informatics (GINI). Various LED lights (420 LED Grow Circle Series 4 (Hurley Enterprises LLC, Hartwell, GA, USA); Roleadro HYG051*200W-W COB LED (Shenzhen Houyi Energy Efficiency Co. Ltd., Shenzhen, China), Lágymányosi LED (Péter Lágymányosi, GINI, Gödöllő)) have been set up in the lab. The plantation design was prepared taking into account the PPFD distribution of LED lighting systems.

The effect of the treatments was tested at each growing station with 7-7 (MINERAL RZ (81-551)) red leafed, non-heading batavia lettuces with wavy leaf-edge.

Table 3: LED light settings during lettuce lighting experiments

Start of experiments	2020.05.04.				2020.06.09.	
Blocks	1	2	3	4	3	4
referred as	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
PPFD*	200±1	200±1	200±1	200±1	200±5	200±5
setup			- UV - IR	+ UV - IR	- UV, +IR, 3 days before taking + UV, - IR	- UV - IR

* Average of measurements per block at seven points of planting sites measured in the PAR range using the mini-quantum sensor PAM-2500 fluorimeter (detailed in Chapter 2.1.1.1.).

2.1. Methods

2.1.1. Instrumental and analytical methods

Non-invasive measurements were carried out every 10 days.

2.1.1.1. Chlorophyll fluorescence measurement (non-invasive method)

Leaf chlorophyll fluorescence was measured after dark adaptation (30 minutes, leaf clip DLC8 (Walz, Germany) with a pulse-amplitude modulated chlorophyll fluorimeter Pam-2500 (Walz, Germany) (2030-B leaf clips, additional: mini-quantum sensor (PAR range), NiCrNi thermocouple) (0.8 s duration (λ≈650 nm) saturation light pulse (1100 µmol photon m^2/s), 20 kHz modulation frequency).

2.1.1.2. Measurement of relative chlorophyll content (non-invasive method)

A Minolta SPAD-502A (Konica Minolta, Tokyo, Japan) (650 nm, 940 nm wavelength measurement, silicone photodiode sensor) was used to measure relative chlorophyll on the leaf plate of the lettuce (4 different leaves).

2.1.1.3. Determination of chlorophyll-a, chlorophyll-b and total carotenoid content

For determination of the extract of the lettuce pigment content there was used UV/VIS spectrophotometer (Walthman, Massachusetts, USA), the method was based on the description of Yang et al. (1998). The equations of Porra et al. (1989) were used for chlorophyll content calculations and Holm's (1954) equation was used in case of carotene content calculation.

2.1.1.4. Determination of minerals by ICP-OES method

All the sample were taken using the inductively coupled plasma optical emission spectrometry (ICP-OES) method (Optima 8000 ICP OES, PerkinElmer, Waltham, MA, USA) (absorption: λ=160-900 nm, injector tube plasma burner (torch) diameter: d=2.0 mm) was analyzed and assessed (WinLab32 software).

2.1.1.5. Determination of total polyphenol content (TPC)

Total polyphenol content (TPC) was determined using the Folin-Ciocalteu reagent, according to the Singleton & Rossi (1965) method.

2.1.1.6. Determination of nitrate content

I measured at least 6 biological replicates of 17 butterhead, 14 batavia, 2 loose-leaf (lollo) and 2 oak leaf lettuce varieties/candidate varieties as independent samples, which came from Felgyő (harvested: 04.04.2017, 09.10.2017).

Nitrate determination was carried out according to the Cataldo et al. (1975) method by modified sample preparation (hot extraction, excavation with Carrez solutions). I made the spectrophotometric measurements (λ=410 nm) with thermo scientific (Walthman, Massachusetts, USA) recording UV/VIS spectrophotometer and valuated the results (VISIONpro V2.02 (Thermo Scientific, Walthman, USA)).

2.1.2. Instrumental color measurement

Colour measurement with a calibrated (white standard) mobile Sheen Micromatch Plus spectrophotometer (model 181/3; Sheen Instruments, United Kingdom) (measuring range: λ=400700 nm, $\Delta\lambda\approx$20 nm).

2.1.3. Consumer tests

2.1.3.1. Focus groups and conjoint analysis

Prior the conjoint study focus group discussions were carried out to determine the factors in Thailand at Mae Fah Luang University. During focus group discussions the aim was the observation of young adults' thinking method, their attitudes and choice criteria that characterize decision making during lettuce shopping. With the exploratory research those factors (product characteristics (5 pcs) and their levels (20 in total)) were successfully determined which are important in terms of lettuce shopping.

2.1.3.2. Eye-tracking tests

Based on the work of Gere (2015), I carried out eye-tracking tests in the Sensory Laboratory of the Faculty of Food Sciences of MATE. A total of 163 participants participated in that research, of which a total of 140 records were perfectly suitable for data analysis of the picture of 6 leafy greens. During the experiment a static eye-tracker (Tobii X2-60) was used to capture gaze data, and Tobii Studio was used to process data (version 3.0.5, Tobii Technology AB, Sweden). Images that provided visual stimuli were displayed on a 1280x1024 Samsung SyncMaster 757 MB monitor.

2.1.3.3. Complex evaluation of phytonutrient values of lettuce types with SRD method

My goal was to determine which is the healthiest lettuce type, based on phytonutrient values from international literature, taking into account all factors. Input data table (based on Kim et al., 2016 data): objects (rows) = phytonutrients; compared elements (columns) = lettuce types; reference column (Max) = best values of lettuce types. I used the Box-cox transformation to transform the data. For the calculation, SRDrepV6T4_CrossVal_V8D.xlsm (written in Visual Basic) software was used (source: http://www.ttk.hu/rolunk/adatbazisok-programok). The ideal (reference) lettuce type gives the SRD zero point, as it does not differ from itself. The rank shows which type or types are the closest (have the smallest number) to the ideal lettuce, and the other SRD values gives the rest of the rank. The SRD value was validated with leave one out (LOO) validation per row, and the significant differences were tested with Sign test.

3. RESULTS

Among the results, there only those are presented which have shown new approaches or significant differences.

3.1. Evaluation of LED lighting systems

Measurements of the spectral composition showed that the Kind LED K5 XL750, 420 LED Grow Circle Series 4 LEDs, Roleadro HYG05 1*200W-W COB LED and Lágymányosi (built) LED lighting systems have different spectral compositions. When setting up the experiment, the wavelength-by-wave photon current density (PPFD) and the PPFD distribution in the area at plant level showed that lighting systems differ in certain parts of the growing area. When viewed by wavelength range, the photon current density distribution was shown to be uniform when examined separately.

3.2. Results of vegetative development

The **average leaf growth** over the time between the measurement days is used to monitor the growth rate. Comparing treatments, it can be said that there was no significant difference between the treatments between 0-10 days. Between 10-20 plants of treatments 1 and 2 developed better, but between 20-30 days treatments 1 and 4 did not show similarity to the previous growth period and lagged behind the average leaf growth intensity of the plants of treatments 5 and 6. Between 0-30. days, the measured average leaf growth did not show significant difference between treatments.

The **average relative chlorophyll content** showed, that only on day 20 was significant difference, between treatment 5 (25.18 SPAD value) which differed significantly from treatment 1 and 2 (21.14, 21.66 SPAD value), these were not significant from each other. Treatment 1 had significantly lower average relative chlorophyll content from treatments 3, 4 and 6 (23.66-24.16 SPAD value).

On day 30th plants of treatment 4 had the highest average relative chlorophyll content (24.44 SPAD value), which was significantly different from treatment 1 and 2 (19.83, 20.80 SPAD value).

Significancy level by treatment shown that there were difference between day 0 and 10, day 10 and 20 or 30 in case of treatments of 1-3.

For the **mean maximum quantum efficiency,** the dark adaptation values F_v/F_m remained within normal range and there was no stress.

The highest **average fresh weight** was in treatment 2 (249 g), which had significant difference from other treatments. Treatment 1 and 3 (210, 209 g) were not different from each other and from treatment 4, but had significantly higher average fresh weight than treatment 5 and 6 (153, 147 g), which did not differ significantly from each other and gave the lowest average fresh weight.

3.3. Amount of phytonutrients

The highest **average nitrate content** was in treatment 1 and 2 (2596, 2591 mg/kg). The lettuces of treatment 6 (2334 mg/kg) with the lowest mean nitrate content were significantly different from all other treatments except from treatment 5 (2414 mg/kg).

The highest **average amount of chlorophyll** was found in treatment 5 (63.07 μg/mg), which had significantly higher level compared to the other treatments. The lowest average chlorophyll contents were in treatment 1 and 2 (38.87; 39.77 μg/mg), which did not differ from each other, but differed significantly from treatment 4 and 6 (54.18; 53.54 μg/mg), which did not differ from each other. The **chlorophyll-b** content was also highest in treatment 5 (30.43 μg/mg), which differed significantly from treatment 3, 1 and 2 (24.88; 21.1; 20.28 μg/mg). Treatment 4 and 6 (28.09; 27.18 μg/mg) had significantly higher values from treatment 1 and 2. The highest **mean carotene level** was in treatment 5 (12.77 μg/mg), compared to that, significantly lower levels were measured in lettuces of treatment 3, 2 and 1 (10.02; 8.93; 8.03 μg/mg).

The **highest mean total polyphenol content** was found in treatment 2 and 1 (101.9; 101.1 μmol GS/100 g), which did not differ from each other but were significantly different from other treatments. The lowest average total amount of TPC was in treatment 4, 3 and 5 (84.4; 86.5; 88.8 μmol GS/100 g), from these, only treatment 6 had significantly higher value (96.9 μmol GS/100 g).

3.4. Results of element content determination

The plants of treatment 4 (4.18 mg/kg fresh weight) had the highest **average iron content**, which differed significantly from lettuces from other treatments. Treatment 2 plants (2.94 mg/kg fresh weight) had the lowest average iron content.

The **average potassium content** was highest for treated plants (4047 mg/kg fresh weight), followed by treatment 6 (3849 mg/kg fresh weight) which differed significantly from each other and from the plants of other treatments. Treatment 4 plants (3523 mg/kg fresh weight) had the lowest average potassium content, while the plants of treatments 5 and 6 and the lettuces of treatment 2 (fresh weight 3595 mg/kg) differed significantly.

The **average phosphorus content** was highest for treated plants (315.3 mg/kg fresh weight), which differed significantly from lettuces from other treatments. Treatment 4 plants (263.9 mg/kg fresh weight) had the lowest phosphorus content, with a significantly higher amount of phosphorus than other treatment plants, with the exception of treatment 6. Furthermore, it differed significantly from the lettuces of treatment 3, 4 and from the plants of treatment 5 to 6.

The **average magnesium content** was highest for treated plants (161.8 mg/kg fresh weight), with significantly lower magnesium levels for all other treatments. The lowest average magnesium content was in treatment 3 plants (fresh weight of 142.9 mg/kg), compared with a significantly higher average magnesium content for lettuces grown in treatments 4 and 5.

The **average calcium content** was highest for treated plants (829.3 mg/kg fresh weight), which was significantly different from lettuces from all other treatments. Treatment 6 had the lowest average calcium content (fresh weight of

727.7 mg/kg), with lettuces of treatment differing significantly from lettuces in treatments 5 and 1.

3.5. Analyze color parameters and coordinates

For the CIE Lab **mean a^*** values, which is the transition between green and red, it was found that treatment 5 plants (-4.6) were significantly reddish than treatment 3 plants (-7.1). For the **mean b^*** values, which is the transition between blue and yellow, the lettuces of treatments 2 and 3 (25.2; 25.9) were significantly yellower than the plants of treatment 5 (20.6).

Based on values derived from L^* a^* b^* coordinates (C^*, h^*), I calculated the differences between treatments (ΔE^*_{ab}, ΔL^*_{ab}, ΔC^*_{ab}, Δh^*_{ab}). For ΔE^*_{ab}, the thresholds described by Mokrzycki & Tatol (2011) can be used to determine whether the color difference is visible to the eye and to what extent it can be detected. Based on the results, the bystander can firmly separate treatment 5 from treatments 2 and 3 ($5<\Delta E$). One can clearly see the difference in color ($3.5<\Delta E<5$) between treatment 4 and treatments 1, 2, 3. Inexperienced observers can see the difference ($2<\Delta E<3,5$) between treatments 3 and 1, treatments 5 and 4, and treatments 6 and 2, 3, 5. For treatments 2 and 1 and treatments 6 and 4, the difference would only be noticed by an experienced observer ($1<\Delta E<2$). In other cases, the difference is no longer visible to the eye ($0<\Delta E<1$).

3.6. Method development for the determination of nitrate content by combining FT-NIR and UV-Vis methods

3.6.1. UV-Vis measurements of nitrate volume

3.6.1.1. Difference between batavia (B) varieties

Among spring-harvested batavia lettuce varieties, B05 and B09 varieties showed a significant difference from B13 and B16 varieties. The other varieties were not significantly different from any group in terms of nitrate content.

3.6.1.2. Difference between butterhead (V) varieties

In the case of spring-harvested butterhead lettuces, the V13 variety had significantly higher nitrate content compared to the varieties V03, V07 and V04, while the V11 varieties differed significantly from those of the V03 and V14 varieties.

3.6.1.3. Difference between butterhead and batavia varieties

When the nitrate content of butterhead and batavia lettuce varieties was compared, the Kruskal-Wallis test showed significant differences only in a few cases. The nitrate content of B16 exceeded significantly that of the varieties V03, B05, V14, V07, and B09. The varieties V03 B05 V14 resulted in significantly lower values than the variety B13. In the other cases, no significant difference could be found.

3.6.1.4. Difference between lettuce types

The nitrate content of the green oak leaf lettuce type was significantly lower than the red oak leaf, green loose-leaf and red loose-leaf lettuce types. There was no significant difference between green butterhead and other lettuce types.

3.6.1.5. Chemometric analysis of FT-NIR spectrum of lettuce types

After the exclusion of spectral outliers, pattern recognition was performed with linear discriminant analysis (LDA); it was found that the studied four variety types diverge from each other, and the lollo type explicitly diverges from batavia and butterhead types. The LDA further revealed, that within variety types, red and green leaved variants of lollo and oak leaf types definitely diverge from each other as well, which was also confirmed in this case by the validation with random grouping.

3.6.1.6. Characterization of quantitative estimation function

The setup of the partial least squares (PLS) estimation function was performed after the exclusion of outliers, with the use of spectral and reference data of 191 samples. For data pre-treatment, multi scattering correction (MSC) was applied. The creation of the model required nine PLS components; the spectral ranges which were taken into consideration for the evaluation were the following: 9558-8100 cm^{-1} 7383-5917 cm^{-1} 5199-4467 cm^{-1}. The coherence was validated by three-segment cross-validation. The 99.4 mg/kg fresh product average error showed a uniform distribution, and no outstanding sample was found.

3.7. Conjoint analysis results

In the conjoint analysis, I analyzed the responses of 252 people (young adults). When analyzing all consumer responses together, the relative importance of the characteristics was given in the following order: lettuce type (38.0 %), appearance (20.5 %), packaging (19.1 %), production system (15.1 %), label (7.4 %).

When analyzing the product evaluation criteria, the highest utility levels were the following values (in descending order). For lettuce types: red loose-leaf lettuce (4.9), green oak leaf lettuce (3.9), green iceberg (2.2). For appearance: leaves (2.1), cutted leaves (1.8). For packaging: unsealed plastic bag (1.9), plastic wrap (1.4). For the label: not labelled (1.4). For cultivation: non-organic soil system (1.9).

Altogether, the analysis of the consumer responses showed that the ideal product combination is the following: red loose-leaf, leaves, unsealed, not labelled, non-organic soil system. Same analysis showed that the least preferred product combination is the red oak leaf lettuce, whole head with root, sealed plastic bag, labelled, hydroponic system.

Based on consumer responses, three consumer segments were defined (Agglomerative hierarchical clustering, Euclidean distance, Ward method).

In the first cluster (N = 82), the following order was given in terms of the relative importance factors of the product characteristics: lettuce type (34.0%), appearance (23.6%), packaging (19.3%), cultivation system (15.3%).), label (7.8%). Analyzing the product evaluation criteria, the following values had the highest utility levels (in descending order). For lettuce types: red loose-leaf lettuce (7.4), green iceberg lettuce (1.9), green oak leaf lettuce (1.7). For appearance: leaves (4.8), cutted leaves (2.8). For packaging: unsealed plastic bag (3.1), plastic wrap (2.3). For the label: without label (1,5). For the cultivation system: non - organic soil system (1,4). The ideal product combination for the first cluster: red loose-leaf lettuce, leaves, unsealed plastic bag, unlabeled, non-organic soil cultivation system.

During shopping, the following factors are very important to them: cleanliness and freshness equally, taste and price equally. Most of them want to see on the label: price, expiration date, nutritional information.

In the second cluster (N = 30), the following order was given in terms of the relative importance factors of the product characteristics: lettuce type (39.5%), appearance (18.9%), packaging (18.7%), cultivation method (14.7%), label (8.2%). Analyzing the product evaluation criteria, the following values had the highest utility levels (in descending order). For lettuce types: green oak lettuce (8.3), red loose-leaf lettuce (2.5), green iceberg lettuce (1.7), green romaine lettuce (1.6). For appearance: cut leaves (1.5), leaves (1.0). For packaging: unsealed plastic bag (3.1). For the label: without label (2.7). For the cultivation method: non-organic soil system (2.9). The ideal product combination for cluster two: red loose-leaf lettuce, leaves, unsealed plastic bag, unlabeled, non-organic soil system.

During shopping, the following factors are very important to them: freshness, cleanliness, visible defects. Most of them want to see on the label: price, expiration date, information about the existence of a GAP certificate.

In the third cluster (N = 140), the following order was obtained in terms of the relative importance factors of the product characteristics: lettuce type (40.0%), packaging (19.0%), appearance (19.0%), cultivation method (15.1%).), label (7.0%). Analyzing the product evaluation criteria, the following values had the highest utility levels (in descending order). For lettuce types: green oak lettuce (4.2), red leaf lettuce (4.0), green iceberg lettuce (2.5). For appearance: cut leaves (1,3). For packaging: plastic wrap (1,1), unsealed plastic bag (1,0). For the label: without label (1,1). For the cultivation method: non-organic soil system (2.9). The ideal product combination for the them: green oak lettuce, cutted leaves, plastic wrap, unlabeled, non-organic soil cultivation system.

During shopping, the following factors are very important to them: freshness and cleanliness. Most of them want to see the following information on the label: price, shelf life, weight.

It was found out, that the least useful product combination for all three clusters is: red oak lettuce, whole head with roots, sealed plastic bag, labeled, hydroponic system.

3.8. Results of eye-tracking tests

Based on the value characteristics, with one exception (enjoyable life), all value characteristics, all eye movement parameters were significant (α=0.05) based on the Kruskal-Wallis test. Based on the characteristic values, 4 clusters were determined (Ward method, Euclidean distance; Dunn's pair-by-pair comparison with Bonferroni correction).

The members of Cluster 1 are conservative family-oriented people with traditional values, but family, friendship, self-realization, financial security and a high level of education are the most important values for them. Based on their eye movement parameters, the images were observed for the longest time, with the number of fixations and the number of visits being the highest.

The most important things for those in Cluster 2 are: friendship, financial security, an enjoyable life, independence. Based on the parameters of eye movement, it can be said that the pictures were observed several times for a short time.

The most important things for cluster 3 are: personal and material security, true friendship and a high level of education. All values are more important to them than medium. The number of visits was medium compared to other clusters, but the length, number of fixations and length of visits were similar to cluster 4.

They are placed in cluster 4, for whom only family life – true friendliness and enjoyable life – is important, all other factors are moderately significant. Based on their eye movement parameters, all their characteristics were the shortest/least.

3.9. Complex evaluation of the production-biology of lettuce types

Based on the phytonutrient values of lettuce types, SRD method was used for ranking. With the consideration of all phytonutrients of each lettuce types, there was Loose-leaf lettuce (red) the best followed by Loose-leaf lettuce (green), Roman lettuce (green), and iceberg and Butterhead lettuce types which two equally were the weakest.

If the uncertainty of the SRD values is also determined with the leave one out (LOO) cross-checking method and the uncertainties are plotted in Box & Whisker plots, then, it is possible to examine each group, based on the overlapped medians and standard deviations between individuals. Elements with overlapped medians and standard deviations formed a group. For a numerical evaluation of the results, the comparison of significant differences was determined by the Sign- and Wilcoxon tests. Based on the results, there were confirmed the previous results, as the rank was the following: Loose-leaf lettuce (red), Loose-leaf lettuce (green), Roman lettuce (green), Iceberg and Butterhead lettuce types. Sign and Wilcoxon tests also demonstrated that each lettuce types differed significantly from each lettuce types ($\alpha=0.05$), except Iceberg and Butterhead lettuces, as they were not different.

4. CONCLUSIONS AND PROPOSALS

During my work, taking into account crop production aspects, I fully described a KindLED K5 XL750 LED panel, I perceived the irradiance of the panel and the spectral quality of the LEDs and were mapped their position in the panel, after all the spectral characterization of the separately adjustable channels. There was examined the luminous flux from a given distance by several precise measurements, which led me to successfully map the distribution of photosynthetic photon flux density (PPFD) of the panel’s lighting coverage area. I used this measurement experience in the future measurements for the 420 LED Grow Circle Series 4 LED, the Roleadro HYG05 1*200W-W COB LED and the built-in LED (Lágymányosi LED) luminaries which were set up for plant experiments. There was pointed out that knowledge of the PPFD distribution contributes to clarify the purpose of the plant experiment and set up the experimental design of the plants.

During the examination of the vegetative parameters and content of phytonutrients of lettuces, there were found that on the 30th growing day were significant differences in average leaf length between the plants of treatment 2 and the plants of treatment 4 (13,6 cm) and 6 (13,7 cm). Non-invasive measurements (maximum quantum efficiency (0.8040.808 F_v/F_m), relative chlorophyll content (19.83-23.38 SPAD value)) did not give any difference between treatments. For the average fresh weight, there was a significant difference between the heaviest lettuces, treatment 2 (249 g) and other treatments, as well as lettuces treatment 1, 3 (210; 209 g) and 5, 6 (153, 147 g). The dry matter content for treatments 1 to 4 differed significantly for plants of treatments 5 and 6. Differences in fresh weight and dry matter content (based on treatments 4 and 6) indicate that lettuces grown at different times have been nutrient-used at different rates, and climate data do not indicate environmental differences. There were significant differences in the amount of phytonutrients in content of nitrate, chlorophyll-a-, chlorophyll-b, chlorophyll-a+b, carotene and TPC. The amount of nitrate (2334-2596 mg/kg) has not reached the limit laid down for this period (4000 mg NO_3/kg fresh weight, non-iceberg lettuce) in directive 1258/2011/EC, for either treatment. In the case of mineral content, the iron content was outstanding for treatment 4, which did not followed by chlorophyll content. There were significant difference between treatments in other mineral contents.

The CIE Lab* data obtained in instrumental colour measurements showed that the treatments showed that the differences in colour were visible on the basis of the derived values (C^*, h^*), calculated on the basis of the statistical evaluation of the parameters of ΔE^*_{ab}. On the basis of the results, it was possible to distinguish in the strongest possible way the plants of treatment 5 from the lettuces of treatment 2 and 3.

Given that in the international literature and lettuce lighting research, I have not found any literature that covers the experimental lighting that I have set up, so I can only make partial and relative comparisons, which need to be treated with reservations.

In these experiments, the differences in the wavelength range of LEDs with a constant mean PPFD (200 μmol/m^2/s) were clearly shown by the average fresh weight, differences in chlorophyll-a, chlorophyll-b, chlorophyll-a+b and carotene, TPC, and the amount of iron, calcium, potassium, phosphorus and magnesium, CIE $\Delta E^*_{ab,}$ are clearly due to the spectral effect. Calcium can also be taken through shoots, so it is used directly at the point of use, I did not assume its modification effect.

Based on UV irradiation tests (treatments 3 and 4 and treatments 5 and 6 combined), the results showed only significant differences in phytonutrient and mineral content and colour variance ($\Delta E_{*ab)}$. It has been shown that combined regulation of UV-A and FR can be used to increase chlorophyll-a, potassium, phosphorus, magnesium, calcium levels significantly, and to create a reddish and yellowish leaf colour based on CIE a^* and b^*.

In the examination of the internal parameters of plants, it has been shown that plant parameters can only be influenced by changing the light environment.

Directive 1258/2011/EC of the European Union defines nitrate content depending on season and production technology. For lettuce grown under cover harvested between 1 October and 31 March, 5000 mg NO_3/kg fresh weight is the permitted limit while in the case of iceberg lettuces produced on an open field the maximum allowed amount is 2000 mg NO_3/kg. The document does not refer to other variety types, nor to color variants. It would be advisable to define this broad range in a variety type-specific manner, in order to reduce food security risks caused by lettuce nitrate intake.

In the present study, a total of 266 lettuce heads were analyzed, in various combinations of seasons and technologies (spring 9 greenhouse, autumn 9 open field) and variety types (batavia, butterhead, loose-leaf (lollo), oak leaf), both red and green colored).

Based on the UV–Vis measurements run on 410 nm, it can be concluded, that the nitrate content of butterhead lettuce did not show remarkable variation in case of different production technologies and seasons. Regarding autumn harvested lettuces produced in the same environmental conditions, it was found, that green oak leaf types accumulated significantly less nitrates, than red oak or lollo types. In the case of spring harvested samples, batavia types accumulated generally more nitrates, than spring harvested butterhead lettuces.

With the analysis of the image with the spectra, it was proven, that the homogenization of the samples and the first derivation function transformation enhances the inspection of the first derivative graph of spectrum averages, and characteristic differences were found in the region between 5000–3900 cm^{-1} wavenumbers (fibers/cellulose, proteins, carbohydrates). According to this, variety types differ from each other; at the same time, oak leaf types, at the same time, do not diverge from each other. Since the water content of the samples is very high, the peaks at 5200–5000, 6900–6800 cm^{-1} cover a lot of information.

In the pattern recognition/classification model of variety types, based on the linear discriminant analysis (LDA) of FT-NIR measurements, it can be concluded that the four variety types investigated separate from each other, where the lollo type explicitly diverges from batavia and butterhead types. The LDA further revealed, that within variety types, red and green leaved varieties of lollo and oak leaf types diverge as well. The results are also validated by random grouping methodology.

A model was successfully built up for the FT-NIR quantification of nitrate content of lettuce types (R2 = 0.95; RMSEE = 74.4 mg/kg fresh product; Q2 = 0.90; RMSECV = 99.4 mg/kg fresh product). The developed model is able to execute the measurement in a quick and chemical free way; the method is suitable for the routine quantification of nitrate content in lettuce samples.

It has been found that the FT-NIR non-invasive quick method has a place in the area of food safety surveillance. It is recommended to examine additional leaf vegetables (e.g. rucola, spinach, baby food raw material) and to create a free-access central database to make the application of the method routine and to further expand the repository.

Through focus group interviews with Thai consumers, I explored the forms of thinking and decision-making processes related to lettuce buying and consumption. Using the orthogonal array method, I created the product combinations to be evaluated, using the attributes previously defined and their factors. I brought product combinations to life and presented them in the form of conjoint cards.

Based on the evaluation of the product combinations, I have segmented 3 homogeneous groups. I defined relative importance factors, utility values, and ideal product combinations by cluster. When analyzing all consumer responses together, the following order was given in terms of the relative importance of the characteristics: lettuce type (38,0 %), appearance (20.5 %), packaging (19,1 %), production system (15,1 %), label (7,4 %). On the basis of the results, the highest utility levels were found in the following factors: red loose-leaf (4,9), green oak leaf (3,9), green iceberg (2,2); for appearance: leaves (2,1), cutted leaves (1.8); for packaging: unsealed plastic bag (1.9), plastic wrap (1.4); for the label: not labelled (1.4); for cultivation: non-organic soil system (1.9). The ideal product combination: red loose-leaf, leaves, unsealed plastic bag, not labelled, non-organic soil system. After delimiting the clusters, it became clear that their utility values were different. Their preferences were as follows: in terms of lettuce type: red loose-leaf lettuce or green oak leaf lettuce; in appearance: leaves or cutted leaves; packaging: unsealed plastic bag or plastic wrap. All three groups preferred not labelled, non-organic soil system grown lettuces.

In eye camera experiments, there were tested leaf vegetables in lettuce mixtures. Respondents answered questions that could be segmented at the end of the stimulus study. The novelty of the experiment is that the combined analysis of values and eye movement characteristics was carried out by combining several statistical methods (cluster analysis, survival analysis, Kruskal-Wallis and Dunn's test). Using the clusters created on the basis of the value segmentation and the eye movement parameters, I defined four clusters, where the group values were consistent with eye movement parameters and decision time. These results can continue to be used as the packaging and the composition of lettuce mixes can be optimized specifically to meet the needs of the clusters.

In general, the international literature evaluates plant parameters separately. In many cases, however, multi-criteria evaluation would be more reasonable, especially for those who do not or have little or no information on the phytonutrients of and their effects on health. The SRD method provides a solution for this as a decision support tool. In this work, it has been shown that by evaluating the phytonutrients of in several aspects, a clear order can be made between lettuce types.

5. NEW SCIENTIFIC RESULTS

1. In my research, I delineated the parameters of LED lighting systems used in crop production that are of primary importance for plants and used them to describe the LED lighting systems used in my experiments. In my work, I explored the spectral composition of several LED lighting systems, delimited the photon current density (PPFD) per wavelength, and mapped the photon current density distribution of lighting systems.
2. In my research, I tested the effects of six different LED light environments on the internal parameters of the batavia lettuce (*Lactuca sativa* L.). I have demonstrated that the treatments have a significant effect on the following measured parameters: relative chlorophyll content, maximum quantum efficiency, fresh mass, nitrate, chlorophyll, carotene, K-, P-, Mg content, CIE a*, b*. As a result, I have demonstrated that only by changing the light environment can the above parameters be influenced.

3. I was the first to develop and use the FT-NIR method to determine the nitrate content of different lettuce types (batavia and butterhead). I developed a new method for determining nitrate content by combining FT-NIR and UV-Vis. PLS model was successfully built for the determination of the nitrate content of lettuce samples for FT-NIR (R^2=0.95).
4. In my research, I used the conjoint analysis method of consumer studies to determine the ideal lettuce product combination, utility levels and importance values (main criteria: lettuce type, appearance, packaging) for Thai young adult target population. I have done the delimitation and characterization of the consumer segments.
5. In my research, I was the first to apply to leafy vegetables a combination of the eye camera test method and value-based segmentation, supplemented by statistical methods. The parameters of eye movement of the clusters created are comparable. Based on the results, the significant difference in the following eye movement parameters was: number of fixations, length of fixations, length of visits, number of visits. I have defined the characterizations of clusters with value characteristics.
6. In my research, I have demonstrated that *Sum of Ranking Differences* **(SRD)** method is suitable for complex evaluation of the phytonutrient values of lettuce types.

6. PUBLICATIONS RELATED TO THE TOPIC OF THE THESIS

Journal articles with Impact Factor:

Boros, I. F., Sipos, L., Kappel, N., Csambalik, L., Fodor, M. (2020) Quantification of nitrate content with FT-NIR technique in lettuce (Lactuca sativa L.) variety types: a statistical approach. JOURNAL OF FOOD SCIENCE AND TECHNOLOGY (NEW DELHI) (Q2, **IF: 1.946** (2019))

Sipos, L., **Boros, I. F.**, Csambalik, L.; Székely, G., Jung, A., Balázs, L. (2020) Horticultural lighting system optimalization: A review. SCIENTIA HORTICULTURAE 273: 109631 (Q1, **IF: 2,769** (2019))

Peer-reviewed journal (MTA list) publications:

Sipos L., **Boros I. F.**, Purcel. Á., Varga. Zs., Szőke A., Székely G. (2017) LED-ek hasznosítási lehetőségei a növénytermesztésben. KERTGAZDASÁG: 49:(3) pp. 11-22.

Conference full paper:

Ombódi, A.; **Boros, I. F.** (2020) Review of LED lighting for leafy vegetables produced in closed plant production systems. In: Jakab, Gusztáv; Csengeri, Erzsébet (szerk.) Water management: Focus on Climate Change, Szarvas, Magyarország: Szent István Egyetem Öntözési és Vízgazdálkodási Intézet, pp. 131-136.

Boros, I. F. (2020) Nitrate level and its main changing factors in leafy vegetables especially in lettuce (*Lactuca sativa* L.) In: Jakab, Gusztáv; Csengeri, Erzsébet (szerk.) Water management: Focus on Climate Change, Szarvas, Magyarország: Szent István Egyetem Öntözési és Vízgazdálkodási Intézet, pp. 41-48.

Boros, I. F., Kappel, N., Gere, A., Sipos L. (2016) Comparison of different lettuce types (*Lactuca sativa* L.) based on their bioactive compounds. 1st International Conference on Biosystems and Food Engineering. 8.12.2016. Budapest, Hungary Proceedings:http://physics2.kee.hu/BiosysFoodEng/cdrom/)

Boros I. F., Sipos L., Gere A. (2017) Eye-tracking analysis of leafy vegetables. Review on agriculture and rural development. 6: 32-37.

Boros I. F., Kappel N., Krisztina Madaras K., Gere A., Sipos L. (2017) Applications of SRD-method in horticultural and food sciences. Review on agriculture and rural development. 6: 38-43.

Conference proceedings (abstracts):

Boros, F., Rakonczay, K., Hetyei, G., Sipos, L., Balázs, L. (2019) Saláták beltartalmi és érzékszervi paramétereinek javítása LED alapú világítórendszerrel. In: Nádas, József (szerk.) X. LED Konferencia: 2019. február 5-6., Programfüzet MEE Világítástechnikai Társaság, pp. 21-22.

Sipos, L., **Boros, F.**, Madaras, K., Csambalik, L., Gere, A. (2019) Multi-criteria decision making–Comparing lettuce types by their phytonutrient content. In: Héberger, Károly (szerk.) Conferentia Chemometrica 2019, pp. P18.

Boros, I. F., Kappel, N., Sipos, L., Fodor, M. (2018) Különböző saláta fajtatípusok (*Lactuca sativa* L.) nitrát felhalmozása. In: Sipos, László; Gere, Attila (szerk.) MTA, Kertészeti és Élelmiszertudományi Bizottság, Élelmiszertudományi Albizottság Workshop. Budapest, Magyarország: MTA, Kertészeti és Élelmiszertudományi Bizottság, Élelmiszertudományi Albizottság, pp. 19-20.

Madaras K., **Boros F.**, Nyitrai Á., Csambalik L., Gere A., Sipos L. (2018) A rangszámkülönbségek összege (Sum of Rank-Differences, SRD) módszerkombináció táplálkozástudományi lehetőségei. In: Gelencsér Éva, Lugasi Andrea (szerk.) Táplálkozástudományi kutatások VIII. PhD konferencia Program és előadás összefoglalók. Konferencia helye, ideje: Budapest, Magyarország, 2018.01.25. (Magyar Táplálkozástudományi Társaság). Budapest: Magyar Táplálkozástudományi Társaság, pp. 18.

Sipos L., **Boros F.**, Madaras K., Gere A. (2017) Statisztikai módszerek a szemkamera kutatások vizsgálati eredményeinek értékelésében. In: Iván Devosa, János Steklács, Zsuzsanna Buzás, Ágnes Maródi. III. Magyar Szemmozgáskutatás Konferencia. 26 p. Konferencia helye, ideje: Kecskemét, Magyarország, 2017.06.01. Kecskemét: Pallasz Athéné Egyetem, 2017. pp. 17.