Detecting color differences in lentil flour samples using an inexpensive, hand-held colorimeter.

Sarahi Viscarra[1*], Ana Vargas[1*], Laura Jardine[1], Mehmet Tulbek[2] and Kirstin E. Bett[1]

[1]Department of Plant Sciences, Univ. of Saskatchewan, 51 Campus Dr., Saskatoon, SK S7N 5A8

[2]Saskatchewan Food Industry Development Centre, 2335 Schuyler Street Saskatoon, SK S7M 5V1

Manuscript received _____; revision accepted _____.

Number of words: 2478

Authors' emails: sarahi.viscarra@usask.ca; a.vargas@usask.ca; laura.jardine@usask.ca; mtulbek@foodcentre.sk.ca; k.bett@usask.ca

Correspondence:
Corresponding author – Ana Vargas
Email: a.vargas@usask.ca

[*]equal efforts

ABSTRACT

Premise: The Nix Spectro 2 was developed to obtain CIEL*a*b* values from small samples and is an affordable alternative to the more expensive spectrophotometers typically used to assess food color.

Methods: To validate the use of the Nix for lentil seed quality research, eight genotypes in three different field trials, were evaluated for flour color using HunterLab (illuminant C) and Nix (illuminants D50 and C) instruments and results compared for consistency.

Results: The a* and b* Nix scores were comparable to those obtained with the HunterLab (r^2 = 0.9-0.95). L* scores had lower correlation values: 0.61 and 0.58 when comparing HunterLab (C) with Nix (D50) and Nix (C), respectively.

Discussion: Lower L* correlations could be a result of the influence of direct light exposure during HunterLab scans. In contrast, the Nix uses more technical repetitions per scan and avoids direct light exposure with the use of an adaptor, suggesting the L* Nix values are more reliable than those from the HunterLab. We observed that the Nix procedures are more repeatable and rigorous, validating its use for assessing color, especially for small samples, a common limitation within breeding programs.

Keywords (4-8): colorimetry, HunterLab, lentil flour, Nix Spectro 2

INTRODUCTION

There is a current demand for alternatives to animal protein for human consumption, and plant-based products have become increasingly desirable. Moreover, pulse-based flours are attractive to industry due to their nutritional value and inexpensive integration to existing product-creation processes. Lentil-based products available to consumers are highly nutritious and meet the demands of specific markers with restricted diets, including gluten-free and low glycemic index products (Campos-Vega et al., 2010). The University of Saskatchewan lentil breeding program is working with food industry partners interested in developing red lentil-based products like dry pasta, fresh pasta, crackers, and snack foods with a characteristic intense red color in the final product. The partnership was established to determine if certain lentil genotypes or growing environments produced lentil seed with a deep red color stable through processing from seed to food products. This work required a method for assessing lentil flour color and color stability across environments cost-effectively for the benefit of breeders and industry partners. In other similar studies, color has been assessed in different steps of product development with the use of colorimeters or spectrophotometers to obtain scores that represent color through a visual color space (Sant'Anna et al., 2014; Švec et al., 2008; Ugarčić-Hardi et al., 1999). The 1979 Commission Internationale d'Eclairae (CIE) color space (e.g., XYZ, sRGB, LAB) is commonly used to represent color in the form of coordinates that correspond to the changes in the visible space (Becker, 2016; Kang, 2011). CIE L*a*b* is a three-dimensional uniform color space L* (lightness), a* (green-red axis), and b* (blue-yellow axis) used to describe color and is the best to describe the perception of color by humans, thus it is often used to characterize food (Kang, 2011). Colorimetric instruments such as those produced by HunterLab (Reston, Virginia, USA),

among others, are reliable but expensive. This can be a limiting factor for studies constrained by funding availability (Cabas-Lühmann & Manthey, 2020; Fratianni et al., 2005). The Nix Spectro 2 colorimeter (Nix Sensor Ltd., Hamilton ON, Canada), referred to throughout this document as Nix, can be purchased online for one fifth the cost of alternatives. The Nix is a user-friendly device with a mobile app that helps control the spectrophotometer and manage data. From the Nix Toolkit app, settings (e.g., illuminant, observant, technical repetitions, and color space values) can be adjusted as desired, and the scanned values can be directly saved in the app and downloaded later as a .CSV file for further processing. The Nix can also be useful when dealing with smaller sample sizes using roughly 0.5 g of lentil flour/sample. The Nix is routinely used for color assessment of paints, coatings, cosmetics, manufacturing, printing, packaging, and textiles. Recent studies with research applications in food science have used the Nix to measure color in beef, fish, and lamb (Dang et al., 2021; Holman et al., 2021; Holman et al., 2018). Jha et al., (2021) used the Nix Pro color sensor to assess color in soil and develop prediction models for soil iron content. Yet, there are no published studies on the use of Nix for evaluating color in legume or cereal flour. The objective of this study was to validate the use of the Nix as an alternative to a HunterLab colorimeter and describe the methodology applied when determining color in lentil flour samples.

METHODS

Lentil Samples

The red lentil samples used in this study were obtained from the breeding programs at the Crop Development Centre (CDC, University of Saskatchewan, Canada) and at the Università

Politecnica delle Marche, Italy. Seeds from genebank accessions were obtained from the International Center for Agricultural Research in Dry Areas (ICARDA) and have been maintained at the CDC over many generations. Three Canadian varieties, one Italian variety, and four genebank accessions were the materials for this study and are described in Table 1. Plots were grown in Osimo, Ancona, Italy (43°45’04” N, 13°49’98” W), and Sutherland (52°09’57.3” N, 106°30’16.1” W) and Rosthern (52°44’31.4” N, 106°14’04.5” W), Saskatchewan, Canada, in 2022. Trials in Canada were grown during the typical growing season – from May to August, in plots of 3 m^2, 3 rows per plot, with inter-row spacing of 30 cm, a row length of 3 m, and a target population of 540 plants per plot. The trial in Italy was grown during February to August in plots of 5 m^2, 5 rows per plot, with an inter-row spacing of 30 cm, a row length of 3 m, and a target population of 900 plants per plot.

Whole seeds were collected from each plot at maturity and dried at 45°C until a moisture content of <13% was reached. Clean, dried seeds were dehulled using a Satake TM05C huller (Satake Engineering Co. Ltd, Stafford, TX, USA) and finely ground using an Udy cyclone sample mill (Udy Corporation, Fort Collins, Co, USA).

Instrument validation and settings

All measurements were taken in a laboratory under consistent light conditions. The general specifications of both equipment are described in Table 2. The HunterLab Mini XE Plus (discontinued product, HunterLab, Reston, VA, USA), referred throughout this document as HunterLab, was used to obtain the first set of color measurements (Figure 1). Approximately 6 g of each lentil sample was placed in a glass Petri dish (75 mm diameter) and measured in triplicate. The HunterLab was standardized using the provided MiniScan XE Plus Reflectance Standard white and black ceramics before every color measurement session. Each slide was

placed over the HunterLab's aperture and scanned (Figure 1, B.2. to B.5). After every scan the generated CIE L*, a*, and b* scores were manually copied to an MS Excel spreadsheet for data analysis and comparison with the Nix results.

A Nix Spectro 2 (Nix Sensor Ltd., Hamilton, ON, Canada) with illuminants D50 and C was used to scan the same set of samples in triplicate (Figure 1, A.1 to A.7). Illuminant D50 is the default setting of the Nix and is meant to represent natural 'horizon' daylight in the early morning or late afternoon, Illuminant C is intended to simulate the average of daylight and commonly used for color measurement in the food and agriculture industry. The data obtained with either, can be transformed to the other illuminant values using a tool on the Nix company website: https://www.nixsensor.com/free-color-converter/ if necessary.

For color analysis, the Nix Toolkit App was downloaded on a Samsung Galaxy Tab mobile device and connected to the Nix via Bluetooth®. Every time the Nix was used, a white tile normalization scan was conducted with the provided ceramic reference tile to standardize the spectrophotometer. About 0.5 g of the lentil flour samples were placed in the opening of a clear slide (Nix Pro 2 low-volume adapter, Nix Sensor Ltd., Hamilton, ON, Canada) (Figure 1, A.1 to A.3). The technique allows the same sample to be scanned twice at different points, and the CIE L*, a*, and b* scores obtained from each scan are averaged by the app and saved. This feature is used to accommodate the presence of uneven surfaces and, or texture. Even though every sample is flattened in the slide during preparation, flour has texture and influences the direction of the light reflected, altering the color values generated – technical repetitions help take these color variations into consideration. The data were collected and stored in a library created in the mobile app and then exported as a .CSV file for data analysis.

Experimental design and statistical analysis

The lentil samples from eight genotypes were obtained from field trials that were arranged in a Randomized Complete Block Design (RCBD) with four (2 trials in Canada) and three (1 trial in Italy) repetitions of each lentil genotype for a total of 87 field plots. Seeds from each plot were scanned to account for environmental effects in color variation. Three slides with lentil flour were prepared for every field plot. Each slide was scanned twice, and values were averaged using the multi-point averaging technique in the Nix Toolkit App. Data were analyzed using the general mixed model in R (R Core Team 2018). Lentil genotypes were fitted as a fixed effect, and plot repetitions and slides from each plot fitted as random effects. Pearson correlation coefficients between data generated with the HunterLab and Nix were also calculated. Plots were generated using the ggplot2 package in R (Wickham 2016).

RESULTS

The mean L*, a* and b* values for all eight lentil genotypes varied between the HunterLab and the Nix and between the two Nix illuminants (Table 3). L* scores were the highest with the HunterLab, and the differences between the two instruments were greater than those observed between the two Nix illuminants. The intensity of red color, as measured by the a* values also varied among instruments and showed greatest variation and highest a* values when samples were evaluated with the Nix using illuminant D50. These were followed by the HunterLab, and the lowest mean was observed with the Nix illuminant C. The b* scores for all lentil genotypes had the lowest variation across instruments, with the HunterLab results having the highest mean. Differences observed among lentil genotypes were significant with both instruments (Table 3, Appendix S1 for genotype means). The differences observed between means from different field

plots were also significant. There were no differences among slide repetitions (technical reps), except for a* and b* obtained with the HunterLab.

Although the absolute values of color space were different between the two instruments, both were able to distinguish color differences among lentil flour from different genotypes, and the scores obtained with both Nix illuminants were positively correlated with those measured by the HunterLab (Figure 2). HunterLab L* scores had similar positive correlations with both illuminants of the Nix instrument; however, the correlations were stronger for a* and b* scores between the two instruments measurements for both illuminants.

Differences established among genotypes with the Nix were consistent with those established with the HunterLab (Figure 3, Appendix S1). Lentil genotypes CDC Robin, ILL 213 and PI 612875, had the lowest L* scores with all three methods, while CDC Redberry, CDC KR-1 and W27760 had the highest values. Similarly, CDC KR-1, ITACA and CDC Redberry, had the lowest b* scores, while CDC Robin, CN 105604 and PI 612875 had the highest b* scores, regardless of the colorimeter used. With all three methods the intensity of red color (a*) was higher for CDC Robin and CN 105604, and the highest for PI 612875 among all genotypes with both instruments (Figure 3).

DISCUSSION

Color is an important characteristic used to assess product quality (Bicho et al., 2014; Cabas-Lühmann & Manthey, 2020; Sant'Anna et al., 2014; Švec et al., 2008). Breeding programs and industry partners benefit from methods that can be used systematically without incurring time-demanding and expensive phenotyping. This study validated the use of the hand-held,

inexpensive Nix instrument and its methodology for assessing color in lentil flour samples in a timely and accurate manner.

Differences in absolute values of L*, a*, and b* scores between instruments and illuminants were expected and previously reported (Dang et al., 2021; Holman et al., 2021; Stiglitz et al., 2016; Ugarčić-Hardi et al., 1999). However, the absolute values are irrelevant in this use-case as the main goal was to differentiate color among lentil samples and to accurately identify genotypes with higher a* scores. The Nix and the HunterLab results ordered the lentil genotypes from the lowest to the highest a* scores and discriminated lentil flour genotypes. Both instruments generating the same order of genotypes, indicate that the Nix is producing the same results as the HunterLab, validating its use as a colorimeter in this application to food color analysis.

The positive correlations observed among L* scores obtained with both instruments were lower than those observed for a* and b* scores. The differences of the L* values found between instruments could be due to differences in methodologies and, or light source differences. It has been reported by Revantino et al., (2022) that temperature and device geometry can influence L* scores; however, the same illuminants are used with both instruments, and 45°/0° is the measurement geometry for both instruments (Table 2.) A brighter light source could explain the differences in L* values. While both LED and Xenon Laser are reliable light sources – both cover similar spectral distribution of visible light: 200~800 nm – LED works best in small spectrophotometers (Gao et al., 2012). The HunterLab uses a Xenon Laser, a brighter light source than broad spectrum LED used by the Nix. Additionally, when scanning samples with the HunterLab the instrument's aperture is directly exposed to the influence of external light, whereas the Nix's aperture is isolated from external light with the help of an adapter. This

difference in methodologies can have a direct impact in the obtained L* values. Brightness is important to score, however, in this case, a* is the most relevant. Based in our observations while using both technologies, it is evident that samples have more exposure to external light when using the HunterLab method; another potential advantage of the Nix over the HunterLab for small samples.

Holman et al., (2021) and Jha et al., (2021) proposed that using more slide repetitions for Nix colorimetric analysis would improve data precision to account for smaller structural dimensions. We also expected to have differences among slides (technical reps) due to flour structural variation; however, these were not found with either illuminant. The flour surface flattening technique used when preparing slides likely helped improve the accuracy of our readings with the Nix, but this difference was not observed in detail during the study. The small variations observed between slides suggest that fewer repetitions can produce a similar outcome, thus opening up the possibility to scan fewer repetitions per sample and generate the same results. This is particularly useful when sample volume is limited. The HunterLab, however, exhibited differences among slide repetitions for a* and b* scores, indicating that multiple repetitions are needed to obtain more accurate results.

The Nix gave us the ability to determine color in much smaller samples than the HunterLab. In breeding research programs, phenotyping with destructive methods can be limited by small amounts of seed. Currently, this type of colorimetry is destructive (i.e., requires seed grinding), so smaller volumes of flour for measurement is desirable. Finally, data is more accurately collected and efficiently managed from the Nix Toolkit app. Manually transferring data from the HunterLab to an MS Excel file increases the risk for error or data loss; whereas the automated

handling of L*a*b* scores via the Nix App reduces human error and simplifies data management.

The Nix Spectro 2 will be used systematically in our breeding program for determining color in lentil flour of populations developed for different research purposes. In addition to validating the use of the Nix, this work identified genotypes PI 612875 and CN 105604 as darker red color lines among evaluated individuals. This will be further evaluated for color stability in final products.

Author contributions

Sarahi Viscarra: investigation, data curation, formal analysis, visualization, writing - original draft preparation.

Ana Vargas: conceptualization, investigation, data curation, formal analysis, methodology, visualization, project administration, writing – original draft preparation, funding acquisition.

Laura Jardine: conceptualization, methodology, project administration, writing – review & editing, funding acquisition.

Mehmet Tulbek: conceptualization, writing – review & editing, funding acquisition.

Kirstin Bett: conceptualization, writing – review & editing, funding acquisition.

Acknowledgments

This research was supported by the 'Enhancing the Value of Lentil Variation for Ecosystem Survival (EVOLVES)', a Genome Prairie managed project funded by Genome Canada [grant: LSP18-16302], with matching financial support from Western Grain Research Foundation [grant: GC1903], the Government of Saskatchewan [grant: 20200026], the University of Saskatchewan

and a Mitacs Accelerate [grant: IT29802]. We thank the Grains Innovation Lab at the University of Saskatchewan for facilitating the use of their HunterLab instrument.

Data Availability Statement

The data supporting this study are available online (https://knowpulse.usask.ca/experiment/phenomics/Detecting-Color-Differences-Lentil-Flour-Using-Inexpensive-Colorimeter)

Supporting Information

Additional supporting information can be found online in the Supporting Information section at the end of this article.

ORCID

Sarahi Viscarra: https://orcid.org/0009-0002-0275-7376

Ana Vargas: https://orcid.org/0000-0003-3293-3641

Laura Jardine: https://orcid.org/0009-0005-7421-1542

Mehmet Tulbek:

Kirstin Bett: https://orcid.org/0000-0001-7959-6959

Conflict of Interest

Authors have no known conflicts of interest with respect to this article.

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Table 1. Lentil genotypes evaluated for flour color.

Accession	Origin	Source
CDC Redberry	Canada	CDC, USask[a]
CDC Robin	Canada	CDC, USask
CDC KR-1	Canada	CDC USask
Itaca	Italy	UNIVPM[b]
CN 105604	Ethiopia	PGRC[c]
ILL 213	Afghanistan	ICARDA[d]
PI 612875	Syria	USDA-ARS[e]
W 27760	Egypt	USDA-ARS

a: Crop Development Centre, University of Saskatchewan, Saskatchewan, Canada, b: Università Politecnica delle Marche, c: Plant Gene Resources of Canada, d: International Centre for Agricultural Research in the Dry Areas, e: United States Department of Agriculture-Agricultural Research Services.

Table 2. General specifications of the HunterLab MiniScan XE Plus and Nix Spectro 2 used to determine lentil flour color.

	HunterLab Mini XE Plus	**Nix Spectro 2**
Aperture	35 mm	5 mm
Light Source	Xenon Light	Broad spectrum LED (white, violet and UV)
Measurement Geometry	45°/0°	45°/0°
Illuminant	C	C (correlated temperature 6774K) D50 (correlated temperature 5003K)
Observant	2°	2°
Colour Space	CIE L*a*b*	CIE L*a*b*
Dimensions (W x H x L)	10.2 cm, 12.7 cm, 25.4 cm	4.5 cm, 6 cm, 6 cm
Weight	1300 g	110 g

Table 3. Color space mean values and significance of effects of eight lentil genotypes evaluated with a HunterLab Mini XE Plus and a Nix Spectro 2 with C and D50 illuminants.

Instrument	**Color Space**		
	L*	**a***	**b***
HunterLab	86.38[a]	7.57[b]	21.5[a]
Lentil genotype	***	***	***
Field Plot	***	***	***
Slide repetition	ns	***	***
Nix - C	82.54[c]	6.58[c]	19.66[c]
Lentil genotype	***	***	***
Field Plot	***	***	***
Slide repetition	ns	ns	ns
Nix - D50	82.86[b]	9.59[a]	20.11[b]
Lentil genotype	***	***	***
Field Plot	***	***	***
Slide repetition	ns	ns	ns
LSD	0.20	0.16	0.25
CV	1.4	11.8	10.2

ANOVA values are ns: not significant, *, **, ***: significant at 0.05, 0.01, 0.001 respectively. Means for L*, a* and b* scores among instruments with different letters are significantly different from each other according to LSD test.

Appendix S1. Significance of effects and means for color space values for 8 lentil genotypes evaluated with HunterLab Mini XE Plus and Nix Spectro 2 with C and D50 illuminants.

	HunterLab Mini XE Plus			**Nix Spectro 2 - C**			**Nix Spectro 2 - D50**		
	L	**a**	**b**	**L**	**a**	**b**	**L**	**a**	**b**
ANOVA									
Lentil genotype	***	***	***	***	***	***	***	***	***
Field Plot	***	***	***	***	***	***	***	***	***
Slide repetition	ns	***	***	ns	ns	ns	ns	ns	ns
Lentil genotypes									
CDC Redberry	87.19^{b}	6.58^{e}	19.95^{g}	83.45^{a}	5.61^{f}	18.12^{g}	83.81^{a}	8.51^{f}	18.41^{e}
CDC Robin	86.10^{e}	7.47^{c}	21.19^{e}	82.29^{cd}	6.52^{d}	19.46^{e}	82.36^{d}	9.49^{d}	19.88^{c}
CDC KR-1	87.49^{a}	6.52^{e}	19.88^{g}	83.42^{a}	5.60^{f}	18.00^{g}	83.78^{a}	8.46^{f}	18.34^{e}
ITACA	86.38^{d}	7.37^{d}	20.41^{f}	82.21^{de}	6.37^{e}	18.60^{f}	82.81^{c}	9.26^{e}	18.90^{d}
CN 105604	86.31^{d}	8.75^{a}	22.39^{b}	82.71^{bc}	7.56^{b}	20.47^{b}	83.04^{bc}	10.62^{b}	20.93^{b}
ILL 213	85.24^{g}	7.70^{b}	21.90^{c}	81.35^{f}	6.84^{c}	20.05^{c}	81.62^{e}	9.95^{c}	20.67^{b}
PI 612875	85.76^{f}	8.75^{a}	24.79^{a}	81.84^{e}	7.70^{a}	22.98^{a}	82.20^{d}	11.06^{a}	23.66^{a}
W27760	86.57^{c}	7.29^{d}	21.56^{d}	83.10^{ab}	6.33^{e}	19.72^{d}	83.29^{b}	9.31^{de}	20.13^{c}
Mean	**86.4**	**7.6**	**21.5**	**82.5**	**6.6**	**19.7**	**82.9**	**9.6**	**20.1**
LSD	0.15	0.08	0.15	0.43	0.13	0.26	0.42	0.18	0.29
CV	0.36	2.09	1.42	1.08	3.84	2.94	1.05	3.94	2.67

ANOVA values are ns: not significant, *, **, ***: significant at 0.05, 0.01, 0.001 respectively. Mean values for each genotype with different letters are significantly different from each other according to LSD test.

Figure 1. Procedure followed when using the HunterLab MiniScan XE Plus (left) and Nix Spectro 2 (right) for instrument validation. A.1: perform white tile normalization; A.2: adjust app settings A.3: place lentil flour in low-volume adapter; A.4: evenly spread and flatten lentil flour with a small spatula; A.5: place low-volume adapter lid; A.6: scan sample with Nix; A.7: label and save color in library; A.8: Export CSV file to device. B.1: Standardize HunterLab; B.2: place lentil flour in Petri dish; B.3: flatten lentil flour by tapping Petri dish on the table; B.4: place Petri dish on top of HunterLab's opening and scan; B.5: obtain L*, a*, and b*; B.6: manually copy L*, a*, and b* scores to an Excel Sheet.

Figure 2. Correlations between L* a* b* color space values of 87 lentil samples evaluated with HunterLab Mini XE Plus and Nix Spectro 2 with C and D50 illuminants.

Figure 3. 3D plots of the mean L*, a*, and b* scores distribution from the eight genotypes with three different instruments HunterLab (top), Nix illuminant C (middle), Nix illuminant D50 (bottom). L* in the y-axis, a* in the x-axis, and b* in the z-axis.

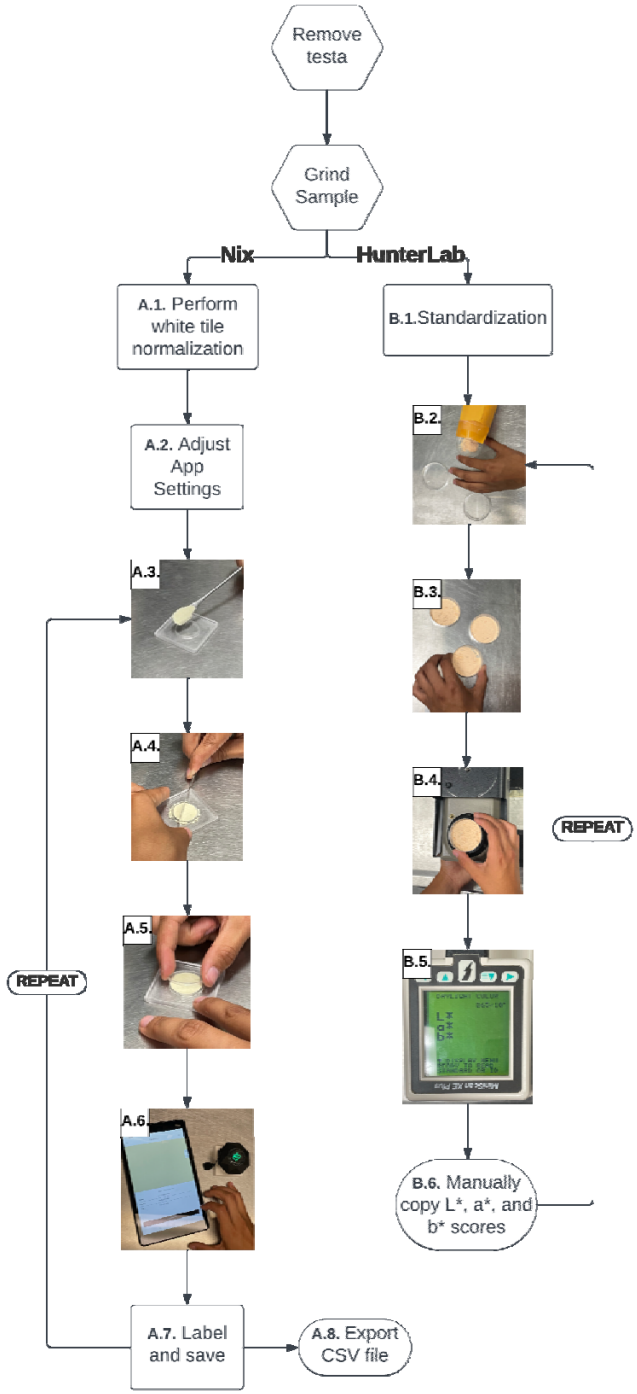
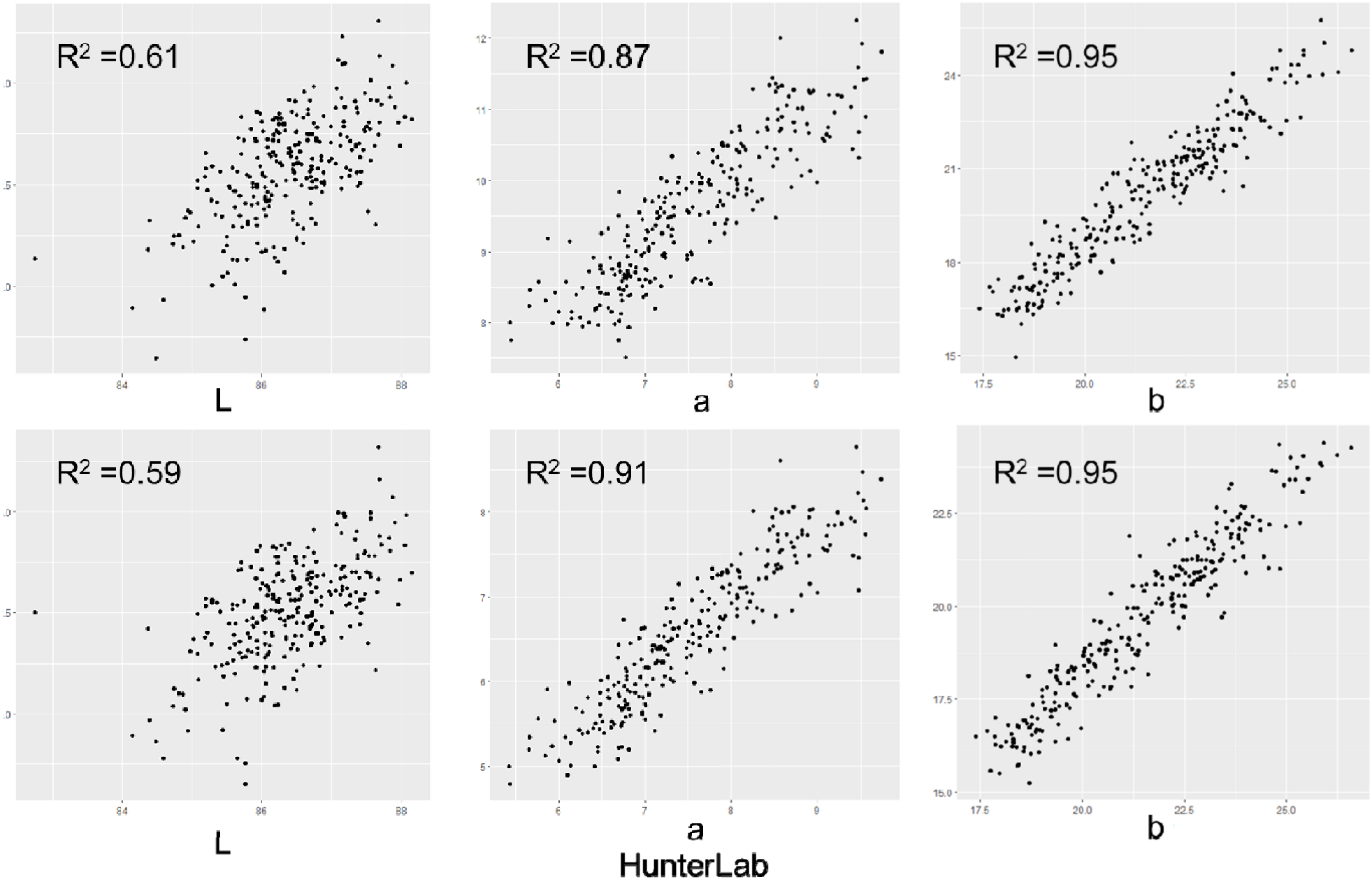
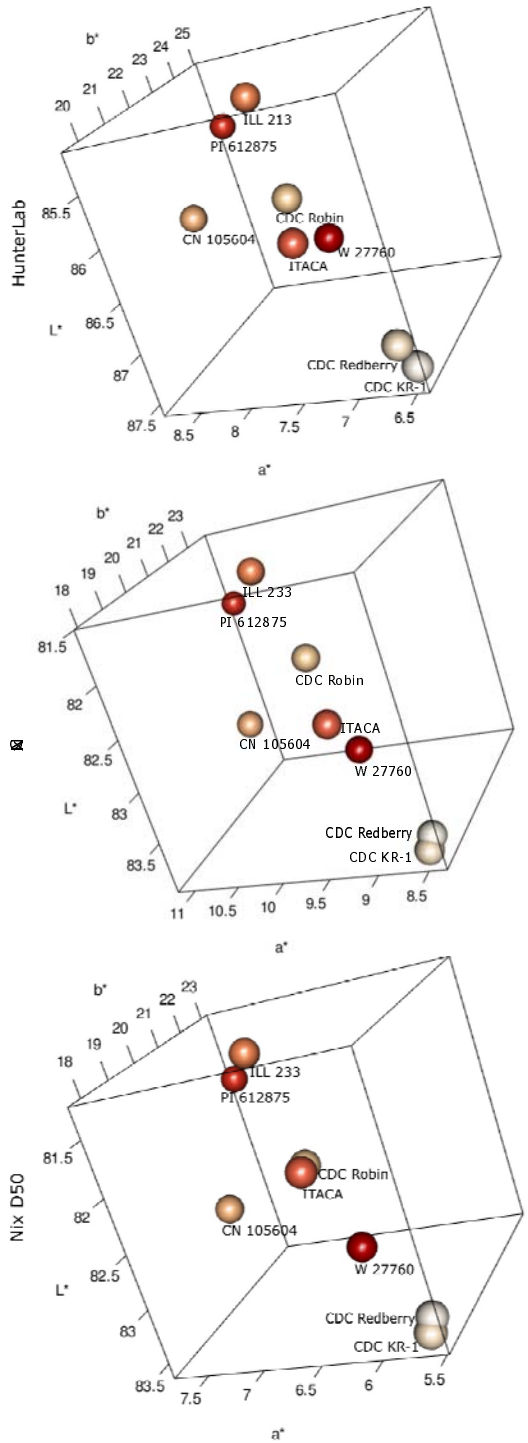


Figure 1. Procedure followed when using the HunterLab MiniScan XE Plus (left) and Nix Spectro 2 (right) for instrument validation. A.1: perform white tile normalization; A.2: adjust app settings A.3: place lentil flour in low-volume adapter; A.4: evenly spread and flatten lentil flour with a small spatula; A.5: place low-volume adapter lid; A.6: scan sample with Nix; A.7: label and save color in library; A.8: Export CSV file to device. B.1: Standardize HunterLab; B.2: place lentil flour in Petri dish; B.3: flatten lentil flour by tapping Petri dish on the table; B.4: place Petri dish on top of HunterLab's opening and scan; B.5: obtain L*, a*, and b*; B.6: manually copy L*, a*, and b* scores to an Excel Sheet.



gure 2. Correlations between L* a* b* color space values of 87 lentil samples evaluated with HunterLab Mini XE Plus and Nix Spectro 2 with C and 50 illuminants.



- **Figure 3.** 3D plots of the mean L*, a*, and b* scores distribution from the ei genotypes with three different instruments HunterLab (top), Nix illuminant C (m Nix illuminant D50 (bottom). L* in the y-axis, a* in the x-axis, and b* in the z-a