RESEARCH ARTICLE

European Journal of
Lipid Science and Technology
www.ejlst.com

Precise Color Communication by Determination of the Color of Vegetable Oils and Fats in the CIELAB 1976 ($L^*a^*b^*$) Color Space

Ludger Brühl and Günter Unbehend*

The precise determination of the colour of oils and fats is a common and important parameter for the production of oils, fats, margarine, and mayonnaise. A new method has been developed and tested in order to improve reliability and reproducibility of the results. It is based on colour values, which describe the colour in a 3D space as defined by the International Commission on Illumination (CIE) as CIE 1976 (L*a*b*). The precision data obtained from a collaborative method validation test show significant improved repeatability with relative coefficients of variation of repeatability between 0.1 and 1.7% except for a very dark pumpkin seed oil (2.7%). The coefficients of variation of reproducibility range from 0.7 to 13.9 %. The signals of very intense colored samples might exceed the linear range of some measuring instruments. Solutions of colour standards tartrazine and Ponceau 4R as pure (>99 %) and non-toxic substances are tested for laboratory calibration covering a colour space from CIE 1976 L* (63–99), a* (−23–67), and b* (0.5–119). A storage experiment with these standard substances is carried out and the results show improved stability over a period of 80 days compared to bromothymol blue and iodine standard solutions.
***Practical Applications*: The results of this study show the suitability of an analysis method for the determination of the colour of oils and fats using the 3D CIE 1976 colour space as an alternative to the common Lovibond system of red and yellow colour values. As the international harmonized approach of the CIE 1976 colour space is widely accepted for the communication of precise colour description and differences between two different colors in many applications all over the world, this will be also an advantage for the communication and specification of colors in the oils and fats industry, market, and trade. In addition, the use of easy to prepare colour standard solutions enables every laboratory to check the suitability of their colour measuring device in a convenient way.**

1. Introduction

Since many years the colour of vegetable oils and fats was often specified for production and trade with Lovibond values of red and yellow.[1–3] In a detailed laboratory collaborative test with all laboratories using the same apparatus and the calibration colour standards from the very same manufacturer reasonable precision data were obtained by Wan in 1997.[3] However, a more recent collaborative trial organized by ISO revealed limited comparability for most samples[4] regardless of the apparatus used or whether automatic or visual colour evaluation was carried out.

On the other hand, there are results of laboratory proficiency tests, which enable the participating laboratories to use their individual apparatus, calibration and method. The proficiency test carried out by the German Society of Fat Research (DGF) showed very limited comparability of results from different laboratories.[5] In addition, experts from industry reported about complaints regarding comparability in the communication of precise colour description of oils and fats especially with discolored oils. The problems with the Lovibond system and green colored olive oils is also reviewed by Moyano.[6]

There had been numerous approaches to improve the determination of the colour. While some relied more on visual colour comparison, there were other approaches to get more comparable results using automated methods. The former visual comparative procedures like the visual Lovibond colour value and the Gardner colour value are still in use due to above mentioned problems and these methods are still available parameters in the yearly laboratory proficiency test in Germany. The results for Lovibond colour index obtained with both procedures as automated and visual determination are both not convincing as there are mostly bimodal distributed results or the standard deviations in relation to the assigned value exceeded 30%, respectively. In addition, the mean results for visual and automated methods deviate significantly.[5]

Dr. L. Brühl, G. Unbehend
Max Rubner-Institut
Federal Research Institute of Nutrition and Food
Institute of Safety and Quality of Cereals
Detmold 32756, Germany
E-mail: Ludger.bruehl@mri.bund.de

DOI: 10.1002/ejlt.202000329

European Journal of Lipid Science and Technology
www.ejlst.com

The colour of olive oils was determined by bromothymol blue (BTB) colour index[7] or the uniform oil colour scale (UOCS).[8] In a later attempt[9] this UOCS was developed further to the modified uniform oil colour scale (MUOCS). While the initial BTB colour standard solutions were used to visually compare olive oils, this concept was based on the more universal system of CIELAB,[10] the recommended system by the International Commission on Illumination (CIE).[11] This system specifies a colour by three parameters L*, a*, and b*, which can be visualized in a 3D space. L* is defined as the lightness from transparent or white to black as the vertical or y-axis, a* ranges from green to red as the x-axis and b* from yellow to blue as the z-axis.

Also, the determination of the colour of palm oil[12] was characterized by several methods in order to improve the common Lovibond system. Therefore, a new alternative method for the determination of the colour also of other oils and fats would be desirable.

The purpose of the present work was to set up an alternative method for the determination of the colour of edible fats and oils based on the CIE 1976 system and to determine the precision data for this method. In addition, a new possibility to improve the calibration and test of the measurement apparatus with defined colour reference solutions was developed and their stability was evaluated in a storage experiment.

2. Experimental Section

Grapeseed oil, extra virgin olive oil, palm oil, pumpkin seed oil, sesame seed oil, sunflower oil, and rapeseed oil were obtained from local markets and stored at 6 °C in the dark until analysis. All oils were filtered and did not contain any sediments. Palm oil was melted at 50 °C and homogenized before measurements. *N*-heptane as solvent for blank/white control, iodine, doubled sublimized, potassium iodine, and bromothymol blue were all of analysis grade and obtained by Merck (Darmstadt, Germany). Tartrazine (Trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(E)-(4-sulfonatophenyl) diazenyl]-1*H*-pyrazole-3-carboxylate, CAS-No. 1934-21-0), and Ponceau 4R (Trisodium (8Z)-7-oxo-8-[(4-sulfonatonaphthalen-1-yl) hydrazinylidene] naphthalene-1, 3-disulfonate, CAS-No. 2611-82-7) were also obtained by Merck (Darmstadt, Germany) as analytical standard substances at purity >99%.

2.1. Iodine Colour Value, Tartrazine and Ponceau 4R Colour Standard Solutions

Iodine colour value (ICV) standard solutions were prepared according to DGF standard method C-IV 4a (20) ICV.[13] Iodine was weighted together with potassium iodide into a volumetric flask and diluted solutions were prepared from this stock solution with concentrations of 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 40, 50, 65, 80, and 100 mg L^{-1} iodine.

Tartrazine and Ponceau 4R colour standard solution were prepared by weighting 10.00 mg of each substance into a 10 mL volumetric flask and filling up to the mark with distilled water. Dilutions were prepared at concentrations of 1, 3, 5, 10, 30, 50, and 100 mg L^{-1} for tartrazine and at concentrations of 1, 5, 10, 14, 20, 30, 50, and 100 mg L^{-1} for Ponceau 4R.

Table 1. Composition of bromothymol blue standard solutions in ml comprising a mix of three solutions: BTB, bromothymol blue 0.4 g L^{-1}; PDHP, potassium dihydrogenphosphate 9.08 g L^{-1}; and DSHP, disodium hydrogenphosphate dihydrate 11.87 g L^{-1} according to ref. [7].

BTB solution	BTB	PDHP	DSHP
4/4	80	900	100
6/3	60	850	150
4/6	120	900	100
2/3	60	950	50
3/8	160	925	75
2/10	200	950	50

The CIELAB values of each of all standard solutions were determined in triplicate according to DGF method C-IV 4f (19) CIELAB colour value of oils and fats.[13]

2.2. Bromothymol Blue Colour Index Standard Solutions

Colour standard solutions were prepared according to González-Quijano.[7] BTB was mixed in a mortar with sodium hydroxide solution and washed quantitatively into a volumetric flask. Aliquots of the BTB stock solution were mixed with varying amounts of buffers solutions in order to obtain colour reference solutions for the comparison of the colour of olive oils. For the purpose of this work the six BTB standard solutions, which represented the colour of most olive oils as described in detail by Melgosa[8] were chosen. The standard solutions tested in this study were 4/4, 6/3, 4/6, 2/3, 3/8, and 2/10. For the detailed composition see **Table 1**. The CIELAB values of each of these colour value standard solutions were determined in triplicate according to DGF method C-IV 4f (19).[13]

2.3. Measurement of the Colour of Oils and Fats as CIELAB Values

For measurements, the completely melted oil with no visible solids or sediments was filled in cuvettes of 1 cm optical path length. CIELAB values are reported based on the definition of a 2° observer and light source C. Colour measurements in the laboratory were all carried out with a colour measurement device (Chromameter CR-5, Minolta, Langenhagen, Germany). The device was equipped with an integrating sphere of 152 mm, a pulsed xenon lamp with UV cut filter and used a measuring time of 1 s.

In addition, the colors of some standard solutions have been measured with a UV–vis photometer (Specord 250, Analytik Jena, Jena, Germany). For measurements with this photometer, sample and reference spectra were recorded at a slit width of 4 nm for wavelengths from 360 to 830 nm using 1 nm intervals, at 0.5 nm s^{-1} scan speed and using *n*-heptane as blank sample. This photometer was able to register data with a linear absorbance up to about three units and was operated without an integrating sphere.

2.4. Collaborative Trial on the Determination of the Colour using Deutsche Gesellschaft für Fettwissenschaft—German Society of Lipid Science Method C-VI 4f (19) CIELAB Colour Value of Oils and Fats

In order to determine precision and reproducibility data for the new method,[13] sets of eight samples coded from A to H were disseminated to the participating laboratories containing cold pressed pumpkin seed oil, virgin rapeseed oil, extra virgin olive oil, refined grapeseed oil, refined sesame seed oil, virgin sunflower seed oil, refined rapeseed oil, and crude palm oil. The participating laboratories used different brands of colour measuring apparatus like LICO 500 and LICO 690 (Hach Lange, Düsseldorf, Germany), Lovibond PFX990, and PFX-i series (Tintometer, Dortmund, Germany), Chromameter CR-5 and Konica CM-5 (Minolta, Langenhagen, Germany), Specord 250 photometer (Analytik Jena, Jena, Germany). Each sample was measured as a coded duplicate by the participating laboratories. For measurements, the completely melted oil with no visible solids or sediments was filled in cuvettes of 1 cm optical path length. CIELAB values are reported based on the definition of a 2° observer and light source C, because many companies still operate colour measurement devices, which are only capable to measure with these definitions. Therefore, the authors were able to evaluate the collaborative trial with the parameters of a 2° observer and light source C, only. Samples were sent out in August and results were obtained until November 2019.

Statistical evaluation was done according to ISO 5725:2019 for accuracy (trueness and precision) of measurement methods and results—Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.[14] Tests for straggler and outlier results was done using Chochran and Grubbs test. The definition of repeatability and reproducibility by ISO is given as follows: Repeatability conditions were defined as conditions under which test results were obtained with the same method, on identical test material, in the same laboratory, by the same operator, using the same equipment and reagents, within a short interval of time while reproducibility conditions are defined as conditions under which test results are obtained with the same method, on identical test material, in different laboratories, by different operators, using different equipment and reagents, within a short interval of time.

3. Results and Discussion

3.1. Collaborative Test for Method Validation with the New Deutsche Gesellschaft für Fettwissenschaft—German Society of Lipid Science Standard Method C-VI 4f (19) CIELAB Colour Value of Oils and Fats

It was an unexpected problem to find a sufficient number of participants for the collaborative trial with an instrument that was able to report CIELAB values based on the newer definitions of a 10° observer and light source D_{65}. Many devices still used in the participating laboratories were only able to report CIELAB values based on the older definitions of a 2° observer and light source C. However, these later results differ from the results obtainable with the newer definitions by applying different data sets for the light source and the observer in the calculation of the final results, only. Since standardization should always reflect the requirements of the practice, the collaborative trial was carried out using the older definitions.

Tables 2–4 show the results obtained for CIELAB values and precision data calculated according to the regulation of ISO 5725. The table showed improved repeatability and reproducibility for this method. A very broad variation of colours of the oils had to be determined in the trial reaching from an almost black pumpkin seed oil, a greenish extra virgin olive oils up to a red palm oil. For most of all parameters valid and good precision data were obtained except for a* and b* values of pumpkin seed oil. These exceptions were attributable to the very strong light absorption of the pumpkin seed oil with a lightness at about 2. In order to give an estimate of the colours of the samples a colour patch is shown in Figure S1, Supporting Information. Most of the common edible oils showed lightness values in the range of 80–100, while the crude palm oil showed also a significantly reduced lightness at 51. The repeatability of the determination was very good in most cases. For the reproducibility in different laboratories we obtained some relative coefficients of variation (RCV) above 10% for lightness and for a* values. However, all RCV were below 15%. The reproducibility limit is a measure how much two results obtained by two laboratories can deviate. For this limit we obtained precision data reaching from 0.5 up to 14. A difference of about 1 unit can be recognized by a trained person depending on the intensity and on the colour.[15,16]

The calculation of Horrat values is not applicable as a unit is not assigned to CIELAB values.[17] However, instead of Horrat values the relative coefficient of the reproducibility standard deviation can serve as a good indicator for comparable and reliable results. Further evaluation of some rejected results showed the need of reference colour standard solutions in the laboratories in order to detect problems with the instrument. In addition, the use of RCV might be of limited significance for very low results as a given deviation compared to a small or a high result will show a relatively high or small coefficient of variation, respectively.

The intense colored oils like pumpkin seed oil and palm oil were beyond the linear range of some apparatus used in this study. Therefore, some results had to be eliminated as straggler or outlier due to significant deviation from the rest of the results by Cochran or Grubbs test. Even other oils showed absorption maxima at the upper end of the linear range of spectrophotometers. While some photometers provide a linear range up to about 3 absorption units, former photometers linear absorptions were obtained from 0.1 to 1, only. These later were not suitable for precise colour measurement of more intense colored samples. The importance of complying with the linear range was also shown by Gomez-Robledo et al.[18] They showed that for some instruments even olive oils needed to be measured in cuvettes of 0.5 cm of optical path length instead of usual used 1 cm cuvettes. However, this can also be attributed to light scattering effects, which increased along with increasing path length. Turbid oils have to be filtered or centrifuged to obtain a clear oil. Any turbidity will affect the result as shown in detail by Gordillo.[19]

The strong light absorption of the pumpkin oil demanded for a high radiation intensity for a linear transmission measurement. In spectrophotometers, a wide slit was used for measurements together with a long integrating time and a slow scan speed in

Table 2. CIE 1976 (L*) results of the statistical evaluation of the collaborative test (CIE values are without dimension). A: pumpkin seed oil; B: virgin rapeseed oil; C: extra virgin olive oil; D: grapeseed oil; E: sesame seed oil; F: sunflower seed oil; G: refined rapeseed oil; H: crude palm oil.

Sample	A	B	C	D	E	F	G	H
Number of participating laboratories (*N*)	10	10	10	10	10	10	10	10
Number of laboratories retained after eliminating outliers (*n*)	8	10	10	10	10	10	10	9
Number of individual test results of all laboratories on each sample (*z*)	16	20	20	20	20	20	20	18
Mean value (*m*)	2.09	91.47	86.81	99.44	97.53	99.42	98.58	51.55
Repeatability standard deviation (s_r)	0.06	0.33	0.26	0.16	0.23	0.18	0.06	0.48
Relative coefficient of variation of repeatability (CVs_r %)	2.7	0.4	0.3	0.2	0.2	0.2	0.1	0.9
Repeatability limit *r* (2.8 s_r)	0.16	0.91	0.73	0.46	0.66	0.50	0.16	1.35
Reproducibility standard deviation (s_R)	0.29	1.77	5.06	1.45	2.03	1.52	1.55	1.28
Relative coefficient of variation of reproducibility (CVs_R %)	13.9	1.9	5.8	1.5	2.1	1.5	1.6	2.5
Reproducibility limit *R* (2.8 s_R)	0.82	4.96	14.15	4.07	5.68	4.26	4.34	3.60

Table 3. CIE 1976 (a*) results of the statistical evaluation of the collaborative test (CIE values are without dimension). A: pumpkin seed oil; B: virgin rapeseed oil; C: extra virgin olive oil; D: grapeseed oil; E: sesame seed oil; F: sunflower seed oil; G: refined rapeseed oil; H: crude palm oil.

Sample	A	B	C	D	E	F	G	H
Number of participating laboratories (*N*)	10	10	10	10	10	10	10	10
Number of laboratories retained after eliminating outliers (*n*)	7	9	10	9	9	9	9	9
Number of individual test results of all laboratories on each sample (*z*)	14	18	20	18	18	18	18	18
Mean value (*m*)	16.35	−6.59	−9.28	−4.68	−6.78	−2.32	−5.58	41.76
Repeatability standard deviation (s_r)	0.25	0.09	0.08	0.04	0.01	0.03	0.05	0.11
Relative coefficient of variation of repeatability (CVs_r %)	1.5	1.4	0.8	0.9	0.2	1.4	0.9	0.3
Repeatability limit *r* (2.8 s_r)	0.69	0.26	0.21	0.12	0.03	0.09	0.15	0.32
Reproducibility standard deviation (s_R)	0.35	0.81	1.22	0.22	0.38	0.20	0.37	1.75
Relative coefficient of variation of reproducibility (CVs_R %)	2.1	12.3	13.2	4.7	5.6	8.4	6.7	4.2
Reproducibility limit *R* (2.8 s_R)	0.97	2.27	3.42	0.62	1.07	0.55	1.05	4.89

order to get more light through the sample and to improve the linear range for intense colored samples. The determination of the colour for tartrazine and Ponceau 4R standard solutions using two different Measurement devices Chromameter CR-5, Minolta, Langenhagen, Germany and the UV–vis photometer (Specord 250, Analytik Jena, Jena, Germany revealed deviations up to four units especially for CIELAB a* and to a lesser extend for CIELAB b* values as can be seen in Figures S6 and S7, Supporting Information. However, the results of the collaborative trial also showed that no brand for colour determination apparatus had to be excluded from the trial.

Wan et al.[3] published the results of a collaborative trial and presented good results for selected oils and fats using automatic and visual Lovibond and AOCS methods, when all laboratories used the same apparatus from the same manufacturer and the same standard glass reference samples which had been provided by the manufacturer of the apparatus especially for this collaborative trial. The results of another collaborative trial carried out by ISO[4] obtained RCV of the reproducibility from 25.3 to 89.9% for results of Lovibond red values of samples like refined palm oil, distilled lauric fatty acids, respectively. For a cold pressed rapeseed oil, it was not possible to obtain a sufficient number of valid results (only five) for automatic instruments.

3.2. Lessons to Learn from Common Practice, For Example, the Deutsche Gesellschaft für Fettwissenschaft—German Society of Lipid Science Laboratory Proficiency Test 2019

There is a significant difference between collaborative trials from a standardization organization and a proficiency test. While for both purposes a set of identical samples will be analyzed by a group of laboratories during a defined time interval, in a collaborative trial the laboratories should carry out the analyses in the same way using the same method for the determination of the

Table 4. CIE 1976 (b*) results of the statistical evaluation of the collaborative test (CIE values are without dimension). A: pumpkin seed oil; B: virgin rapeseed oil; C: extra virgin olive oil; D: grapeseed oil; E: sesame seed oil; F: sunflower seed oil; G: refined rapeseed oil; H: crude palm oil.

Sample	A	B	C	D	E	F	G	H
Number of participating laboratories (*N*)	10	10	10	10	10	10	10	10
Number of laboratories retained after eliminating outliers (*n*)	7	9	9	8	8	9	9	9
Number of individual test results of all laboratories on each sample (*z*)	14	18	18	16	16	18	18	18
Mean value (*m*)	3.53	133.12	112.13	12.49	29.23	6.64	14.77	87.70
Repeatability standard deviation (s_r)	0.06	0.18	0.30	0.14	0.18	0.07	0.13	0.86
Relative coefficient of variation of repeatability (CVs_r %)	1.7	0.1	0.3	1.1	0.6	1.1	0.9	1.0
Repeatability limit *r* (2.8 s_r)	0.17	0.51	0.84	0.40	0.51	0.20	0.36	2.41
Reproducibility standard deviation (s_R)	0.27	2.05	0.75	0.27	0.45	0.60	1.34	2.26
Relative coefficient of variation of reproducibility (CVs_R %)	7.7	1.5	0.7	2.2	1.5	9.1	9.1	2.6
Reproducibility limit *R* (2.8 s_R)	0.76	5.73	2.11	0.76	1.27	1.68	3.76	6.32

Table 5. Assigned values (AV), standard deviation for proficiency assessment (SD), and relative coefficient of variation (RCV) of results from three samples (S) coded A to C of Lovibond red and yellow values for automatic and visual determination and of Gardner colour index from laboratory comparison test 2019 (LVU). Number of results obtained for the three samples for automated Lovibond 29–33, for visual Lovibond 12–13, and for Gardner colour index 25–29[5].

S	Lovibond												Gardner colour index		
	Red						Yellow								
	Automatic			Visual			Automatic			Visual					
	AV	SD	RCV	AV	SD	RCV	AV	SD	RCV	AV	SD	RCV	AV	SD	RCV
A	6.6	1.7	26	3.5	1.1	31	nd	nd	nd	41.2	21.9	53	6.21	0.44	7
B	nd	nd	nd	4.8	2	42	nd	nd	nd	52.3	28.4	54	9.67	0.84	9
C	10	3.1	31	7.6	2.3	30	nd	nd	nd	58.8	14.3	24	6.83	0.82	12

nd: not determined due to bimodal distribution.

precision data of a certain method, but in a proficiency test the laboratories are free to apply their own methods. Collaborative trials are carried out to show the obtainable precision under best conditions, while proficiency tests reflect more the situation in daily practice.

In 2019 the 25th laboratory proficiency test was carried out in Germany by DGF using three oil samples coded A to C. Sample A was a vegetable oil mix, sample B an extra virgin olive oil and sample C a used frying oil. All samples contained some small additions in order to modify the different parameters to be analyzed. In the proficiency test all participating laboratories (n = 101) had free choice of the method, apparatus and parameters to be reported. Amongst other parameters, laboratories were able to report on Lovibond red and yellow values by automatic or visual determination. In addition, the Gardner colour index could be specified. For each of these parameters a number of laboratories, which are usually doing such determinations in their routine work, have send in their results and got an evaluation in return. For each result the applied method could be specified, too. Due to this specification the evaluation of the data was divided into visual and automated determinations. **Table 5** shows the results obtained for the different methods of colour determination. The automatic determination of Lovibond colour scale data often yielded in a bimodal distribution of the results, while the RCV of the visual Lovibond results were quite high in a range from 26% to 54%. In addition, substantial differences between some visual and automated Lovibond results can be observed. For the parameter Lovibond yellow, all automated Lovibond yellow results were bimodal distributed with maxima in the frequency distribution at Lovibond yellow 70 and around 90, while visual results showed a normal distribution and ranged from 41 to 59. The bimodal distribution of the data showed a problem with automated determination of Lovibond values even for common oils, while the comparability of the evaluation with the human eye by trained persons was limited.

For the Gardner colour index the results varied significantly less with RCV from 7% to 12%. However, the Gardner colour index is usually not used in practice for edible oils and fats, but for technical products. The results for Gardner colour index, automated and manual Lovibond obtained in 2019 were comparable to those obtained in previous years (data not shown) and underline the limited reproducibility of results from different

Table 6. Iodine colour value (ICV), mean, standard deviation (SD), and relative coefficient of variation (RCV) of CIELAB values of ICV standard solutions ($n = 6$).

ICV [mg I_2/l*10]	L* mean	SD	RCV [%]	a* mean	SD	RCV [%]	b* mean	SD	RCV [%]
1	99.7	0.6	0.6	−2.1	0.9	−44.4	5.7	1.4	25.3
2	99.4	0.5	0.5	−4.4	1.2	−26.6	11.8	1.7	14.4
3	98.9	0.4	0.4	−6.5	1.2	−19.2	17.8	1.8	10.3
4	98.3	0.4	0.5	−8.0	1.4	−17.5	23.2	2.2	9.3
5	98.0	0.4	0.4	−9.5	1.4	−14.5	28.5	2.4	8.3
7	97.0	0.5	0.5	−11.6	1.1	−9.7	38.3	1.9	5.0
10	95.4	1.3	1.4	−12.8	0.9	−7.0	49.0	2.5	5.0
15	94.0	0.4	0.4	−13.0	0.8	−5.9	63.3	2.3	3.7
20	92.2	0.3	0.4	−11.7	0.9	−8.0	73.4	2.3	3.2
30	89.0	0.4	0.5	−8.0	0.5	−6.2	86.0	2.1	2.4
40	85.9	0.4	0.5	−3.1	0.3	−10.5	93.3	1.9	2.0
50	83.2	0.7	0.9	1.6	0.4	23.2	98.0	1.5	1.5
65	79.0	1.2	1.5	8.1	0.4	5.1	101.5	0.8	0.8
80	75.5	1.4	1.8	14.0	0.4	2.5	103.2	0.5	0.5
100	71.2	1.7	2.4	23.2	2.9	12.4	102.6	1.7	1.6

laboratories in modern benchmark proficiency tests with the methods in use so far and the urgent need for a more precise procedure for colour determination.

Having regarded the systematic differences between laboratory proficiency tests and method validation collaborative tests, the much lower RCV results of the DGF standard method underlined its usefulness with RCVs ranging from 0.7 to 13.9 % while most RCVs were well below 10%. Another advantage of the DGF standard method compared to the gardner colour index is the more precise description of the colour hue. The gardner colour index is a 1D colour scale from light yellow to dark brownish hues and therefore, green, red, or blue off-hues are not properly covered by this evaluation.

3.3. Determination of CIELAB Values for Reference Colour Standard Solutions

During the first stage of the method development, preliminary collaborative tests still revealed some problems with CIELAB values comparing results from different laboratories even with same devices. Therefore, it was a crucial step in the new developed method to implement a possibility to check each measurement apparatus for exact colour measurements to ensure the proper working of the instrument. For this reason, some experiments were carried out to offer a simple procedure with easy to obtain reference materials, which should be non-toxic. Some former methods used toxic substances for this purpose like cobalt chloride, which is suspected of causing cancer, is highly allergenic and classified as carcinogenic, mutagenic, and toxic for reproduction material.[20] Other methods use indicator solutions with a buffer to obtain a huge set of different colored solutions depending on the pH and the indicator concentration.[9] Therefore, we looked for substances, which are safe to use, stable, readily soluble, and obtainable in at high purity. Tartrazine and Ponceau 4R are food grade colorants in the European Union, but in other countries like Norway they have been banned as food ingredients. However, they could be used safely in the laboratory and were supplied as standards at high purity of >99%. It was not necessary to focus on oil-soluble dyes, because calibration of the instrument could also be obtained using water-soluble reference materials. Of course, water-soluble dyes had to be used with distilled water as reference blank sample in order to provide samples with the same refractive index in both cuvettes for measurement and for reference. In contrast to the standards, the oils and fats were measured with *n*-heptane as reference blank sample in order to use a readily available and safely to handle liquid with similar refractive index to oils and fats.

The different colour standard solutions cover different areas of the 3D CIELAB colour space and improve the possibility to check the colour measurement devices in the laboratory as compared to a 1D procedure. The measured edible oils and fats are also well covered with these standards.

Table 6 shows the CIELAB values of the ICV standard solutions. The solutions of this set of reference standard colour solutions showed a coloration from nearly colorless at ICV 1 to light yellow at ICV 10 and turning to an intense brownish-yellow at ICV 50. Lightness (L*), which is defined from black = 0 to colorless/white 100, decreased from 99.4 at ICV 0 to about 70 at IVC 50 (see also **Figure 1**). For the a* value (see **Figure 2**), which described the hue intensity from green (−120) to red (120), a trend reversal was obtained starting from −1, decreasing to −12, and increasing back to +25. The b* values started rising continuously in relation to concentration, while at higher concentrations b* values tended to converge at a constant value at 105. For a first impression a photo of the set of ICV standards was taken by a cell phone at daylight with the solutions filled in 1 cm cuvettes with a sheet of white paper as background (see Figure S3, Supporting Information).

The CIELAB values obtained for Ponceau 4R and tartrazine are shown in **Tables 7** and **8**, respectively. While tartrazine standard solutions showed only a small decrease of lightness from about

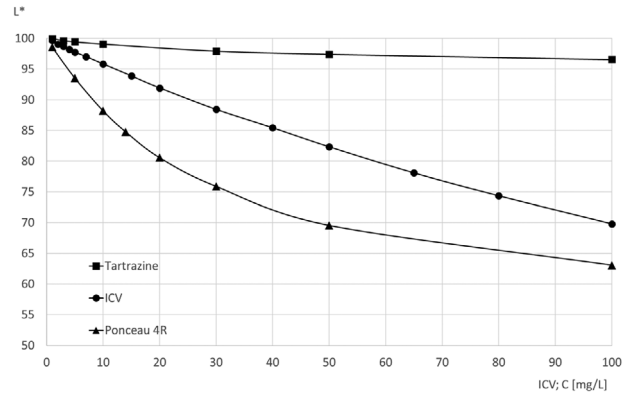

Figure 1. CIELAB 1976 (L*) values of calibration series of iodine colour value (ICV), tartrazine, and Ponceau 4R standards.

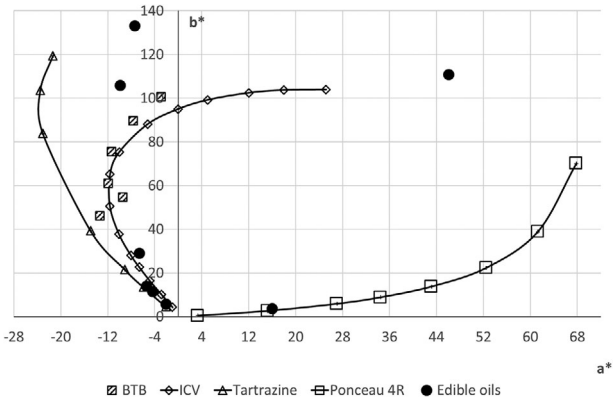

Figure 2. CIELAB 1976 (a* and b*) values of calibration series of iodine colour value (ICV), tartrazine, Ponceau 4R, and selected bromothymol blue (BTB) standards. The position of measured edible oils and fats used in the collaborative trial in relation to the calibration series is also indicated.

100 to 97 compared to the ICV scale, the lightness values of the Ponceau 4R standard solutions cover a range from about 99 to 63. In addition, tartrazine standards showed some more intense green hues reaching an a* value down to −23.5 and Ponceau 4R covered the red hues up to a* value of 68. The BTB standard solutions do not form a line or a curve in Figure 2, because the hue of each solution depends not only on BTB concentration but also on pH of the buffer. Therefore, they form in Figure 2 only six points in a plane with no exact continuous trend in a curve. Their CIELAB values are listed in **Table 9**. For each of the sets of standards also a photo was taken by a cell phone at daylight with the solutions filled in 1 cm cuvettes and a sheet of white paper as background (see Figures S2–S5, Supporting Information).

Figures 1 and 2 show the widened range of lightness, the range from red to green and the range of blue to yellow hues by the two additional sets of Ponceau 4R and tartrazine standard solutions in the CIELAB colour space. In contrast, the BTB standard solutions in test were all very close to the ICV. Most of the oils used in the collaborative trial were also covered well by these new proposed standards except the very low lightness values of pumpkin seed oil and palm oil of 2 and 51, respectively, and a very high b* value for virgin rapeseed oil at 133. Colour patches of the oils are provided in Figure S1, Supporting Information. With the additional standards we were able to provide

Table 7. Concentration of Ponceau 4R (C_P), mean, standard deviation (SD), and relative coefficient of variation (RCV) of CIELAB values of Ponceau 4R standard solutions ($n = 3$).

C_P [mg L^{-1}]	L* mean	SD	RCV [%]	a* mean	SD	RCV [%]	b* mean	SD	RCV [%]
1	98.58	0.035	0.04	3.30	0.046	1.4	0.45	0.017	3.8
5	93.57	0.094	0.10	15.02	0.364	2.4	2.59	0.061	2.3
10	88.24	0.090	0.10	26.76	0.439	1.6	5.73	0.283	4.9
14	84.81	0.058	0.07	34.47	0.100	0.3	8.74	0.205	2.3
20	80.59	0.995	1.23	43.13	0.125	0.3	13.61	0.271	2.0
30	75.91	0.814	1.07	52.45	0.205	0.4	22.14	0.289	1.3
50	69.56	1.053	1.51	61.03	0.404	0.7	38.66	0.326	0.8
100	63.07	1.006	1.60	67.63	0.335	0.5	69.46	1.077	1.6

Table 8. Concentration of tartrazine (C_T) versus mean, standard deviation (SD), and relative coefficient of variation (RCV) of CIELAB values of tartrazine standard solutions ($n = 3$).

C_T [mg L^{-1}]	L* mean	SD	RCV [%]	a* mean	SD	RCV [%]	b* mean	SD	RCV [%]
1	99.96	0.114	0.1	−2.17	0.113	5.2	4.68	0.242	5.2
3	99.61	0.087	0.1	−5.98	0.061	1.0	13.31	0.266	2.0
5	99.41	0.087	0.1	−9.12	0.012	0.1	21.60	0.075	0.3
10	99.05	0.007	0.1	−15.02	0.096	0.6	39.38	0.032	0.1
30	97.91	1.018	1.0	−23.08	0.012	0.1	83.91	0.078	0.1
50	97.39	1.003	1.0	−23.52	0.015	0.1	103.35	0.295	0.3
100	96.54	1.011	1.0	−21.32	0.048	0.2	119.15	0.528	0.4

Table 9. BTB index, mean, standard deviation (SD), and relative coefficient of variation (RCV) of CIELAB values of BTB colour standard solutions (n = 3). For composition of BTB standard solutions see Table 1.

BTB	L* mean	SD	RCV [%]	a* mean	SD	RCV [%]	b* mean	SD	RCV [%]
4/4	88.55	0.107	0.1	−11.86	0.098	0.8	60.82	0.442	0.7
6/3	89.10	0.167	0.2	−13.28	0.165	1.2	46.01	0.361	0.8
4/6	84.51	0.277	0.3	−11.41	0.044	0.4	75.35	0.384	0.5
2/3	93.00	0.081	0.1	−9.41	0.038	0.4	54.45	0.488	0.9
3/8	83.19	0.121	0.1	−7.66	0.006	0.1	89.44	0.243	0.3
2/10	83.22	0.150	0.2	−2.97	0.104	3.5	100.33	0.748	0.7

calibration points in the 3D space of the different colour values L*, a*, and b*. This is of special importance in case of off-hues, which were difficult to describe by a 1D calibration. While in the work of González-Quijano, Melgosa, Salmeron, and Moyano,[7–10,21] who tried to cover the whole gamut (the entire colour space of about 1700 olive oils in the study was covered) with about 60 individual standard solutions, our approach was a more general system to check the suitability of the results for the three dimensions without covering the colour of all possible samples. Otherwise, this would have needed a very high number of individual standard solutions and seemed to be impractical. Therefore, it was not intended to replace the detailed procedure established especially for olive oils by our procedure. However, these sets of standard solutions will enable an easy check for the analysis of many other oils like rapeseed oils, sunflower oils, and all others with a more or less yellow appearance. Oils with a more greenish colour correspond more closely to the tartrazine or BTB standards. The Ponceau 4R standard solutions will improve the control especially for high carotenoid containing oils like red or crude palm oil, virgin wheat germ oils, and others.

While Salmeron used 60 virtually defined colour reference points in the 3D CIELAB colour space to determine the theoretical best match of a given sample of extra virgin olive oil to one of these defined reference points of the latest colour index for olive oils, the modified uniform colour scale,[9] the physical standard solutions presented here enabled the check of the trueness of the results. In case, that the linear range of an instrument is not sufficient for the selected samples this can be easily identified by comparing the obtained CIELAB parameters with the parameters reported in this study.

3.4. Storage Experiment using Iodine Colour Value, Tartrazine, Ponceau 4R, and Bromothymol Blue Standard Solutions

Solutions of the different colour scales were stored in the dark and at ambient light at room temperature for about 80 days in order to evaluate the stability of the solutions. While the neat colour substances can be stored in the dark without any degradation, solutions of the standard substances may show some changes during storage as reported for BTB standards.[22] In our storage experiment we determined the colour of six standard solutions of BTB and ICV, and four solutions of Ponceau 4R and tartrazine in regular intervals for 80 days. L*-, a*-, and b*-values for BTB standards varied by 0 to 2 units, 0 to 3 units and 2 to 4 units at storage in the light, respectively. However, these values were stable in-between a range of 1 unit at storage in the dark. The colour standard solutions of the IVC series showed most alterations and L*-, a*-, and b*-values differed by 0 to 3, 0 to 4, and 1 to 20 units at storage in the light, respectively. Also in the dark, significant changes were observed during the first interval of 12 days, while changes were less than 1 unit until the end of the storage test. This might be attributed to an oxidation reaction taking place during the first storage interval. The two sets of standard solutions prepared with Ponceau 4R and tartrazine showed a better stability when stored in light or in the dark with changes in the range of up to 1 unit.

As colour differences are often noticeable by comparison with the human eye at about one unit for most cases the standard solutions should be prepared freshly to calibrate the system. Otherwise, standard solutions should be stored in the dark. Oxidation reactions may affect especially ICV standard solutions. In the storage experiments of Melgosa[22] they recognized also a drift of the colour of the BTB standard solutions and recommended to use these standards after a period of three month of stabilizing with a shift of the colour by about up to three CIELAB units in the first 150 days compared to the initial results.

4. Conclusion

In this study a new method for the determination of the colour of oils and fats was tested and resulted in comparable results for oils like refined rapeseed, sunflower or grapeseed oil. In addition, also virgin oils like rapeseed, sesame, pumpkin, or crude palm oil with intense hues were determined successfully with acceptable precision data. However, the determination of the colour of intense colored oils and fats is still a challenging task and care should be taken to avoid measurements beyond the linear range of the absorption maxima of the colour measurement devices. The use of common standard solutions like the BTB or the ICV colour standards was compared to new standard colour reference solutions using Ponceau 4R or tartrazine, which are an easy, safe and affordable way to check the instrument in the laboratory. Standard colour reference solutions should be prepared freshly. A storage test revealed some recognizable colour changes during storage in day light, but also during storage in the dark oxidation reactions may affect especially ICV standard solutions. The new standard method can provide an alternative way for precise colour communication in trade and quality control and will enable reliable and comparable results for the determination of the colour of oils and fats.

Abbreviations

BTB, Bromothymol blue; CAS, Chemical abstracts service; CIE, Commission Internationale de l'Éclairage – International commission of illumination; CMR, carcinogenic, mutagenic and toxic for reproduction; DGF, Deutsche Gesellschaft für Fettwissenschaft – German Society of Lipid Science; ICV, iodine colour value; MUOCS, modified uniform oil colour scale; p.A., pro analysii – analysis grade; UOCS, Uniform oil colour scale; UV-VIS, spectral range from ultra violet- to visible light.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The skillful assistance of Mrs. Sena Poyraz for the precise measurement of oils, fats, and standard solutions in the course of her bachelor thesis at the University of Applied Sciences and Arts, Ostwestfalen-Lippe is gratefully acknowledged. In addition, the following laboratories participated in the DGF collaborative trial to determine the precision data for the development of the DGF standard method C-IV 4f (20) Color of oils and fats:[13] Archer Daniels Midland GmbH, Mainz, Germany; Archer Daniels Midland Research GmbH, Hamburg, Germany; Brökelmann & Co. Ölmühle GmbH & Co, Hamm, Germany; Bunge Deutschland GmbH, Mannheim, Germany; Cargil GmbH, Hamburg, Germany; Cargil Texturising Solutions Deutschland GmbH, Hamburg, Germany; C. Thywissen GmbH, Neuss, Germany; Gustav Heess GmbH, Leonberg, Germany; Henry Lamotte Oils GmbH, Bremen, Germany; Max Rubner-Institut, Detmold, Germany; O. & L. Sels GmbH & Co KG, Neuss, Germany; Pilot Projekt, Magdeburg, Germany; SGS Germany GmbH, Hamburg, Germany.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

L.B.: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing-original draft, writing-review & editing. G.U.: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing-original draft, writing-review & editing.

Keywords

CIE 1976 (L*a*b*), collaborative trials, vegetable oil colors, proficiency test, standard methods, vegetable oils

Received: October 26, 2020
Revised: March 30, 2021
Published online: April 26, 2021

[1] D. Berner, *J. Am. Oil Chem. Soc.* **1989**, *66*, 313.
[2] A. A. Belbin, *Fett Wiss. Technol.* **1994**, *96*, 113.
[3] P. J. Wan, T. W. Hurley, J. D. Guy, D. L. Berner, *J. Am. Oil Chem. Soc.* **1997**, *74*, 731.
[4] International Standard Organization (ISO), ISO 27608:2010, Animal and vegetable fats and oils – Determination of Lovibond (R) colour – Automatic method, **2010**.
[5] F. Amoneit, *25th DGF Proficiency Test on Fat Analysis – Final Report*, Deutsche Gesellschaft für Fettwissenschaft, Frankfurt, Germany **2019**.
[6] M. J. Moyano, F. J. Heredia, A. J. Meléndez-Martínez, *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 278.
[7] R. G. González-Quijano, F. G. Rosales, *Grasas Aceites* **1986**, *37*, 282.
[8] M. Melgosa, R. Huertas, E. Hita, J. M. Roa, F. J. Heredia, J. Alba, M. J. Moyano, *J. Am. Oil Chem. Soc.* **2004**, *81*, 323.
[9] J. F. Salmerón, L. Gómez-Robledo, M. A. Carvajal, R. Huertas, M. J. Moyano, B. Gordillo, A. J. Palma, F. J. Heredia, M. Melgosa, *J. Food Eng.* **2012**, *111*, 247.
[10] M. J. Moyano, M. Melgosa, J. Alba, E. Hita, F. J. Heredia, *J. Am. Oil Chem. Soc.* **1999**, *76*, 687.
[11] Commission Internationale de l'Eclairage (CIE), CIE 015:2018, Colorimetry 4th ed, **2018**.
[12] Y. A. Tan, A. Kuntom, C. K. Lee, K. S. Low, *J. Am. Oil Chem. Soc.* **2004**, *81*, 733.
[13] D. G. f. Fettwissenschaft, *(DGF), Deutsche Einheitsmethoden zur Untersuchung von Fetten, Fettprodukten, Tensiden und verwandten Stoffen*, Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany **2020**.
[14] International Standard Organization (ISO), ISO 5725:2019, Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method, **2019**.
[15] M. Huang, H. X. Liu, G. H. Cui, M. R. Luo, M. Melgosa, *J. Opt. Soc. Am. A* **2012**, *29*, 883.
[16] M. Melgosa, E. Hita, J. Romero, L. J. Delbarco, *J. Opt. Soc. Am. A* **1992**, *9*, 1247.
[17] W. Horwitz, R. Albert, *J. AOAC Int.* **2006**, *89*, 1095.
[18] L. Gómez-Robledo, M. Melgosa, R. Huertas, R. Roa, M. J. Moyano, F. J. Heredia, *J. Am. Oil Chem. Soc.* **2008**, *85*, 1063.
[19] B. Gordillo, L. Ciaccheri, A. G. Mignani, M. L. Gonzalez-Miret, F. J. Heredia, *J. Am. Oil Chem. Soc.* **2011**, *88*, 1317.
[20] *European Pharmacopoeia Commission, European Pharmacopoeia*, Council of Europe, Strasbourg, France **2020**.
[21] M. J. Moyano, A. J. Meléndez-Martínez, J. Alba, F. J. Heredia, *Food Res. Int.* **2008**, *41*, 513.
[22] M. Melgosa, M. M. Perez, E. Hita, F. J. Heredia, J. Alba, M. J. Moyano, *J. Am. Oil Chem. Soc.* **2001**, *78*, 265.