Correlation between olive oil color and present pigments: case study with color methods

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Abstract

The aim of the study was to find correlation between olive oil color and pigments present in olive oil. The material of the experiment consisted out of different olive oil types (n = 22), including olive oil mixtures (refined olive oil + extra virgin olive oil) and extra virgin olive oils (multivarietal and monovarietal). RGB parameters were determined by the digital camera and LAB bya spectrophotometer. Following pigments were estimated in the samples of olive oil: chlorophylls, carotenoids, phenols and β-carotene. The highest correlation between RGB parameters and pigments was observed with total carotenoid contents (-95.5%, $p < 0.05$). Total carotenoid contents correlated highly with LAB parameters too, especially with "b" value (yellowness) (94%, $p < 0.05$). The study unequivocally showed high correlation between pigments in olive oil and olive oil color, which is giving the possibility for the estimation of pigments' content with less time consuming method that does not include the usage of chemicals.

Industrial camera, olive oil color, personal camera, pigments

Introduction

Pigments present in olive oil represent a valuable source of antioxidants for consumers and protect the olive oil from oxidative-hydrolytic deterioration. The content of pigments is dependable on many factors such as: olive cultivar, weather, growing conditions, harvest time, mill processing, storage conditions and others. Antioxidant content analysis requires the use of chemical, expensive devices, staff education and is usually time consuming (Kosma et al. 2016).

Color can be measured by spectrophotometer and expressed as "L" (lightness), "a" (redness) and "b" (yellowness) values and or by digital camera and expressed as "R" (red), "G" (green) and "B" (blue). The relationship between Lab values and β-carotene content has been observed (Takahata et al. 1993 and Marchal et al. 2013). The correlation between olive oil color and pigment contents is possible to evaluate using the Pearson correlation coefficient, due to its ability to measure the strength and direction of two variables' correlation (linear relationship). The correlation is considered to be strong when R^2 (the squared of Pearson correlation) is greater than 80 and weak if R^2 is less than 50 (Bolboaca and Jantschi 2006).

Beside characteristic aroma, taste and nutritional profile, olive oil color is another property that distinguishes olive oil from other types of edible oils (Morello et al. 2004). The color is probably one of the most important sensorial parameters of olive oil (especially extra virgin olive oil) that attracts consumers (Mendez and Falque 2007 and Stoll et al. 2017).

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The color of olive oil is defined as green-yellowish and is determined mainly by chlorophyll and carotenoid contents (Gandul-Rojas et al. 2000). Pigments are mainly responsible for olive oil color which attracts consumers and can be used for finding correlations between olive oil color and quantitative pigments' content. This kind of method would not include the usage of chemicals and it would certainly be less time consuming (Cayuela et al. 2014 and Reboredo-Rodriguez et al. 2016). The content of chlorophyll and carotenoid pigments in olive oil plays an important role due to their antioxidant capacity and due to their influence on olive oil color. Chlorophyll gives green and carotenoid yellow color to olive oil. Phenols are natural antioxidants which influence olive oil oxidation stability, and give a bitter taste to olive oil. Olive oil with high contents of phenols, carotenoids, tocopherols and chlorophyll is stabile towards the oxidation processes (Reboredo-Rodriguez et al. 2016). The method for pigments' content estimation, according to olive oil color, could be also a good indicator during industrial production of olive oil which would quickly estimate production process on its antioxidant profile (Moyano et al. 2008).

Relationship and correlation between color parameters and certain pigments in the food system, such as olive oil, represents the potential application in qualitative and quantitative measurement of pigments by faster and cheaper colorimetric methods. The relation between pigments and color measurement have already been found in following food systems: parsley leaves, blueberries, salmon, red pepper, grapes, peaches, sweet potatoes, carrots, meat, Swiss chard, squash, cranberries and wines (Arias et al. 2000).

The aim of the study was to establish the correlations between pigments (chlorophylls, carotenoids and phenols) content in different types of olive oil and their color determined by digital camera, spectrophotometer and image analysis.

Material and Methods

The total number of samples (n = 22) used for the experiments consisted of olive oil mixtures (refined olive oil + extra virgin olive oil) (n = 4), multivarietal extra virgin olive oil (olive oil bought in retail markets in the Czech Republic, originating from Spain and Greece) (n = 6) and monovarietal extra virgin olive oil (Oblica olive cultivar, originating from Bosnia and Herzegovina) (n = 12). Ten ml of each olive oil sample was poured onto 10 petri dishes with 45 mm diameter and measured separately.

The color of olive oil samples was estimated as RGB and LAB parameters. RGB parameters were measured with industrial – DFK 23U 274 camera (Imagine Source GmbH, Bremen GER). LAB values were estimated using USB4000-UV-VUS-ES spectrophotometer (Ocean Optics, Largo, FL, USA) in the range of wavelength from 180 to 880 nm and 15 ms exposition time. The assessment of RGB and LAB values was done using NIS-Elements BR ver. 4.5 software (Laboratory Imaging, Prague, Czech Republic).

The color analysis was also done by personal camera EOS600D (Canon, JPN). The scanning was done under standard light conditions with 2 lamps Delux L – 1 x 18 W lamps (OSRAM, GER). The shooting mode was in manual setting: exposure time 1/80, aperture F 5.0, image size L, sensitivity ISO 100. Subsequently, color analysis were processed and analyzed by the NIS-Elements BR 4.13.04 image analysis software. Square area of 2.500 x 2.000 px, in the middle of petri dish, was selected for the evaluation. Subsequently, the selected parameters for color and brightness were measured.

The total contents of chlorophylls, phenols and carotenoids were estimated using UV/VIS spectrophotometer (CE7210, Cecil Instruments, UK). The content of β-carotene was estimated on HPLC 1260 Infinity (Agilent Technologies, Santa Clara, CA, USA), with the usage of isocratic mobile phase acetonitrile (70%), methanol (18%) and dichloromethane (12%). The used column was Zorbax SB-C18 4.6 x 250 mm (Agilent Technologies, USA).

Statistical analysis included Pearson's correlation analysis, for finding the relationship between pigment contents and olive oil samples color. SPSS 20 statistical software (IBM Corporation, Armonk, USA) was used.

Results and Discussion

Table 1 shows RGB values analyzed by industrial camera and their correlations with total chlorophylls, carotenoids, phenols and β-carotene contents.

Statistically very significant ($p < 0.01$) correlations were found between total carotenoids content and following RGB parameters: mean intensity (-90.9%), min intensity (-90.0%), max intensity (-87.5%), mean blue (-95.5%), mean saturation (95.0%), mean brightness (-90.9%), mean density (92.8%), mean green (-79.8%) and sum blue (-65.3%). Correlations ($p < 0.01$) were also observed with other RGB parameters but under 60%. The correlations with chlorophylls content were also significant, though slightly lower. It was observed for following RGB parameters: mean saturation (87.9%), mean blue (-84.9%) and mean density (82.4%). With all other RGB parameters and total chlorophyll contents were found statistically very significant correlations ($p < 0.01$), though they accounted for less than 60% of the variations. According to Cayuela et al. (2014) chlorophylls and carotenoids significantly influence olive oil color which is confirmed by our results. It was also observed that color changes of olive oil correspond with deterioration of its quality, especially due to decrease of carotenoids and chlorophylls content (Mendez and Falque 2007).

Total phenols content in olive oil samples correlated ($p < 0.01$) over 60% only with sum density (63.5%), while with the rest of measured RGB parameters correlated ($p < 0.05$) under 60% or correlations were not observed at all, as with sum intensity, intensity variations, sum blue, hue typical, hue variations and bright intensity.

The contents of β-carotene in investigated olive oil samples strongly correlated ($p < 0.01$) with following RGB parameters: mean intensity (-70.0%), min intensity (-69.4%), max intensity (-66.7%), mean blue (-78.1%), mean saturation (80.2%), mean brightness (-70.0%) and mean density (72.1%). Density variations were not correlated ($p > 0.05$) with β-carotene content, but other RGB parameters correlated ($p < 0.01$) and accounted for less than 60% of β-carotene variations.

Table 1. Relationship between antioxidant content (total chlorophylls, carotenoids, phenols and β-carotene) in olive oil and RGB values (estimated by industrial camera)

RGB values		Total chlorophylls		Total carotenoids		Total phenols		β-carotene	
		R^2	p	R^2	p	R^2	p	R^2	p
Mean intensity	187.08 ± 22.91	-78.2	0.000	-90.9	0.000	-36.2	0.000	-70.0	0.000
Sum intensity	343503632.7 ± 113796909.1	-42.4	0.000	-41.6	0.000	11.7	0.084	-29.2	0.000
Intensity variation	3.34 ± 1.69	-22.2	0.001	-30.6	0.000	-8.8	0.192	-23.9	0.000
Min. intensity	174.90 ± 20.08	-78.6	0.000	-90.0	0.000	-37.7	0.000	-69.4	0.000
Max. intensity	200.01 ± 25.61	-76.3	0.000	-87.5	0.000	-29.8	0.000	-66.7	0.000
Mean red	220.49 ± 13.40	-45.7	0.000	-59.1	0.000	-22.9	0.001	-35.9	0.000
Mean green	206.34 ± 16.44	-65.5	0.000	-79.8	0.000	-33.5	0.000	-57.3	0.000
Mean blue	139.69 ± 42.36	-84.9	0.000	-95.5	0.000	-37.6	0.000	-78.1	0.000
Sum red	331994919.95 ± 164318946.26	-30.7	0.000	-28.1	0.000	20.5	0.002	-16.9	0.012
Sum green	311354625.27 ± 155478676.58	-35.5	0.000	-33.6	0.000	16.8	0.012	-21.9	0.001
Sum blue	217504214.40 ± 129620883.82	-62.3	0.000	-65.3	0.000	-6.6	0.330	-51.3	0.000
Hue typical	37.10 ± 6.46	-19.7	0.003	-31.1	0.000	-9.6	0.156	-22.2	0.001
Hue variation	5.30 ± 16.38	-30.2	0.000	-40.7	0.000	-10.7	0.112	-30.9	0.000
Mean saturation	70.23 ± 35.83	87.9	0.000	95.0	0.000	37.8	0.000	80.2	0.000
Mean brightness	74.05 ± 8.84	-78.2	0.000	-90.9	0.000	-36.2	0.000	-70.0	0.000
Sum brightness	112529903.26 ± 57440444.97	-42.4	0.000	-41.6	0.000	11.7	0.084	-29.2	0.000
Bright variation	1.31 ± 0.66	-22.3	0.001	-30.6	0.000	-8.8	0.194	-23.9	0.000
Mean density	0.15 ± 0.06	82.4	0.000	92.8	0.000	36.8	0.000	72.1	0.000
Density variation	0.01 ± 0.00	-18.8	0.005	-18.2	0.007	20.5	0.002	-11.3	0.095
Sum density	195921.52 ± 126355.74	31.5	0.000	42.1	0.000	63.5	0.000	40.1	0.000

Table 2. Relationship between antioxidant content (total chlorophyll, carotenoids, phenols and β-carotene) in olive oil and RGB values (personal camera)

	RGB values	Total chlorophylls		Total carotenoids		Total phenols		β-carotene	
		R^2	p	R^2	p	R^2	p	R^2	p
Mean intensity	133.93 ± 29.14	-62.3	0.000	-52.4	0.000	8.2	0.224	-70.0	0.000
Sum intensity	842919260.3 ± 328004774.5	-41.3	0.000	-27.8	0.000	25.0	0.000	-29.2	0.000
Min. intensity	94.01 ± 29.45	-33.2	0.000	-33.8	0.000	0.2	0.973	-69.4	0.000
Max. intensity	149.04 ± 30.75	-55.3	0.000	-42.6	0.000	8.6	0.201	-66.7	0.000
Mean red	172.05 ± 22.86	-14.5	0.031	5.2	0.446	43.7	0.000	-35.9	0.000
Mean green	159.41 ± 24.33	-41.1	0.000	-25.1	0.000	29.1	0.000	-57.3	0.000
Mean blue	70.34 ± 51.12	-80.4	0.000	-79.9	0.000	-19.3	0.004	-67.4	0.000
Sum red	1074383123.82 ± 348858428.42	-13.3	0.049	8.6	0.200	45.8	0.000	13.8	0.042
Sum green	997796843.51 ± 338974782.69	-25.3	0.000	-8.6	0.203	38.7	0.000	1.3	0.852
Sum blue	456577813.58 ± 383827764.22	-71.5	0.000	-68.1	0.000	-11.7	0.084	-57.4	0.000
Hue typical	38.50 ± 1.58	-74.3	0.000	-84.6	0.000	-5.5	0.418	-72.2	0.000
Hue variation	0.93 ± 1.00	-44.3	0.000	-56.0	0.000	-18.3	0.006	-45.7	0.000
Mean saturation	133.23 ± 69.23	86.3	0.000	87.3	0.000	22.9	0.001	71.9	0.000
Mean brightness	52.52 ± 11.43	-62.9	0.000	-52.4	0.000	8.2	0.223	-38.2	0.000
Sum brightness	330556572.67 ± 128629323.32	-42.4	0.000	-27.8	0.000	25.0	0.000	-16.9	0.012
Bright variation	0.66 ± 0.14	-15.9	0.019	-26.9	0.000	-46.3	0.000	-18.4	0.006
Mean density	0.37 ± 0.18	73.4	0.000	65.2	0.000	-6.3	0.355	50.1	0.000
Density variation	0.02 ± 0.03	37.6	0.000	39.3	0.000	-12.8	0.059	-11.3	0.095
Sum density	2119207.37 ± 809286.41	86.8	0.000	85.9	0.000	22.7	0.001	70.9	0.000

RGB values measured by personal camera are shown in Table 2. In comparison with industrial digital camera (camera – DFK 23U 274), personal camera showed high (> 60%) correlation with less RGB values.

The total chlorophylls content correlated (both positively and negatively) highly (> 60%) with following RGB parameters: mean intensity (-62.3%), mean blue (-80.4%), sum blue (-71.5%), hue typical (-74.3%), mean saturation (86.3%), mean brightness (-62.9%), mean density (73.4%) and sum density (86.8%). Total carotenoids correlated with following parameters: mean intensity (-90.9%), min. intensity (-90.0%), max. intensity (-87.5%), mean green (-79.8%), mean blue (95.5%), sum blue (-65.3%), mean saturation (95.0%), mean brightness (-90.9%) and mean density (92.8%). β-carotene content in olive oil samples correlated with following RGB parameters: mean intensity (-70.0%), min. intensity (-69.4%), max. intensity (-66.7%), mean blue (-67.4%), hue typical (-72.2%), mean saturation (71.9%) and sum density (70.9%) (Table 2). These results are indicating that low cost camera system can be used for the determination of olive oil pigments. The usage of low cost camera for olive oil evaluation was confirmed by Salmeron et al. (2012).

Table 3. The color of olive oil samples expressed in LAB units

	Lab values	Total chlorophylls		Total carotenoids		Total phenols		β-carotene	
		R^2	p	R^2	p	R^2	p	R^2	p
L (lightness)	97.23 ± 1.57	-82.7	< 0.001	-88.5	< 0.001	-53.1	< 0.001	-64.8	< 0.001
a (redness)	-5.86 ± 2.16	-81.3	< 0.001	-92.7	< 0.001	-42.4	< 0.001	-78.7	< 0.001
b (yellowness)	32.98 ± 17.38	85.5	< 0.001	94.0	< 0.001	38.4	< 0.001	79.5	< 0.001

Table 3 shows the relation between color of olive oil samples (expressed as LAB parameters) and total chlorophylls, carotenoids, phenols and β-carotene contents. The highest negative ($p < 0.01$) correlation was found between total carotenoids content and "a" (redness) parameter where "a" values accounted for 92.7% of variations. Positive high correlations ($p < 0.01$) were observed between "b" (yellowness) values and measured pigments content in olive oil samples. The highest correlation was observed between total carotenoids content and "b" values (R2 = 94.0%; $p < 0.001$). Our findings are in accordance with previous observations that the decrease of "b" value means decrease in yellowness and subsequently carotenoids content reduction, while lower values of "a" parameter means the reduction of green color and consequently chlorophyll degradation (Stoll et al. 2017). The color of olive oil ranges from green-yellow to golden-yellow, depending on olive variety and fruit ripeness. Our results are close to yellow color (yellow-green) (industrial camera: 221, 206,140; personal camera: 173, 159, 70). In RGB space is yellow rgb (255, 255, 0), the green color is in RGB (0, 128, 0). Kosma et al. (2016) studied the color of 6 olive varieties and found "L" values range from 72.25 ± 3.24 to 65.92 ± 2.83. our "L" values were higher (97.23 ± 1.57). Our findings show a mean "a" parameter of -5.86 ± 2.16, while the values for "a" reported by Kosma et al. (2016) ranges from -4.60 ± 2.03 to -9.20 ± 1.60. The mean "b" value in our samples was lower (32.98 ± 17.38) in comparison with authors (from 69.05 ± 18.27 to 98.30 ± 1.25), due to lower total carotenoids content in the samples of olive oil where refined olive oil was included (Kosma et al. 2016). Ameny and Wilson (1997) also found "b" value to be in best correlation with carotenoids content, while Takahat et al. (1993) found best relation of carotenoids with "a" value in the samples of sweet potatoes. The best correlation between "b" and total carotenoids content was also observed in the work of Minguez-Mosquera et al. (1991), who found a high correlation between "b" and pheophytins as major components of chlorophyll.

The color of olive oil is predominantly influenced by the contents of chlorophyll, carotenoids and certain compounds formed during olive fruits milling processes. During extraction chlorophyll is transformed to pheophytins and this process is changing the color from bright green to olive brown. The color of olive oil is in relation with the olive variety, but also with the degree of fruit ripeness. It is known that higher degree of ripeness causes decrease in chlorophylls and carotenoids content in olive fruits. At the beginning of the harvest when the green fruits dominate (chlorophyll pigments are dominant), olive oil is greenish, but after when the domination is taken by dark and purple fruits, olive oil becomes more gold in color (decrease of chlorophyll/carotenoid ratio) (Gandul-Rojas et al. 2000).

Table 4. Total chlorophylls, carotenoids, phenols and β-carotene contents in olive oil

	Chlorophylls	Carotenoids	Phenols	β-carotene
	[mg·kg^{-1}]			
Mixture* [n = 4]	0.41 ± 0.68	158.88 ± 69.55	43.38 ± 8.80	0.10 ± 0.01
Extra virgin olive oil I** [n = 6]	21.29 ± 11.87	633.08 ± 131.66	726.17 ± 589.81	1.21 ± 0.30
Extra virgin olive oil II*** [n = 12]	15.87 ± 11.15	412.36 ± 201.86	36.10 ± 10.26	0.64 ± 0.38
mean±sd [n = 22]	14.54 ± 12.26	426.47 ± 229.25	225.62 ± 433.80	0.70 ± 0.49

*the mixture of extra virgin olive oil and refined olive oil, bought in retail markets in the Czech Republic (Spain and Greece provenience)
**extra virgin olive oil bought in retail markets in the Czech Republic (Spain and Greece provenience)
***extra virgin olive oil produced in Bosnia and Herzegovina from Oblica olive cultivar

Table 4 shows the average contents of total chlorophylls, carotenoids, phenols and β-carotene with standard deviations in the samples of olive oil (three types of olive oil), which were used for finding statistically significant ($p < 0.05$) correlations with color parameters.

Conclusions

The study showed high correlation between present pigments in olive oil (chlorophyllsa, carotenoids, phenols) and its color. The color of olive oil can be used for the measurement of pigments, not only as qualitative but also as a quantitative method due to the statistically very significant correlations which were established. Among RGB values, mean blue correlated the most with total carotenoid contents, while "b" value (yellowness) among LAB parameters related the most with total carotenoids content. The color parameters measured by industrial digital camera and personal camera can serve as fast, cheap and reliable method, during industrial processing of olive oil.

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