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THE AUTHENTICITY OF VEGETABLE OIL USING SMARTPHONE-BASED IMAGE

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Abstract
Chlorophylls, tocopherols, and phenolic compounds are fluorescent substances found in vegetable oils. As a result, fluorescence analysis can be used to authenticate vegetable oils in a simple, effective, and non-destructive manner. The goal of the study was to determine the authenticity of vegetable oil using smartphone-based image analysis in fluorescence light. The image was taken with a Huawei P30 Lite and saved as an 8-bit JPG file. Mactronic PHH0062 390 nm-LED flashlight was employed as a light source. Due to the Hue spectral histograms, the image was analyzed in the HSV (Hue, Saturation, Value) color model. Eleven vegetable oils were chosen for testing: sea buckthorn, sunflower, rice, macadamia nut, hemp, corn, grape, linseed, rapeseed, olive, and milk thistle. Because vegetable oils have a diverse Hue spectrum, fluorescence examination can be used to verify their authenticity.
Key words: fluorescence analysis, HSV color, Hue spectral histogram, smartphone, vegetable oil.

Introduction

Vegetable oils are abundant in fatty acids and are one of the nutrients required for normal human function. Carotenoids, chlorophylls, tocopherols, and phenolic compounds are biologically active fluorescent chemicals that play a vital role in human health. Carotenoids are isoprenoids with eight isoprene units that are the primary source of yellow, orange, and red colors in nature. According to the study, carotene is the most studied carotenoids; however, other carotenoids in vegetable oils include lycopene, lutein, and cryptoxanthin. According to scientific literature, carotenoids are not only precursors of vitamin A provitamins and antioxidants, but they also limit the formation of toxic compounds in the cell, use in cancer therapy, and reduce risk of chronic illnesses associated with coronary heart disease (Fakourelis, Lee, & Min, 1987; Anguelova & Warthesen, 2000; Huang *et al*., 2003; Prakash & Gupta, 2014).

Plants and cyanobacteria contain a green pigment – chlorophyll, which consists of a chlorin or porphyrin ring and magnesium ion in the center (Pareek *et al*., 2017). Chlorophyll improves human health by boosting the immune system, purifying the body of toxins, decreasing blood pressure, detoxifying the liver, and defending against skin rashes, sinusitis, fluid buildup, and other ailments (Inanc, 2011). Tocopherols, which have a chromanol ring and a hydrophobic side chain, are fat-soluble antioxidants. They are classified into four congeners (vitamers): α-, β-, γ-, and δ-, which differ in the benzopyran ring methylation pattern (Boschin & Arnoldi, 2011; Schwarz *et al*., 2008). Vitamin E in the form of α-tocopherol is the most active form, and it is thought to reduce risk in the body from degenerative diseases including cancer and cardiovascular disease (Hasani *et al*., 2008; Zingg, 2007). Researchers discovered that γ-tocopherol was superior to α-tocopherol in terms of lowering platelet aggregation, delaying intra-arterial thrombus formation, and slowing low-density lipoprotein (LDL) oxidation (Li *et al*., 1999; Saldeen, Li, & Mehta, 1999). As a result, research into these luminous pigments for human health is crucial. Vegetable oils can be classified using fluorescent pigment molecules.

Traditional for classification and authenticating vegetable oils several analytical techniques, for example, high-performance liquid chromatography (Fasciotti & Pereira Netto, 2010; Lerma-García *et al*., 2007), gas chromatography (Li *et al*., 2016; Troya *et al*., 2015), visible spectrophotometry (Pizarro *et al*., 2013), near and mid-infrared spectroscopy (Sinelli *et al*., 2010), nuclear magnetic resonance spectroscopy (Vlahov, Del Re, & Simone, 2003; Rezzia *et al*., 2005), Fourier transform infrared spectroscopy (Maggio *et al*., 2010; Jiménez-Carvelo *et al*., 2017), ultraviolet photoionization ion mobility spectrometry (Garrido-Delgado, Muñoz-Pérez & Arce, 2018), Raman spectroscopy (Jiménez-Carvelo *et al*., 2017), laser-induced breakdown spectroscopy (Mbesse Kongbonga *et al*., 2014) and microwave reflectometry (Cataldo *et al*., 2012) were used. The majority of these analytical techniques and methodologies necessitate the use of damaging chemicals, highly trained staff, and high operating and maintenance costs. When compared to other techniques, fluorescence spectroscopy for analytical measurements of vegetable oils is simpler and less expensive (Poulli, Mousdis, & Georgiou, 2005; Guimet, Boqué, & Ferré, 2006; da Silva *et al*., 2015). However, there is simpler spectroscopy that is more mobile, has lower operational and maintenance expenses, and requires less sample preparation. Fluorescence spectroscopy can be replaced with smartphone-based image analysis thanks to ultra-high optical and light sensors and high-resolution cameras. The goal of the study was to determine the authenticity of vegetable oil using smartphone-based image analysis in fluorescence light.

Table 1

Vegetable oil sample information

Sample / Type	Brand	Origin	Peroxide value Meq O_2 kg^{-1}
Sea buckthorn/cold pressed	Z/S Extra	Latvia	4.9
Macadamia nut	SIA 'Oil Tree'	Germany	4.2
Hemp/cold pressed	SIA 'Spelta'	Latvia	2.5
Corn seed / extracted	Basso Fedele e figli S.r.l.	Italy	1.2
Grapeseed / extracted	Carapelli	Italy	2.1
Linseed/cold pressed	Ruata F.lli S.p.A	Italy	2.1
Rapeseed/cold pressed	SIA 'Iecavnieks & Co'	Latvia	1.1
Olive / Extra virgin	GOCCIA D'ORO	Italy	2.9
Milk thistle/cold pressed	Z/S Extra	Latvia	3.4
Rice bran oil / extracted	OLITALIA S.r.l.	Italy	1.7
Sunflower/cold pressed	UAB 'FORSAS'	Slovakia	2.0

Materials and Methods

The principle of the classification of vegetable oils by fluorescence analysis of research is a digital imaging of vegetable oils by smartphone in 390 nm LED light spectra.

Samples of research: A total of eleven vegetable oils in their original commercial packaging were purchased in Latvia in 2020 and chosen for analysis: (sea buckthorn, sunflower, rice, macadamia nut, hemp, corn, grape, linseed, rapeseed, olive, and milk thistle) (Table 1).

Equipment for analysis: For digital imaging, the smartphone Huawei P30 Lite (Huawei Technologies Co., Ltd., China) year of issue 2019, April 25, 48-megapixel triple camera, Android 10 – operating system EMUI version 10.0.0, processor Hisilicon Kirin 710, RAM 4.0 GB were used. Light source 390 nm-LED flashlight Mactronic PHH0062 (MACTRONIC Spółka z o.o. Sp.k., Poland) was used.

The system of Image acquisition: The digital image acquisition system is comprised of a black color printed white paper box (C=40%, M=30%, Y=0%) and black color printed white paper (K=100%) (24 x 32 x 38 cm^3). A light-emitting diode (LED) flashlight Mactronic PHH0062 (MACTRONIC Spóka z o.o. Sp.k., Poland) with 390 nm illumination was used. Inside the box, the LED flashlight was situated 21 cm to the front of the oil sample. To take a digital image, a smartphone with a 48-megapixel camera (Huawei P30 Lite) was placed outside in front of the open side of the box, 17 cm away from the PMMA 2.5 mL macro disposable cuvettes (BrandTech Scientific, Inc., US) with vegetable oil. Analyses were carried out in a dark room.

Imaging and image analysis: The image was taken with a smartphone camera and saved in 8-bit JPG format with an average size of 7.0 (8000 x 6000 pixels), ISO auto, f/1.8, 27 mm (wide), 1/2.0″, 0.8 μm, PDAF (Phase Detection Autofocus). The image was analyzed by the open-source software ImageJ. Samples were analyzed in ten repetitions.

Calculation method: For the transformation of the digitally obtained image RGB (Red, Green, and Blue) to HSV (Hue, Saturation, and Value) color the following formula was used, equation 1 (Zhang *et al*., 2016).

$$H = \begin{cases} \cos^{-1} \dfrac{(R-G)+(R-B)}{2\sqrt{(R-G)^2+(R-B)(G-B)}} & if\ (B \le G) \\ 360^0 - \cos^{-1} \dfrac{(R-G)+(R-B)}{2\sqrt{(R-G)^2+(R-B)(G-B)}} & if (B > G) \end{cases}$$

$$S = \frac{\max(R,G,B) - \min(R,G,B)}{\max(R,G,B)} \qquad (1)$$

$$V = \frac{\max(R,G,B)}{255}$$

where: R – red, G – green, B – blue, H – Hue, S – saturation, V – value

Data processing/Statistical analysis

The data of the research was analysed by mathematical and statistical methods (standard deviation, mean, Two-Way ANOVA). For the data analysis the Microsoft Excel software of the version 2019 and ImageJ was used.

Results and Discussion

For obtaining the Hue histograms, the smartphone digital RGB images needed to be converted to Hue images by HSV color space according equation 1. The differences between the RGB and Hue images are illustrated in Figure 1.

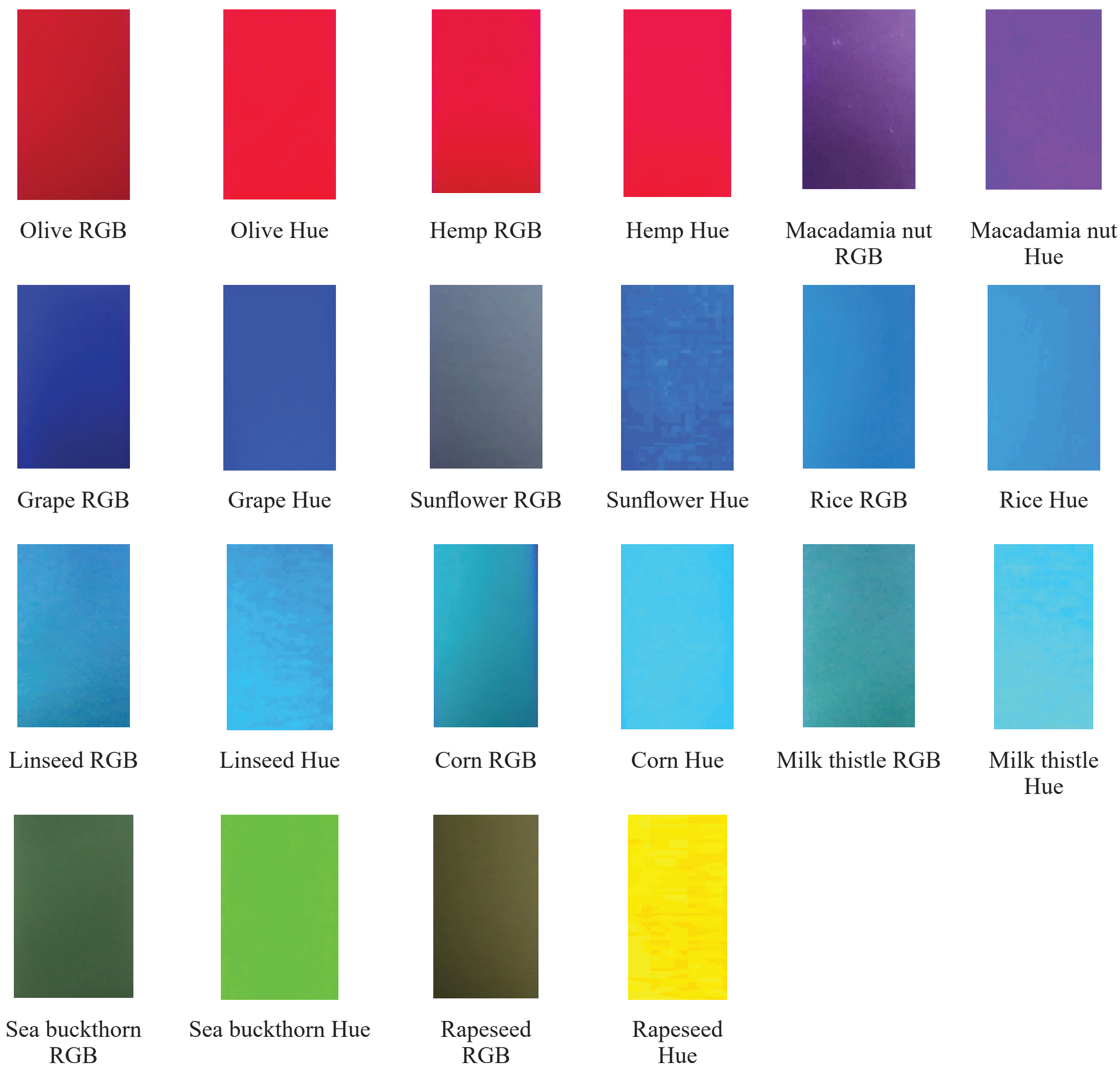

Figure 1. Sample RGB and Hue image color difference in vegetable oils.

Despite the fact that the RGB colour model correlates with biochemical processing of human visual system, it does not appear to connect with human color perception. The HSV color model is based on Hue, Saturation and Value characteristics, which corresponds to human color distinction. The separation of achromatic (Value) and chromatic (Hue, Saturation) information is a benefit of this space (Loesdau, Chabrier, & Gabillon, 2014). The obtained Hue color information from RGB image (Figure 1) can be used for generating Hue histograms (Figure 2), which shows fluorescent reflective color characterized by mean, min and max and StdDev of each vegetable oils.

Hue histograms show that large fluorescence differences are found among olive, hemp, macadamia nut oils and most common vegetable oils. Hue histogram includes Hue parameter for quantitative analysis to identify the vegetable oils. Obtained Hue values in Figure 3 show the apparent color similarity of the following samples of vegetable oils: olive-hemp, grape-sunflower, rice-linseed and corn-milk thistler; however, evaluating the calculated t-test and p-values, it can be stated despite the fact that visually similar colors are visible, the t-test value is well above this limit for all mentioned pairs of vegetable oils. As the t-test value is greater than 5.11 (<0.001), for olive-hemp, grape-sunflower, rice-linseed and corn-milk thistle oils, there is a significant difference between all samples of vegetable oil pairs.

Although there is a significant difference between the pairs of vegetable oils, the studied oils can be divided according to color boundaries, according to Wolfa's scientific data (Woolf *et al*., 2021), images converted to the 8-bit system have a defined Hue color circle that ranges from 0 to 255 px, thus according to this Hue color wheel, vegetable oils can be classified by color based on fluorescent molecules that have been studied in the scientific literature. Cluster analysis (Figure 4) shows and Hue value confirms that Olive and hemp oils are in the red color range 235.0 to 255.0,

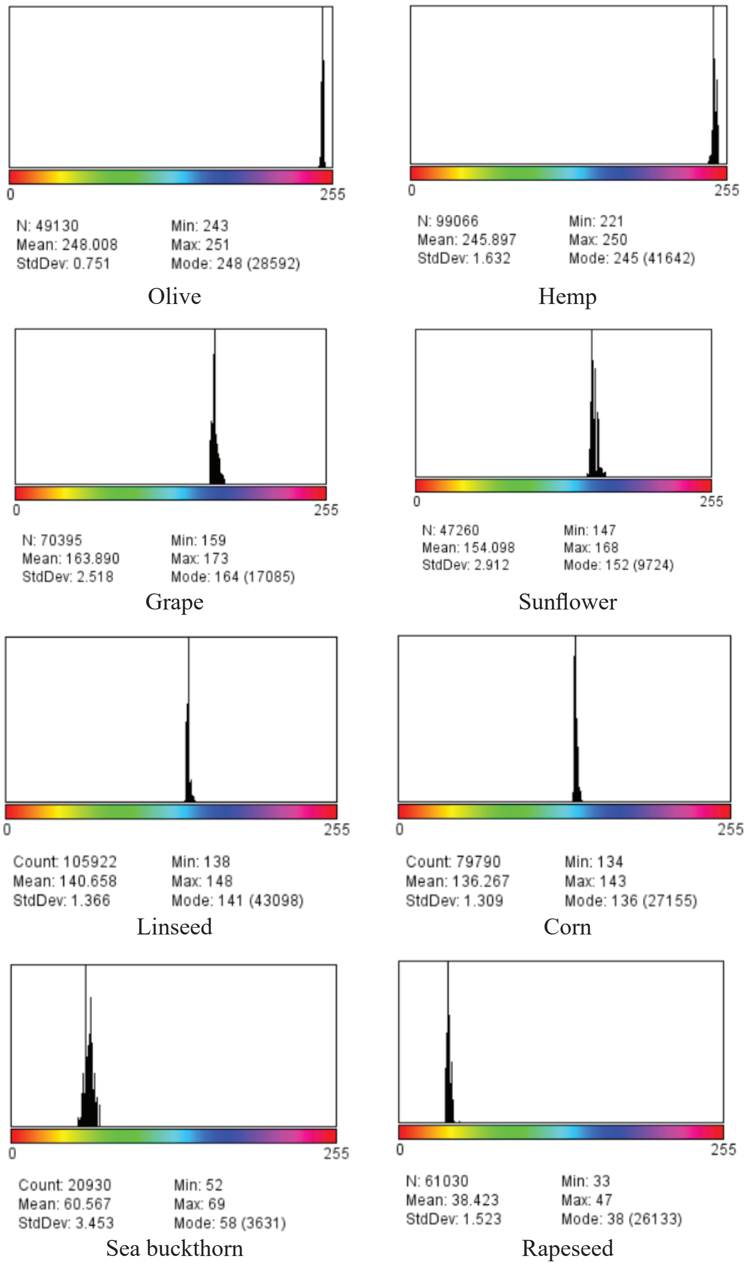

Figure 2. Hue histograms of vegetable oils.

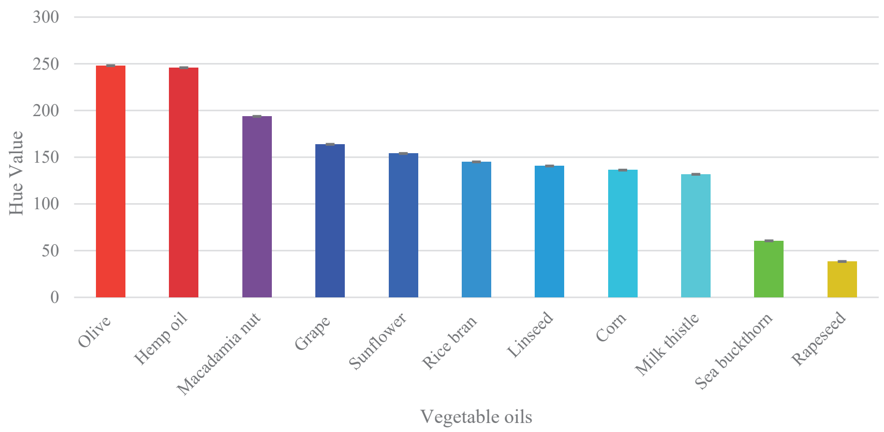

Figure 3. Hue values of vegetable oils.

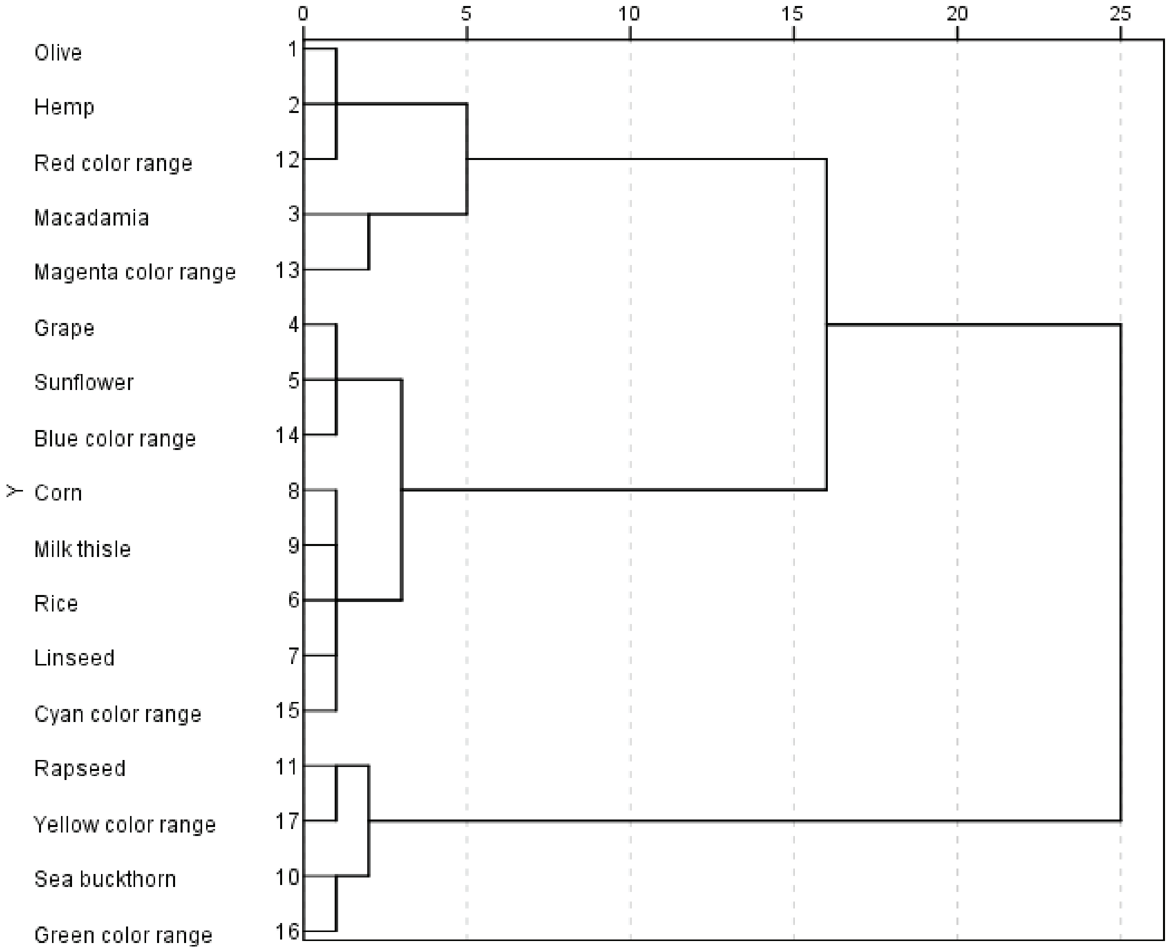

Figure 4. Cluster analysis of color range of vegetable oils by Hue values.

respectively 248.0±0.7 and 245.8±1.6, macadamia nut oil in the magenta color range 175.0 to 231.0, or 193.7±1.3, grape and sunflower oils in the blue color range 150.0 to 175.0, or 163.8±2.5 and 154.0±2.9, rice bran, linseed, corn, and milk thistle oils in the cyan color range 120.0 to 150.0, or 145.1±1.1, 140.6±1.3, 136.2±1.3 and 131.7±2.3, sea buckthorn in the green color range 41.0 to 85.0, or 60.5±3.4 and rapeseed oil in yellow color range, or 38.4±1.5.

Hue values of vegetable oils are in the range from 0 to 255. In order to group vegetable oils according to their Hue values obtained fluorescent colors, hierarchical cluster analysis was used (Figure 4). The first cluster includes olive and hemp oils, which indicate the formation of a similar red color. The second cluster contains only one vegetable oil, macadamia nut because none of the other vegetable oils produced the magenta color tone. The third cluster contains grape and sunflower oils with blue color formation. The fourth cluster includes four vegetable oils – corn, milk thistle, rice bran, and linseed, which by analogy form a cyan color. The fifth and sixth clusters contain one vegetable oil each – rapeseed and sea buckthorn, which form different color tones of yellow and green. Vegetable oils can be classified into 6 hierarchical clusters by the color – red, magenta, blue, cyan, yellow, and green – using fluorescent light and the obtained value of the Hue histograms.

According to scientific articles, it has been discovered that the strong fluorescence of olive oil is due to the natural fluorescent molecules like tocopherols, pheophytins, chlorophylls, chlorophylls, phenolic compounds, and their oxidations products. Fluorescence of phenolic compounds in olive oil was found using excitation with the maximum in the 362-400 nm range (Tena, Garcia-Gonzalez, & Aparicio, 2009). Hemp oil contains phenolic compounds, chlorophylls, carotenoids, and tocopherols which give the same strong red fluorescence as olive oil. The fluorescence of the red color indicates that the olive and hemp oils are high with chlorophyll compounds (Kleinegris *et al*., 2010). Blue to cyano color characterized by a decrease in vitamin E (tocopherol) concentrations in the cyano direction, it means blue is the highest concentration and cyano with lowest concentration of tocopherols. The clear green color in sea buckthorn indicates the presence of a pronounced carotenoid content (Kleinegris *et al*., 2010).

Conclusions

With a simple method using a smartphone digital image and a cheap 390 nm flashlight, vegetable oils can be classified and authenticated by fluorescence converted RGB mode to HSV – Hue histograms. The benefit of such a method is to control fraud of more expensive and valuable oils. The advantage over classical analytical methods is that no chemical solvents, reagents, optical filters, prisms, or grating are used.

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