

METHOD FOR DETERMINING COLOR CHARACTERISTICS OF PLANT PIGMENTS

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Pigments from petals of Ranunculus repens L. and Calendula officinalis L. are used to describe a method for determining color characteristics of pigments that is based on scanning the studied object on a flat scanner and defining the colors using Adobe Photoshop 5.0. Ten yellow pigments were found in R. repens; 15 yellow and orange, in C. officinalis.

Key words: pigments, determination method, *Ranunculus repens L.*, *Calendula officinalis L.*

Pigments of plant origin are very interesting for use in food products. Coloring agents from berries have mainly been studied [1, 2]. However, the most promising sources for obtaining them are flowers because the pigments isolated from them contain less ballast compared with other plant parts. They are brighter and richer in color [3].

The determination of color is of great significance in choosing a plant material for isolating dyes. Visual evaluation is subjective and not always suitable whereas special instruments that are used, tonometers, can work only with dried material or a dye solution [4, 5]. However, pure pigments, the color of which can differ from that of the whole plant, are often present. It became necessary to develop a method for determining the color characteristics of individual pigments.

We have previously developed a method for analyzing synthetic food dyes [6]. The present article reports results from a study of the ability to use this method to analyze pigments isolated from plants and to investigate the color characteristics of individual pigments.

We investigated petals of *Ranunculus repens L.* (Ranunculaceae) and *Calendula officinalis L.* (Asteraceae), which have intense yellow and orange colors, respectively.

Petals that were dried in the shade at room temperature were ground and extracted with *n*-hexane for 24 h at the same temperature by standing with periodic stirring. Mechanical impurities were carefully removed by centrifugation. The UV spectra were then recorded.

The color characteristics of the studied petals and pure pigments were compared (Tables 1 and 2). It was found that the tones of *R. repens* are yellow; of *C. officinalis*, orange.

Furthermore, the absorption maximum of the *C. officinalis* extract is red-shifted (λ_{\max} 480 nm) compared with analogous values of the *R. repens* extract (λ_{\max} 430 nm) although the spectrum of the latter contains a shoulder near 480 nm.

Separation of the pigments by TLC gave the best results with stepwise gradient elution using a benzene—ethylacetate (95:5) mixture. Components that were weakly retained on the sorbent were analyzed using hexane—benzene (9:1).

Analysis of densitograms indicated that the composition of both plants is qualitatively the same (Fig. 1).

We also studied peak 11, which moves with the solvent front (Fig. 1). It was found to contain one pigment for *R. repens* (Fig. 2a) and also a group of different pigments for *C. officinalis* (Fig. 2c and d). The retention indices of the pigments and their presence in the studied plants are given in Table 3. *R. repens* and *C. officinalis* contain 10 and 13 pigments, respectively. The results agree with the literature in which other plant materials are described [7].

The color characteristics of the separated components of the studied flowers (Tables 4 and 5) confirm that the orange hue of *C. officinalis* is due mainly to components 12-14, which are absent in *R. repens*.

TABLE 1. Color Characteristics of Petals of *Ranunculus repens* and *Calendula officinalis*

P	Hue (H), °	Saturation (S), %	Brightness (B), %	Lightness (L), %	Characteristics by light regimes						
					RGB			CMYK			
					R scale (Red)	G scale (Green)	B scale (Blue)	C scale (Cyan)	M scale (Magenta)	Y scale (Yellow)	K scale (Black)
I	55.4 ± 0.48	98.3 ± 0.15	99.7 ± 0.07	92.6 ± 0.42	254.4 ± 0.08	235.4 ± 1.01	3.9 ± 2.30	0 ± 0.00	10.1 ± 1.86	93.2 ± 0.06	0 ± 0.00
II	29.4 ± 0.94	98 ± 0.22	99.5 ± 0.05	67.7 ± 0.55	254.3 ± 0.05	123.8 ± 1.97	3.1 ± 1.18	0 ± 0.00	61.9 ± 1.28	97.7 ± 0.21	0 ± 0.00

P- plants: *Ranunculus repens* (I); *Calendula officinalis* (II).

TABLE 2. Characteristics of Certain "Pure" Color Hues of RGB, CMYK, and Lab (CIEL *a*b) Regimes

Regime	Scale	Hue (H), °	Saturation (S), %	Brightness (B), %	Lightness (L), %	Characteristics by light regimes						
						RGB			CMYK			
						R scale	G scale	B scale	C scale	M scale	Y scale	K scale
RGB	R (red)	0	100	100	54	255	0	0	0	87	99	0
	G (green)	120	100	100	88	0	255	0	56	0	88	0
	B (blue)	240	100	100	29	0	0	255	100	79	0	0
CMYK	C (blue)	195	100	92	62	0	178	235	100	0	0	0
	M (red)	326	100	89	48	226	0	127	0	100	0	0
	Y (yellow)	59	100	98	94	249	244	0	0	0	100	0
Lab	+a (red)	335	69	100	100	255	78	152	0	64	4	0
	-a (green)	173	100	100	100	0	255	224	46	0	28	0
	+b (yellow)	52	100	100	100	255	221	0	0	39	93	0
	-b (blue)	219	41	100	100	151	187	255	42	20	0	0

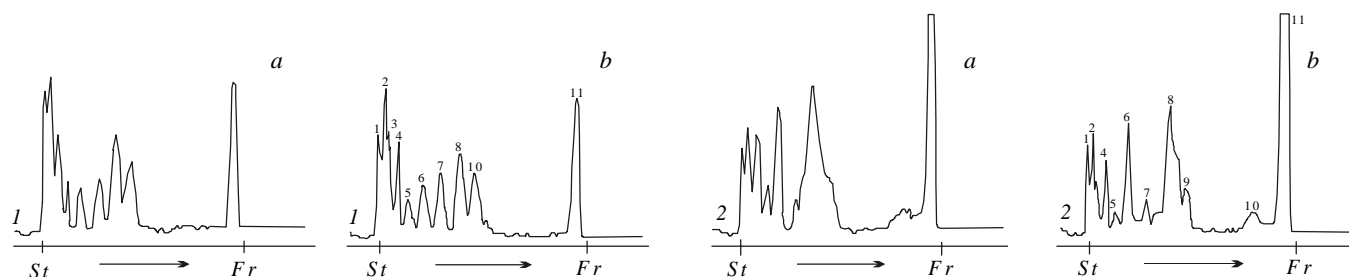


Fig. 1. Densitograms of pigments from *Ranunculus repens* L. (1) and *Calendula officinalis* L. (2): eluent benzene—ethylacetate 95:5 (a); repeated gradient elution, step I, benzene eluent, height of eluent rise, 2 cm from origin; step II, benzene—ethylacetate 97.5:2.5, 4 cm; step III, benzene—ethylacetate 95:5, 7 cm (b) (peak designations here and in Figs. 2 and 3 correspond to those given in the tables).

The color characteristics of the pigments with the strongest peaks were more accurately evaluated using comparative color intensities in the CMYK regime. This method was used previously for synthetic dyes.

All components have a distinct peak in the yellow region (Y scale). The color of peak No. 6 lies wholly in the yellow region. The characteristics of peaks 1, 3-7, and 9-11 are analogous. A reddish hue is clearly noted for peak No. 8 (M scale). This component together with the substance in peak 2 probably explains the shoulder at 480 nm that was mentioned above. Pigments 12-14 have distinct colors. These substances of these peaks impart the orange color to *C. officinalis*.

Programmed gradient elution was used to separate compounds in the *C. officinalis* extract [8]. This established that peaks 12-14 contain two more components 15 and 16. The color characteristics of these are analogous to the corresponding values for pigments 13 and 14 (Fig. 3).

TABLE 3. Chromatographic Parameters of Plant Pigments

Pigment No.	Presence		R_f value in systems*			
	<i>Ranunculus repens</i>	<i>Calendula officinalis</i>	hx	hx—bz, 9:1	hx—bz, 1:1	hx—ea, 95:5
1	+	+	0	0	0	0.02
2	+	+	0	0	0	0.05
3	+	-	0	0	0	0.05
4	+	+	0	0	0	0.08
5	+	+	0	0	0	0.13
6	+	+	0	0	0	0.18
7	+	+	0	0	0	0.33
8	+	+	0	0	0	0.36
9	+	+	0	0	0.01	0.46
10	-	+	0	0	0.03	0.90
11	+	+	0	0.02	0.50	1
12	-	+	0.08	0.47	1	1
13	-	+	0.70	0.70	1	1
14	-	+	0.87	0.87	1	1

*hx, *n*-hexane; bz, benzene; ea, ethylacetate.

TABLE 4. Color Characteristics of Pigments

No.*	Hue (H), °	Saturation (S), %	Brightness (B), %	Lightness (L), %
1	52.5 ± 0.5	24.5 ± 0.4	90.3 ± 0.2	88.3 ± 0.1
2	47.3 ± 0.8	46.2 ± 0.3	93.7 ± 0.1	86.6 ± 0.1
3	58.6 ± 0.2	21.5 ± 0.7	92.7 ± 0.1	92.3 ± 0.1
4	49.7 ± 0.2	19.4 ± 0.5	93.6 ± 0.1	90.6 ± 0.1
5	58.2 ± 1.2	5.5 ± 0.3	93.3 ± 0.1	93.1 ± 0.1
6	35.2 ± 0.2	25.3 ± 0.2	93.5 ± 0.1	86.7 ± 0.1
7	62.1 ± 0.1	14.0 ± 0.2	93.2 ± 0.1	93.3 ± 0.1
8	46.6 ± 0.2	24.1 ± 0.2	92.7 ± 0.1	89.6 ± 0.1
9	47.4 ± 0.2	15.5 ± 0.2	93.6 ± 0.1	91.6 ± 0.1
10	72.1 ± 0.2	1.7 ± 0.6	93.3 ± 0.1	93.5 ± 0.1
11	67.3 ± 0.2	8.0 ± 0.6	89.6 ± 0.1	90.7 ± 0.1
12	38.3 ± 0.2	21.0 ± 0.2	86.7 ± 0.1	83.6 ± 0.1
13	31.2 ± 0.2	33.1 ± 0.5	88.4 ± 0.1	79.1 ± 0.1
14	28.8 ± 0.3	31.9 ± 0.2	87.7 ± 0.1	81.5 ± 0.1

*Pigment numbers correspond to those given in the text and figures.

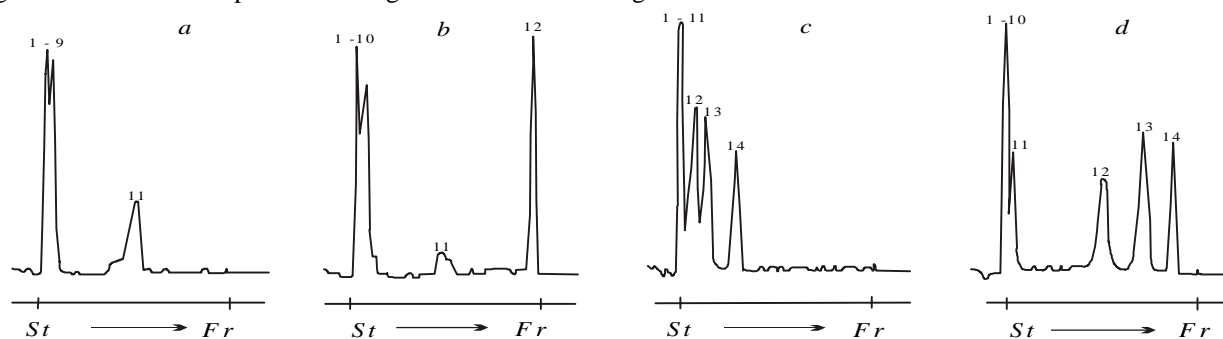


Fig. 2. Densitograms of pigments: *Ranunculus repens*, eluent hexane—benzene 1:1 (a); *Calendula officinalis*, eluent hexane—benzene 1:1 (b); *Calendula officinalis*, eluent hexane (c); *Calendula officinalis*, eluent hexane—benzene 9:1 (d).

TABLE 5. Color Characteristics of Pigments in CMYK and RGB Regimes

No.	Characteristics by color regimes						
	RGB			CMYK			
	R scale	G scale	B scale	C scale	M scale	Y scale	K scale
Background	235.8 ±0.2	240.9±0.4	243.0 ±0.3	9.1 ±0.4	3.6 ±0.5	3.2 ±0.4	0.0 ±0.0
1	229.1 ±0.8	222.0 ±0.6	174.3 ±0.8	9.7 ±0.9	10.5 ±0.4	37.0 ±0.3	0.2 ±0.1
	227.0 ±0.6	231.8 ±0.6	203.3 ±0.4	8.4 ±0.4	4.3 ±0.2	24.67 ±0.3	0.0 ±0.0
2	47.2 ±0.4	215.9 ±0.6	128.3 ±0.7	9.6 ±0.3	15.7 ±0.2	27.8 ±0.5	0.3 ±0.1
	234.3 ±0.4	235.1 ±0.7	207.7 ±0.5	9.4 ±0.3	4.5 ±0.3	22.2 ±0.2	0.0 ±0.0
3	235.7 ±0.6	235.0 ±0.7	185.1 ±0.6	8.8 ±0.2	4.5 ±0.3	34.8 ±0.6	0.0 ±0.0
	-	-	-	-	-	-	-
4	234.2 ±0.4	229.3 ±0.4	191.0 ±0.7	8.8 ±0.4	8.3 ±0.2	29.8 ±0.4	0.0 ±0.0
	233.3 ±0.4	236.5 ±0.6	209.9 ±0.4	8.9 ±0.5	4.5 ±0.3	21.0 ±0.4	0.0 ±0.0
5	58.4 ±0.4	236.2 ±0.6	225.0 ±0.6	8.7 ±0.2	5.4 ±0.5	12.2 ±0.2	0.0 ±0.0
	236.7 ±0.3	245.2 ±0.5	236.4 ±0.4	8.6 ±0.3	3.8 ±0.3	7.3 ±0.2	0.0 ±0.0
6	232.4 ±0.4	238.1 ±0.4	217.0 ±0.4	8.3 ±0.4	4.2 ±0.4	17.3 ±0.2	0.0 ±0.0
	235.1 ±0.4	235.7 ±0.8	201.1 ±0.6	9.3 ±0.3	4.3 ±0.5	29.4 ±0.3	0.0 ±0.0
7	236.0 ±0.5	238.1 ±0.6	204.8 ±0.6	8.7 ±0.2	3.2 ±0.3	24.4 ±0.2	0.0 ±0.0
	235.2 ±0.6	240.7 ±0.6	235.0 ±0.4	8.8 ±0.4	3.1 ±0.2	8.0 ±0.1	0.0 ±0.0
8	234.6 ±0.6	223.7 ±0.7	180.0 ±0.4	8.4 ±0.2	11.2 ±0.2	34.0 ±0.2	0.0 ±0.0
	235.7 ±0.7	210.3 ±0.4	173.9 ±0.7	8.7 ±0.3	19.1 ±0.3	39.8 ±0.3	0.4 ±0.1
9	235.8 ±0.6	230.1 ±0.5	201.8 ±0.4	8.0 ±0.4	8.3 ±0.2	23.9 ±0.2	0.0 ±0.0
	235.9 ±0.4	238.1 ±0.4	224.8 ±0.6	9.5 ±0.4	4.0 ±0.1	13.6 ±0.3	0.0 ±0.0
10	-	-	-	-	-	-	-
	234.7 ±0.6	239.0 ±0.6	229.3 ±0.4	8.9 ±0.2	3.4 ±0.1	12.6 ±0.2	0.0 ±0.0
11	228.5 ±0.6	229.1 ±0.5	210.9 ±0.4	7.9 ±0.2	6.6 ±0.3	18.9 ±0.2	0.0 ±0.0
	234.9 ±0.7	236.5 ±0.4	223.4 ±0.4	8.7 ±0.3	3.1 ±0.1	11.5 ±0.2	0.0 ±0.0
12	-	-	-	-	-	-	-
	222.8 ±0.6	207.9 ±0.6	176.3 ±0.4	8.2 ±0.4	18.6 ±0.3	31.1 ±0.2	0.0 ±0.0
13	-	-	-	-	-	-	-
	225.3 ±0.4	189.8 ±0.6	148.2 ±0.6	8.1 ±0.2	28.6 ±0.4	40.7 ±0.5	0.0 ±0.0
14	-	-	-	-	-	-	-
	225.1 ±0.4	200.3 ±0.4	152.4 ±0.4	8.2 ±0.3	22.0 ±0.2	42.4 ±0.3	0.0 ±0.0

Values were measured near peaks. Upper value refers to *Ranunculus repens*; lower, *Calendula officinalis*.

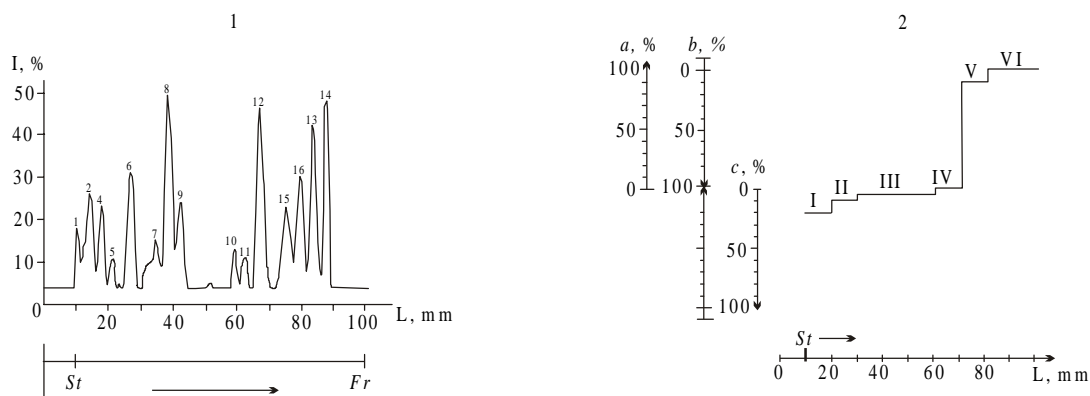


Fig. 3. Densitograms of *C. officinalis* pigments prepared by repeated gradient elution (1) and the elution scheme (2). Color intensity, % (I); distance along the plate, mm (L), origin (St), solvent front of the last step (Fr). *n*-Hexane (a), benzene (b), ethylacetate (c). Steps I-IV.

EXPERIMENTAL

Absorption spectra were recorded on an SF-46 spectrophotometer in quartz cuvettes. Preparative TLC was performed on Sorbfil plates (AO Sorbpolimer, Krasnodar). Samples were scanned on a flat Master Scan Express 6000P scanner at 600 dpi.

Color characteristics were determined using the color definitions on the instrument panel in Adobe Photoshop 5.0 in the following color regimes: HSB, hue, saturation, brightness; RGB, red, green, blue; CMYK, cyan, magenta, yellow, black; Lab (CIE) L^*a^*b , lightness, green into red, blue into yellow. Color characteristics were determined using ten iterations. Statistical treatment was carried out by standard methods [9]. The confidence level was set at 0.95.

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