

Determination of essential and toxic elements, ascorbic acid content and color of different leaves in two cabbage varieties

Ana LEAHU,* Cristina GHINEA, Mircea-Adrian OROIAN, and Cristina DAMIAN

Stefan cel Mare University of Suceava, Romania, Faculty of Food Engineering, 13th University Street, Suceava, Romania

Abstract. The main goal of this paper was to evaluate total macro- and microelement contents of different cabbage leaves of two varieties of cabbage (*Brassica oleracea* var. *capitata* f. *alba* and *Brassica oleracea* var. *capitata* f. *rubra*) and to determine the ascorbic acid content and color parameters (L^* , a^* , b^* and $-\Delta E$). Also, the anthocyanins content of *Brassica oleracea* var. *capitata* f. *rubra* was investigated. The highest mean contribution of elemental interactions regarding total macroelements in white cabbage leaves from inside (14-16 leaf), was observed for calcium and selenium, whereas the lowest was found for heavy metals, nickel, cadmium, and cobalt. The total contents of calcium and selenium in red cabbage leaves from inside (14-16 leaf) were highest. Results showed that the red-purple hue is more pronounced outside of the red cabbage than inside and it was also found that there are losses of yellowish hues for white cabbage from the outer leaves to the inner ones. Regarding the content of anthocyanins, it was obtained a value of 65.124 mg/100 g fresh weigh for outer leaves of red cabbage. Our results indicated that ascorbic acid content of red cabbage is approximately 3 times higher than in the white cabbage.

Keywords: cabbage leaves, heavy metals, ascorbic acid, color parameters, total anthocyanins content.

1. Introduction

In recent decades, fresh vegetables have been investigated, due to their potential as health promoting phytochemicals, such as polyphenols, ascorbic acid which prevent cell damage caused by free radicals and dietary fiber and therefore responsible for this protective effect. The antioxidant properties of cabbage polyphenols are responsible for their antiviral and anti-inflammatory properties and reduce the risk of heart disease, neurodegenerative diseases, and diabetes [1]. Important sources of dietary polyphenols are beverages, fruits and vegetables. It is considered that the average daily intake of polyphenols is 1 g per person [2, 3]. Brassica vegetables consumption has significantly benefits on human health by reducing the risk of chronic diseases, having potential anticarcinogenic and being extensively researched in recent years [4 - 6].

White cabbage (*Brassica oleracea* L. var. *Capitata* f. *Alba*) has its origins in the Mediterranean region and since ancient times has been considered a health-promoting vegetables. Today, this dietary vegetable is consumed in large quantities both in Europe and around the world being preferred by consumers due to its availability and low price. Besides that, brassica vegetables contain health-promoting compounds: high levels of glucosinolates (GLS), polyphenols, carotenoids, tocopherols, and vitamins [5]. Bioactive compounds present in white and red cabbage, such as water-soluble vitamin C and phenolic compounds (lignans, flavonoids and phenolic acids), as well as lipid-soluble vitamin E and carotenoids, are important contributors to the defense against oxidative stress [7]. According to the Kusznierevicz *et al.* [4] who studied the antioxidative

compounds of white cabbage samples derived from four European countries, the highest total polyphenols content was identified for cabbage from Belgium (4.917±0.52 mg gallic acid equivalents (GAE)/g dry weight) comparative with the samples from other regions. Tanongkankit *et al.* [8], studied the effect of vacuum drying at 60, 70 and 80°C on the phenolic compounds and vitamin C, in white cabbage outer leaves. They stated that under all conditions tested there were no differences in antioxidant retention.

Consumption of dietary fiber has an important role in many physiological processes and in the prevention of diseases such as constipation, hemorrhoids, irritable colon, colon cancer, obesity and diabetes. It has been scientifically proven that cabbage is a good source of dietary fiber. Considering the benefit of dietary fiber, the World Health Organization (WHO) recommends for adults an intake of 25 g total fiber/day [9]. The cabbage outer leaves that usually are removed during industrial processing are in fact an important source of dietary fiber, having also a high content of vitamin C and polyphenols [8].

The essential mineral elements content of cabbage is represented by calcium (Ca), potassium (K), magnesium (Mg) and phosphorous (P), and essential or potentially essential trace elements: cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), selenium (Se) and zinc (Zn). Copper, chromium, iron and zinc play an important role in human, animal and plant cell metabolism. Copper can be found in many enzymes, some of which are essential for Fe metabolism and various studies have reported a direct correlation between the dietary Zn/Cu ratio and the incidence of cardiovascular disease [10]. Iron is constituent of catalase

*Corresponding author. *E-mail address:* analeahu@gmail.com (Ana Leahu)

(a heme protein) and is essential for the transport of oxygen from the lungs to the tissues as well as the transport of carbon dioxide. Zinc enzymes participate in a wide variety of metabolic processes including carbohydrate, lipid and protein synthesis or degradation [10]. It has been demonstrated that a high intake of vegetables through high potassium content reduces the risk of coronary heart disease (CHD), high blood pressure and stroke [11]. Bvenura and Afolayan investigated the accumulation of Cu, Mn, Zn, Pb and Cd in different vegetables (including cabbage). Their results showed that the type of cabbage analyzed contained: 28.85 mg/kg Mn, 27.38 mg/kg Zn, 0.62 mg/kg Cu, 0.24 mg/kg Cd and Pb concentration below the detection limit [12]. Stančić *et al.* investigated the heavy metals content in the vegetables like lettuce, cabbage, potato and others from one Croatian market. The results of their study indicated that Cd was presented in cabbage in concentration between 0.10-0.24 mg/kg, Cu between 2.1-3.4 mg/kg and Mn from 8.7 to 21.9 mg/kg [13]. Radulescu *et al.* determined the bioaccumulation of heavy metals in *Brassica oleracea L. var. capitata*, considering different parts of the plant: outer and interior leaves, core, interior and exterior stem, root. According to their results Cu concentration exceeds the value (5 mg/kg) admitted by Romanian legislation for all samples, while Cd accumulates in high concentration in all samples excepting the exterior stem and root of cabbage [14].

The aim of this study was to characterize mineral content, to determine ascorbic acid content, to establish color parameters of different white and red cabbage leaves, and to investigate the anthocyanins content of red cabbage leaves.

2. Experimental

2.1. Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol, monopotassium phosphate, orthophosphoric acid, nitric acid and standard L- ascorbic acid were purchased from Sigma (Germany). Deionized water was used throughout.



Figure 1. Samples for analysis of white and red cabbage leaves (outer (S1, S4), mid (S2, S5) and inner layer (S3, S6)).

2.2. Samples

The samples (*Brassica oleracea var. capitata f. alba* and *Brassica oleracea var. capitata f. rubra*) were harvested in autumn 2017 at the site Milisauti. Figure 1 shows the layers of cabbage leaves. For the determinations, the leaves 1-3 were used from the outside of the cabbage head (S1 and S4), after removed a few leaves. From the center of the cabbage, leaves were taken corresponding

to the leaves 6-8 (S2 and S5), and from the inside of the vert, the leaves 14-16 (S3 and S6). The cabbage extracts were prepared in the laboratory immediately after collection and transport.

2.3. Chemical analyses

Color measurements. Minolta Chroma Meter (Model CR 310, Minolta Camera Co. Ltd., Japan) device was used to calculate directly three variables L^* (lightness), a^* (redness), and b^* (yellowness) of the samples, using the average of 3 readings per sample [15].

HPLC analyses. High performance liquid chromatography was used in order to determine the ascorbic acid content of the samples. For this purpose, 4 g of cabbage samples was extracted with 12 ml of acidified solutions (perchloric acid and *o*-phosphoric acid 1%). The extraction was considered to be complete when solvents became colorless (the total solvent volume was 50 ml). Filter paper Whatman no. 1 was used for extracts filtration, after that the extracts were maintained at -20°C [15].

Ascorbic acid was determined by using SHMADZU system coupled with UV-VIS detector (DAD). ZORBAX - C18 column ($5\mu\text{m}$, 250×4.6) was eluted in isocratic system with a mobile phase consisted of phosphate buffer pH = 3.5 (TFA): solution 0.02 ml of monopotassium phosphate and orthophosphoric acid 10%, adjusted to pH = 3.5 at a flow rate of 0.6 ml/min. The chromatograms were registered at 245 nm.

Sigma 99% standard *L* ascorbic acid was used for the identification and a calibration curve was obtained based on dilutions of this standard solution in bidistilled water [15].

Total anthocyanins content in red cabbage was determined by UV-VIS spectrophotometer, according to Harzallah *et al.* [16]. Absorbance was measured at 530 and 657 nm, and pigment content was expressed as cyanidin-3-glucoside (cyd-glu, molar extinction coefficient of $26,900 \text{ L cm}^{-1} \text{ mol}^{-1}$ and molecular weight of 449.2 g mol^{-1}).

Four grams of sample were vortexed for 1 min in 40 mL of 85:15 (v/v) methanol/0.1 M HCl) and incubated for 24 h at room temperature in the dark. The mixture was then centrifuged at 10,000 rpm for 10 min, and the supernatant was recovered. The absorbance of the resulting solutions was measured at 530 and 657 nm using a spectrophotometer T70 UV-VIS PG Instruments Ltd. Samples were diluted so that absorbance readings at 530 nm were less than 1.2. They were allowed to equilibrate for 15 min before absorbance at 530 and 657 nm and the control was conducted in the same manner, except that the distilled water was used instead of sample. All determinations were carried out in triplicate.

The difference in absorbance (A) between 530 nm ($A_{530 \text{ nm}}$) and 657 nm ($A_{657 \text{ nm}}$) wavelengths was calculated (Eq. 1):

$$A = A_{530 \text{ nm}} - 0.25 \times A_{657 \text{ nm}} \quad (1)$$

The concentration of monomeric anthocyanin pigment was obtained (Eq. 2):

$$\text{Anthocyanins content (mg/g)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times l} \quad (2)$$

where *MW* is the molecular weight (449.2 g/mol), *DF* is the dilution factor, ϵ is the molar absorptivity ($\epsilon = 26.900$ L/cm/mg) for cyanidin-3-glucoside was used, and *l* is for a standard 1 cm path length. Total monomeric anthocyanins were reported as milligrams anthocyanins per 100 g fresh weight (mg cyanidin-3-glucoside/100 g fw) [16].

Total ash composition was obtained by calcination of 5 g of sample at 600 °C for 240 min in an electric furnace (SR ISO 763: 2008). The resulted ash was transferred into a 25 mL volumetric flask, where it was dissolved by adding a mixture of nitric acid 65% and deionized water.

Concentrations of mineral content in the filtrate of ash samples were estimated by using a mass spectrometer with inductively coupled plasma (ICP-MS) Agilent Technologies 7500 Series (Agilent, USA). The ICP-MS parameters were: nebulizer 0.9 mL/min, RF power 1500 W, carrier gas 0.92 L/min, makeup gas 0.17 L/min, mass range 7–205 a.m.u. (atomic mass unit), integration time 0.1 s, acquisition 22.76 s. Detector parameters were: discriminator 8 mV, analogue HV 1770 V and pulse HV 1070 V.

2.4. Statistical analyses

Three samples of each white or red cabbage were analyzed in order to obtain the contents of ascorbic acid, anthocyanins, minerals and color. All assays were carried out in triplicate. The obtained data were analyzed by using Minitab software version 17, one – way ANOVA was applied for statistical evaluation and Tukey method was used for comparisons.

3. Results and discussion

Color analysis. The chromatic characteristics of the white and red cabbage leaves (outer, central and inner layer) samples shown in Tables 1 and 2. A color parameter studied was brightness, L^* , the colorimetric parameter used to characterize the color variation of food during processing.

Table 1. Color parameters in white cabbage samples

	Color parameters			
	L^{*1}	a^{*2}	b^{*3}	ΔE^4
S1	80.8	-5	32.81	32.48
S2	84.6	-1.95	18.14	17.34
S3	86.13	-2.08	17.84	16.22

L^{*1} - lightness; a^{*2} - indicates red for positive value and green for negative value; b^{*3} - indicates yellow for positive value and blue for negative value; ΔE^4 - total change in color.

Table 2. Color parameters and total monomeric anthocyanins in red cabbage samples

	Color parameters				<i>TMA</i> ¹
	L^{*3}	a^{*4}	b^{*5}	ΔE^6	
S4	23.96	3.59	-1.04	71.5	65.124
S5	24.89	6.84	-3.43	71.4	52.874
S6	25.87	8.84	-3.91	71.9	21.541

*TMA*¹ - total monomeric anthocyanins mg cy-3-gluE, mg/100g fw (fresh weight of cabbage); L^{*3} - lightness; a^{*4} - indicates red for positive value and green for negative value; b^{*5} - indicates yellow for positive value and blue for negative value; ΔE^6 - total change in color.

As expected, the L^* value is different for the three sample leaves of the white cabbage raw materials. Inside samples in cabbage induced slight color changes with ΔE

values for S2 and S3 cabbage leaves of 17.34 and 16.22, respectively. The reduction in b^* from 32.81 to 17.84 indicated losses of yellowish hues.

One of the parameters used to characterize the color variation is the total color difference ΔE^* , since it is a combination of the parameters L^* , a^* and b^* .

It was observed that the total change in color ΔE^* increased from the outer leaves to the inner layer red cabbage (Table 2).

The lightness (L^*) for the samples showed an increasing tendency to 25.87, which means that the red-purple hue is more pronounced outside the red cabbage than inside it. The outer leaves of red cabbage always showed lower total color difference ΔE^* and L^* , and higher anthocyanin levels, a synonym of redder and darker leaves.

Ascorbic acid C levels in white cabbage leaves vary between 158.965 (S1) and 163.52 mg/100 kg fw of cabbage (S3), whereas for red cabbage the concentration is 490.214 mg/kg of S4 and for S6, 487.003 mg/kg of cabbage (Figure 2).

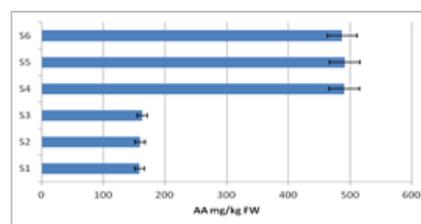


Figure 2. Ascorbic acid (mg/kg fw) – of white and red cabbage leaves (outer (S1, S4), mid (S2, S5) and inner layer (S3, S6) used for analysis.

The vitamin C content of red cabbage is approximately 3 times higher than in the white cabbage (Figure 2). White cabbage cultivated in summer are rich source of ascorbic acid (37.3 mg/100 g fw) compared to cabbage cultivated in winter (27.9 mg/100 g fw) [5].

Anthocyanin levels in red cabbage were as follows: 65.124 mg/ 100 g fw for outer leaves (S4) and 21.541 mg/ 100 g for inner leaves. Podsedek *et al.* [7] reported the anthocyanin levels for red cabbage (40.53 mg/ 100 g FW - 76.16 mg/ 100 g FW). The anthocyanin rich red cabbage showed high vitamin C contents and correspondingly high anti-oxidant activity.

Table 3. Limit of detection (LOD), limit of quantification (LOQ), precision, recovery for the 13 elements analyzed using ICP-MS.

Analyte	LOD ($\mu\text{g/L}$)	LOQ ($\mu\text{g/L}$)	Precision (CV %)	Recovery (%)
Na	115.125	348.829	3.89	98
Mg	1.212	3.672	4.05	99
Al	3.812	11.55	3.21	97
Ca	3.156	9.563	4.87	96
Cr	0.592	1.794	2.93	97
Mn	0.456	1.382	4.21	99
Fe	0.829	2.512	4.87	99
Co	86.254	261.35	4.09	103
Ni	0.261	0.791	3.26	95
Cu	0.346	1.048	4.21	98
Zn	22.659	68.657	2.98	98
Se	1.61	4.878	1.92	104
Cd	62.624	189.751	1.95	104

Method of validation. Determination of all 13 elements was performed simultaneously with ICP-MS after acid mineralization. According to [17], two important performance characteristics were calculated: limits of detection (LOD) and limits of quantification (LOQ). The calculation of LOD and LOQ was performed considering three and ten times the standard deviation of

the blank divided by the slope of the analytical curve, respectively [17]. LOD, LOQ, precision and recovery values for the analyzed elements are presented in Table 3.

The elemental concentrations. In recent years, a much discussed subject is the human health risk due to the bioaccumulation of heavy metals in the vegetables.

Table 4. Selected metal concentrations (mg/kg) in cabbage leaves

Element	Concentration [mg/kg]					
	White cabbage			Red cabbage		
	S1	S2	S3	S4	S5	S6
Na	38.5	49.0	55.0	50.0	46.5	55.0
Mg	11.0	14.0	18.0	18.0	18.0	19.5
Al	1.65	1.65	1.6	1.6	1.65	2.1
Ca	650.0	700.0	750.0	1250.0	1200.0	1350.0
Cr	55.0	100.0	85.0	95.0	95.0	105.0
Mn	6.5	11.0	9.0	11.5	11.0	12.0
Fe	4.9	8.57	6.3	6.75	7.47	6.85
Co	0.16	0.225	0.185	0.215	0.230	0.215
Ni	31.0	6.0	4.350	6.0	5.5	55.0
Cu	8.0	3.3	3.15	4.0	3.45	3.6
Zn	2.2	2.9	3.05	4.4	2.95	4.45
Se	230.0	375.0	415.0	405.0	285.0	360.0
Cd	0.750	1.100	1.250	1.450	1.350	1.550

The elemental concentrations in the different leaves of white and red cabbage are summarized in Table 4. The white and red cabbage had the highest mean concentrations of Ca, Se, and Na. The Ca concentrations in the inner leaves were higher than in outer leaves. The highest concentrations of Cr were determined in the inner layer of red cabbage leaves and in the mid layer of white cabbage leaves. Cr concentrations in the other layers of red cabbage (outer, mid) are equal, while the lowest concentration of Cr was registered for outer layer of white cabbage. It can be seen that Co accumulates more in the mid layers of both cabbage types and also that red cabbage has a higher capacity to accumulate Cd compared to white cabbage (Table 4).

Statistical evaluation. One-way analysis of variance (ANOVA) was applied to determine if the group means are different. The α -level used is the common one of 0.05. p -values obtained were compared with α -value. In Table 5 are presented the values obtained for Cr, Co, Ni, Cu, Zn and Cd. It can be observed that p -value for each element is 0.000 which means that some of the cabbage leaves have different means.

In Figure 3, in the interval plot S6 has the highest mean while S1 has the lowest mean. It can be observed that in Fig. 3b S5 has the highest mean, followed by S2.

The highest mean in the case of Ni investigation was registered for S6 followed by S1, while the other samples are almost in the same grouping categories (Fig. 3c).

In Fig. 3d is shown that S1 has the highest mean, while S3 has the lowest mean. In the case of Zn and Cd, S6 has the higher mean, while S1 the lowest one. The highest concentration of Cr, Ni, Zn and Cd is in S6 (red cabbage, inner leaves), while the lowest in S1 (white cabbage, outer leaves). Co is accumulating more in S5 (red cabbage, mid leaves) and less in S2 (white cabbage, mid leaves).

The means were compared by using Tukey Pairwise Comparisons in order to establish the group of means. In Table 6 were presented the values for means (when the amounts of Cr included in each sample were investigated) and the grouping categories for each sample. Samples S1, S2, S3, and S6 are significantly different while S4 and S5 are in the same group (the differences between the amount of Cr present in these samples are not statistically significant). The confidence interval includes zero only for S5-S4 (-1.374; 0.974) indicating also that the differences are not statistically significant. It was observed that for the remaining pairs of means all the confidence intervals do not include zero value

Table 5. Analysis of variance

Source	DF (total degrees of freedom)	Adj SS (Adjusted sums)	Adj MS (Adjusted mean)	F-value	p-value
Cr					
Type of sample (TS)	5	4830.32	966.065	5269.45	0.000
Error (E)	12	2.20	0.183		
Total (T)	17	4832.52			
Co					
TS	5	0.0100	0.0020	22.02	0.000
E	12	0.0011	0.00009		
T	17	0.0111			

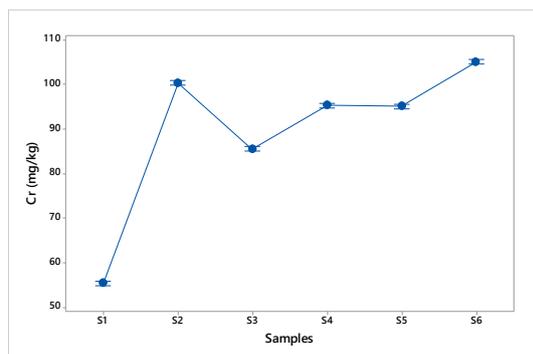
Source	DF (total degrees of freedom)	Adj SS (Adjusted sums)	Adj MS (Adjusted mean)	F-value	p-value
Ni					
TS	5	6478	1295	2342	0.000
E	12	6.64	0.55		
T	17	6485			
Cu					
TS	5	44.34	8.86	37.98	0.000
E	12	2.80	0.23		
T	17	47.14			
Zn					
TS	5	12.77	2.55	100.22	0.000
E	12	0.30	0.025		
T	17	13.08			
Cd					
TS	5	1.228	0.2456	884.3	0.000
E	12	0.003	0.0002		
T	17				

In Table 6 are presented the mean values for each sample and the grouping categories obtained for the other investigated elements (Cr, Co, Ni, Cu, Zn, and Cd) by using Tukey Method and 95% confidence interval. It is considered that means which do not share a letter are significantly different. In order to determine how well the model fits the data S and R^2 were calculated. It is considered that the model describes better the response when the value for S is lower ($S = 0.42$ for Cr, $S = 0.09$ for Co, $S = 0.74$ for Ni, $S = 0.48$ for Cu and $S = 0.15$ for Zn).

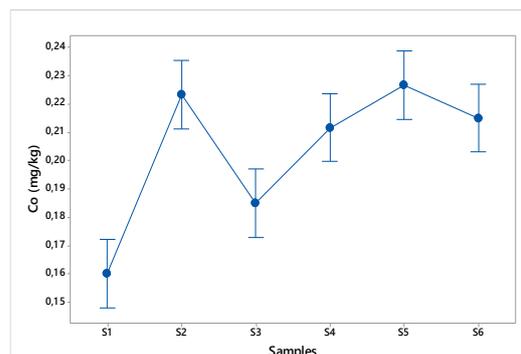
Table 6. Mean values and grouping categories for each sample obtained when Cr, Co, Ni, Cu, Zn and Cd values are investigated.

Factor	N	Mean	Grouping
Cr			
S6	3	105.13	A
S2	3	100.33	B
S4	3	95.3	C
S5	3	95.1	C
S3	3	85.5	D
S1	3	55.33	E
Co			
S5	3	0.226	A
S2	3	0.223	A
S6	3	0.215	A
S4	3	0.211	A
S3	3	0.185	B

Factor	N	Mean	Grouping
Ni			
S6	3	55	A
S1	3	31	B
S2	3	6.5	C
S4	3	6.0	C D
S5	3	5.4	C D
S3	3	4.2	D
Cu			
S1	3	7.66	A
S4	3	4.00	B
S6	3	3.63	B
S5	3	3.45	B
S2	3	3.33	B
S3	3	3.15	B
Zn			
S6	3	4.42	A
S4	3	4.40	A
S3	3	3.06	B
S2	3	2.90	B
S5	3	2.70	B
S1	3	2.20	C
Cd			
S6	3	1.54	A
S4	3	1.45	B
S5	3	1.34	C
S3	3	1.25	D
S2	3	1.08	E
S1	3	0.75	F



a)



b)

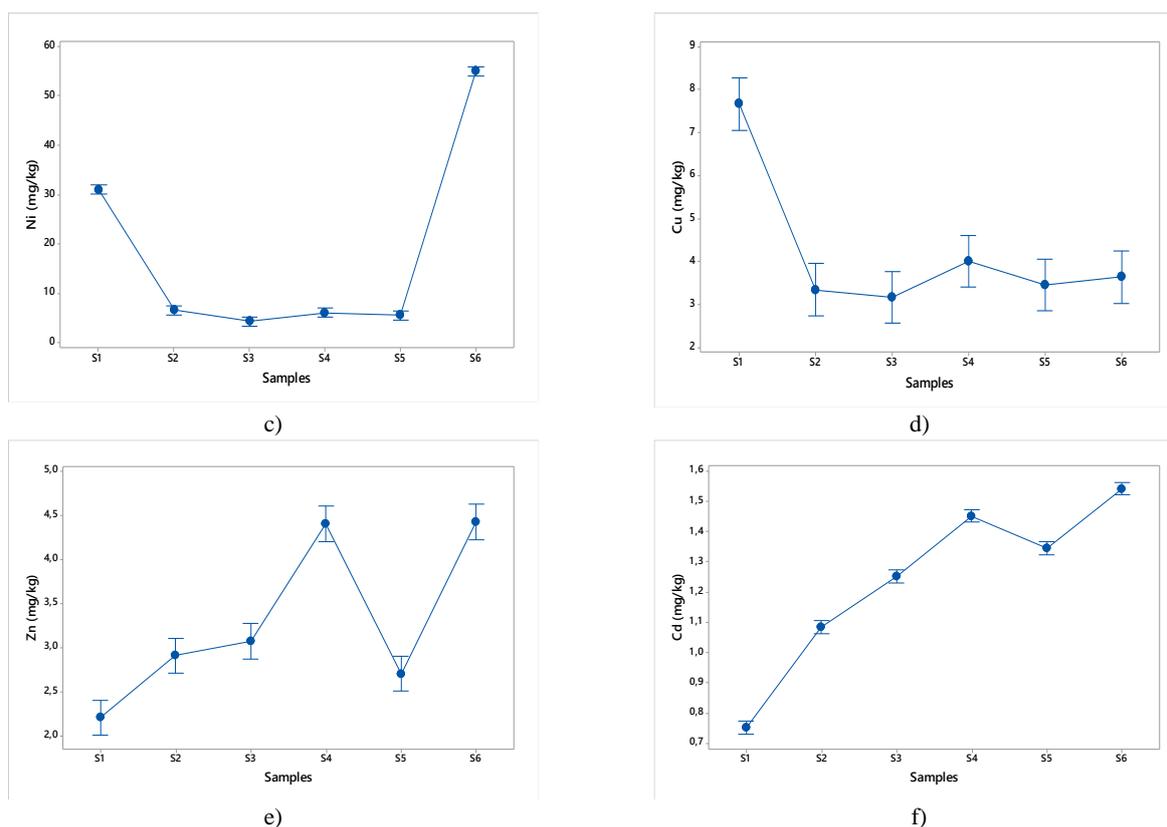


Figure 3. Interval plot of S1, S2, S3, S4, S5, S6 for six elements (Cr, Co, Ni, Cu, Zn, Cd)

Also, when high values were obtained for R^2 it can be assumed that the model fits the data. The values obtained for R^2 were: 99.9% for Cr, 90.17% for Co, 99.9% for Ni, 94% for Cu, 97.6% for Zn. These means that the values obtained for R^2 indicate that the predictors explain 99.9 % of the variance in Cr and Ni, and only 90.17% of the variance in Co.

4. Conclusions

In this work, acid ascorbic, anthocyanins, macroelements, microelements, and heavy metal contents in different types of cabbage were determined. Color parameters were also investigated. Brassica vegetables have been used in human nutrition due to their rich nutritional value and low-calorie content, being grown in relatively cold areas. In red cabbage the evolution of color is a direct result of an increase in the levels of anthocyanins. The anthocyanins content of red cabbage will decrease for the inner layers as expected.

Results showed that the highest concentration of Cr, Ni, Zn and Cd is in red cabbage, inner leaves, while the lowest in white cabbage, outer leaves. In red cabbage, mid leaves a higher concentration of Co accumulates compared to the Co concentration that accumulate in white cabbage (mid leaves). After applying the Tukey Pairwise Comparisons results indicates that depending on the studied elements and especially on the concentrations in which they accumulate in different parts of the cabbage the samples may be significantly different or may be in the same group. It can be assumed based on the values obtained for S that the model describes well the response. Higher values for R^2 were obtained which means that the model fits well with the data.

Conflict of interest

Authors declare no conflict of interest.

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