BIOLOGICA NYSSANA
4 (1-2) • December 2013: 49-55
11th SFSES • 13-16 June 2013, Vlasina lake

Original Article

Chemometric Analysis of Chlorophyll a, b and Carotenoid Content in Green Leafy Vegetables

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***Abstract*:**
Mitić, V., Stankov Jovanović, V., Dimitrijević, M., Cvetković, J., Petrović, G., Stojanović, G.: ***Chemometric Analysis of Chlorophyll a, b and Carotenoid Content in Green Leafy Vegetables. Biologica Nyssana, 4 (1-2), December 2013: 49-55.***

Effects of cooking on chlorophyll a and b and total carotenoid content in seven leafy green vegetables (Brussels sprout, white cabbage, kale, chard, garden patience, broccoli and spinach) were evaluated. Pigment content varied between species. Chlorophyll a content was higher in all analyzed vegetables, compared to chlorophyll b and carotenoid content. Boiling caused significant loss of chlorophyll a, chlorophyll b and carotenoids in kale (90.30%, 96.32% and 98.09%, respectively). Cluster analysis was applied on pigment content in fresh and boiled vegetables and two statistically significant clusters were obtained, with difference in spinach position.

Key words: Carotenoid content, chlorophyll content, chemometrics, cluster analysis, leafy green vegetables

Introduction

Leafy green vegetables are widely consumed in Serbia. Most of the vegetables are cooked by boiling in water or consumed fresh. Fresh leafy vegetables contain valuable food components, such as chlorophylls, carotenoids, vitamins and phenolic compounds. These compounds are associated with dietary activities (Kimura & Rodriguez-Amaya, 2003; Gutierrez et al., 2008). Leafy green vegetables are well known for their characteristic flavor, color, and therapeutic value (Faller & Fialho, 2009). These vegetables also contain chlorophylls and carotenoids (Kimura & Rodriguez-Amaya, 2002), which are responsible for visual quality attributes (Xue & Yang, 2009). Chlorophylls are the earth's most important organic molecules as they are necessary for photosynthesis (Marković et al., 2012). Chlorophyll is green pigment, which contain magnesium ion at the center of the porphyrin ring. There are various types of chlorophyll structure (chlorophyll a, chlorophyll b, chlorophyll c1, chlorophyll c2, chlorophyll d), but plants contain chlorophyll a and b. Chlorophyll b differs from chlorophyll a only in aldehyde group bonded to the porphyrin, compared to the methyl group for chlorophyll a. Chlorophyll a is photosynthetic pigment and absorbs blue, red and violet wavelengths in the visible spectrum, while chlorophyll b absorbs blue light and is used to complete the absorption spectrum of chlorophyll a. Carotenoids are an important group of pigments in bacteria, algae and higher plants, where they function as accessory photosynthetic pigments covering regions of the visible spectrum not utilized by chlorophylls. Carotenoids belong to tetraterpenoids, and they are split into two classes:

xanthophylls (which contain oxygen) and carotenes (hydrocarbons, contain no oxygen). Chlorophylls and carotenoids have an important role in the prevention of various diseases such as cancer, cardiovascular diseases and other chronic diseases (Sangeetha & Baskaran, 2010).

Cooking process can cause changes in physical characteristics and chemical composition of vegetables (Zhang & Hamauzu, 2004). Chlorophylls are known to be easily degraded by acids, heat and light (Tonucci & Von Elbe, 1992). Food color plays important role in product acceptability, so it is important to prevent chlorophyll loss. The reason for the green color loss during processing is attributed to conversion of chlorophylls to pheophytins.

This study was undertaken to evaluate pigment content in fresh and boiled broccoli (*Brassica oleracea* var. *silvestris*), Brussels sprouts (*Brassica oleracea* var. *gemmifera*), white cabbage (*Brassica oleracea* var. *capitata*), kale (*Brassica oleracea* var. *sabauda*), chard (*Beta vulgaris*), spinach (*Spinacia oleracea*) and garden patience (*Rumex patientia*). Cluster analysis was applied to group vegetables based on their pigment content.

Material and methods

Fresh broccoli, Brussels sprouts, white cabbage, kale, chard, spinach and garden patience were purchased from local market in Niš – Serbia. The contents of chlorophyll and carotenoids were determined spectrophotometrically. Pigment content was determined in fresh and boiled vegetables. Vegetable (10 g) was added to 50 mL of water and cooked for 5 minutes. Samples were drained off and cooled. Vegetables were cut into small pieces and weighed to 0.5000 g. Measured vegetable material was mixed with acetone and homogenised in a mortar. $MgCO_3$ was added before vegetable homogenization to prevent chlorophyll pheophytinization. Mixtures were filtered, mortar and pestle were washed several times with acetone, and the content was quantitatively transferred to the filter. The filter was washed with acetone so that the rest of the filter was completely white. Filtrate was diluted with acetone to a total volume of 25 ml. Apsorbance of prepared mixtures was recorded at 662, 644 and 440 nm using acetone as blank and pigment content was calculated using the formula of Holm and Wetsttein:

Chlorophyll a = $9.784 \cdot A_{662} - 0.990 \cdot A_{644}$

Chlorophyll b = $21.426 \cdot A_{644} - 4.650 \cdot A_{662}$

Chlorophyll a + b = $5.134 \cdot A_{662} + 20.436 \cdot A_{644}$

Carotenoids = $4.695 \cdot A_{440} - 0.268 \cdot (a + b)$;

A = absorbency at corresponding wave length, values 9.784, 0.990, 21.426, 4.650 and 0.288 is the molar absorptivity coefficient according to Holm (1954) and Wetsttein (1957) for acetone (absorption of 1 cm). After calculating the concentrations, the amounts of pigment per g of fresh matter were calculated applying the formula:

$$c = \frac{c_1 V r}{m}$$

C = content of pigment (mg/g) of fresh matter;

C_1 = the concentration of pigment calculated by the previous formula (mg/l);

v = the starting volume of extract (ml);

r = dilution;

m = the weighed fresh plant (g).

Statistical analysis

All results are expressed as mean of three determinations. Arithmetic mean, the standard deviation and the coefficient of variation and correlations were calculated applying software Statistica 7 (StatSoft, Inc., Tulsa, OK, USA).

Cluster analysis (CA) is a multivariate technique, with the purpose of classifying the objects of the system into categories or clusters based on their similarities (Richard & Dean, 2002). CA is reported in several publications for the analysis of foodstuff (Cam et al., 2009, Tomsone et al., 2012). Results obtained by cluster analysis are typically illustrated by a dendrogram. Cluster analysis was done by means of Ward's method using Euclidean distances as a measure of similarity. Ward's method attempts to minimize the sum of squares of any two (hypothetical) clusters that can be formed at each step. Euclidean distance is most common way to measure distance between objects.

$$\text{Distance}(x,y) = \sqrt{\sum_{i=1}^{n} (x_i - y_i)^2}$$

The distances can be affected by differences in scale among the dimensions from which the distances are computed, so the variables must be standardized. The linkage distance is reported as D_{link}/D_{max}, which represents the quotient between the linkage distances for a particular case divided by the maximal linkage distance. The data derived from this study were originally centered by logarithmic transformation with all variables standardized. Statistica 7 software was used to compile the dendrogram.

Results and discussion

Analyses performed on selected vegetables showed a large variety of chlorophyll a, b and carotenoid content. Chlorophyll and carotenoid content in green leafy vegetables was measured in fresh and boiled vegetables and the results were presented in Figure1, 2 and 3.

Chlorophyll a content varied between species, from 0.047 mg/g fresh vegetables (f.v.) in Brussels sprout, to 0.837 mg/g f.v. in garden patience (Figure 1). Amount of chlorophyll a was higher than chlorophyll b and carotenoids in all selected vegetables, which is in agreement with literature (Lopez-Ayerra et al., 1998). In fresh vegetables chlorophyll b ranking was: spinach > kale > garden patience > chard > broccoli > cabbage > Brussels sprout (Figure 2). These results showed that the chlorophyll concentration in the leafy green vegetables is dependent on species. The highest carotenoid content (Figure 3) in spinach (0.191 mg/g f.v.) was seven times higher than than the lowest content in Brussels sprout (0.024 mg/g f.v.).

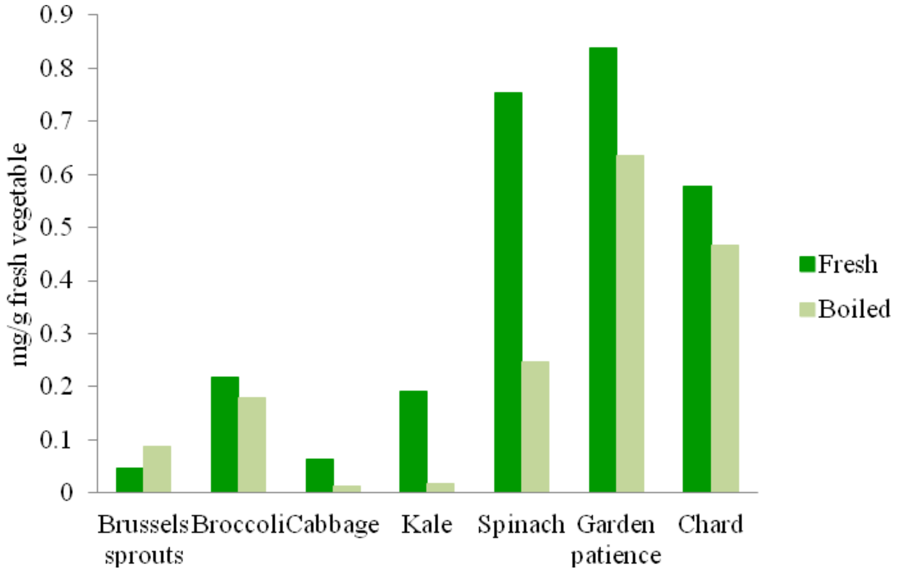

Fig. 1. Chlorophyll a content in fresh and boiled vegetables

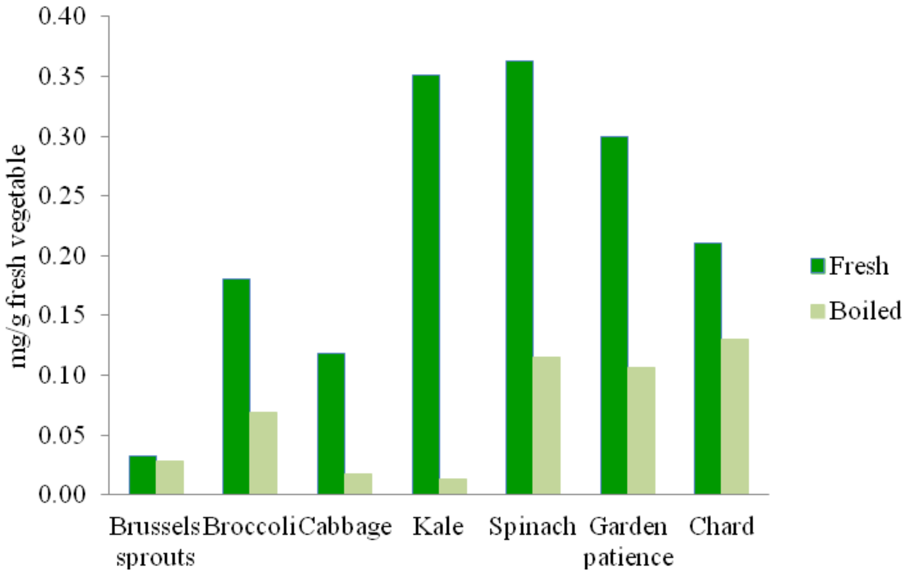

Fig. 2 Chlorophyll b content in fresh and boiled vegetables

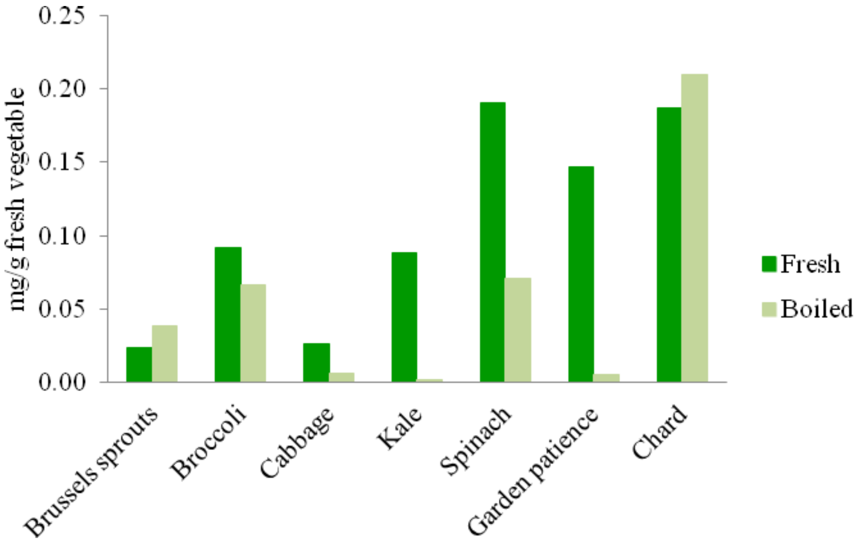

Fig. 3. Carotenoid content in fresh and boiled vegetables

Most vegetables are cooked by boiling before consumed. Cooking process has influence on chemical composition of vegetables (Ismail et al., 2004; Zhang and Hamauzu; 2004). Sahlin et al. (2004) concluded that boiling, baking and frying had effect on the ascorbic acid, total phenolic, lycopene and antioxidant activity of the tomatoes. Few data on the chlorophyll and carotenoid content of these vegetables are found in the literature, and disagreement with literature data may be caused by differences in varieties used and analytical methods applied. Turkmen et al. (2006) reported that chlorophyll a+b content in boiled spinach and broccoli was lower than in fresh vegetables, which is in agreement with our study. Boiling caused significant loss of chlorophyll a, chlorophyll b and carotenoids in kale (90.30%, 96.32% and 98.09%, respectively). Reduction of chlorophyll a and b content is attributed to the degradation of chlorophylls in their main derivatives pheophytin a and b, respectively (Boekel, 1999). Pheophytin formation is result of Mg^{2+} eliminiation of chlorophyll. Pheophytin can be produced by an acidic cooking environment or by prolonged cooking.

In boiled vegetables, chlorophyll a content was lower than in fresh ones, except in Brussels sprout. Boiled Brussels sprout contained 0,534 mg/g f.v. chlorophyll a, which is almost twice higher than chlorophyll a content in fresh vegetables. Chlorophyll b content was lower in boiled than in fresh vegetables. Chlorophyll a and b content decreased the most in kale (96.32% and 90.30%, respectively). Carotenoid content was higher in boiled Brussels sprout and chard than in fresh ones. In other vegetables tested, carotenoid content decreased, ranged from 28.07% in broccoli to 98.09% in kale.

Chemometrics is the branch of chemistry dealing with the analysis of chemical data (extracting information from data) and ensuring that experimental data contain maximum information (the design of experiments) (Wold, 1995). Chemometrics is used to classify of food products based on their main compounds (Woodcock et al., 2007). In cluster analysis (CA), samples are grouped based on similarities. In this study CA was performed to the standardised data, on chlorophyll a, chlorophyll b and carotenoid content in fresh and boiled vegetables. Two clusters were formed after applying cluster analysis to pigment content in fresh vegetables. Brussels sprout, cabbage, broccoili and kale formed one cluster, while spinach, garden patience and chard grouped in second cluster. Brussels sprout and cabbage showed lowest pigment content, so it was expected that they are „nearest neighbors“. Euclidean distance between this two species was lowest (0.09). Largest distance was observed between Brussels sprout and garden patience (0.84), and accordingly differences between their pigment content was highest.

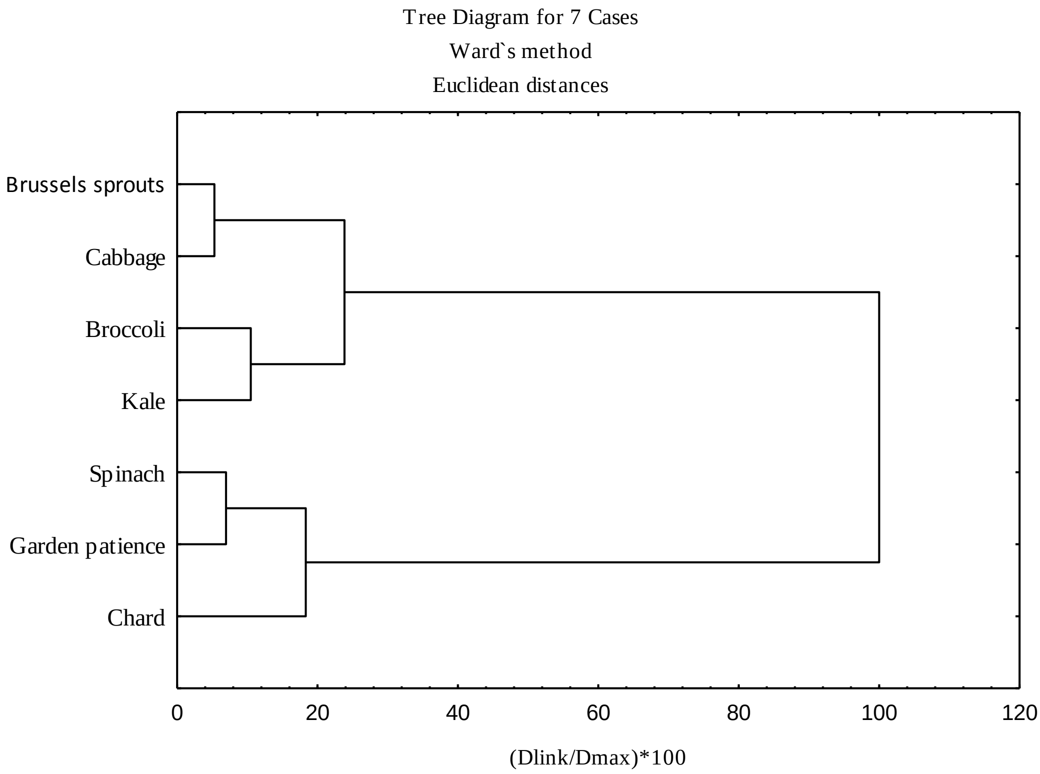

Fig. 4. Dendrogram obtained by cluster analysis using means of pigment content in fresh vegetables

Table 1. Euclidean distances among analyzed fresh vegetables

Euclidean distances (Fresh vegetables)							
	Brussels sprouts	Broccoli	Cabbage	Kale	Spinach	Garden patience	Chard
Brussels sprouts	0.00	0.24	0.09	0.36	0.80	0.84	0.58
Broccoli	0.24	0.00	0.18	0.17	0.57	0.63	0.37
Cabbage	0.09	0.18	0.00	0.27	0.75	0.80	0.55
Kale	0.36	0.17	0.27	0.00	0.57	0.65	0.42
Spinach	0.80	0.57	0.75	0.57	0.00	0.11	0.23
Garden patience	0.84	0.63	0.80	0.65	0.11	0.00	0.28
Chard	0.58	0.37	0.55	0.42	0.23	0.28	0.00

CA was performed on pigment content in boiled vegetables (Figure 5). According to CA, boiled leafy green vegetables can be grouped as follows: Brussels sprout, cabbage, kale, broccoli and spinach (cluster 1) and garden patience and chard (cluster 2). Clustering according boiled vegetables pigment content shows different grouping of vegetables. Compared to dendrogram compiled using fresh vegetables pigment content, this one differs in spinach position. Boiled spinach have similar pigment content as Brussels sprout, cabbage, kale and broccoli, while fresh spinach is more similar to garden patience and chard. Nearest neighbors are boiled cabbage and kale, with Euclidean distance of 0.01.

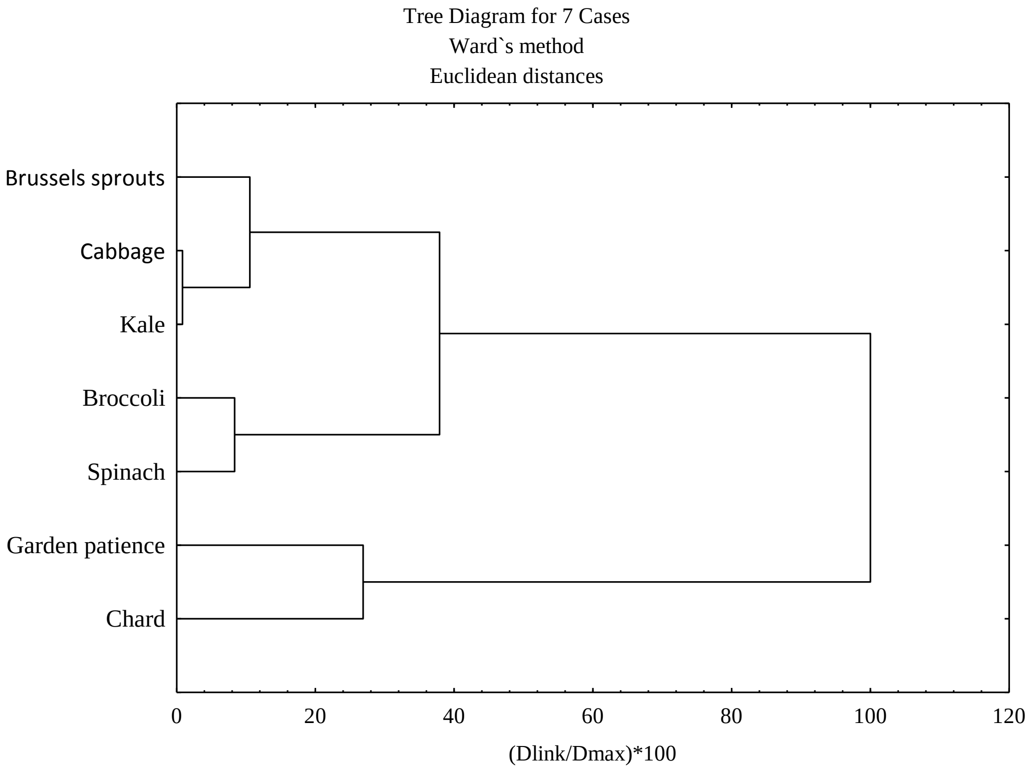

Fig. 5. Dendrogram obtained by cluster analysis using means of pigment content in boiled vegetables

Table 2. Euclidean distances among analyzed boiled vegetables

Euclidean distances (Fresh vegetables)							
	Brussels sprouts	Broccoli	Cabbage	Kale	Spinach	Garden patience	Chard
Brussels sprouts	0.00	0.10	0.08	0.08	0.18	0.55	0.43
Broccoli	0.10	0.00	0.18	0.18	0.08	0.46	0.33
Cabbage	0.08	0.18	0.00	0.01	0.26	0.63	0.51
Kale	0.08	0.18	0.01	0.00	0.26	0.62	0.51
Spinach	0.18	0.08	0.26	0.26	0.00	0.39	0.26
Garden patience	0.55	0.46	0.63	0.62	0.39	0.00	0.27
Chard	0.43	0.33	0.51	0.51	0.26	0.27	0.00

Conclusion

Chlorophyll a, b and carotenoid content varied between species. Chlorophylls and carotenoids have significant role in human diet, so it is important to determine their loss during cooking process. Largest decrease of all analysed pigments was recorded in kale. Cluster analysis can be used to classify vegetables according to certain properties. According to pigment content in fresh vegetables, two statistical significant clusters were obtained. Pigment content in boiled vegetables also yelds dendrogram with two clusterts, but with different grouping.

Acknowledgements: The research was supported by Ministry of Education, Science and Technological Development of the Republic of Serbia [OI 172047; OI 172051].

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