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Chemometric tools in the investigation of the mineral composition of food products derived from seaweed

Metody chemometryczne w badaniu składu produktów spożywczych wytworzonych z alg

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ABSTRACT: The work presents a new chemometric-assisted approach to distinguish commercially available food products based on their chemical composition. The analysed material consisted of 15 seaweeds (red *Rhodophyta* and brown *Phaeophyta* macroalgae) of various origin. The concentrations of the main nutrients (K, Na, Ca, and Mg) and essential trace elements (Fe, Mn, and Zn) were determined using flame atomic emission spectroscopy and atomic absorption spectrometry. The highest concentrations of nutrients were found in the products of brown algae (for example: the highest concentration of Ca was determined in the *Kombu* algae product – 13.92 mg/g dr.wt.; Mg – in *Wakame* – 9.85 mg / g dr.wt.) compared to the products of red algae (the lowest concentrations of Ca and Mg were found in *Dulce* algae – 1.87 mg / g dr.wt. and 2.83 mg / g dr.wt., respectively).
Chemometric tools, i.e. principal components analysis and cluster analysis combined with heat maps allowed to distinguish samples clearly by species, red algae (*Nori, Dulse, Irish moss)* from brown ones (*Wakame*, *Kombu*). However, neither the place of harvest (country of origin) nor the food processing has allowed the separation of the food samples into individual groups. It was proven that the nutritional properties of food derived from naturally grown sea algae depend on the characteristic of the species, rather than on the place of harvest. Furthermore, the method of food processing changes its mineral composition to a very limited degree.

Keywords: seaweeds, algae, microelements, macroelements, flame atomic emission spectroscopy and atomic absorption spectrometry, chemometrics

1. Introduction

The interest of people in algae has begun in ancient times due to the possibility of its use as food and for medical purposes [1,2]. Due to their bioactivity and functional properties, they are important resources in numerous commercial applications, particularly in the production of food, pharmaceutical formulations, nutraceuticals [3–5] or bioethanol [6]. Macroalgae are a renewable source of biomass in marine ecosystems. They convert light energy and carbon dioxide into biomass which includes carbohydrates, protein, and lipids, thus, algae have crucial importance for the food chain. Among cultivated marine organisms, seaweeds play an important role as rich sources of natural food, containing several nutrients and functional ingredients, including minerals and vitamins [4,5,7]. Seaweeds species also contain a variety of minerals in high concentrations, that is, 8-36% of dry matter in some species [8]. Mineral macronutrients include sodium, calcium, magnesium, potassium, chlorine, sulfur, and phosphorus, while micronutrients include iodine, iron, zinc, copper, selenium, molybdenum, fluoride, manganese, boron, nickel, and cobalt. Among these minerals, calcium accounts for 4–7% of dry matter and therefore seaweeds are also the most important vegetal source of Ca [5]. Among the bioactive compounds identified in algae, special attention has been paid to phenolic compounds due to their health benefits [9]. These include phenolic acids, tannins, flavonoids, and phlorotannins [5,10].

Regarding specific benefits in human health, seaweeds have been shown to exert preventive effects against several non-transmissible diseases such as cardiovascular disease, antihypertensive, anti-obesity and anti-diabetic effects, anticancer or antioxidant activity [1,4,5,11–14]. Seaweeds products

are used as a food supplement to replace many functional ingredients in the food processing industry. It is used in natural forms, processed in various ways, or powdered as an additive in many dishes [10].

The biodiversity of seaweeds, that is, their availability as red (*Rhodophyta*), green (*Chlorophyta*), brown (*Ochrophyta*), and blue-green (*Cyanobacteria*) macroalgae [1, 4, 15], offers the possibility of finding a wide variety of natural compounds with interesting properties [3]. Therefore, increasing attention has been paid to macroalgae metabolites of industries from different branches (textile, fuel, plastics, paint, varnish, cosmetics, pharmaceuticals and food) in recent years [15]. Algae are also widely used in the animal breeding sector [8].

Seaweeds or marine algae are harvested either naturally or from cultivated crops. Commercial seaweeds harvesting are carried out in approximately 35 countries, in waters ranging from cold, to temperate, to tropical [16–18]. As reported by Global Market Insights Inc. the value of the commercial seaweeds market is expected to rise over $85 billion by 2026.

Chemometrics is the scientific application of mathematical and statistical methods designed to extract useful information from instrumental analytical data. These algorithms are tools commonly used in the evaluation of food origin and quality, and their use has an impact on food safety. Many studies also describe the use of chemometric methods in assessing the quality and source of algae or products made of these plants. For example, 31 samples from different macroalgae species have been studied to determine the influence of several parameters such as the harvesting season, the geographical origin, the species, or a pretreatment procedure on their volatile composition [15]. The multivariate study includes principal component analysis (PCA) and cluster analysis (CA). In [10] the chemometric techniques were used to discriminate two Indonesian seaweeds cultivars based on their physicofunctional properties, processed using multivariate analysis, discriminant analysis, principal component analysis, and cluster analysis. The functional chemical substances and the antioxidant activity of lipids were investigated in 21 marine algae along the Japanese coast. Principal component analysis was performed to detect any correlation between chemical substances and the algae phylum [18]. Non-destructive and fast analyzes of different morphological structures of two brown algae, which were collected during different seasons, were performed using Fourier transform infrared techniques in combination with chemometric methods and described in [19].

Seaweeds are known to be an abundant source of minerals. The mineral composition of seaweeds is very changeable due to many exogenous and also endogenous factors and differs also within the same species. Six representatives of edible seaweeds from the central west Portuguese coast were harvested from the Buarcos bay, Portugal, and their chemical characterization was determined. The elemental composition was determined using inductively coupled plasma optical emission spectrometry for Mo, B, Zn, P, Cd, Co, Ni, Mn, Fe, Mg, Ca, Cu, Na, Al, and K [8]. The paper [20] provides one of the most comprehensive studies of metal distributions (Pb, Zn, As, Cd, Co, Cr, Cu, Mn, Ni) in three main macroalgae species. Chapter [21] focuses on some trace elements I, Fe, Zn, and Mg that are abundant in seaweeds and also describes factors that influence the mineral content in seaweeds.

The main objective of this study was to investigate the elemental composition of 15 seaweed-derived food products (red and brown macroalgae), produced in Ireland, Scotland, and Spain. The elemental compositions including macroelements Mg, Ca, Na, K, and microelements Fe, Mn, and Zn, after proper preparation of the samples for analysis, were done using flame atomic emission (FAES) and atomic absorption spectrometry (AAS). To determine the variability of the chemical composition, various clustering methods with heat maps and principal component analysis using the nonlinear iterative partial least squares (NIPALS) algorithm were applied for the exploratory analysis of the data.

The research presented in this paper may enhance the practical application of chemometrics to the study of the origin and quality of algae, optimize food processing technology, and promote the use of seaweeds in the development of new food products.

2. Experimental

2.1. Instrumentation and reagents

The determination of the chosen elements (Mg, Ca, K, Na, Fe, Mn, and Zn) in commercially available food samples - edible algae – was made by means of flame atomic emission spectroscopy and atomic absorption spectrometry methods using a PerkinElmer spectrometer Model 3110 (USA). Prior to quantitative analyzes, the samples were digested wet in a microwave system. The microwave digestion process was performed using the Anton Paar Multiwave 3000 system (Swiss made).

During the digestion process, hydrogen peroxide (30% suprapur, Merck, Germany) and concentrated nitric acid (V) (suprapur, Merck, Germany) were applied. Standard stock solutions of Mg, Ca, Fe, Mn, Zn, Na and K (1000 mg/l, Merck, Germany) were used in quantitative experiments. For dilutions, double-distilled water was used.

2.2. Algae samples

In this work, the commercial food products derived from *Wakame*, *Kombu*, *Nori*, *Dulse,* and *Irish moss* were measured and described. *Wakame* is a typical brown variety, often referred to as light brownish blue. It is the second most consumed seafood in the world. It has a very smooth texture and a delicate taste, which makes it such a popular type of algae [22,23]. *Kombu* is a species of brown seaweed. It has anti-inflammatory and antirheumatic properties, supports weight loss, and regulates blood pressure. Due to the high content of magnesium, it also prevents myocardial infarctions and muscle cramps. *Kombu* regulates blood flow and therefore supports the fight against atherosclerosis and diseases of the vascular system [22].

Table 1. Origin and information about the tested samples (red *Rhodophyta* and brown *Phaeophyta* macroalgae). The date of production and the use date of the commercial product was given from which the samples were taken for testing.

No	Label	Product name	Algae color	Company	Country	Production date	Expiration date
1	K-PM-E	*Kombu*	*Brown*	Porto-Muiños [24]	Spain	09.07.2018	30.04.2021
2	N-PM-E	*Nori*	*Red*			13.09.2017	31.10.2020
3	W-PM-E	*Wakame*	*Brown*			10.09.2018	30.04.2021
4	D-PM-E	*Dulse*	*Red*			21.05.2018	31.10.2020
5	K-A-E	*Kombu*	*Brown*	AlgAran [25]		-	31.05.2021
6	W-A-E	*Wakame*	*Brown*			-	30.04.2020
7	D-A-E	*Dulse*	*Red*			-	31.12.2019
8	K-MS-S	*Kombu*	*Brown*	Mara Seaweed [26]	Scotland	25.10.2018	31.03.2020
9	D-MS-S	*Dulse*	*Red*			15.10.2018	31.03.2020
10	D-CF-I	*Dulse*	*Red*	Carraig Fhada Seaweed [27]	Ireland	-	28.08.2019
11	C-CF-I	*Irish moss*	*Red / brown*			-	28.01.2020
12	K-WISV-I	*Wakame*	*Brown*	Wild Irish Seaweed [28]		-	03.11.2019
13	W-WISV-I	*Dulse*	*Red*			-	11.08.2020
14	D-WISV-I	*Kombu*	*Brown*			-	15.10.2020
15	C-WISV-I	*Irish moss*	*Red / brown*			-	02.09.2020

Nori belongs to the group of red seaweeds. It can be used in the treatment and prevention of anemia. Due to the rich composition of vitamins and minerals, they have a positive effect on eyesight, nourishing the mucosa and skin around the eyes and improving the ability to see at night. Due to the protein content, *Nori* is recommended as a supplement to a balanced diet for pregnant women, children, adolescents, and people who practice sports. This type of seaweeds helps fight bacterial infections. It is an excellent food for lowering cholesterol and preventing atherosclerosis (it contains unsaturated fatty acids and taurine). Consuming a small amount of *Nori* aids digestion and helps eliminate stored fat. Due to the content of antioxidant substances, these algae are able to neutralize the action of free radicals responsible for tissue degeneration and aging [2,22]. *Dulse* is a typical variety of edible red algae. The color is often referred to as reddish brown. *Dulse* seaweed is perfect as a dietary supplement for people with anemia, weakness, or postoperative conditions. Consuming this species of algae has a positive effect on eyesight, strengthening it. Often, this species of seaweeds is recommended in stomach and intestinal disorders and for the regeneration of the gastric membrane. Like other edible varieties of red algae, it acts as an antiseptic to parasites and supports the intestinal flora [22]. *Irish moss* belongs to the group of red seaweeds. The color of this algae comes in shades of dark purple, yellow, or green. Plants immersed in water only take on a purple color. *Irish moss* contains carrageenan (E407) which is a thickener, emulsifier, and gelling agent. In medicine, this species is used in constipation and diarrhea because it can absorb water and regulate intestinal flow. Algae the have also ability to absorb X-rays and other radioactive elements in the body [23].

Figure 1. Photographs of the selected algae studied in our laboratory.

The tested material consisted of 15 food preparations from 8 red and 7 brown sea algae (**Table 1**) [24–28]. It was a biological material with a complex composition, characterized by high inhomogeneity. One of the most important factors that caused the difficulty of homogenization were visible fragments of tissues with different salinity. As a result of the manufacturing method, commercial food products were in various forms: in the form of powder, in the form of pieces of leaves, and in the form of pressed parchment. All the algae tested came from the Atlantic Ocean. A significant difference between the formulations (**Figure 1**) was already evident in the packaging method and the material used for packaging (for example, cling film or paper). There was also a significant difference in smell. In some cases, it was intense seafood aroma or very delicate.

2.3. Standard procedure of measurement

Samples preparation

The edible algae samples were initially dried to a constant weight (in a laboratory drier at 50 °C). The samples were then shredded into pieces with the use of stainless-steel scissors and powdered in an agate mortar to homogenize the material. The prepared algae were stored in tightly closed plastic containers in a desiccator.

Microwave digestion of the samples

The algae samples (~200 mg) were placed in Teflon vessels for digestion. To each vessel, 2 cm^3 of hydrogen peroxide and 6 cm^3 of concentrated nitric acid (V) were added. The microwave digestion process lasts 120 minutes. The sample solution obtained was quantitatively transferred to 50 cm^3 volumetric flasks and filled on the line with double distilled water [29]. Each sample was processed in two independent repetitions. The analysis of each taken sample was repeated three times. All biological material has decomposed and dissolved. The microwave wet digestion was carried out in accordance with the assumptions, as a clear solution was obtained each time. To check the possibility of sample contamination, blank samples were prepared during each step of the prepared analytical procedure. The blank samples contained very small amounts of micro and macroelements. The blank concentrations determined were found to be too low to significantly influence the result of the quantitative analysis.

Quantitative determination of the elements

Two different analytical methods were used to quantify the concentrations of the selected metals in the samples. Measurements of sodium and potassium concentrations were carried out by means of the atomic emission spectrometry method, and measurements of calcium, magnesium, iron, manganese, and zinc were made using the atomic absorption spectrometry method. The measurement parameters appropriate for each of the elements have been optimized and summarized in **Table 2**. Regardless of the method, acetylene-air flame was used, and the determinations were made under standard conditions. The standard solutions for calibration were prepared by dilution (in double distilled water) of the standard stock solutions. Calibration was performed separately for each element based on appropriate standard solutions. All quantitative determinations were made based on the obtained calibration curves, and, if necessary, the sample solutions were diluted appropriately to fit the linear calibration range of the method. Before each batch of samples, recalibration was performed based on standard solutions characteristic for each metal.

Table 2. Operating conditions of the instrument (AAS and FAES).

Analyte	Method	Hollow cathode lamp (HCL)	Wavelength / nm	Monochromator slit size / nm
Mg	AAS	Magnesium	285.2	0.7
Ca	AAS	Calcium	422.7	0.7
Fe	AAS	Ferric	248.3	0.2
Mn	AAS	Manganese	279.5	0.2
Zn	AAS	Zinc	213.9	0.7
K	FAES	-	766.5	0.7
Na	FAES	-	589.0	0.2

2.4. Chemometric analysis

Chemometric calculations using clustering methods and PCA were performed using Matlab 2014b with *PLS Toolbox*. Multivariate data modelling was implemented in Python 3.8 with the *Scikit-learn* module. *Scikit-learn* is a free software machine learning library for the *Python* programming language. Calculations were made using *Google Colab* platform, which is a free Python notebook that does not require setup and runs entirely in the cloud.

3. Results and discussion

The analysis of the elemental composition in samples of edible species of sea algae showed the high biodiversity of the biological material. Various ranges of individual analysed metals were determined. The results of the elemental analysis of the tested samples are presented in **Tables 3** and **4**. A large variation was found in the concentrations of individual macroelements in the algae food preparations considered. Magnesium concentration changed in the whole set of the tested preparations from 2.11 to 9.85 mg/g, calcium concentration from 1.87 to 21.11 mg/g, potassium concentration from 11.3 to 135.4 mg/g and sodium concentration from 18.8 to 109.0 mg/g. It has been observed that there was a relationship between the macronutrients studied. The concentrations of these elements can be arranged in order from the highest to the lowest average weight fractions. The relation of concentrations K > Na > Ca > Mg was observed. Iron concentration in the entire set of preparations ranged from 34.3 to 197.0 μg/g, manganese concentration from 0.58 to 86.11 μg/g, and zinc concentration from 0.65 to 47.66 μg/g. The relation of average concentrations of the microelements can then be presented as Fe > Mn > Zn.

The analysis of the concentrations of macroelements Mg, Ca, K, Na and the microelements of the Fe, Mn, Zn in food preparations of sea algae, depending on the color and species of algae analysed, is presented in **Table 5**. Brown species of sea algae were characterized by higher values of Ca, K, Na, and Mg concentrations. In terms of species, the highest concentrations of macronutrients were measured for brown Wakame algae.

Table 3. Quantitative analysis of nutrient minerals in seaweed-derived food products (n = 6). Results are given per gram of dry matter.

No.	Label	Mg / mg/g	Ca / mg/g	K / mg/g	Na / mg/g
1	K-PM-E	5.53 ± 0.01	13.92 ± 0.23	94.1 ± 1.4	23.0 ± 1.0
2	N-PM-E	5.54 ± 0.30	3.01 ± 0.27	26.6 ± 1.8	39.5 ± 2.4
3	W-PM-E	7.66 ± 0.55	10.53 ± 1.34	92.3 ± 5.5	46.6 ± 4.3
4	D-PM-E	2.76 ± 0.27	11.62 ± 1.02	23.1 ± 1.6	109.0 ± 1.3
5	K-AR-E	6.15 ± 1.46	10.67 ± 0.12	123.5 ± 6.7	32.7 ± 0.6
6	W-AR-E	7.94 ± 1.41	11.76 ± 0.30	84.4 ± 1.7	60.6 ± 4.6
7	D-AR-E	2.11 ± 0.23	6.14 ± 0.14	72.4 ± 1.1	18.8 ± 1.2
8	K-MS-S	7.12 ± 0.04	10.35 ± 0.06	53.8 ± 1.6	49.7 ± 0.5
9	D-MS-S	2.83 ± 0.01	2.33 ± 0.04	79.0 ± 0.8	23.5 ± 1.0
10	D-CF-I	4.01 ± 0.02	2.78 ± 0.06	67.1 ± 4.5	35.6 ± 0.9
11	C-CF-I	6.16 ± 0.18	4.44 ± 0.15	11.3 ± 0.4	30.5 ± 1.2
12	K-WISV-I	5.71 ± 0.35	8.31 ± 0.75	25.0 ± 0.8	32.0 ± 1.8
13	W-WISV-I	9.85 ± 0.34	21.11 ± 0.12	75.7 ± 2.7	63.8 ± 2.2
14	D-WISV-I	2.87 ± 0.01	1.87 ± 0.01	135.4 ± 6.7	29.4 ± 3.6
15	C-WISV-I	6.98 ± 0.12	9.30 ± 1.20	16.4 ± 0.2	29.5 ± 0.1

As for the weight fraction of individual metals in algae, it should be mentioned that the following decreasing dependence of the concentrations of K > Na > Ca > Mg was again maintained (except for alga Nori Na > K > Mg > Ca). Red algae (Nori, Dulse, Irish Moss) have higher concentrations of manganese than brown algae. For red algae, the following decreasing dependence of the mass fractions of Fe > Mn > Zn was maintained. On the other hand, for brown algae (Wakame, Kombu), the concentrations of individual elements in the descending order were as follows: Fe > Zn > Mn.

Table 4. Quantitative analysis of traces of metals in seaweed-derived food products (n = 6). Results are given per gram of dry matter.

No.	Label	Fe / µg/g	Mn / µg/g	Zn / µg/g
1	K-PM-E	47.2 ± 4.3	2.66 ± 3.18	17.29 ± 0.45
2	N-PM-E	183.0 ± 10.9	28.89 ± 1.32	13.51 ± 0.66
3	W-PM-E	42.1 ± 13.1	5.19 ± 2.84	16.02 ± 4.89
4	D-PM-E	189.5 ± 18.0	57.45 ± 11.75	0.65 ± 0.57
5	K-AR-E	34.3 ± 3.8	1.74 ± 0.96	19.64 ± 3.39
6	W-AR-E	85.4 ± 7.8	9.78 ± 0.06	12.88 ± 0.57
7	D-AR-E	106.0 ± 3.2	68.43 ± 21.09	18.81 ± 0.27
8	K-MS-S	54.5 ± 5.1	0.58 ± 0.17	23.37 ± 1.01
9	D-MS-S	109.4 ± 3.9	57.32 ± 4.42	22.09 ± 0.06
10	D-CF-I	164.6 ± 1.6	44.99 ± 2.60	17.71 ± 1.58
11	C-CF-I	66.4 ± 8.5	6.40 ± 1.78	33.13 ± 2.34
12	K-WISV-I	179.4 ± 6.2	4.37 ± 0.52	23.89 ± 1.14
13	W-WISV-I	197.0 ± 33.1	17.07 ± 1.29	47.66 ± 0.34
14	D-WISV-I	99.3 ± 11.1	26.96 ± 10.08	20.15 ± 4.70
15	C-WISV-I	130.9 ± 16.1	86.11 ± 9.89	39.93 ± 9.40

The range of variation of the analyzed microelements and macroelements for *Wakame*, *Kombu*, *Dulse*, *Nori* and *Irish Moss* species are in most cases consistent with the literature values. The occurring minor deviations from the literature values resulted from the high variability of the elemental composition of the biological material caused by the environmental conditions of the algae. An important aspect influencing the nutritional composition of algae is also the place and time of harvesting and age of the plant. A comparative analysis of the concentrations of micro and macroelements in food preparations from sea algae with literature data is also given in **Table 5**.

Table 5. Comparison of the concentrations of the macroelements and microelements determined compared with the data from the literature.

Conc. mg/100 g of the product	*Brown*		*Red*			Ref.
	Wakame	*Kombu*	*Nori*	*Dulse*	*Irish moss*	
Mg	727 - 1009	511 - 718	533 - 575	195 - 402	603 - 706	This work
	680 - 3000	384 - 2000	200 - 576	154 - 610	600 - 835	algae producers, (Saá, 2002)
Ca	958 - 2119	778 - 1408	268 - 322	187 - 1264	433 - 1014	This work
	670 - 3000	500 - 3000	200 - 800	200 - 1200	398 - 1300	Algae producers, (Saá, 2002)
K	7384 - 9621	2436 - 12820	2535 - 2793	2150 - 13060	1105 - 1652	This work
	5500 - 8843	4330 - 11707	1300 - 3615	4400 - 9000	1350 - 3400	Algae producers, (Saá, 2002)
Na	4355 - 6537	3225 - 5007	3777 - 4122	1794 - 11050	2946 - 3132	This work
	1600 - 7230	900 - 6000	400 - 3742	270 - 3000	1200 - 4332	Algae producers, (Saá, 2002)
Fe	3.29 - 22.04	3.15 - 18.37	17.53 -19.07	9.15 - 20.17	6.05 - 14.23	This work
	5 - 40	4 - 80	5.6 - 35	5.6 - 140	3.86 - 21	Algae producers, (Saá, 2002)
Mn	0.32 - 1.80	0.04 - 0.49	2.80 - 2.98	1.98 - 8.33	0.52 - 9.31	This work
	0.1 - 1.4	0.1 - 1.6	5.6 - 140	1 - 15.5	0.2 - 1.32	Algae producers, (Saá, 2002)
Zn	1.25 - 4.74	1.72 - 2.47	1.30 - 1.40	0.02 - 2.35	3.15 - 4.66	This work
	0.4 - 3.4	0.49 - 2.86	3.9 - 21	0.3 - 2.86	7.01 - 7.27	Algae producers, (Saá, 2002)

For chemometric analysis, the data obtained during the tests were collected in a data matrix together with the labels (color, species, origin) differentiating the samples. At the beginning of the chemometric approach, the dataset was analyzed using PCA (**Figure 2**).

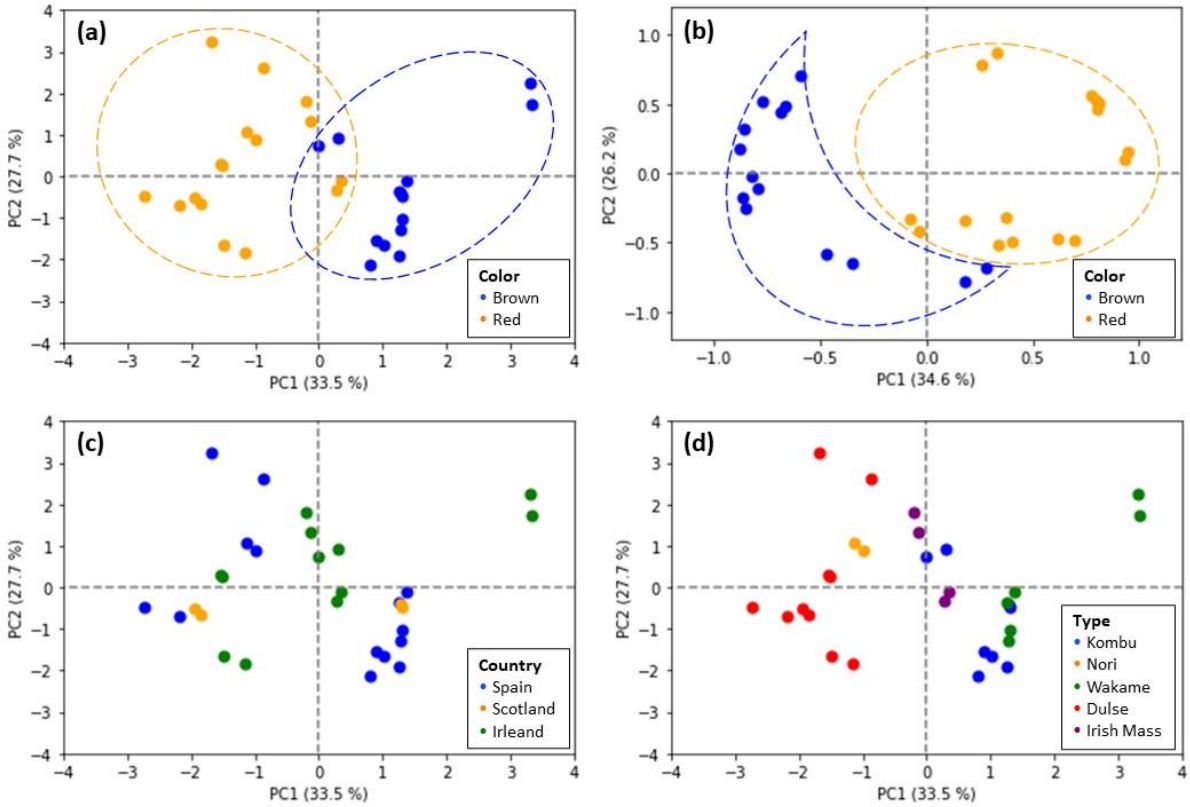

Figure 2. PCA scores on the PC1/PC2 plane, subsets extracted by: **a)** color, **c)** type of algae, and **d)** country of origin of the algae. For comparison: **b)** KPCA with cosine kernel, scores on the subsets of the PC1 / PC2 plane extracted by color.

The results were projected onto the PC1/PC2 plane. The calculation showed the best separation of the samples in terms of color (**Figure 2a**) and a good disparity in terms of algae species (**Figure 2d**), but very little in terms of country of origin (**Figure 2c**). Regarding the possibility of distinguishing red and brown algae, it was observed that the division is based on the PC1 values. When analyzing the values of the component weights, it was noticed that the greatest absolute values of the weights are assumed for calcium, magnesium, and manganese. Therefore, it was found that the concentration of these elements was closely related to the color of the seaweed. Continuing analysis of the samples divided into algae color classes Kernel PCA was applied which changed kernel from linear to cosine. The Kernel PCA is a non-linear extension of PCA [30]. This technique allows the kernel to be changed from the linear one used in classical PCA analysis to other kernels. The examples of kernel that can be used in KPCA are sigmoid, cosine, or defined by the user. The use of such a solution significantly improved the ability of the method to discriminate between samples (**Figure 2b**). To distinguish the country where the algae were harvested, it is also necessary to use PC2, for which the highest absolute values of the input weights for iron, potassium and sodium were calculated. Therefore, considering these elements made it possible to indicate the country of origin of the algae. This conclusion was problematic for seaweeds harvested off the coast of Scotland. The samples did not form a homogeneous cluster. This was probably because they are both species of seaweeds, *Kombu* and *Dulse*. Furthermore, two distinct subsets of algae from Spain were related to the color of the algae. Figure 2d supports the conclusion that the study of the concentration of seven elements was sufficient to distinguish the species of algae. Here you can see the cluster formed by the *Nori* and *Dulse* samples, the second cluster contains *IrishMass* (in the center of the PC1/PC2 plane), and the third cluster contains two other algae: *Kombu* and *Wakame*. These three subsets can be distinguished from each other and are primarily determined by the first principal component, which is related to the concentrations of Ca, Mg, and Mn.

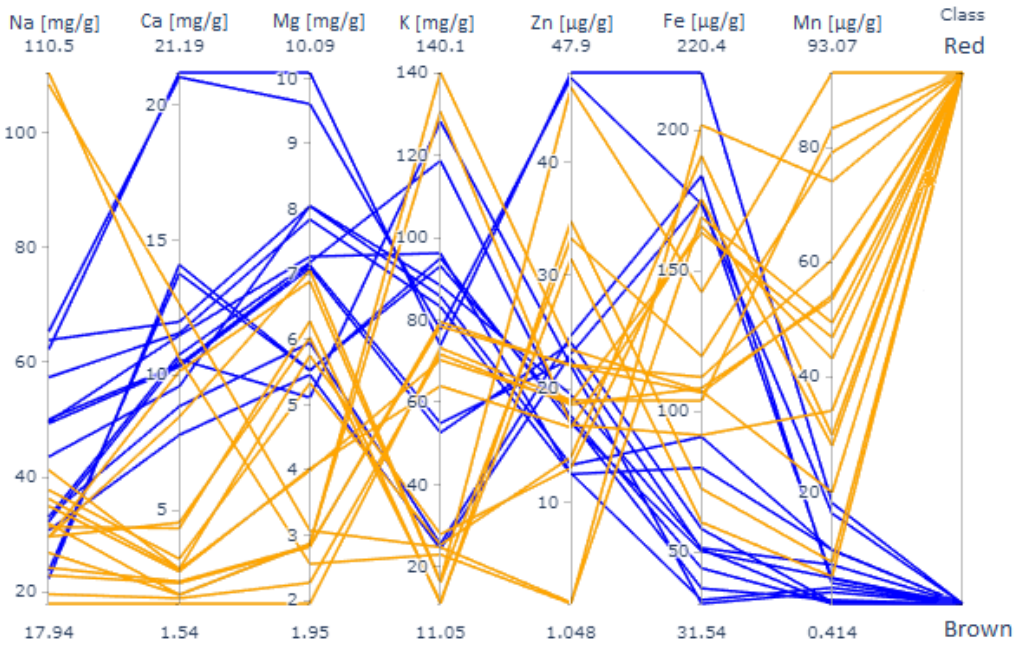

Figure 3. Concentration of the chemical elements using the parallel coordinates, subsets extracted by algae color.

In the case of the analysis with parallel coordinates (**Figure 3**), the order in which the axes were set on the graph was crucial. The layout of the axes makes the compiling of the data clearer. This plot was made considering the color of the algae. Therefore, it can be concluded that the analysed brown seaweeds are characterized by a higher content of magnesium and calcium, but a low manganese content. However, red algae have a higher concentration of manganese compared to brown algae, a lower concentration of calcium and magnesium, and mostly low concentrations of sodium and potassium (except for two samples). It is important to observe which elements differentiate the algae by color. These are Ca, Mg, and Mn, which confirms the earlier conclusions obtained during the PCA analysis.

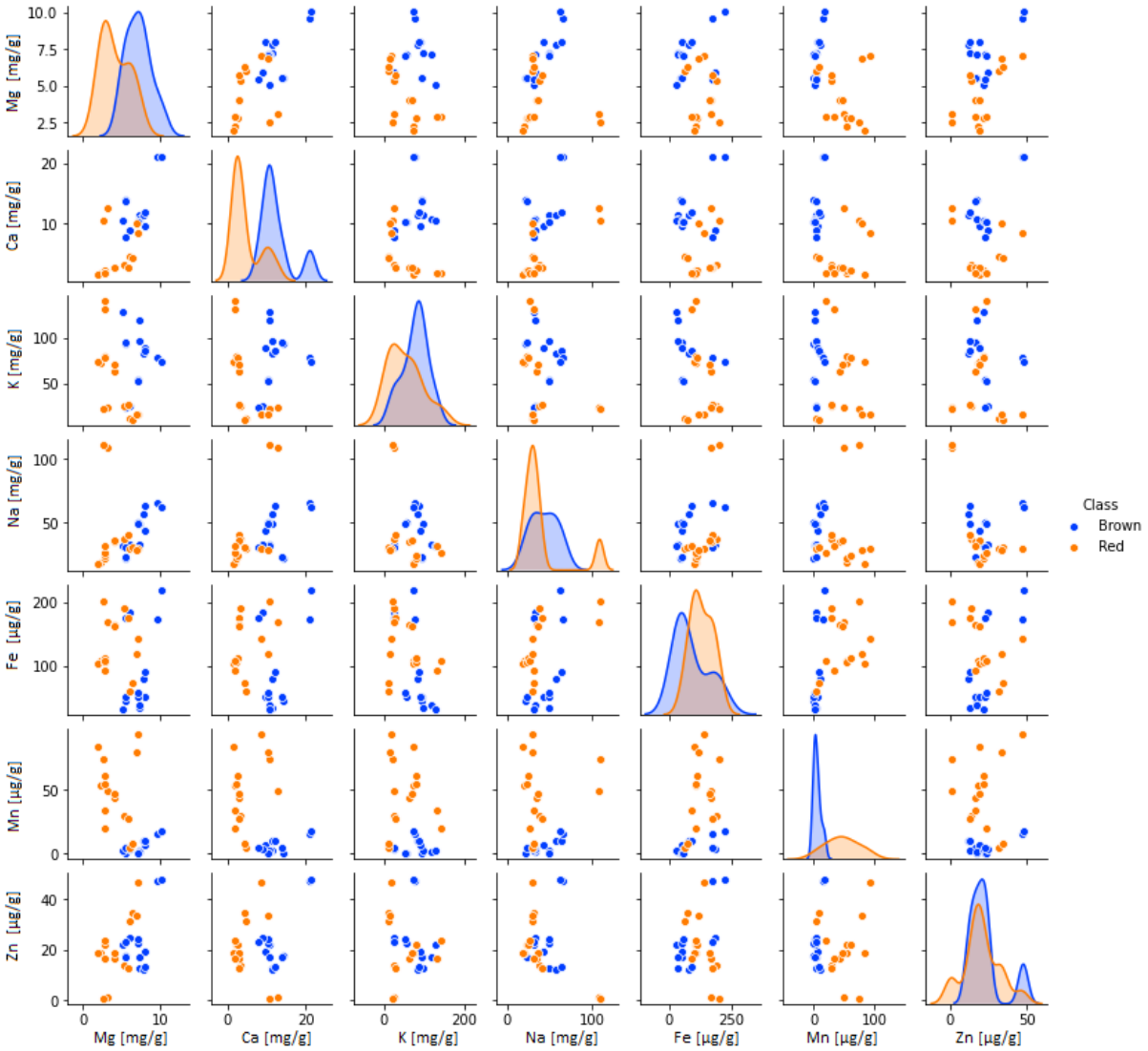

Figure 4. Concentrations of the elements using scatter matrix, subsets extracted by algae color.

The presentation of the data using a scatter matrix (**Figure 4**) confirmed the previous observations. The diagonal shows distribution of the variability of individual variables (the tested concentrations of elements) divided into two groups, red and brown algae. The distributions were shifted for elements such as magnesium, calcium, and manganese. When the entire set of objects was examined, it was confirmed that these variables allow differentiation of the classes of objects. By analysing the pairs of individual variables, it was indicated how they affect the diversity of the analysed samples. It was noticed that the comparison of manganese concentration with concentrations of the individual analysed elements had the greatest impact on the distinction of the samples. To a lesser extent, the distinction of the samples was influenced by the concentrations of sodium and calcium compared to the other elements analysed.

Using dendrograms combined with a heat map, a general relationship between micro- and macro-elements in sea vegetables was demonstrated (**Figure 5**). This diagram, made for auto-scaled data, shown the values of the features in the central part by the intensity of color. On the left side there was a dendrogram showing the grouping of objects, and on the top side a dendrogram illustrating the similarity of the variability of features. The heat map confirmed that brown algae contained relatively more macronutrients and less micronutrients compared to red algae. The dendrogram on the left indicated that there were two homogeneous clusters in the sample set, containing red algae and the

other brown algae. Only two samples did not belong to the correct cluster. This was *Kombu* seaweed harvested at the coast of Ireland. Low concentrations of most elements (except iron), including Ca and Mg, resulted in incorrect classification of brown algae as red algae. The use of the dendrogram combined with the heat map allowed to determine to what extent concentration of a given element affects the position of a given sample in the dendrogram.

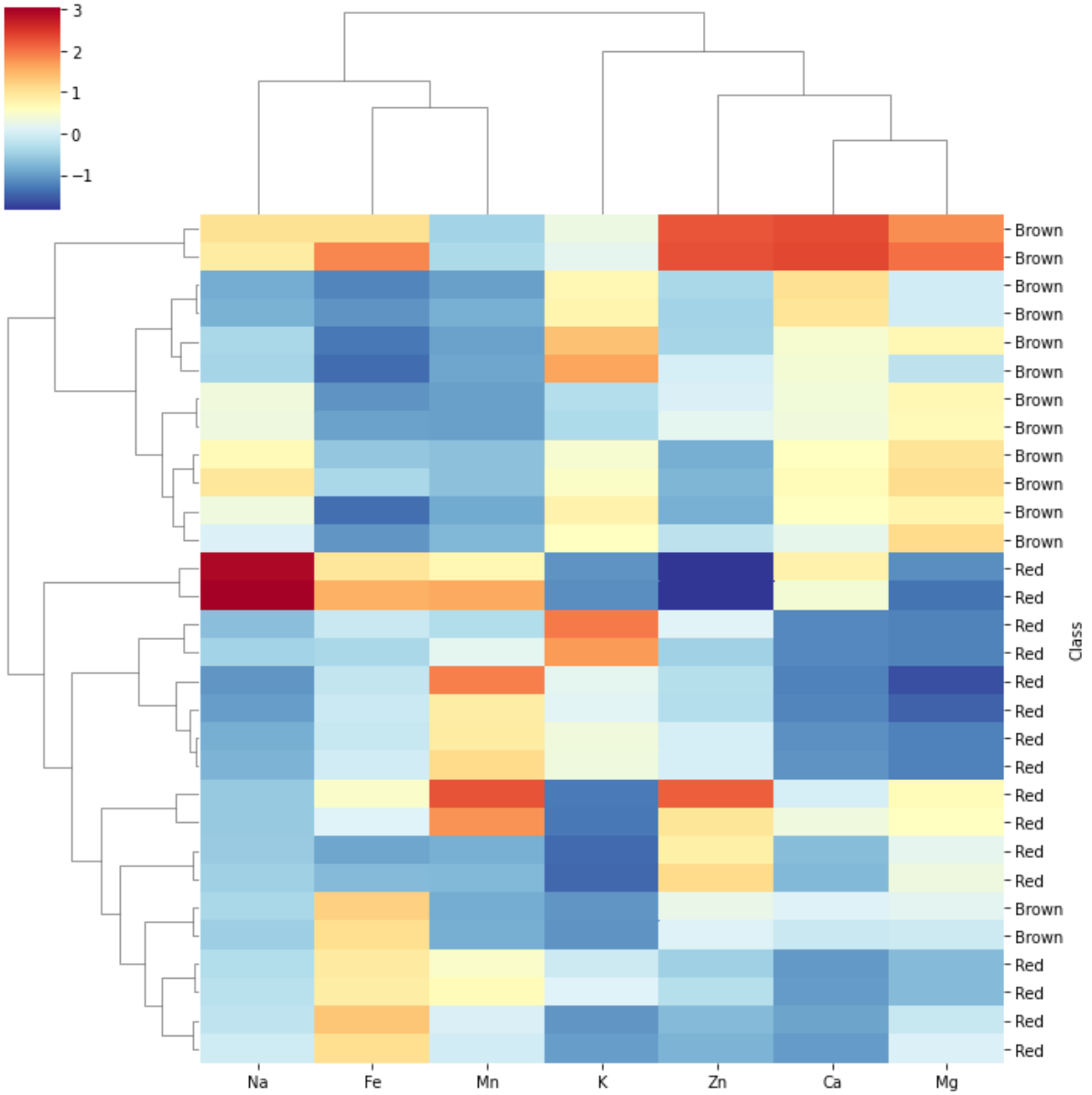

Figure 5. Dendrogram combined with a heat map based on the autoscaled concentrations of the elements, subsets extracted by the algae color.

Using the same techniques, but this time with reference to the species of algae (**Figure 6**), it was observed how micro and macroelements affected individual species of algae. Alga *Wakame* was characterized by high concentrations of Mg and Ca, and low concentrations of Mn compared to other species of algae and algae products under consideration. *Kombu* seaweed contained medium or high concentrations of Mg and Ca, while low concentrations of Fe and Mn microelements. In the composition of the *Dulse* algae, high Mn concentration and low Mg and Ca concentrations was determined. *Irish Moss* seaweed contained a relatively high level of Zn compared to other algae species, and very low levels of K and Na. Algae *Nori* contained a high concentration of Fe and low

concentrations of Ca, K, and Na. Considering all the features at the same time made it possible to indicate clusters of objects that to some extent coincide with the types of algae considered.

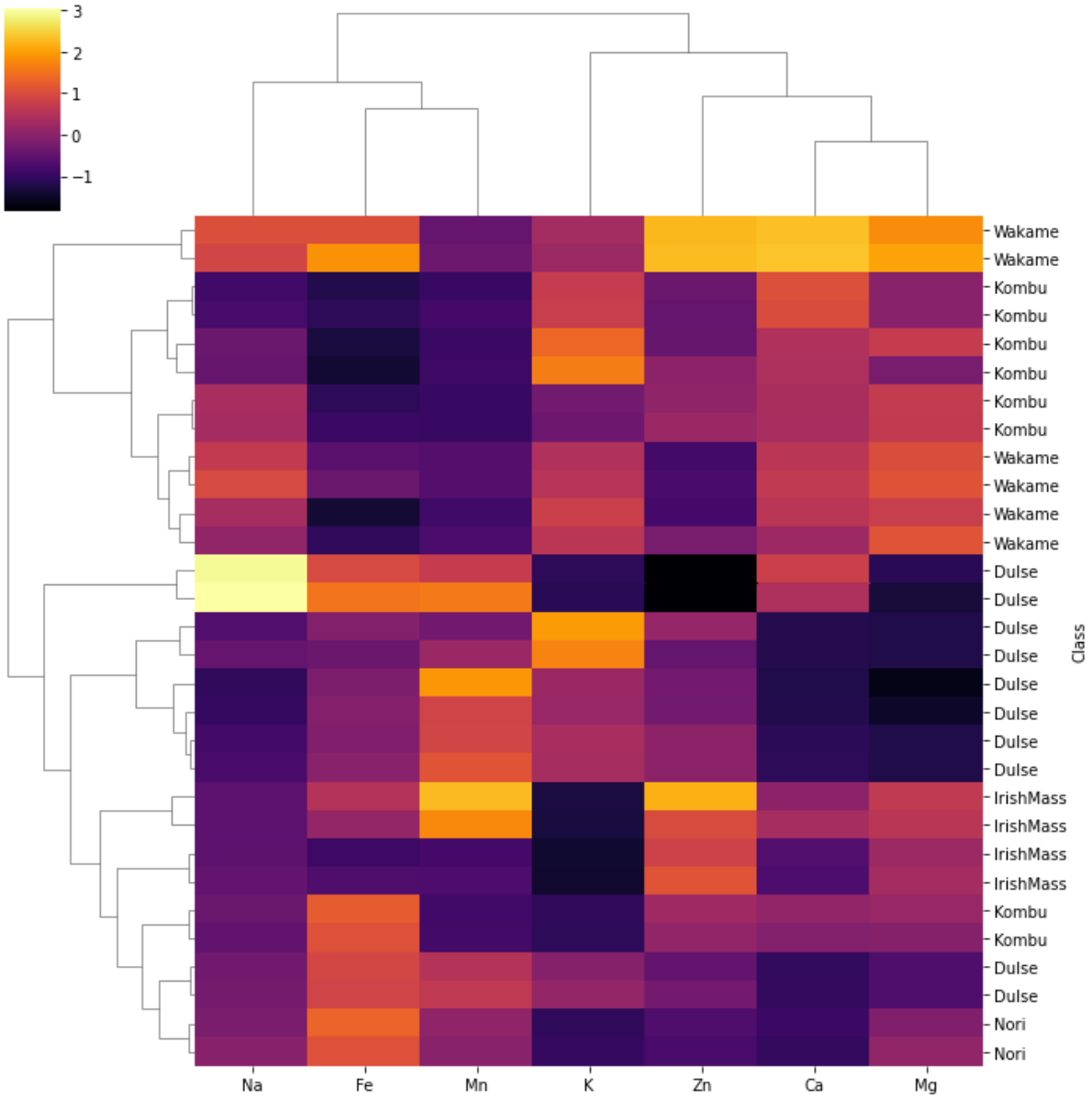

Figure 6. Dendrogram combined with a heat map based on the autoscaled concentrations of elements, subsets extracted by the algae species.

4. Conclusions

In the presented work, quantitative methods of elemental analysis combined with the use of chemometric tools were presented, in relation to edible seaweeds products. Before quantitative elemental analysis, the samples were digested wet in the microwave digestion system. The results obtained showed high biodiversity of the biological material. Various concentration ranges for the analysed metals were determined. The country of origin and the production process did not significantly affect the concentration values of the elements analysed. The characteristics of the investigated seaweeds include the color and species of algae for which the chemometric analysis was carried out. The algorithms used included PCA analysis in different kernels, parallel coordinate analysis, scatter matrix analysis, and dendrograms with heat maps.

Projection of the objects on the PC1/PC2 plane showed that this method differentiates the samples due to the color of the algae using the linear and cosine kernels. A similar effect was obtained when the samples were grouped according to the species of algae. This effect was not noticed when the algae were grouped according to their country of origin. This observation proved that the biological characteristics of seaweeds were decisive in their classification. The different processing methods carried out by producers in several countries did not affect the quality of the available preparation in terms of the content of the metals tested.

The use of the parallel coordinate method allowed to demonstrate the relationships between the concentrations of individual elements in the tested samples. The appropriate axis system allowed to observe the importance of the sodium, calcium, and manganese concentrations to distinguish between red and brown algae. This observation was confirmed by the use of scatter matrix analysis. It also showed that the concentrations of these three elements are crucial for the differentiation of seaweeds in terms of their colors. The prepared dendrograms, which were presented in conjunction with the heat maps (in two variants), suggestively showed information about each work and variable tested, after their appropriate arrangement. The diagrams confirmed the possibility of distinguishing algae by determining the concentration of metals. This effect was unequivocal when the objective was to distinguish between red and black algae. When considering the species of algae, a certain order was also visible. The analysis of heat maps allowed for the indication of the outliers, i.e. samples with a special composition.

The tests performed confirm that determination of the metal content carried out in conjunction with the chemometric approach is useful for assessing the color and species of seaweeds. This work can be classified as an important trend in science, which concerns the search for quick and reliable methods of assessing the quality and authenticity of food.

Funding

This work was supported from the subsidy of the Ministry of Education and Science for the AGH University of Science and Technology in Kraków (Project No 16.16.160.557).

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