Discrimination of mineral waters using near-infrared spectroscopy and aquaphotomics

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Abstract
Despite that water is one of the most studied materials today its dynamic properties are still not well understood. Water state in human organism is of high importance for normal healthy functioning of human body. Different kinds of water are usually classified according to their present solutes and concentrations of these solutes, but though it is known that water molecules can form clusters around present solutes, the classification of waters based on types of water molecular organization and present clusters is not present in current literature. In this study the multivariate analysis is used for classification of commercial mineral waters based on their near-infrared spectra (NIR). Further, the aquaphotomics has been applied, a new approach for interpretation of near-infrared spectra of water, that gives insight into organization of water molecules in each of these waters.

Keywords: water, discrimination, near infrared spectroscopy, aquaphotomics, multivariate analysis.

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Water is maybe one of the most studied materials today; it has been studied with different tools and methods [1–7], but its behaviour is still the subject of intensive scientific research. On nano- and microlevels, like in biological cellular and extracellular spaces, water is not a homogeneous structure, but rather dynamic equilibrium among changing percentages of assemblages of different oligomers and polymer species [8]. The structure and these assemblages or units themselves are dependent on its chemical contents, temperature and pressure [8,9].

Spring water is consumed as a drink beneficial for human health and is used for therapeutic purpose. Despite mineral water being associated with safer and healthier drink than tap water, the quality and composition of mineral water vary with its origin and require careful monitoring. When it comes to quality of drinking waters, it is usually considered in the terms of the concentrations of different solutes, cations and anions, the ratio of certain ions, etc., with regard to human organism functioning. But little is said about the structure of the water itself, even though with approximately 65–70%, water is the most abundant chemical in the human body and it plays a central role in the regulation of cell volume, nutrient transport, waste removal and thermal regulation [10].

Aquaphotomics [11] is a term, recently proposed to describe the concept in which water as multi-element system could be well described by its multi-dimensional spectra. Aquaphotomics is based on visible-near infrared spectroscopy (Vis-NIRS) and multivariate analysis. It discovers new properties of water hydrogen bonds in different aqueous systems under various perturbations.

Based on years of experience in vis-NIR spectroscopy research of water and different aqueous systems under various perturbations, 12 characteristic wavelength ranges (6–20 nm width each) have been identified in the area of the first overtone of the water NIR spectra, where despite the type of perturbation the observed systems showed predictable spectral variations. To present these changes of water absorbance pattern a star chart named "Aquagram" is used [6]. Aquagram displays normalized absorbance values at several water bands on the axis originating from the center of the graph. Absorbance values at specific water absorbance bands were placed on the respective radial axes. Aquagrams were used in studying aqueous and biological systems to obtain more information on the matter (ions, molecules, etc.) present in the water. In this approach, water is used as a "mirror", which can reflect functionality of the present structures [12].

Aquaphotomics has been successfully applied in various fields from water characterization, food quality control to early diagnosis of disease [11–17], but very low number of publications exists on the subject of using NIR spectra of waters for its discrimination [1].

A quite number of papers are published on the subject of different chemical composition of water and the respective bio-functionality from the aspect of the concentration of the present ions, but there is virtually none about the bio-functionality of the water struc-

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tured around the present solutes. Thus, the aim of this study was to determine the structural organization of the various mineral and tap waters regularly used in human diet. In this study, several commercial mineral waters and tap waters were investigated by the means of NIR spectroscopy with novel aquaphotomics approach [11]. Potential of multivariate analysis applied on the NIR spectra of waters and Aquaphotomics in discrimination of different types of mineral waters which are commercially available was also assessed. The purpose of applying aquaphotomics was to look for unique aquaphotomic fingerprint for each of these waters which can subsequently be used as a distinctive criterion for their identification.

MATERIAL AND METHODS

Materials

The pure water samples were investigated (Aqua purificata sterilisata, Pharma product, Serbia) and seven commercial mineral waters available on the market, which were purchased in the local shop and stored in dark ambient before analysis. Concentration of some typical cations and anions for analysed waters are presented in Table 1. The sources of information on physicochemical properties presented in this table were labels on the bottles, as well as the information from the manufacturers' websites [18–21]. For waters Aqua viva, Aqua Una and Belgrade tap water, the analysis of physicochemical properties was performed by the Knjaz Miloš laboratory and published in [22]. All commercial mineral waters were non-carbonated.

Methods

NIR Spectra of waters were acquired in transmittance mode, using mini-spectrometer Hamamatsu (TG – Cooled NIR-IC9913GC, Hamamatsu, Japan) in the range from 900 to 1700 nm. A quartz liquid sample cell was used as a container. Order of recorded spectra for water samples was random, and for each water sample 10 consecutive spectra were recorded. For each water sample, at least 10 spectra were acquired, with a total of 80 spectra. Only the region of the first overtone of water (1300–1600nm) was used in further analysis. The temperature in laboratory was 24.4 °C, and humidity was 61%.

Multivariate analysis

All multivariate analysis was carried out by Pirouette ver. 4.0 (Infometrics, USA) software. Multivariate data analysis in the form of Hierarchical Cluster Analysis (HCA) and Soft Modelling of Class Analogies (SIMCA) was applied.

Hierarchical Cluster Analysis (HCA) is unsupervised pattern recognition method. It calculates the distances (or correlation) between all samples using a defined metric such as Euclidean distance. The most similar objects are first grouped [23]. Euclidean distance was here used as a criterion. The results are presented in a form of dendogram.

Soft Independent Modelling of Class Analogies (SIMCA) employs principal components analysis of spectra for the construction of mathematical models for each class to be analysed. This analysis is a supervised method for sample classification which consists of assigning training sets to classes and then a principal component model is formed for each class with different confidence regions. Interclass distances are calculated using between class residuals and variable importance is determined by comparing average residual variance of each class to all classes and residual variance of all classes to themselves. Variable importance, known as discriminating power can be used to define variables with predominant effect on the samples classification [24]. The models were validated using cross-validation (leave-ten-out).

Aquaphotomics

Aquaphotomics is based on visible-near infrared spectroscopy (Vis-NIR) and it uses a part of the water NIR region from 1300 to 1600 nm. The WAMACS coordinates [11] are the 12 wavelength ranges found in this part of the water spectrum, for which specific water vibrations were assigned [11]. The acquired spectra of all waters were normalized, the pure water spectrum was subtracted and the values of the normalized absorbance in 12 WAMACS coordinates are extracted and presented in aquagram.

Table 1. Concentration of some typical cations and anions in investigated waters (mg/L)

Water	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	Reference
Prolom	43,9	0.5	1	1	5	16	[18]
Gala	18.15	1.65	64	29	0.8	14	[19]
Rosa	2.7	1	10.4	0.9	2	6.4	[20]
Zlatibor	4.51	0.9	62.7	30.5	1.4	17.6	[21]
Beograd tap	6.5	1.1	61.1	11.1	11.3	37.5	[22]
Aqua viva	13.7	2.1	90.1	13.3	20.6	28.1	[22]
Aqua una	8.8	1	88.2	19.5	2.8	9.9	[22]

Aquagram displays normalized absorbance values at several water bands on the axis originating from the center of the graph. Absorbance values at specific water absorbance bands were placed on the respective radial axes.

RESULTS AND DISCUSSION

Figure 1 presents results of hierarchical cluster analysis applied on the NIR spectra of analysed waters. The results show grouping of waters in three categories of similar waters. First category is comprised of Aqua Una, Rosa and Zlatibor, the second is comprised of Aqua Viva water samples taken from different bottles (volume 0.5 and 0.33 L) and Prolom, and the third is comprised of Aqua Purificata, Belgrade tap water and Gala.

Figure 2 presents results of the hierarchical cluster analysis applied on the chemical contents data (from Table 1). The clusters of similar waters presented in dendogram in Figure 2, are clearly different from the clusters identified and presented in dendogram in Figure 1.

This clearly illustrates that it is not only the content of different elements what makes one water unique and distinctive comparing to other waters (or on the other way similar), but is the organization of water molecules around this molecules and ions, which is considered when the analysis is applied on the NIR spectra. The spectrum which shows the information on

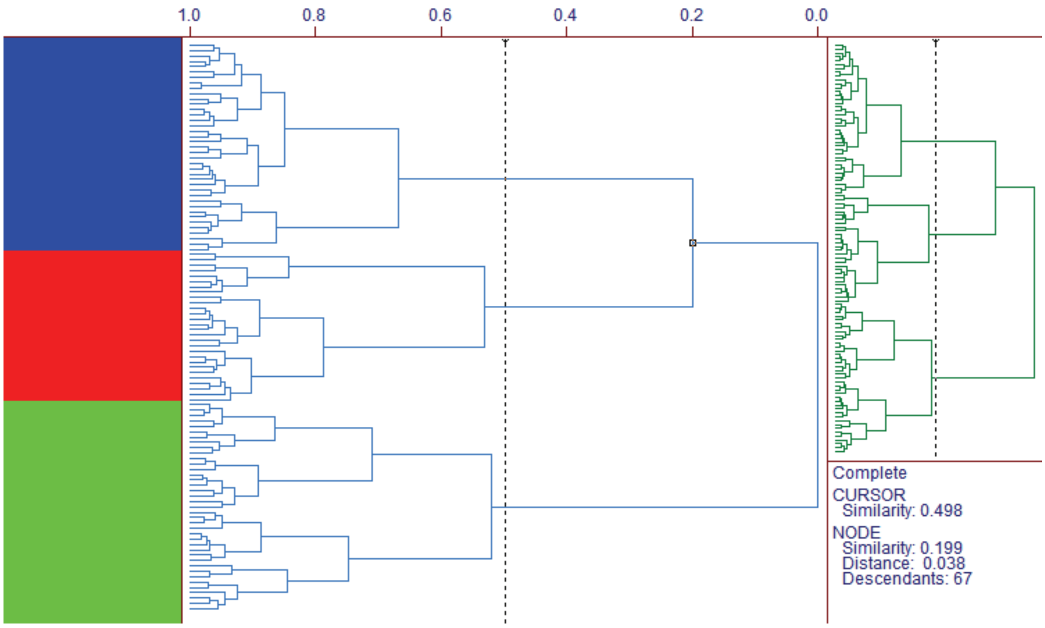

Figure 1. Dendogram showing clustering of NIR spectra of analyzed waters shows three distinctive clusters: blue – Aqua una, Rosa and Zlatibor; red – Aqua viva and Prolom, green – Aqua purificata, Gala and Belgrade tap water.

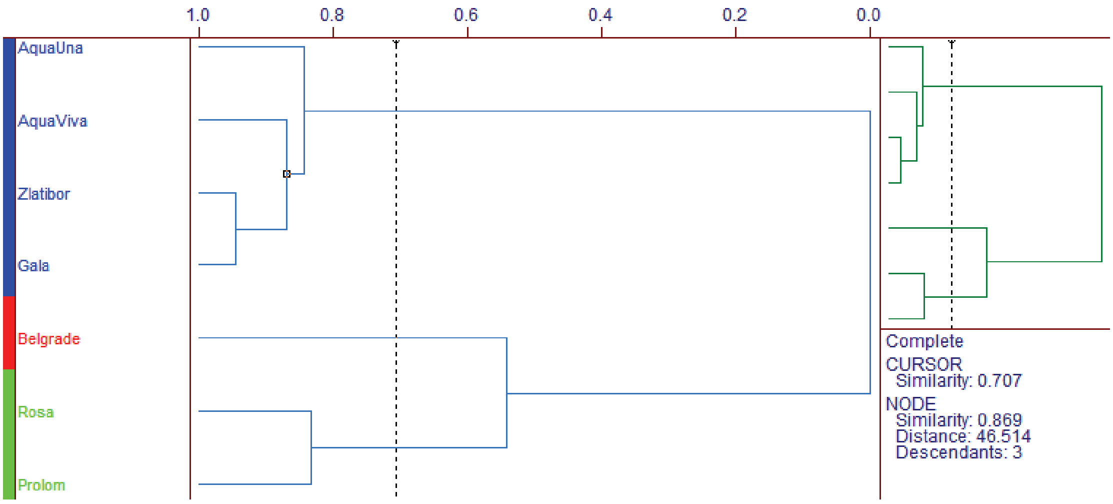

Figure 2. Dendogram of chemical contents of analyzed waters shows three distinctive categories: blue – Aqua una, Aqua viva, Zlatibor and Gala; red – Belgrade tap water; green – Rosa and Prolom.

vibration of water molecular bonds, thus takes into account a parameter which the analysis based on the mineral content of waters are clearly missing.

Figures 3–5 present aquagrams of analysed waters. Similar aquagrams are presented together on one star chart. From these aquagrams, it is obvious that the groups of similar looking aquagrams are in agreement with the clusters identified in dendogram from Figure 1. Thus, the aquagrams of Belgrade tap water and Gala water is presented in Figure 3, Aqua viva and Prolom water in Figure 4, and Aqua una, Zlatibor and Rosa in Figure 5.

The aquagrams are showing how the water molecules are arranged in these waters around present solutes. Thus, aquagrams give insight into water organization. This organization is what makes each and every one of these waters distinctive and different comparing to others, thus this can be viewed as a unique fingerprint for each of these waters under specified conditions.

This fingerprint property of aquagrams is evident on the example of water Aqua viva aquagram (Figure 4). The samples of Aqua viva water are taken from randomly chosen two bottles with different volumes (0.33 and 0.5 L). As it can be seen from the aquagrams of these waters, the aquagram lines are almost identical. However, it should be noted, that the aquagram of any analysed water will be different if the experiment conditions were different. Therefore, any hereby presented aquagram of water should be considered as a fingerprint of water under exactly these conditions (same temperature, atmosphere pressure, humidity etc.).

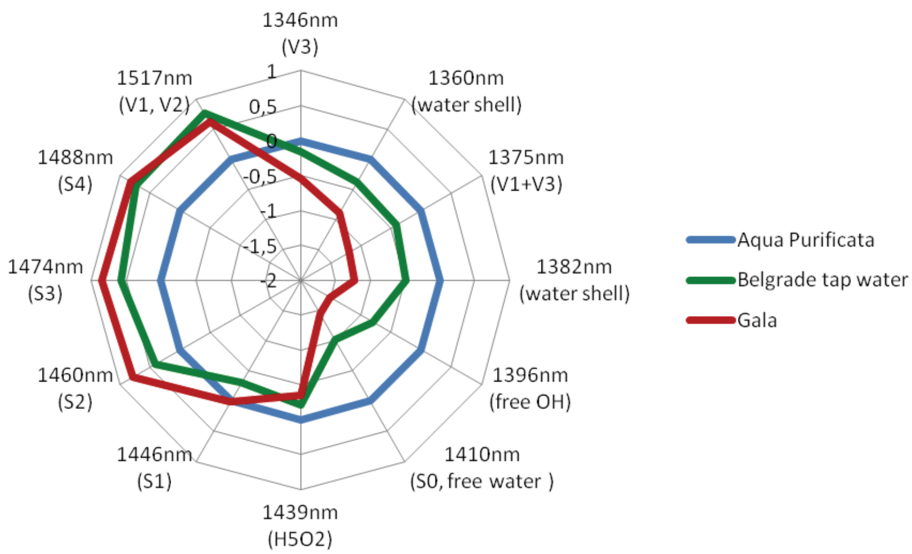

Figure 3. Aquagrams for waters Aqua purificata (AP), Belgrade tap water and Gala water.

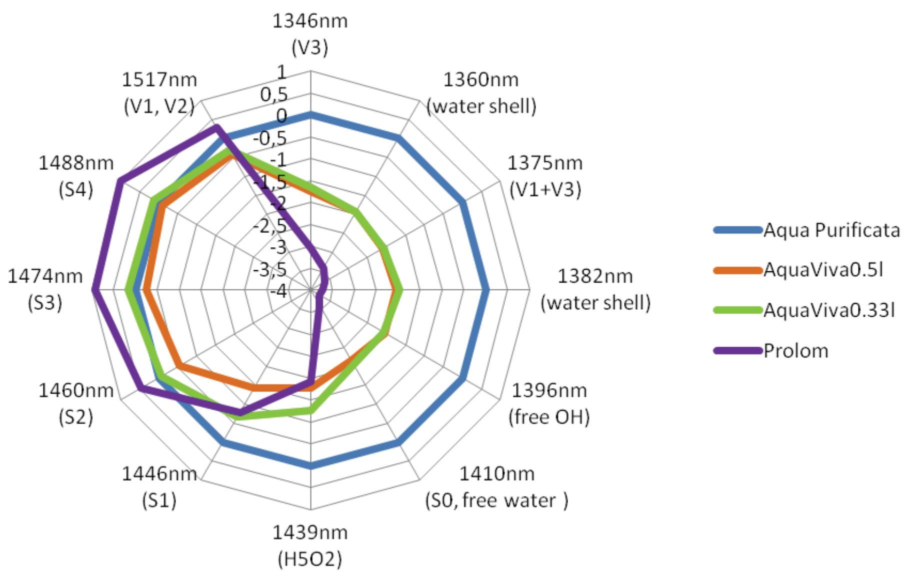

Figure 4. Aquagrams for waters Aqua purificata (AP), Aqua viva water from two bottles (vol. 0.33 and 0.5 L) and Prolom water.

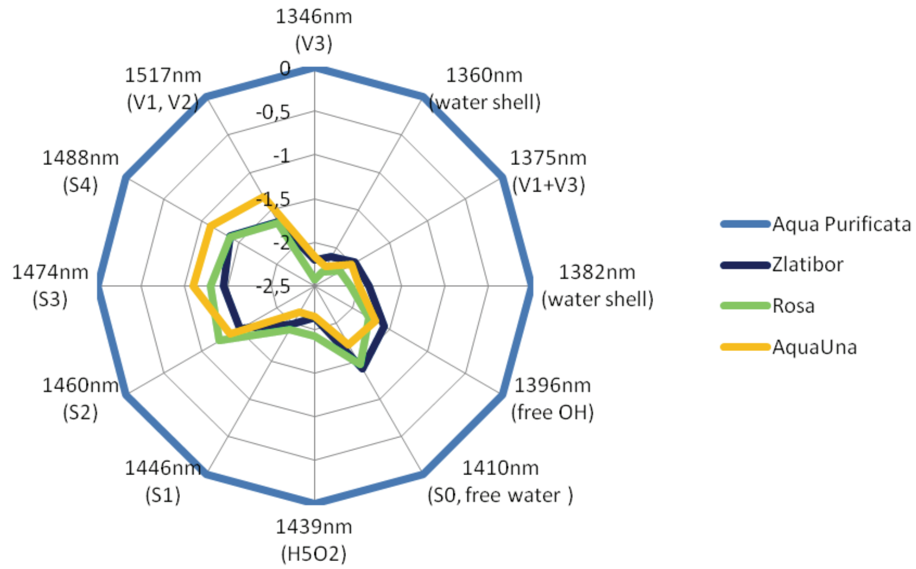

Figure 5. Aquagrams for waters Aqua purificata (AP), Aqua una, Rosa and Zlatibor water.

From aquagrams on Figure 3, it can be seen that common features of Belgrade tap water and Gala water are absorbances at 1517nm (v1,v2) which is a strongly bound water, 1488nm (S4) – water molecules making 4 hydrogen bonds (water pentamers), 1474nm (S3) – water molecules making 3 hydrogen bonds (water tetramers), 1460nm (S2) – water molecules making 2 hydrogen bonds (water trimers). The absorbances at these water matrix coordinates (WAMACS [11]) suggest that water molecules in these two waters are strongly bound to each other – the water molecules are connecting with one another. The absorbance at these water matrix coordinates is higher comparing to pure water which means higher number of these water species comparing to pure water.

Figure 4 presents aquagrams for Aqua viva water and Prolom water, which have similar water organization to previously described waters – water molecules are making hydrogen bonds between themselves creating dimers, trimers, tetramers, pentamers and strongly bound water. However, the intensity of absorbance at these WAMACS is much lower in Aqua viva water, and higher in Prolom water. Prolom water has highest absorbance in these WAMACS, which means that this water has highest number of water molecules bound in dimers, trimers, tetramers pentamers and strongly bound water, with highest number of tetramers and pentamers. For comparison, the number of these larger water formations is highest in Prolom water, then little bit lower in Belgrade tap water and Gala water, and very small in Aqua viva, where it is almost the same as in pure water.

The aquagrams for last three waters – Aqua una, Zlatibor and Rosa are similar and are presented in Figure 4. Their aquagrams are different comparing to other waters. The absorbances are lower in all WAMAC comparing to pure water, and apart from bonded water (1460 nm – S1, 1474 nm – S2, 1488 nm – S3 and 1517 nm (v1,v2)), there is also absorbance at 1410 nm – free water (free water molecules) and at 1396 nm (free OH, water molecules with free OH^{-}).

Further, we applied method SIMCA for discrimination of analysed waters based on its NIR spectra. A method of internal validation is applied using cross validation (leave-ten-out).

The waters are well separated and the interclass distance is for all waters well over 3, as it can be seen from Table 2, which is a criterion for good separation of classes [25].

All waters were properly classified, based on the developed SIMCA model into previously assigned classes, with no misclassified samples and results are presented in Table 3.

Table 2. Interclass distance between 8 different waters based on SIMCA class projections of the NIR spectra in 1300–1600 nm region (CS1 – Aqua purificata, CS2 – Aqua una, CS3 – Belgrade tap water, CS4 – Gala, CS5 – Prolom, CS6 – Rosa, CS7 – Zlatibor)

	CS1(4)	CS2(4)	CS3 (5)	CS4(4)	CS5(4)	CS6(4)	CS7(3)	CS8(4)
CS1	0							
CS2	43.02	0						
CS3	37.38	12.53	0					
CS4	15.48	46.88	33.32	0				

Table 2. Continued

	CS1(4)	CS2(4)	CS3 (5)	CS4(4)	CS5(4)	CS6(4)	CS7(3)	CS8(4)
CS5	13.64	31.20	22.63	13.80	0			
CS6	53.45	21.57	21.86	56.01	36.40	0		
CS7	46.61	**6.76**	18.68	52.87	36.08	20.15	0	
CS8	42.59	**5.27**	15.63	47.68	31.69	18.64	**4.63**	0

Table 3. Number of misclassified samples based on SIMCA model

Assigned category	Predicted category based on the NIR spectra of water								
	Aqua purificata	Aqua una	Aqua viva	Belgrade tap water	Gala	Prolom	Rosa	Zlatibor	No match
Aqua purificata	10	0	0	0	0	0	0	0	0
Aqua una	0	10	0	0	0	0	0	0	0
Aqua viva	0	0	20	0	0	0	0	0	0
Belgrade tap water	0	0	0	10	0	0	0	0	0
Gala	0	0	0	0	10	0	0	0	0
Prolom	0	0	0	0	0	10	0	0	0
Rosa	0	0	0	0	0	0	10	0	0
Zlatibor	0	0	0	0	0	0	0	10	0

The highest discriminating power was attributed to 1346 nm wavelength in spectra (Figure 6). This wavelength is one of the WAMACS coordinates with assigned vibration mode of v3.

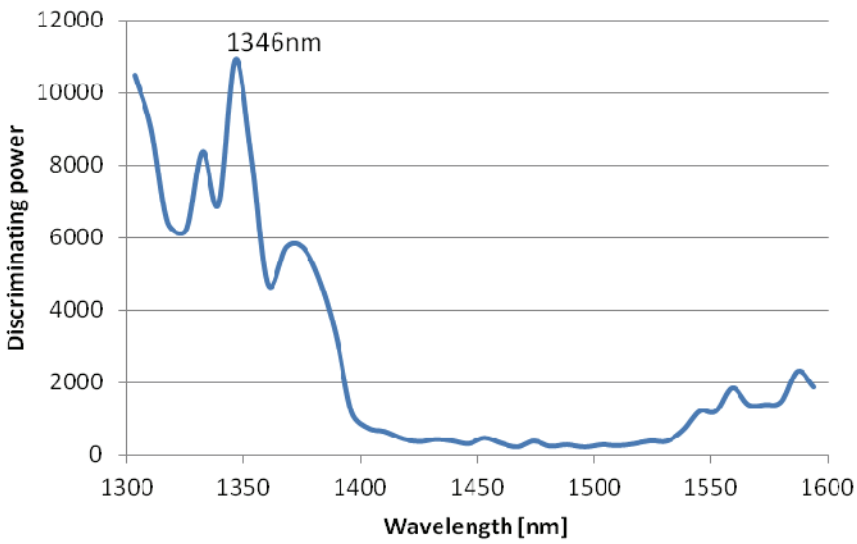

Figure 6. Discriminative power of individual wavelengths of the spectra in SIMCA model. Highest discriminating power has the 1346 nm wavelength.

CONCLUSION

The present study confirms the potential of NIR spectroscopy in discrimination and classification of commercial mineral waters. This shows that apart from the present solutes and their concentration, these waters can be classified according to their NIR spectra, by the means of the multivariate analysis and Aquaphotomics, which are actually giving information about organization of water molecules. This organization of water may have influence on the biomolecular information processing, so in our future study we would investigate if the organization of water molecules has also some health effects.

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IZVOD

DISKRIMINACIJA MINERALNIH VODA UZ POMOĆ BLISKE INFRACRVENE SPEKTROSKOPIJE I AKVAFOTOMIKE

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(Stručni rad)

Voda je jedan od najčešće proučavanih materijala danas, ali uprkos tome mnoga njena svojstva i dalje ostaju nerazjašnjena i neiskorišćena. Voda je neophodna za normalno funkcionisanje ljudskog organizma, između ostalog zbog toga, poremećaji homeostaze vode u ljudskom telu leže u osnovi mnogih bolesti. Analiza vode i njene ispravnosti za upotrebu u ljudskoj ishrani uglavnom se bavi onim što je prisutno u vodi – koncentracijama prisutnih anjona i katjona, prisustvu mikroorganizama i tome slično. Različite vrste voda se uglavnom i klasifikuju upravo prema vrsti elemenata koje sadrže, koncentraciji prisutnih elemenata, ili pak odnosu između koncentracije pojedinih jona i njihov efekat na ljudski organizam razmatra se isključivo sa stanovišta elemenata koji su prisutni u njoj. Međutim, iako je poznato da voda formira različite tipove klastera i može da se organizuje oko prisutnih elemenata na različite načine, klasifikacija voda na osnovu organizacije vodenih molekula, kao i efekti različito klasterizovanih voda na ljudski organizam, za sada ne postoje u literauri. Predmet ovog rada je diskriminacija različitih tipova voda na osnovu njihovog spektra u bliskoj infracrvenoj oblasti, primenom multivarijacione analize i novog pristupa za tumačenje spektara vode u ovoj oblasti, poznatog pod nazivom Akvafotomika. Akvafotomika interpretira spektar vode u bliskoj infracrvenoj oblasti preko posebno definisanih koordinata vodene mreže (*water matrix coordinates* – WAMACS) kojima su pripisani tačno određeni vibracioni modovi molekula vode preko kojih se može zaključiti kako se molekuli vode organizuju. Na taj način, primenom saznanja akvafotomike, voda se može opisati i sa aspekta njene organizacije u klastere, i time se omogućiti i diskriminacija voda na osnovu prisutnih tipova klastera što je prikazano u ovom radu.

Ključne reči: voda • diskriminacija • bliska infracrvena spektroskopija • Akvafotomika • multivarijaciona analiza