

Potentiometric sensor containing set of ion selective electrodes with lipid modified membranes for quality assessment of tested non-alcoholic beverages

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ABSTRACT

Potentiometric sensors may be a good tool for fast quality control of commercial drinks. In this work, such a sensor, containing a set of ion-selective electrodes with lipid-modified membranes (benzyl-hexadecyldimethylammonium chloride monohydrate, hexadecylamine, 1-dodecanol, elaidic acid, cholesterol) was used for discrimination and quality control of non-alcoholic beverages, mainly composed of sugar and citric acid. It was found that the electrodes stability, membrane reproducibility and sensitivity to acetic, hydrochloric and citric acid concentration were very good. On the contrary, ion selective electrodes were not sensitive to sweet substances (glucose, fructose, sucrose) concentration changes. The potentiometric sensor consisting of ion-selective electrodes was applied to commercially available non-alcoholic beverages, a reference drink and to di-component mixtures of sucrose and citric acid. A database of its responses to those mixtures was created. The possibility of using a “fingerprint” method for recognition and quality control of unknown beverages was discussed. The sensor responses to non-alcoholic beverages were transformed by principal component analysis and agglomerative hierarchical clustering analysis and compared to the results obtained with sensory analysis. The tested drinks can be divided into two groups of similar sour taste intensity (lemonades and orangeades). The beverage of low sour taste intensity and the non-carbonated drink were outside these groups. The obtained results show that the potentiometric sensor with lipid-modified ion-selective electrodes may be a useful tool for quality control of unknown drinks on the last step of a production line.

Keywords: Quality assessment; Potentiometric sensor; Ion selective electrodes; Lipophilic compound-polymer membrane; Non-alcoholic beverages; Principal component analysis; Agglomerative hierarchical clustering

1. Introduction

The quality of liquid foodstuffs is one of the most important consumer's interests. Among the variety of products, consumers choose those that are of good quality for an appropriate price. The quality of food products is determined mainly by their composition and taste [1]. For that reason, the development of rapid and low-cost methods of food quality assessment is very desirable. Such methods are based on different types of sensors used for characterization, taste and quality estimation of liquid foodstuffs [2–7]. However,

elaborating a new taste sensor called “electronic tongue” [7,8] is no easy task, because the food quality depends on the many components which affect each other.

Electrochemical sensors (e.g., potentiometric [2,4–24], voltammetric [3]) are one of the most promising tools for quality assurance in the food industry. Such sensors have been developed worldwide and some of them have been successfully used for quality assessment. Among them, potentiometric sensors are composed of a set of electrodes with lipid-polymer membranes (ion selective electrodes, ISEs) and a reference electrode connected to a multi-channel meter and

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computer [2,4–21] are commonly used. The ISEs play the role of a transducer, transforming taste information generated by chemical substances, present in a solution, into electrical signals. Such sensor is characterized by so-called global selectivity, which means that it may act as the human gustatory system with the selectivity to given taste rather than to individual chemical substances [9]. Different compositions of a solution, containing chemical substances producing taste, affect their interaction with lipid-polymer membrane and cause the difference in electrode responses [5,9].

To find taste information of examined samples, different responses from ISEs of the potentiometric sensor are analyzed using the following methods: pattern recognition [8,15] or multivariate analysis methods (e.g., principal component analysis, PCA [2,8,17,20,21,25,26], agglomerative hierarchical clustering (AHC) [26] or partial least square, PLS regression [26]).

Potentiometric sensors based on ISEs were used in laboratory conditions for taste substances recognition and discrimination of several liquid products such as beer, coffee, tea, milk, tomatoes, wine, mineral water, rice, sake, juices and tonics [2,6–9,12,15–17,20–23]. Such sensors were characterized by good stability and selectivity to sour, bitter and salty taste substances [2,10,13,14,17,19] and were not sensitive to sweet substances. The commercialized taste sensing system with ISEs, called TS-5000Z (Intelligent Sensor Technology, Inc.) [5,10,14] or SA402B (Intelligent Sensor Technology, Inc.) [13] were also elaborated by Japanese scientists and successfully used for taste and quality assessment.

It should be noted that more complicated electronic tongues consisting of many working electrodes in an array have been proposed [15,16]. However, it was shown by using artificial neural networks as a classifier, that the device is capable of reliable discrimination between different brands of mineral waters and apple juices with a reduced number of electrodes in the array [16]. It means that it is not necessary to use complicated devices for taste recognition or liquid food classification.

Therefore, the potentiometric sensor with five ISEs modified by appropriate lipid (benzylhexadecyldimethylammonium chloride, hexadecylamine, elaidic acid, 1-dodecanol or cholesterol) and a reference electrode was elaborated in our laboratory.

On the basis of impedance [18] and potentiometric measurements [19], it can be stated that five membranes in ISEs of the sensor can be divided into two groups:

- positively charged membranes containing benzylhexadecyldimethylammonium chloride monohydrate or hexadecylamine,
- negatively charged membranes with elaidic acid and with 1-dodecanol or cholesterol. The last two compounds have a neutral structure but behave as negatively charged membranes [19].

It was already found that ISE with cholesterol [17] and ISE with elaidic acid [19] were very stable for at least 5 days of the experiment. Moreover, the reproducibility of the elaidic membrane was also good [19]. The obtained results showed that this sensor with five ISEs can be used as a sour taste sensor [19]. That sensor was also successfully applied

for the discrimination of different kinds of commercial tonics [17,20].

In this work, the possibility of applying potentiometric sensor with five ISEs modified by lipids (benzylhexadecyldimethylammonium chloride monohydrate, hexadecylamine, elaidic acid, 1-dodecanol or cholesterol) for quality assessment of commercial non-alcoholic beverages (orangeades, lemonades), mainly composed of citric acid, sucrose and carbon dioxide was discussed. The PCA and AHC were performed for the discrimination of selected commercial drinks. The possibility of applying the so-called “fingerprint” method for taste recognition of unknown non-alcoholic beverages was discussed. The sensitivity and stability of ISEs were also examined.

2. Experimental

2.1. Materials

Benzylhexadecyldimethylammonium chloride monohydrate, hexadecylamine, elaidic acid, 1-dodecanol, cholesterol, dioctophenyl phosphonate (DOPP), high molecular weight PVC, citric acid, caffeine, sucrose, glucose fructose were Fluka Selectrophore® reagents of analytical grade purity (>99%). The monomer 3,4-ethylenedioxythiophene (EDOT) was obtained from Bayer AG and distilled under vacuum before use. Poly(sodium4-styrenesulfonate (NaPSS, MM ~70 000 g/mol, purity 99%) was obtained from Aldrich (Poznań, Poland). Acetic and hydrochloric acids were obtained from POCH (Gliwice, Poland). Tetrahydrofuran (THF, POCH, Gliwice) was distilled under LiAlH_4 and triple distilled water was used for preparation of solutions of the taste substances. Non-alcoholic beverages (orangeades and lemonades) were commercially available products. Reference drink prepared from sucrose, citric acid and non-carbonated water was also examined.

2.2. Electrode preparation (ISE)

The following materials: PVC (300 mg), DOPP (0.5 mL) and appropriate ion-selective lipophilic compound (0.5% w/w) were dissolved in THF and poured into a glass plate surrounded by glass rings. After the solvent evaporation, the polymer film (200 μm thickness) was separated from the plate in the form of a transparent and colourless membrane. The membrane was cut into discs (7 mm in diameter) and attached to the electrode bodies (type IS 561) made by Moeller AG. The obtained electrodes were filled with KCl solution (0.1 M) and used with the reference electrode Ag/AgCl/Cl⁻ (3 M KCl) in the experimental set-up (Fig. 1).

2.3. Potentiometric measurements

The potentiometric sensor consisted of five ISEs with a membrane containing an appropriate ion-selective lipophilic compound (benzylhexadecyldimethylammonium chloride monohydrate, hexadecylamine, elaidic acid, 1-dodecanol or cholesterol), the reference electrode Ag/AgCl/Cl⁻ and a voltmeter (Fig. 1). The electrodes were immersed in the examined solution. The electrode potentials were measured using high-input-impedance voltmeter HACH EC30 and transferred into PC. The ISEs were conditioned in KCl

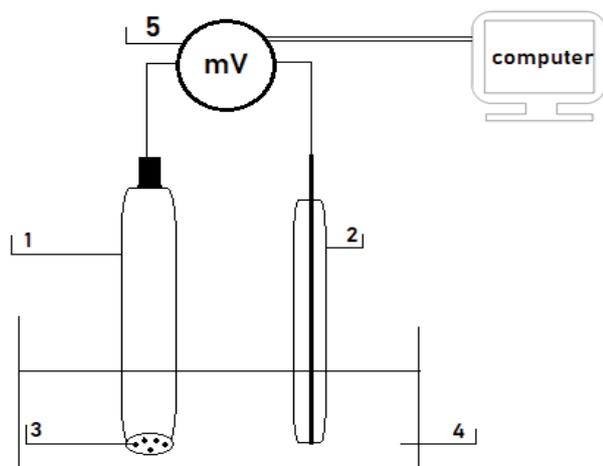


Fig. 1. Experimental set-up: 1 – set of five working ISEs; 2 – reference electrode Ag/AgCl/Cl⁻; 3 – one from five ISEs; 4 – substance solution; 5 – voltmeter.

solution (0.01 M) before and between measurements. All experiments were carried out at a temperature of $23^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Each measurement was repeated twice.

2.4. Sensory analysis

The sensory analysis of the sour taste intensity of tested non-alcoholic beverages was performed by a group of selected consumers (ten persons). Those with a high sensory sensitivity to sour and sweet taste were selected from the group of 25 consumers. The testers compared the intensity of sour taste of the non-alcoholic beverages to a set of reference solutions, containing different amounts of citric acid (from 0.1% to 0.4%) in the presence of an appropriate amount of sucrose (from 4.0% to 12.0%). The intensity of a sour taste was established on the scale of 1 – 5 (where 1 – very slightly sour, 5 – very strongly sour). The taste intensity estimation was carried out in standard conditions [27].

2.5. Tested non-alcoholic beverages

The composition of six commercially available non-alcoholic beverages (Jurajska Lemon, Sprite, Ustronianka Citric, Hoop Premium Lemon, Hellena Orangeade, Nata Orangeade) was taken from the manufacturers' information (Table 1). Unfortunately, there is no data about their acidity. Therefore, the sour taste intensity of non-alcoholic beverages in the presence of sweet substances was established by testers (Table 1). The mean value and standard deviation (SD) of sour taste intensity on the scale 1 – 5 are given in Table 1.

As it can be seen (Table 1) the Hoop Premium Lemon has the highest sugar content and Ustronianka Citric the lowest. The most intensive sour taste is represented by Jurajska Lemon (No. 1), meanwhile the least intensive by Hoop Premium Lemon (No. 4).

2.6. Mathematical methods

The ISE sensor results of the non-alcoholic beverages were transformed with PCA [25,26], which reduces

information included in five-dimensional data space to that in two-dimensional space without losing information. The AHC was also used for the interpretation of the obtained data [26].

3. Results and discussion

The stability of each ISE in a given solution and the reproducibility of membranes preparation are very important features of the potentiometric sensor. The stability of ISE with benzylhexadecyldimethylammonium chloride monohydrate (positively charged membrane), and ISE with 1-dodecanol (negatively behaving charged membrane) in a citric acid solution of the range $1\text{--}10^{-4}$ M is presented in Fig. 2a and b.

As it can be seen the ISE with benzylhexadecyldimethylammonium chloride monohydrate in a citric acid solution of 1×10^{-1} M– 1×10^{-3} M concentration range is stable, even during three weeks within the experimental error (Fig. 2a). The ISE modified by 1-dodecanol membrane is stable in the same range during only 5 d (Fig. 2b). The same effect can be observed for the other negatively behaving charged membranes of ISEs containing elaidic acid [19] or cholesterol [17]. These membranes are characterized also by good reproducibility since the results obtained with freshly prepared electrode membranes were the same within experimental error [19].

The tested non-alcoholic beverages are composed mainly of sweet (sucrose or other sugars), sour (mainly citric acid) substances and carbon dioxide. Therefore, the electrode responses in mono, and di-component taste solution have been investigated.

The radar chart showing changes of ISE potentials with citric acid concentration is presented in [17]. The radar charts for the other sour substances (acetic and hydrochloric acid) are shown in Fig. 3.

The radar chart shape for hydrochloric acid solutions (Fig. 3b) is completely different than in the case of acetic acid (Fig. 3a). Since ISE potentials change linearly with the solute concentration increase in the range 10^{-1} – 10^{-3} M, the slopes of $E = f(c)$ could be calculated. The results are presented in Table 2.

The ISEs with negatively charged membranes (Nos. 3–5) are more sensitive than ISEs with positively charged membranes (Nos. 1 and 2) in hydrochloric and acetic acid solution. This is not the case for citric acid solution.

Taking into account the mean slope of $E = f(c)$ for all ISEs it may be stated that the potentiometric sensor is the most sensitive to hydrochloric acid concentration (mean slope = 44 mV, Table 2) and the least sensitive to acetic acid concentration (mean slope = 19 mV). Its global sensitivity for citric acid (mean slope = 26 mV) is lower than in hydrochloric acid but higher than in acetic acid.

The sensitivity of ISEs responses to glucose and fructose concentration in the range $1\text{ M--}1 \times 10^{-3}$ M is presented in Fig. 4 and b and for sucrose in the reference [17]. It is seen that all the membrane electrodes were almost not sensitive to these sweet substances' concentration changes in the given range. The exception is the ISE with cholesterol membrane potentials obtained in a very high glucose concentration (1 M, Fig. 4a).

Table 1
The characteristic of tested non-alcoholic beverages

No.	Name	Color	Content	Sour taste intensity with SD
1	Jurajska Lemon	Yellow	- sucrose 5%; - ascorbic acid; - citric acid; - lemon flavor; - juices: grape (8%), apple (8%), lemon (2.5%), lime (1.5%) - natural sparkling mineral water	4.6 [\pm 0.4]
2	Sprite	Colorless	- sucrose or glucose – fructose syrup 10% - citric acid - flavor - potassium citrate (acidity regulator) - sparkling water	3.5 [\pm 0.2]
3	Ustronianka Citric	Yellow	- sucrose 4.0% - acidity regulator - citric acid - flavor - sparkling water	3.5 [\pm 0.1]
4	Hoop Premium Lemon	Yellow	- sucrose 11.2% - citric acid - lemon juice 1% - sparkling water	1.5 [\pm 0.2]
5	Hellena Orangeade	Slightly orange	- sucrose or sucrose – fructose syrup, 9.1% - citric acid - carotene dye - flavor - potassium sorbate - sparkling water	2.0 [\pm 0.1]
6	Nata Orangeade	Orange	- fructose, sucrose 9,3% - orange juice 25% - citric acid - sodium benzoate - dye - sparkling water	2.1 [\pm 0.1]
7	Reference drink	Colorless	- sucrose 9% - citric acid 0.2% - non-carbonated water	1.9 [\pm 0.1]

Source: from the manufacturers' information.

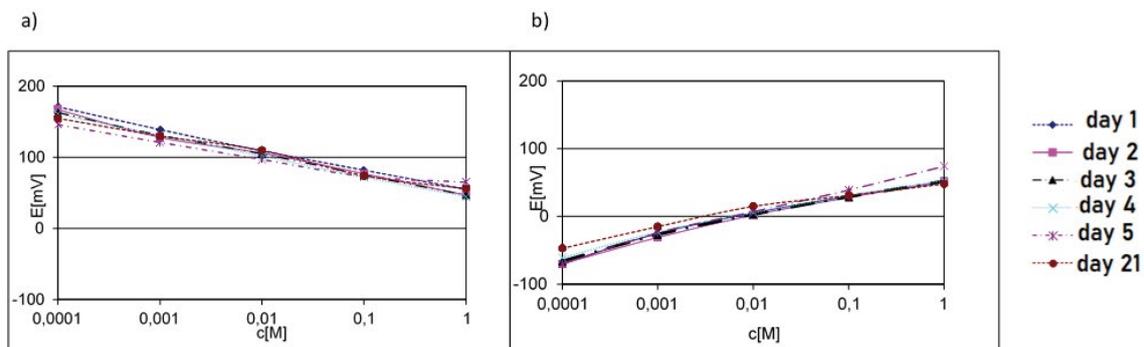


Fig. 2. Stability of ISE containing: (a) benzylhexadecyldimethylammonium chloride monohydrate and (b) 1-dodecanol in citric acid solution at given concentration.

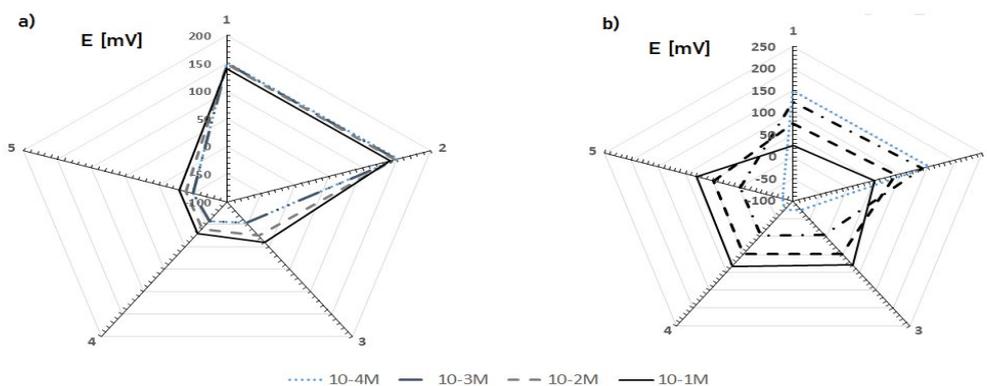


Fig. 3. Radar charts obtained by potentiometric sensor with ISEs in solution of (a) acetic acid and (b) hydrochloric acid; Electrodes with: (1) benzylhexadecyldimethylammonium chloride monohydrate, (2) hexadecylamine, (3) 1-dodecanol, (4) elaidic acid, (5) cholesterol.

Table 2
Slopes of $E = f(c)$ for ISEs of potentiometric sensor in sour substances

No ISE	Lipid in ISE	Slope S (mV/c) ^a		
		Citric acid	Acetic acid	HCl
1	Benzylhexadecyldimethylammonium chloride monohydrate	29	15	36
2	Hexadecylamine	21	8	41
3	1-dodecanol	29	21	49
4	Elaidic acid	28	26	48
5	Cholesterol	24	25	44
	Mean values	26 [±3]	19 [±6]	44 [±6]

^ain the range from 10⁻¹ to 10⁻³ M.

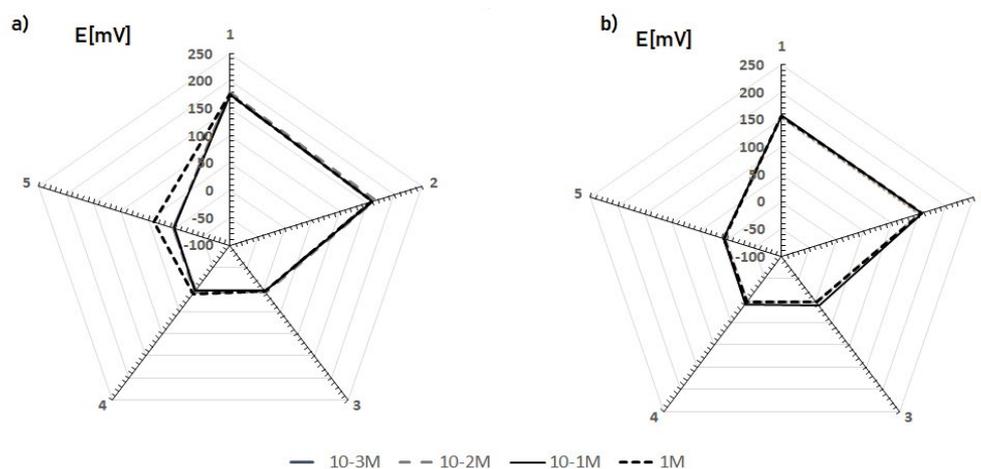


Fig. 4. Radar charts obtained by potentiometric sensor with ISEs in solution of (a) glucose and (b) fructose; Electrodes with: (1) benzylhexadecyldimethylammonium chloride monohydrate, (2) hexadecylamine, (3) 1-dodecanol, (4) elaidic acid, (5) cholesterol.

It seems that the responses of the ISEs with lipid-modified membranes are not only linked to charge transfer across the membranes but also to the changes of double-layer capacity and potential as well as they are influenced by adsorption processes at the membrane interface. Therefore, a lack of sensitivity for the system with sweet substances may be caused by their non-ionic nature.

Since sweet substances and citric acid are the main components in the tested non-alcoholic beverages, the ISEs responses of the potentiometric sensor to the di-component solution (sucrose and citric acid) were examined (Fig. 5a–h). The range of solution component concentrations was chosen by taking into account the composition of commercial tested non-alcoholic beverages.

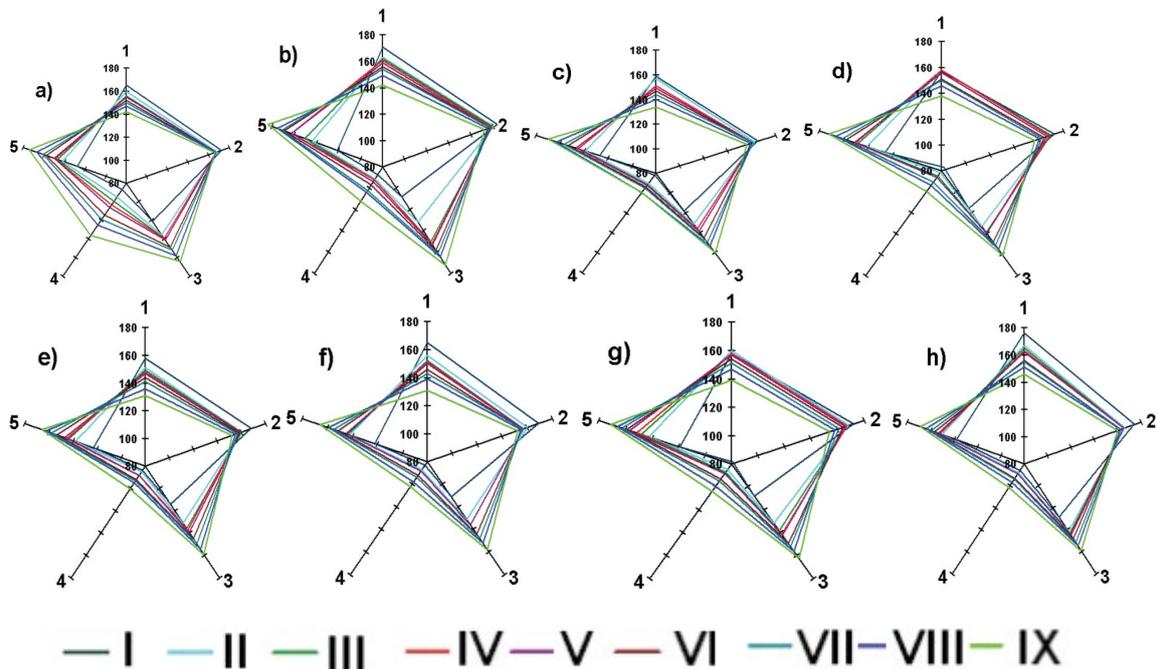


Fig. 5. Radar charts obtained by sensor with ISEs in two-component mixtures: sucrose (%): (a) 0.3, (b) 5.8, (c) 6.8, (d) 7.8, (e) 8.9, (f) 9.9, (g) 10.95 and (h) 11.98; citric acid (%): I-0.019, II-0.096, III-0.1537, IV-0.1729, V-0.1921, VI-0.3842, VII-0.5764, VIII-0.9606, IX-1.9212; Electrodes with: (1) benzylhexadecyldimethylammonium chloride monohydrate, (2) hexadecylamine, (3) 1-dodecanol, (4) elaidic acid, (5) cholesterol.

As it can be seen (Fig. 5a–h) the shape of the radar charts is changing not only with sucrose but also with citric acid concentration. At the lower constant sucrose concentration (0.3% or 5.8%; Fig. 5a and b), the citric acid concentration changes influence the negatively charged membrane electrodes (nos. 3–5) more than positively charged ones (nos. 1,2). This effect is less visible at higher constant sucrose concentrations (Fig. 5f–h).

It is seen that at a constant lower citric acid concentration (e.g., I – 0.019%), the sucrose concentration changes influence slightly the shape of the radar chart. This effect is even less observed at higher sucrose concentrations (Fig. 5e–h). It is in coherence with the general observation that sweet substances are masking the acid taste [28]. The observed changes are due to the fact that the sweet agent reduces the sour taste intensity as it was confirmed by sensory analysis (Table 1).

The radar charts for the tested non-alcoholic beverages (Jurajska Lemon, Sprite, Ustronianka Citric, Hoop Premium Lemon, Hellena Orangeade, Nata Orangeade) containing mainly sweet compounds (sucrose or other sweeteners), citric acid and/or lemon juice and carbon dioxide are presented in Fig. 6. A reference drink made from citric acid, sucrose and distilled water was added to this radar chart.

It should be noted that higher potential differences are in the case of positively charged electrodes (Nos. 1 and 2), meanwhile, the lowest are in the case of ISE modified by elaidic acid (negatively charged membrane, No. 4). This effect is probably due to weak interactions of this ISE membrane with the acid compounds of the tested drink.

As it can be seen the shapes of the radar chart of the reference drink and Hoop Premium Lemon differ significantly

from the others. In the case of the reference drink, non-carbonated water used for preparing the solution may be the reason. On the contrary, the higher amount of sucrose (11.2%) masking the sour taste (Table 1) of Hoop Premium Lemon seems to be responsible for a different radar chart shape in comparison to the other drinks.

The shape of the radar chart of a given beverage might be compared to sensor data for di-component mixtures presented in Fig. 6. In this way, the composition of an unknown drink might be estimated. Such a method is called the “fingerprint” method. Of course, the database should include radar chart shapes of solutions not only with sucrose and citric acid but also with other ingredients present in the appropriate non-alcoholic beverage (juices, dyes, flavour regulators, preservatives). It means that the “fingerprint” method requires the creation of a large reference database. Such a method could be used for quality control of unknown drinks on the last step of the production line.

The ISE responses of the potentiometric sensor concerning non-alcoholic beverages were transformed by multivariate analysis, PCA and AHC (Fig. 7a and b) and compared with the results obtained by sensory analysis. As it can be seen two principal components (PC1 and PC2) explain 94.87% of the data variability. This suggests that these two components play a significant role in the model.

The points representing beverages of high sour intensity (Nos. 1, 2, 3) are on the left at the bottom of the PCA graph while point of Hoop Premium Lemon characterized by low sour taste intensity (No. 4) is laying at the top right of the diagram.

It was found that non-alcoholic beverages (Nos. 1, 2, 3, 5, 6) could be classified into two groups of similar sour

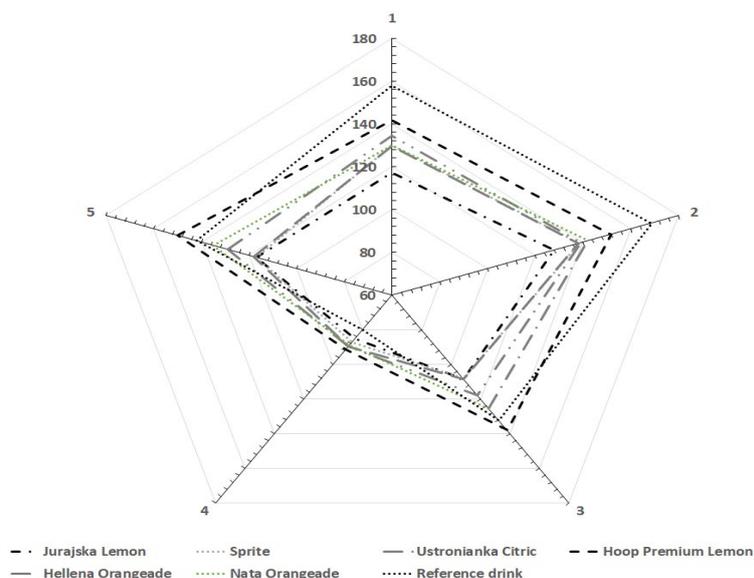


Fig. 6. Radar charts obtained by ISEs in tested non-alcoholic beverages.

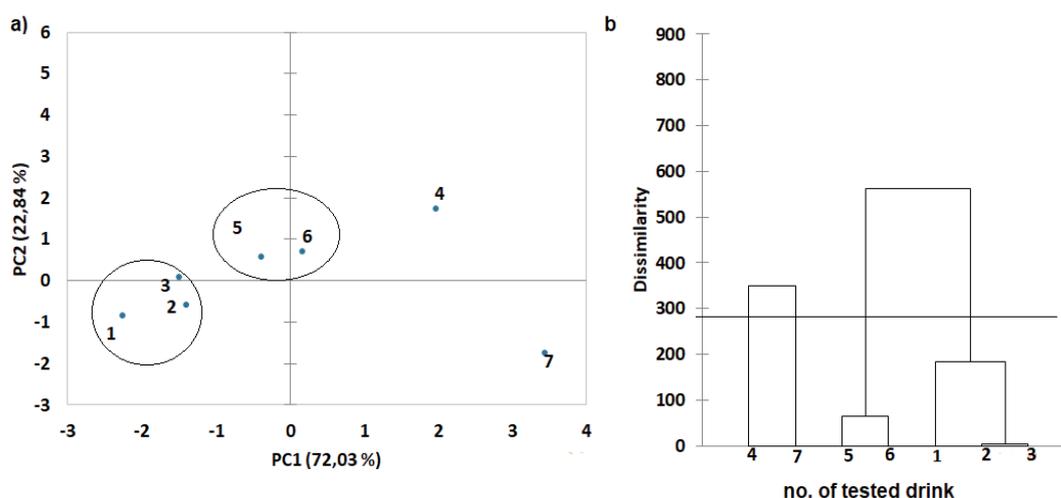


Fig. 7. (a) PCA and (b) AHC of the data obtained by potentiometric sensor with ISEs for tested non-alcoholic beverages, 1 – Jurajska Lemon, 2 – Sprite, 3 – Ustronianka Citric, 4 – Hoop Premium Lemon, 5 – Hellena Orangeade, 6 – Nata Orangeade and 7 – reference drink.

taste intensity (Fig. 7a and b) as it was found by sensory analysis (Table 1). The first group is made up from Jurajska Lemon (No. 1), Ustronianka Citric (No. 3) and Sprite (No. 2), since they have intensive lemon citric taste (Table 1). The next group (Nos. 5 and 6) is made up from typical orangeades characterized by an orange flavor and similar intensity of sour taste. These drinks contain more sucrose which reduces the intensity of sour taste (sensory results in Table 1).

The Hoop Premium Lemon beverage (No. 4) contains the most sucrose and is characterized by the lowest sour taste (sensory result in Table 1). Therefore, the point representing this beverage is outside of these two groups.

The reference drink (No. 7) of a similar sugar and citric acid composition as orangeades (Nos. 5 and 6) did not

contain carbon dioxide. Therefore, the point representing this drink is laying far from the second group (Nos. 5 and 6). It means, that carbon dioxide content strongly influences the sour taste intensity, which results in different electrode responses. This effect was already observed in tonics [23].

Thus, the PCA and AHC analysis indicate that non-alcoholic beverages with a similar intensity of taste are grouped together. These results suggest that the potentiometric sensor with five ISEs may be used for recognition of unknown non-alcoholic beverages.

The obtained results show that potentiometric sensor with electrodes modified by lipids might be used for quality assessment of non-alcoholic drinks containing carbon dioxide.

4. Conclusion

Potentiometric sensor containing a set of ISEs with lipid modified membranes (benzylhexadecyldimethylammonium chloride monohydrate, hexadecylamine, 1-dodecanol, elaidic acid, cholesterol) was used for discrimination and quality control of non-alcoholic beverages mainly composed of sugar and citric acid (lemonades, orangeades and Sprite).

It was found that the stability of ISEs with positively charged membrane (benzylhexadecyldimethylammonium chloride monohydrate) and negatively charged one (1-dodecanol) in citric acid solution was very good at least within 5 days of the experiment. The sensitivity of ISEs to acetic, hydrochloric and citric acid is also quite good. The mean slope values of $E = f(c)$ show that sensitivity of ISEs in acid solution changes in the order: hydrochloric acid > citric acid > acetic acid. On the contrary all the ISEs were almost not sensitive to sweet substances (glucose, fructose, sucrose) concentration changes.

The potentiometric sensor was used to examine non-alcoholic beverages (Jurajska Lemon, Sprite, Ustronianka Citric, Hoop Premium Lemon, Hellena Orangeade, Nata Orangeade) and to the reference drink. The shapes of radar charts are different mainly for Hoop Premium Lemon and the reference drink. The sensor results were transformed by PCA and AHC analysis and compared with sensory analysis results. The tested drinks can be divided into two groups: first one containing drinks of intensive lemon taste (Jurajska Lemon and Ustronianka Citric and Sprite) and the second one containing orangeades of similar sweetness, sour taste and an orange flavor. Hoop Premium Lemon drink is outside these groups, because it is characterized by the lowest sour taste intensity. It is probably due to sugar reduction of sour taste intensity. The point representing the reference drink is also outside of these both groups since it does not contain carbon dioxide. This effect was already observed in tonics [23].

The potentiometric sensor was also used to test di-component mixtures of sucrose and citric acid creating a database. The shape of the radar chart of an unknown beverage might be compared with those in the database. Such so called "fingerprint" method might be used for quality control of unknown drinks.

The obtained results show that the potentiometric sensor with lipid-modified ion selective electrodes might be used for quality control of unknown drinks containing carbon dioxide, for example, at the last step of production line.

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