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The possibility of application of the potentiometric sensor with all-solid-state electrodes containing lipid-polymer membranes for classification of tested black leaf teas in terms of their quality

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ABSTRACT

Potentiometric sensor with all-solid-state electrodes containing lipid-polymer membranes for a set of black leaf teas coming from four countries (China, India, Sri Lanka and Kenia) classification was applied. The tested tea samples were reassigned to quality classes marked with numbers I–V according to the information available. The sensor results of tea samples are presented in the form of radar charts. It was observed that, in the majority of cases samples with a similar radar chart shape belong to the same quality class. Based on principal component analysis and agglomerative hierarchical clustering analyses four groups of leaf tea samples and two separate items were distinguished. The first group was constituted by the highest quality Yunnan teas. The second group is made up of high-quality Indian teas and one tea sample of undeclared quality. The third group includes tea samples of unknown quality assigned to quality Class IV. The last group distinguished is constituted by the sample of Indian Madras and Ceylon tea, which are imported for retail trade for blends. This grouping largely corresponds to the quality classes of tested tea samples. The obtained results suggest that potentiometric sensor with all-solid-state electrodes containing lipid-polymer membranes might be used for discrimination of black leaf teas of different quality and also for determination of quality class of an unknown tea sample.

Keywords: Quality estimation; Lipid-polymer membranes; Leaf teas quality; All-solid-state electrodes; Principal component analysis and agglomerative hierarchical cluster analysis

1. Introduction

Food and Agriculture Organization (FAO), defines tea as the product obtained from plant species called: *Camellia sinensis* (Linnaeus) O. Kuntze *var. Sinensis* and *var. assamica.* It includes green tea (unfermented), black tea (fermented) and partially fermented tea [1]. Precise entries, indicating the botanical origin of the plant along with the varieties allowing it to be considered as tea are also included.

The definition of tea is also presented in the Compendium of Guidelines for Tea (*Camellia sinensis*) prepared by the European Tea Committee (ETC) and the European Herbal Infusions Association (EHIA) [2]. Only the delicate buds and leaves of different varieties of *Camellia sinensis* (Linnaeus) O. Kuntze, processed according to recognized production methods and suitable for the preparation of an infusion for consumption, are considered as tea under this definition.

The tea adulterations, that occur today, are mainly related to blending the certified tea from the geographical

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tea-growing region with cheaper and uncertified teas from other plantations [3,4]. Such practice was pointed out by Wang et al. [5] and Ma et al. [6] proving that in China there are cases of adulterating teas protected by a geographical region of origin, the so-called Geographical Indication (GI), which include, inter alia, *Dongting Biluochun* or *Xihu Longjing* teas, with non-certified teas. In turn, Kovács et al. [7] drew attention to the adulteration of tea by adding tea leaves from lowland plantations (usually cheaper) to high-quality teas obtained from mountain plantations. Similar practices have also been reported in the Polish tea market, where tea adulteration consists of false information or lack of information about the origin of the product.

There is a need for the development of a fast and effective mechanism to ensure the authenticity of tea and other food products. These products are considered the best quality based on their authenticity [8]. The application of electrochemical taste sensors could be a way to meet this need. Such sensors (potentiometric and voltammetric) have already been successfully applied for the analysis of various food products [9–22]. Diverse, modified taste sensors combining various measurement techniques were also used for selected black tea classification. The Chinese group of He et al. [23] has applied a potentiometric sensor array with a set of non-specific all-solid-state electrodes for the classification of tea samples from different geographical origins. Indian group of Bhattacharyya et al. [24] used cyclic voltammetry with platinum and glassy carbon working electrodes for the classification of selected black tea samples. There was also a voltammetry electronic tongue system with gold, silver and palladium working electrodes applied to collect signals for further classification of tea by convolutional neural network-based auto features extraction method [25]. Some multi-electrode sensors based on electrochemical impedance spectroscopy for discrimination and characterization of selected black and green tea samples were also developed [26–28]. Attempts were also made to combine taste sensors with electronic noses for tea characterization [7,29–31].

In this paper, we focus on the possibility of the application of the potentiometric sensor with all-solid-state electrodes (ASSEs) containing lipid-modified membranes for tea quality classification. The principle of operation of such sensors is based on measuring the difference in electrical potential between the working electrodes (ASSEs) and the reference electrode. The ASSEs contain conducting polymer instead of an inner electrolyte as an ion to electron transducer. Due to the application of working electrodes with lipid-polymer membranes potentiometric sensor is characterized by global selectivity [9,11,15,32]. This means that such a sensor's mechanism of operation is similar to the one of a human tongue. The interactions between different substances present in the solution are detected by such a sensor due to the presence of lipid-polymer membranes. For this reason selectivity to specific substances responsible for the taste is not a desirable feature of such a sensor [32].

The objective of this study was to evaluate the applicability of the elaborated potentiometric sensor with ASSEs containing lipid-polymer membranes to classify the tested black tea leaf samples from different countries (China, Indie, Sri Lanka, Kenia) in terms of their quality.

2. Experimental

2.1. Materials

The reagents used for ASSEs preparation were as follows: benzyldimethyltetradecylammonium chloride (BDMTACl, 98%, Aldrich), dodecyltrimethylammonium bromide (DTMABr, 98%, Aldrich), palmitic acid (PA), stearic acid (SA > 99%, Fluka), phytol (P, 97% – mixture of isomers, Sigma-Aldrich), 3,4-ethylenedioxythiophene (EDOT, Aldrich), poly(sodium 4-styrenesulfonate) (NaPSS, Aldrich), polyvinyl chloride (PVC) and dioctyl phenylphosphonate (DOPP, 95% Aldrich). Demineralized water distilled over $KMnO_4$ and tetrahydrofuran (THF) distilled under $LiAlH_4$ were used in all experiments.

2.2. Experimental set-up

The measurement set-up consisted of five ASSEs with different lipid compound: E.1 – BDMTACl, E.2 – DTMABr, E.3 – PA, E.4 – SA, E.5 – P, reference electrode: Ag/AgCl/Cl^- (10–1 M KCl) and a voltmeter connected to a computer. All electrodes have been immersed in tested tea infusions (Fig. 1a).

Each ASSE involves a conducting polymer layer (No. 4 in Fig. 1b) covering glassy carbon support (No. 3) and a lipid-polymer membrane (No. 5) The detailed preparation of each ASSE is described in [21,22,34].

The electrode potentials were measured using a high-input-impedance voltmeter HACH EC30 and transferred into a computer. The ASSEs were conditioned in KCl solution (0.01 M) before and between measurements. The experiments were carried out at a temperature of 23°C ± 0.5°C. The determination was performed in four replications for each tea infusion and mean potential values (±1%) were taken.

2.3. Tested black teas

The set of black leaf teas coming from four countries (China, India, Sri Lanka and Kenia) was examined. These were teas obtained directly from the ship's holds. The authenticity of the country of origin of the analysed leaf teas was confirmed based on information from the shipping documents. However, due to trade secrets, access to this information was often limited only to the tea country of origin. In some cases, the declared quality was contained in the relevant documents. Such leaf teas (eleven samples) were chosen for examination. According to the documents provided, three of the tested samples were teas used to compose tea blends. The characteristics of the analysed tea samples are presented in Table 1. Tea samples for testing were prepared in the form of 2% infusions at a temperature of 95°C ± 2°C. Before the measurements, the samples were cooled down to the temperature of 23°C ± 2°C. For each experiment, the new infusion was prepared and cooled to the appropriate temperature.

The tea samples were reassigned to classes intending to reflect their overall quality. The quality Class I is constituted by the highest quality leaf teas. These are Chinese Yunnan leaf teas of well-defined quality (superior and Flowery Orange Pekoe, FOP). These teas are characterized by dark infusion, slightly spicy taste and smoky aroma. There are

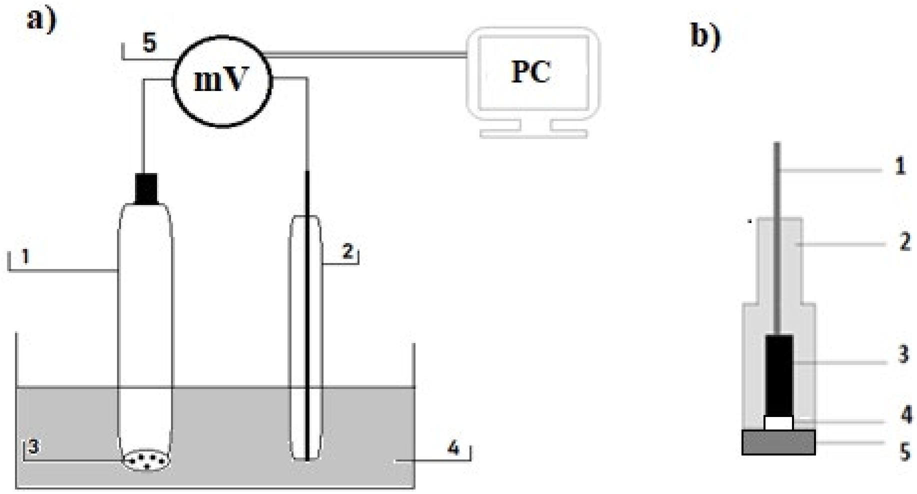

Fig. 1. (a) The measurement set-up: 1 – five working ASSEs with polymer membranes; 2 – reference electrode Ag/AgCl/Cl⁻; 3 – single ASSE with lipid-polymer membranes immersed in tested infusion; 4 – tested tea infusion; 5 – voltmeter connected to PC and (b) ASSE structure: 1 – copper wire; 2 – Teflon casing; 3 – glassy carbon; 4 – conducting polymer; d_{PEDOT/PSS^-} = 0.5 μm; 5 – modified lipid-polymer membrane; d_{PVC} = 10 μm.

high quality, Indian leaf teas in quality Class II. These are Darjeeling Himalaya Tippy Golden Flowery Orange Pekoe (TGFOP) and Assam Ambaguri Finest Tippy Golden Flowery Orange Pekoe (FTGFOP) teas in this class. The first one is very characteristic black tea of geographically protected area of origin. The latter one is also considered a high quality Indian black tea. Kenyan Milma black tea of broken leaves, Golden Flowery Broken Orange Pekoe (GFBOP) is considered to be a second-grade black tea and was assigned to a quality Class III. All the leaf teas which quality was not declared (mainly due to trade secrets) were assigned to quality Class IV. All the tea samples which quality was not described (due to trade secrets) but it is known that they were imported as a component of tea blends, were assigned to quality Class V. It is known that the quality of teas imported for the preparation of tea blends for retail trade is lower [33,35].

2.4. Mathematical analysis

The obtained data were analyzed by multivariate analytical methods: principal component analysis (PCA) by Pearson Algorithm and Agglomerative Hierarchical Clustering (AHC) by Ward Algorithm with Euclidean distance for similarity scale [36]. XLSTAT-Base software ver. 2015.1.05 by Addinsoft (Faber & Bro, 2002) was applied for mathematical modelling.

3. Results and discussion

The raw potentiometric data obtained by the potentiometric sensor with the appropriate lipid-polymer membrane in ASSEs according to assigned quality classes are presented in radar charts (Fig. 2a–e).

It can be observed that the shapes of curves in the radar chart representing the tea samples of quality Class I (Fig. 2a) are similar to each other. It is also the case of quality Class II (Fig. 2b). However, in the case of quality Classes IV and V (Fig. 2d and e), some differences in radar chart shapes may be observed. Samples No. 4 and 8 belonging to quality Class IV are of a similar shape as opposed to Sample 9. It is also the case of sample 3 assigned to quality Class V (Fig. 2e). It can be seen that the sensor results obtained for the only one Kenyan tea (No. 11 in Fig. 2c) belonging to quality Class III differ from all other samples. In order to divide the tested teas into groups by potentiometric sensor, the PCA and AHC analyses were performed (Fig. 3).

Two principal components (PC1 and PC2) of PCA explain over 78% of the variability of the model (Fig. 3b). This indicates that there exists a strong pattern by which the tested samples are grouped. There were four different groups of tested samples and two tea samples outside these groups (Fig. 3a). These groups are marked with circles in the PCA graph (Fig. 3b). Samples 1 and 2, previously assigned to quality Class I, are grouped together (Fig. 3a and b). These are both Yunnan Gold black leaf teas of the well-defined, highest quality.

The next AHC group is constituted by Samples No. 6, 7, 9. Tea Samples No. 6 and 7 were initially assigned to quality Class II. They are both well described, high-quality Indian teas (6 – Darjeeling Himalaya and 7 – Assam Ambaguri). However, Sample No. 9 (Ceylon Malatiyana tea), for which quality of leaves was not declared, was initially assigned to quality Class IV. It is known that Ceylon teas are often compared to Indian teas and they are considered to be of equally high quality [35]. The analysis of the radar charts representing the raw sensor data for Samples 6, 7 and 9 also reveals similarities (Fig. 2b and d). These results may lead to the conclusion that the quality of tea Sample No. 9 is similar to the quality of Samples 6 and 7.

The other group is constituted by Samples 4 (Indian tea) and 8 (Ceylon tea) (Fig. 3a and b). These samples were initially assigned to the quality Class IV due to the lack of information on their quality (Table 1).

Table 1
Characteristics of the tested black leaf teas

No.	Country of origin	Declared commercial quality	Leaves quality	Sensory characteristics	Botanical species	Quality description	Quality class
1.	China	Yunnan Gold	Superior; long wiry leaves without tips	Dark infusion; fresh, slightly spicy falvour	*Camellia sinensis*	Main grade, high quality	I
2.	China	Yunnan Gold	FOP; full leaves in good condition	Dark infusion; mild flavor; smoky aroma	*Camellia sinensis*	High-quality; the first grade in China	I
3.	China	Yunnan (imported by Polish tea brand)	Quality of leaves not declared		*Camellia sinensis*	Not enough information; imported for blends	V
4.	Indie	Assam Dikam	Quality of leaves not declared		*Camellia assamica*	Not enough information	IV
5.	Indie	Madras (imported by Polish tea brand)	Quality of leaves not declared		*Camellia assamica*	Not enough information; imported for blends	V
6.	Indie	Darjeeling Himalaya	TGFOP; pointed leaves on the upper part and increased number of golden buds	Bright, golden infusion; characteristic notes of sweet spring greens and muscatel. Dry, rewarding finish.	*Camellia sinensis*	Main grade in Darjeeling; geographically protected area of origin	II
7.	Indie	Assam Ambaguri	FTGFOP; mixture of whole leaves, small and medium-sized, from the first harvest, with an admixture of a large number of leaf tips	dark amber infusion, not too strong; noble taste and aroma with little bitterness	*Camellia assamica*	High quality	II
8.	Sri Lanka	No information	FBOP; broken long and thick leaves and the youngest tips	Not declared	No information	Not enough information	IV
9.	Sri Lanka	Ceylon Malatiyana	Quality of leaves not declared		*Camellia sinensis*	Not enough information	IV
10.	Sri Lanka	Ceylon Supreme (imported by Polish tea brand)	Quality of leaves not declared		*Camellia sinensis*	Not enough information; imported for blends	V
11.	Kenya	Milma	GFBOP; broken pointed leaves on the upper part and golden buds	Copper colour infusion; intense flavor and delicate aroma	*Camellia sinensis*	Second grade	III

GFBOP – Golden Flowery Broken Orange Pekoe; FOP – Flowery Orange Pekoe; TGFOP – Tippy Golden Flowery Orange Pekoe; FTGFOP – Finest Tippy Golden Flowery Orange Pekoe; FBOP – Flowery Broken Orange Pekoe.
Source: shipping documents and producers' declarations.

As it was previously mentioned, Ceylon and Indian teas are considered to have similar sensory properties [35]. Tea Sample No. 4 is known to be *Camellia assamica*, which is often used to refine lower-quality compositions [37]. There is no information on tea Sample No. 8 except for the quality of leaves, which are broken (FBOP). The shapes of radar for these two samples (Fig. 2d) are almost the same, which might indicate that both tea samples (Nos. 4 and 8) are of similar quality.

The last group is constituted by Madras tea (No. 5) and Ceylon tea (No. 10), which are characterized by undefined quality and imported for tea blends. These teas were assigned to quality Class V. However, Yunnan tea (No.3) also of quality Class V is outside this group. It might be due to the different sensory properties of this tea from previously discussed teas and also from Yunnan well-defined, high-quality samples (Nos. 1 and 2 in Fig. 3b). The analysis of radar charts of those three samples (Fig. 2e) also shows the significant shape differences of Sample No. 3 in comparison to Sample Nos. 5 and 10.

Kenyan tea (No. 11, Table 1) is outside these groups (Fig. 3b) due to specific climatic conditions during the cultivation of the plants which result in different sensory properties.

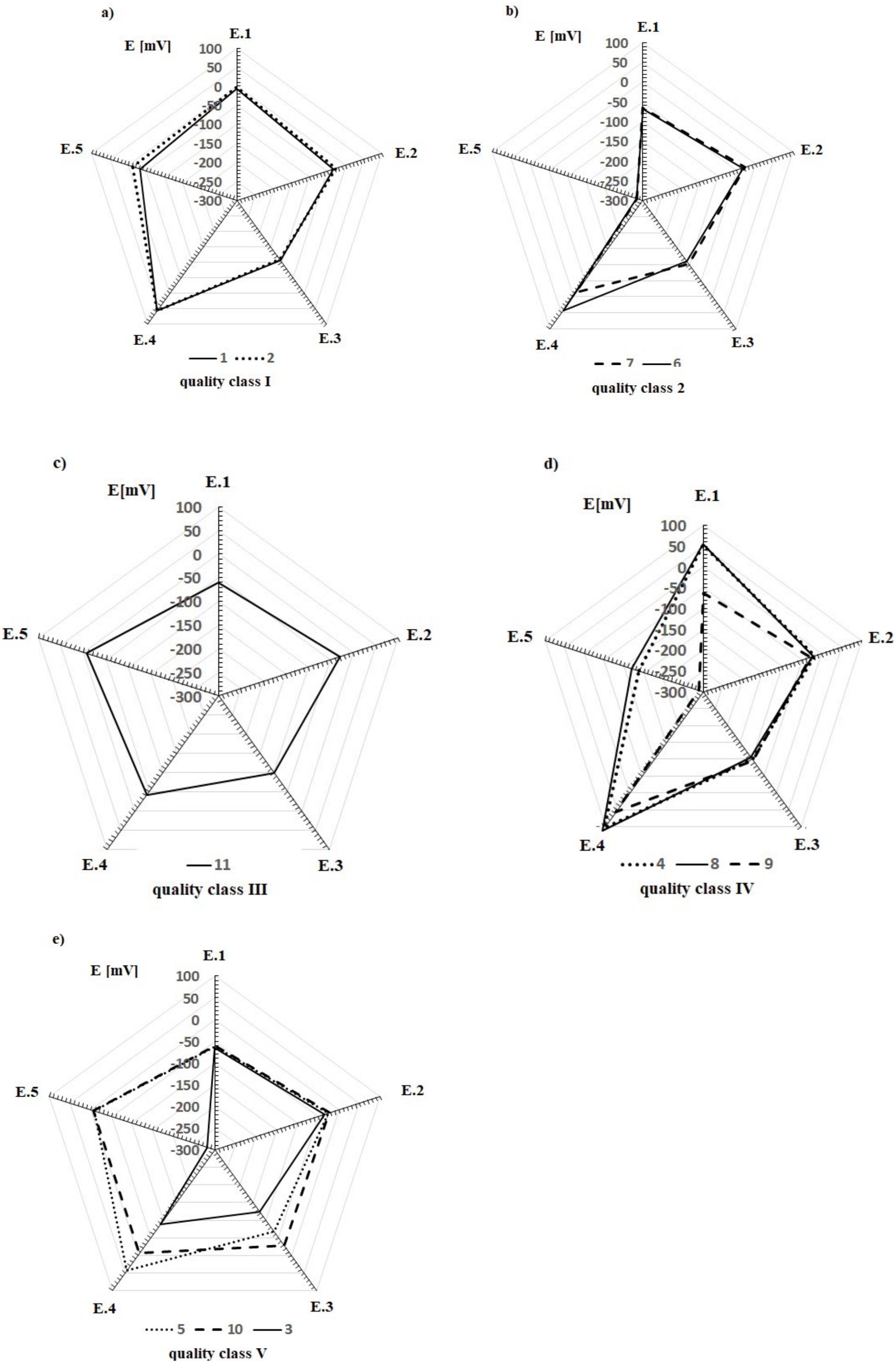

Fig. 2. Radar charts representing the raw potentiometric results obtained by sensor with ASSEs; (a) quality Class I: 1 – Yunnan Gold Superior; 2 – Yunnan Gold FOP; (b) quality Class II: 6 – Darjeeling Himalaya TGFOP; 7 – Assam Ambaguri FTGFOP, (c) quality Class III: 11 – Milma GFBOP, (d) quality Class IV: 4 – Assam Dikam; 8 – Sri Lanka black tea FTGFOP1; 9 – Ceylon Malatiyana, (e) quality Class V: 3 – Yunnan; 5 – Madras; 10 – Ceylon Supreme (Table 1).

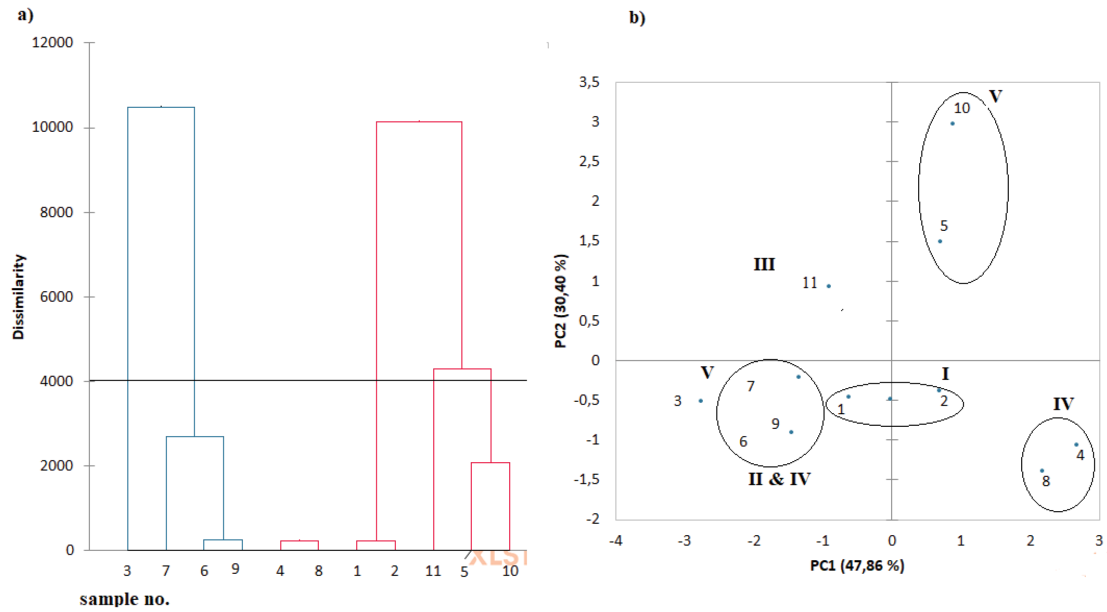

Fig. 3. Results of (a) AHC and (b) PCA analyses of raw sensor results of tested tea samples; the groups were marked based on AHC; 1–11 – tested tea samples; I–V – quality classes (Table 1).

The obtained results indicate the possibility of the application of the potentiometric sensor with ASSEs containing lipid-polymer membranes for quality identification of tested black leaf teas. This would be important when evaluating teas of unknown quality.

4. Conclusions

The elaborated in our laboratory potentiometric sensor with ASSEs containing lipid-polymer membranes was applied for a set of black leaf teas classification. Eleven black leaf tea samples were coming from four countries (China, India, Sri Lanka and Kenia). Due to trade secrets, the quality of six out of eleven tea samples was not declared. The tea samples were reassigned to appropriate quality classes marked with numbers I–V.

The results obtained by the sensor were presented in the form of radar charts. It can be seen that the samples assigned to a given quality class have similar radar chart shapes.

The obtained results were analyzed using mathematical methods (PCA and AHC). There were four groups of teas and two separate items distinguished. The first group was constituted by the highest quality Yunnan teas (Class I). In the second group, there were two high-quality Indian teas (Class II) and one tea sample of undeclared quality. There were two samples of unknown quality in the third group (Class IV). However, the partial information available about the quality of these samples suggests that there are some similarities between them. The last group distinguished was constituted by the sample of Indian Madras and Ceylon tea (Class V). These two samples were also lacking in quality information, but they are both known to be imported for retail trade in the form of blends. It is known, that for this purpose, teas of a lower quality are usually used [33,35].

Attention should be paid to the fact, that the mathematically (AHC) obtained groups largely correspond to the quality classes of tested tea samples. This might suggest that there is a potential possibility of using the potentiometric sensor with ASSEs containing lipid-polymer membranes for discrimination of black leaf teas of different quality. Moreover, it has been shown that our potentiometric sensor may be applied for the determination of the quality class of an unknown tea sample.

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