Computers and Electronics in Agriculture 191 (2021) 106545

Contents lists available at ScienceDirect

Computers and Electronics in Agriculture

journal homepage: www.elsevier.com/locate/compag

Impedance analysis of milk quality using functionalized polyamide textile-based sensor

Goran M. Stojanović [a,*], Ankita Sinha [a], Adnan E. Ali [a], Varun Jeoti [a], Marija B. Radoičić [b], Darka D. Marković [c], Maja M. Radetić [d]

[a] University of Novi Sad, Faculty of Technical Sciences, Trg Dositeja Obradovića 6, 21000 Novi Sad, Serbia
[b] University of Belgrade, "Vinča" Institute of Nuclear Sciences, P.O. Box 522, 11001 Belgrade, Serbia
[c] University of Belgrade, Innovation Centre of the Faculty of Technology and Metallurgy, Karnegijeva 4, 11120 Belgrade, Serbia
[d] University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11120 Belgrade, Serbia

ARTICLE INFO

Keywords:
Textile
PANI/TiO_2 nanocomposite
Impedance
Milk

ABSTRACT

Present paper demonstrates design and characterization of a textile based microfluidic chip sensor for the detection of milk adulteration through measuring the real part of the impedance and impedance phase angle. Polyamide (PA) based textile fabric was chemically functionalized with polyaniline and titanium dioxide nanoparticles (PANI/TiO_2) nanocomposite and embedded in the microfluidic chip. Prototyping of microfluidic chip was performed by xurography and hot lamination using polyvinyl chloride foils. Morphological and chemical properties of fabricated textile-based PA-PANI/TiO_2 chip sensor were evaluated by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). Quality of milk was accessed with fabricated textile sensor using cow and goat milk samples. The electrochemical impedance spectroscopy (EIS) was applied to detect the change in resistance and phase angle of pure and diluted milk. The developed PA-PANI/TiO_2 chip sensor acted as a variable resistor that was able to identify adulterations and spoilage of the milk samples with sensitivity of 0.06 degrees of phase angle variation per % of water dilution. Our work promises that application of textile electronics could be efficiently exploited for food safety, point-of-care and environment monitoring applications.

1. Introduction

Milk and dairy products are important sources of energy due to the presence of high content of essential nutrients (Choudhary et al., 2019; Govindarajalu et al., 2019). Therefore, milk quality has always been a matter of global concern to ensure good public health (Bhadra et al., 2012; Lima et al., 2018). More often, the spoilage and contamination of milk are caused due to microorganisms which results in milk-borne infections threatening the health of the consumer and lead to a decrease in the overall quality of milk. Furthermore, milk purity is another matter of big concern due to the increased practices of adulteration. The water added into milk increases its volume for sale and hence ensures larger profit (Handford et al., 2016). Milk dilution can be usually detected by determining the changes in milk freezing point or by detecting changes in the refraction of light after fat extraction. However, these methods are costly and time-consuming. In cryoscopic method, the freezing point of diluted milk is normally observed closer to the freezing point of water (Das et al., 2011; Salmaz et al., 2019; Nascimento et al., 2013). However, pouring salt into milk (Handford et al., 2016) has also become a general practice as the original freezing point of milk can be recovered in this way. Such practices do not make the cryoscopic method fraud-proof as it is limited to the detection of water dilution to milk by measuring the freezing point (Nascimento et al., 2013). Thus, it is difficult for the dairy industry to confirm the quality of supplied milk and consequently to ensure its quality to consumers (Ghasemi-Varnamkhasti et al., 2017; Durante et al., 2016).

There are various indicators to determine milk spoilage and degradation such as chemical composition, physical properties and change in its taste and hygiene. However, measuring a change in its electrical properties is considered as one the possible ways to check quality of milk. Water addition to milk results in the decrement of mineral salt concentrations which impacts its electrical properties such as a decrease in electrical conductivity or an increase in impedance. In this regard, EIS technique has demonstrated high potential for its implications in real-

* Corresponding author.
E-mail address: sgoran@uns.ac.rs (G.M. Stojanović).

https://doi.org/10.1016/j.compag.2021.106545
Received 2 April 2021; Received in revised form 14 July 2021; Accepted 1 November 2021
Available online 12 November 2021

time analysis of food products and has been utilized extensively in past few decades to characterize milk samples (Dias et al., 2009; Mabrook et al., 2006). Impedance measurements provide a basic information on electrical conduction behaviour of milk using both real and imaginary parts of impedance over a wide range of frequencies. Numerous other methods can be found in open literature towards measuring the correlation of milk dilution and thermal parameters of raw milk using an interdigital capacitor sensor (Phimphisan, 2015). Further, a moisture sensor based on microwave absorption was also proposed for detecting milk quality (Agranovich et al., 2016). These studies indicated that electrical resistance and capacitance of raw milk increased together with water content in the milk, regardless of the electrical frequency (Agranovich et al., 2016).

As milk is prone to adulteration, there is a need for its careful testing through a suitable, sensitive and cost-effective method to guarantee its quality and thus public safety (Das et al., 2011; Salmaz et al., 2019). A growing need for the fabrication of low-cost, portable and flexible detection platforms has enabled microfluidic technology to flourish in the past few years. In microfluidics, easy and rapid fabrication of chips depends on the choice of substrates as well as on the prototyping method. Xurography has been proved to be an efficient technique which utilizes an inexpensive cutter plotter and hot lamination machine for facile prototyping of microfluidic chips. Multiple approaches to fabricate flexible microfluidic chips have been demonstrated in previous years which varied from plastic to paper based sensing devices. Textile based flexible substrate have been also utilized with miniaturized structures which have shown minimal deterioration and high sensing performances. Despite benefits such as large surface area and its ability to be integrated into electronic devices, implications of fabric based microfluidic analytical devices is rarely reported (Malon et al., 2017).

Basically, the electrical conductivity of any textile material is the property that transforms it into a sensing material and delivers a key role in the development of textile-based electronic systems. A variety of methods have been used to develop electrically conductive textile such as by integration of conductive nanoparticles into fibres or by enrichment of the fibres with conductive polymers. Moreover, it can be done by the printing of conductive threads onto the fibre surface or by chemical functionalization of fibres (Poghossian et al., 2019; Kaur et al., 2015; Ismar et al., 2020).

The polyamide (PA) based microfluidic sensor used in the present study represented a novel two-dimensional resistor which provided large sampling zone and thus the surface area for transduction of electric signals during milk interaction and detection. Moreover, fabricated PA based sensor successfully demonstrated its implications towards analytical sensing of food and beverages which was previously limited to biomolecules for point-of-care diagnostics. When compared to paper based microfluidic devices, textile substrates show high mechanical strength which do not require hydrophobic barriers for creating microchannels making them a suitable platform for milk analysis requiring a low volume of samples (Poghossian et al., 2019). The main idea in the present paper was to demonstrate a possible fabrication and exploitation of a textile-based compact and portable microfluidic platform for detection of milk quality and adulteration using real cow and goat milk samples. Overall, PA textile fabric was chemically functionalised with PANI/TiO_2 nanocomposite with an aim to detect a change in its electrical impedance and phase angle of milk samples to quantify its quality and adulteration.

2. Experimental

2.1. Materials and instruments

Polyamide (PA 6.6, 140 g/m^2) woven fabric was used as a textile substrate. Felosan RG-N (CHT) was applied as a washing agent for PA fabric. Titanium tetrachloride ($TiCl_4$, ≥99%, Fluka), hydrogen peroxide (H_2O_2, 30%, JT Baker), aniline (p.a. > 99.5%, Centrohem), ammonium persulphate (APS, p.a. > 98%, Centrohem) were used for the synthesis of nanocomposite. Hydrochloric acid (HCl, HPLC grade, 36.5–38.0%) and sulphuric acid (H_2SO_4, HPLC grade, 95.0–98.0%) were obtained from JT Baker. NaCl salt was purchased from a local store. PVC A4 hot lamination foil from MBL® 125MIC, Belgrade, Serbia was utilized for microfluidic chip fabrication.

Spectrophotometer Thermo Scientific Evolution 600 UV–Vis spectrophotometer was employed for indirect determination of concentration of colloidal solution comprising TiO_2 nanoparticles (NPs). Morphology of chemically functionalized PA fibres was analysed by FESEM (Tescan Mira3 FEG). The samples were coated with a thin layer of Au prior to analysis. Fourier transform infrared (FTIR) spectra of neat PA fibre and functionalized PA fibre were recorded and compared in the ATR mode using a Nicolet 6700 FTIR Spectrometer (Thermo Scientific) at 2 cm^{-1} resolution over a wavenumber range of 500–4000 cm^{-1}. Cutter plotter (CE6000-60 PLUS, Graphtec America, Inc., USA) with 45° cutting blade (CB09U) was applied to cut the PVC foils for microfluidic chip fabrication. Bonding of carved microchannels on PVC foils was performed using a hot lamination with an A4 card laminator (FG320, Minoan Binding Laminating doo, Serbia). Design of a microfluidic component was performed using an AutoCAD software. Electrochemical measurements were accomplished using a Palmsense4, Potentiostat/Galvanostat/Impedance Analyzer connected with a laptop equipped with PSTrace 5.8 software.

2.2. Preparation of conductive textile samples

Firstly, PA woven fabric was cleaned in a bath containing 0.05 w/v % non-ionic washing agent Felosan RG-N at 50 °C for 15 min (liquor-to-fabric ratio 50:1). The fabric was then thoroughly rinsed with distilled water several times and dried completely at room temperature. Synthesis of 0.2 M colloidal solution of TiO_2 NPs was performed by acid hydrolysis of $TiCl_4$. In detail, 6 mL of $TiCl_4$ solution cooled down to −20 °C was added drop-wise to 200 mL of cooled water (4 °C) under vigorous stirring and then kept at the same temperature for 30 min. pH of the solution was between 0 and 1, which was dependant on $TiCl_4$ concentration. The slow growth of nanoparticles was achieved by running dialysis of a colloidal solution against water at 4 °C using a Spectra/Por dialysis membrane (molecular weight cutoff (MWCO) 3500 Da (Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA). Water was changed on a daily basis and the dialysis process was continued till pH of the solution reached 3. The concentration of TiO_2 colloidal solution was determined using spectrophotometer from peroxide complex ($\lambda = 410$ nm, $\varepsilon_{410} = 710$ M^{-1} cm^{-1}) concentration (Tompson, 1984) which was formed after adding 2 mL of H_2O_2 into the solution comprising 20.9 mL of water, 2 mL of 96 wt% H_2SO_4 and 0.1 mL of colloidal TiO_2 NPs (Radoičić, 2013).

In the next step, PA fabric was functionalized with PANI by in situ polymerization of aniline with APS in the presence of colloidal TiO_2 NPs in an acidic medium at room temperature. Specifically, 3.7 mL of 0.2 M TiO_2 colloidal solution was added to 1.2 M solution of HCl in 50 mL volumetric flask. 0.50 g of PA fabric was then immersed into this solution and was stirred for 10 min followed by the addition of APS and aniline to the reaction mixture. Dispersion of aniline was prepared by putting 0.73 mL of aniline to 25 mL of 1.2 M HCl. APS solution was made by dissolving 2.282 g of APS in 25 mL of 1.2 M solution of HCl. Polymerization reaction lasted one hour and fabricated PA fabric was subsequently rinsed with HCl solution (pH 3). Finally, obtained functionalized PA fabric was designated as PA-PANI/TiO_2.

2.3. Preparation of milk samples

Raw (pure) cow milk and goat milk samples were purchased from a local farmer (village Despotovo, near Novi Sad, Serbia). Different milk samples were prepared for electrochemical measurements. The samples of raw milk were diluted with tap water in different volume ratios 90:10,

85:15 and 80:20 (v/v%). In the next set of diluted samples of cow milk 10 mg of NaCl was added. Milk samples were stored at room temperature for few days and designated as the spoiled milk sample which was also tested within the electrochemical studies.

2.4. Design of the microfluidic chip

The fabricated microfluidic chip is shown in Fig. 1, which represents a multi-layered structure comprising three polyvinyl chloride (PVC) foils (as cost-effective sealing material) and functionalized conductive PA fabric (PA-PANI/TiO_2) in the middle of the chip.

PVC transparent foil with total dimensions 4 cm × 2 cm was used as the bottom layer of the microfluidic chip. PVC foil with the channel in its central part served as a middle layer. A rectangular piece of PA-PANI/TiO_2 textile fabric (2.5 cm × 0.8 cm) was inserted into the channel. PVC foil on the top was perforated in the centre of the structure. A hole with a diameter of 5 mm enabled injection of the tested milk samples. In all layers of the PVC foils, the space for attaching conventional alligator's clips was carved by cutter plotter machine. These accesses are connections between the ends of the piece of the conductive textile and they represent terminals for testing the component and performing the electrical measurements. In order to obtain a compact portable microfluidic platform for easy handling and manipulation of the entire chip, three PVC layers were laminated together at 130 °C using a Card hot Laminator. A precise micropipette was used to inject 5 μL of milk into the textile part of the chip and measurement was conducted after the injected milk was soaked by the fabric. This could be visually observed because the colour of the fabric changed. As soon as the drop reached the terminals, the measurement started.

2.5. Experimental setup

For testing the prepared milk samples, electrochemical impedance spectroscopy was performed using the PalmSens4 instrument. The Bluetooth wireless connection was established between the hardware part of the electrochemical interface and the laptop computer. This experimental setup is depicted in Fig. 2.

Software tool PSTrace 5.8 was used for controlling the whole measurement process and for setting measurement parameters. The PalmSens4 system was implemented in a two-electrode configuration using alligator connectors to establish electrical contact with two sides of functionalised PA fabric. The frequency range from 1 Hz to 100 kHz was applied for EIS measurements. All electrochemical measurements were performed in triplicate and mean values are presented.

3. Results and discussion

3.1. FESEM and FTIR analysis of textile nanocomposites

FESEM images of neat PA fibre and PA fibre functionalised with PANI/TiO_2 nanocomposite are presented in Fig. 3. In order to get insight

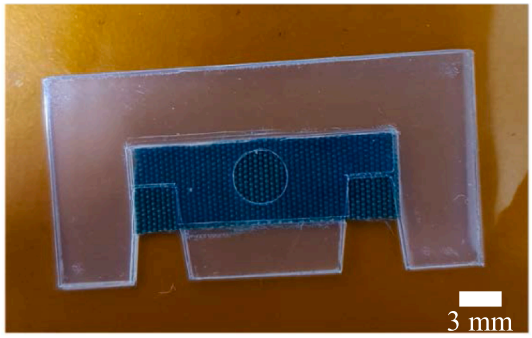

Fig. 1. Fabricated PA-PANI/TiO_2 textile-based microfluidic chip.

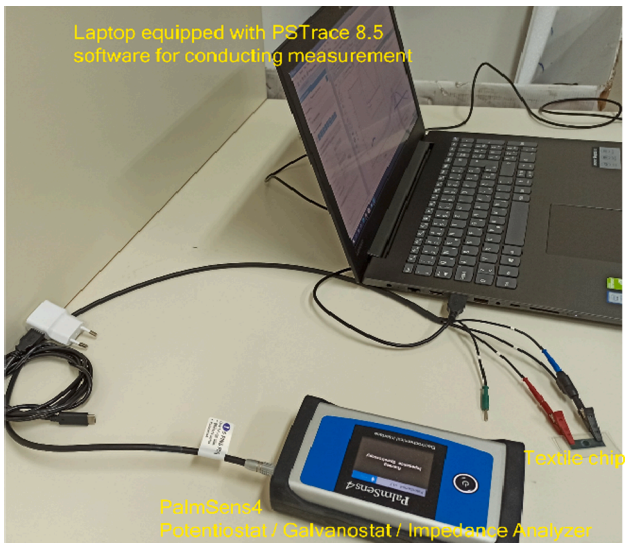

Fig. 2. Experimental setup for performing electrical characterization of milk samples with textile-based microfluidic chip.

into change in fibre surface morphology in more detail, FESEM images of both samples were taken under magnifications of ×5000, ×10000 and ×100000. As shown in Fig. 3, neat PA fibre has characteristic smooth surface. In situ polymerization of aniline on PA fibre resulted in the formation of PANI/TiO_2 nanocomposite which unevenly covered the surface of PA fibre (Fig. 3).

Apparently, some areas were fully covered with a quite thick layer of the nanocomposite, while some parts were left barely uncovered. In addition, the uniformity of functionalised material on the substrate considerably varies for different fibre materials. Such tendency was also noticed on different fibres functionalised in the same manner (Stojanović et al., 2020; Radoičić et al., 2015). It is worth mentioning that despite the less uniform coating, achieved results were remarkably better than those obtained on PET fibres, where in situ polymerization of aniline took place in the absence of TiO_2 NPs (Radoičić et al., 2015). In that case, only small patches of polymer were scattered over the fibre surface suggesting that TiO_2 NPs play a significant role in polymerization of aniline and strongly influence the fibre surface morphology. Namely, the problem of faster polymerization compared to monomer adsorption (Ferrero et al., 2006) can be successfully overcome by initial immersion of PA fabric into TiO_2 NPs colloidal solution as described in experimental part. Such order of actions along with the presence of TiO_2 NPs throughout the polymerization reaction leads to an increase of monomer adsorption on the fibres surface and eventually results in a more uniform coating of final nanocomposite (Radoičić et al., 2020). FESEM image taken under the largest magnification clearly revealed a network of ribbon-like nanostructures of PANI/TiO_2 on PA fibre surface. Noticed network is a characteristic feature of PANI and has been well documented in the literature (Radoičić et al., 2013). The average diameter of nanoribbons was approximately 70 nm.

The changes in the chemistry of PA fibre surface induced by functionalization with PANI/TiO_2 nanocomposite were further evaluated by FTIR spectroscopy. FTIR spectra of neat PA fabric and PA-PANI/TiO_2 fabric are shown in Fig. 4. The peaks at 1625 cm^{-1} and 1537 cm^{-1} detected in the spectrum of neat PA fabric were attributed to N—C═O and C—N—H vibrations in the amide group, respectively (Díaz-Alejo et al., 2013). They were commonly identified as amide I and amide II modes in secondary amides. In addition evident bands at ~1450 cm^{-1} could be assigned to the stretching, deformation and wagging vibrations of N—H bonds. The band corresponding to wagging vibrations of NH amide groups was observed at 1365 cm^{-1}. Two bands occurring at 1200 cm^{-1} and 1188 cm^{-1} originate from amide mode III. The bands located

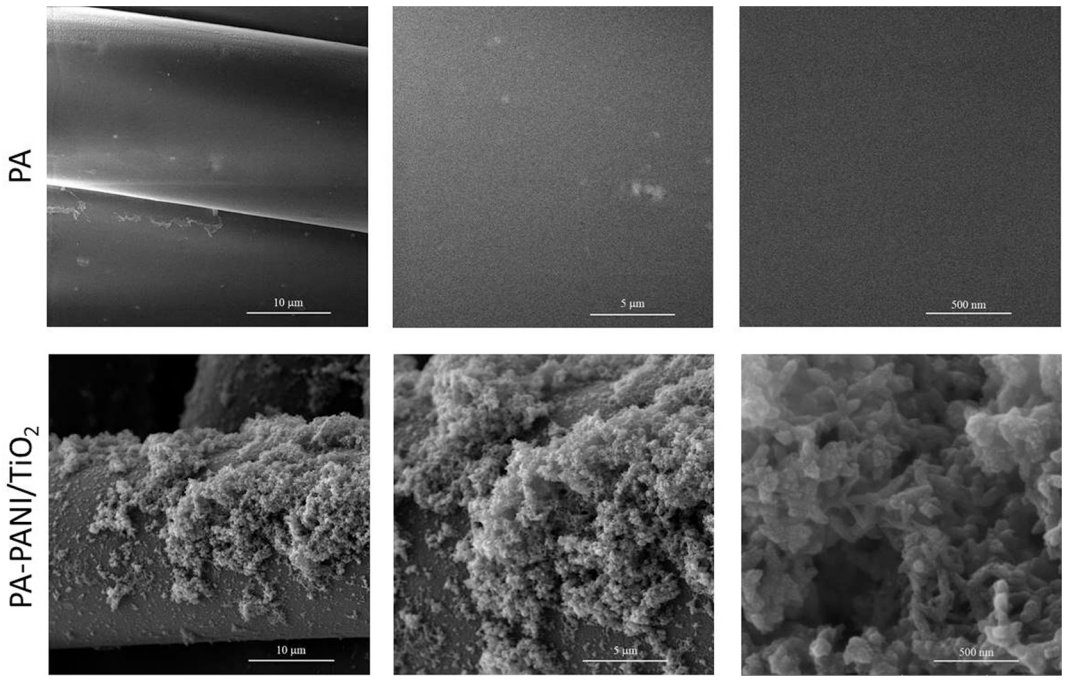

Fig. 3. FESEM images of neat PA and PA-PANI/TiO_2 fibres.

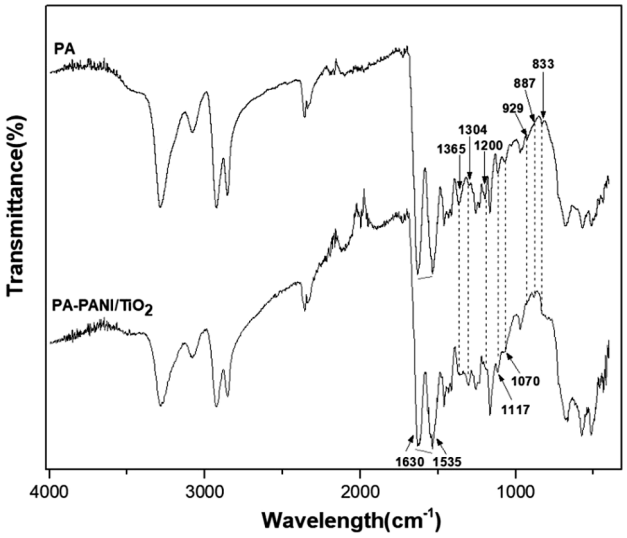

Fig. 4. FTIR spectra of neat PA and PA-PANI/TiO_2 fabrics.

at approximately 1170 cm^{-1} were due to CO—CH symmetric bending vibration combined with CH_2 twisting. The bands appearing at 1117 cm^{-1}, 1070 cm^{-1} and 830 cm^{-1} were related to primary (NH_2) and secondary (—NH—) amines (Díaz-Alejo et al., 2013).

It is well known that neat PANI emeraldine salt has intensive vibration bands at ~1570 cm^{-1} and ~1490 cm^{-1} due to quinonoid (Q) and benzenoid (B) ring stretching vibrations, respectively (Ćirić-Marjanović et al., 2008). Thus, slight changes in the intensity ratio of bands at 1325 cm^{-1} and 1537 cm^{-1} in PA-PANI/TiO_2 nanocomposite compared to the same bands in a neat PA sample were evident (marked red in Fig. 4). This change could indicate the presence of PANI emeraldine salt in synthesized nanocomposite formed because of the overlapping of bands of neat PANI and neat PA fabric. In addition, the presence of PANI in PA-PANI/TiO_2 sample was confirmed by a significant increase of band at 1304 cm^{-1} corresponding to C—N stretching vibration of a secondary aromatic amine of PANI (Trchová et al., 2006). Formation of a new band at 887 cm^{-1} in PA-PANI/TiO_2 sample was assigned to γ(C—H) vibration of 1,2,4-trisubstituted benzene ring which indicated the branching of PANI chains. Complete disappearance or significant intensity decrease of vibration bands at 1365 cm^{-1}, 1200 cm^{-1}, 1117 cm^{-1}, 1070 cm^{-1}, 929 cm^{-1} and 830 cm^{-1} corresponding to amide groups of PA (described above) also implied the presence of PANI/TiO_2 coating on PA surface which suppressed the vibrational response of neat PA fabric.

3.2. Electrochemical studies of milk samples using PA-PANI/TiO_2 textile-based chip

Basically, we fabricated a variable resistor in the form of described fabric. The resistance of this fabric was changed when different fluids, in this case milk in different forms, were dropped on the textile material, as a major part of the proposed microfluidic chip. Consequently, a variation of resistance as well as the phase angle was measured as a response of the proposed textile-based sensor.

3.2.1. EIS of pure and diluted cow milk

To investigate milk adulteration with water, impedance measurements were carried out with PA-PANI/TiO_2 fabric-based sensor. The addition of water into milk led to a decrease in the concentrations of mineral salts and thus, ions concentration, resulting in an increase of resistance. In order to prove this fact, the electrical resistance of pure and diluted cow milk was measured. To determine the resistance, firstly a definite volume of pure cow milk (5 μL) was dropped on the sensor into the fabric-based chip. The variation of the resistance was verified as the frequency was increased from 1 Hz to 100 kHz. Further, the samples diluted in volume ratios 90:10 and 80:20 (5 μL) were dropped on the sensor and change in resistance was observed over this frequency range. The change in resistive part of the impedance of raw and diluted cow milk as a function of frequency is presented in Fig. 5a. It can be seen that by raising the milk dilution ratios from 90:10 to 80:20, electrical resistance values increased from 19.5 kΩ to 31.5 kΩ, at a frequency of 10 kHz (Fig. 5b). The reason for such behaviour was expected from the fact that conductivity of milk was attributed mainly to the presence of Na^+-, K^+-, Ca^{2+}-, Mg^{2+}- and Cl^--ions. However, the addition of water decreased ions concentration in milk and thus changed its conductivity/resistivity.

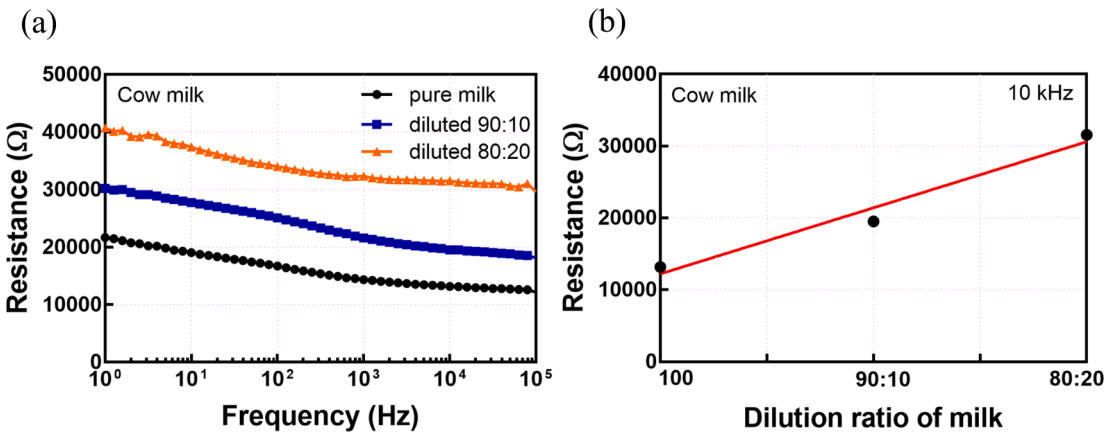

Fig. 5. (a) Change in resistance of pure cow milk and water diluted samples as a function of frequency, (b) Correlation between electrical resistance of milk and milk dilution.

Our findings are in line with previously reported papers, which demonstrated that an increase in the dilution of milk with water brings about considerable increase of the resistance values of milk over a wide frequency range (Banach et al., 2012; Henningsson et al., 2005; Mabrook and Petty, 2003; Sadat et al., 2006; Abdelkafi et al., 2015). The results confirmed that the conductivity of pure milk remained high compared to adulterated milk, which was also reported in the literature (Sadat et al., 2006). Based on the obtained resistance values, the correlation between the electrical resistance of raw milk and the volume ratio of milk dilution at a frequency of 10 kHz was investigated as shown in Fig. 5b. The trend demonstrated an increase in resistance of pure milk as dilution increases that can be ascribed to decrease in salt (ions) concentration in milk induced by adding of tap water. This correlation is represented by linear regression equation $y = 919 \cdot x + 1223$, with the regression coefficient (r^2) equal to 0.96.

3.2.2. *Phase angle studies of the sensor to detect milk adulteration*

In the next step of our research, the change in impedance phase angle of the prepared PA-PANI/TiO_2 fabric chip resistor was investigated with an aim to detect milk adulteration. The reason for this part of the study is threefold: (a) the instrument PalmSens4 originally measures modulus of impedance and phase angle, (b) detection of adulteration in milk by measuring phase angle shift which was well supported by the literature (Das et al., 2011; Salmaz et al., 2019) and (c) the measuring phase angle can provide the precise information on deviation from a perfect resistor, the phase angle of which is equal to 0°. In this part of the research, raw cow milk, raw goat milk and mixed sample (50:50 v/v%) were used as test samples. The variation in phase angle of the fabric-based sensor was analysed for all three samples over a frequency range from 1 Hz to 100 kHz (Fig. 6). In comparison to cow milk, the goat milk samples demonstrated lower values of phase angle which means that the proposed sensor is closer to the perfect resistor. This might be the consequence of higher content of non-protein nitrogenous compounds (cow – 2.63, goat – 2.90) and milk fat (cow – 3.67, goat – 3.8) in goat milk, indicating that it would be more difficult to deviate the goat samples from the perfect resistor. Actually, our sensor was modelled as a variable resistor measured between the two electrodes, but very small parasitic capacitor between those two electrodes also exists. The capacitor reflects the milk fat, protein and other non-conducting attributes of the milk and its adulterated versions. The value of capacitive reactance is expected to be negative and thus, it should appear as a negative phase angle, as can be seen in Fig. 6.

The correlation between the phase angle of the sensor and adulteration of milk with added tap water was also established. Determination of phase angle allows identification of the phase shift between the voltage and current through the component. In the first set of experiments, a variation in phase angle of the fabric-based component was evaluated as a function of cow milk dilution. It was observed that an increase in milk dilution ratio from 90:10 to 80:20 resulted in decrease in phase angle from −2.7° to −3.3° (Fig. 7). In this case, a decrease in phase angle implied the drop in conductivity of the sensor and an increase in resistance values. This is in good agreement with previous works (Phimphisan, 2015; Liu et al., 2015). Moreover, it was also reported that the larger the percentage of dilution, the smaller the phase angle of the sensor.

It is well known that the addition of water to milk causes an increase in its freezing point, which is normally compensated by adding small

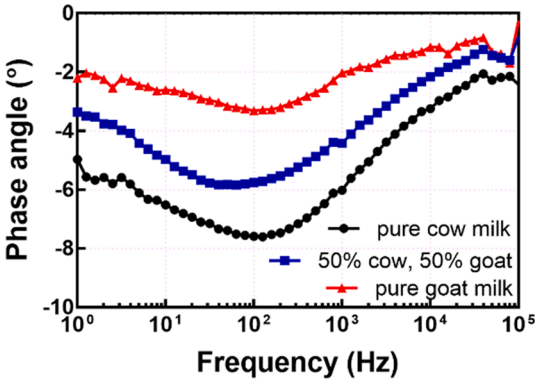

Fig. 6. Comparison of change in phase shift of raw cow, goat and mixed cow and goat milk as a function of frequency.

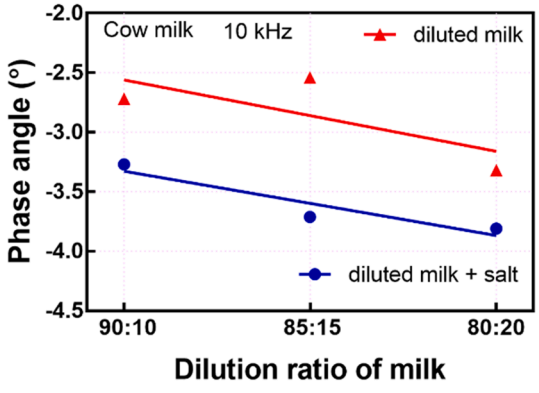

Fig. 7. Correlation plot showing variation in phase angle of the sensor as a function of increasing dilution of milk with water (the weight of added salt was constant).

amounts of salt. In order to detect this adulteration, diluted milk samples with added salt (10 mg) were prepared and a change in phase angle of the sensor for each sample was measured as a function of milk dilution with water at 10 kHz. The phase shift between milk samples with added salt and milk samples without salt was about 2°. Obviously, fabricated sensor was able to differentiate the pure milk from other adulterations. A linear decrease in phase angle of the samples with added salt from −3.2° to −3.8° was evident when dilution ratio increased from 90:10 to 80:20. This is based on the fact that the phase angle of the sensor changes with variation in the ion concentration in the milk as it is highly affected by adding tap water.

The correlation plot between the phase angle of the sensor for diluted cow milk with and without salt and milk dilution ratio is shown in Fig. 7. The correlation between milk dilution ratio and shifting of the phase angle for diluted cow milk with and without salt can be represented by regression equations $y = -0.06 \cdot x - 1.96$ and $y = -0.05 \cdot x - 2.78$, respectively. Moreover, the performances of the prepared PA-PANI/TiO_2 fabric-based sensor were quantitatively evaluated by calculating the sensitivity, limit of detection (LOD), and limit of quantification (LOQ) values for diluted cow milk with and without salt from the correlation plot. The regression statistics parameters of the proposed sensor are presented in Table 1. The LOD was calculated as $3.3 \cdot s/m$ and LOQ as $10 \cdot s/m$, where s represents the standard error from regression statistics and m is the slope coefficient of the correlation curve of the linear regression lines. The confidence interval of 95% was implemented.

3.2.3. Phase angle studies of the sensor to detect milk spoilage

The goal of this systematic study was to demonstrate that the proposed textile-based sensing platform can be used also for detection of milk spoilage. Therefore, the electrical properties of spoiled milk were investigated by measuring the changes in the phase angle of the prepared PA-PANI/TiO_2 sensor as a function of frequency and compared with results for fresh milk. A slight shift of the phase angle was verified for fresh milk and spoiled milk as the frequency was increased from 10^3 to 10^4 Hz. The focus was on this range of frequencies for two reasons: (a) the linear dependence as can be seen also in Fig. 6, and (b) this range would be of practical importance if the platform would be connected as an input component on microprocessors-based electronic system. Spoiled milk, which was stored at room temperature since the day it was received, showed higher variation in phase angle shift than fresh milk as shown in Fig. 8. This could be attributed to the change in the ion concentration and composition of the spoiled milk. Namely, an increase in the metabolic activities in the spoiled milk caused by microorganisms may result in the conversion of weakly bonded proteins or lipids into charged metabolic products e.g. amino acids, lactate, acetate (Liu et al., 2015).

3.2.4. Reproducibility studies of PA-PANI/TiO_2 fabric-based sensor

The impedance phase angle was measured in order to evaluate the robustness of the fabricated PA-PANI/TiO_2 sensor using same cow milk sample within different time intervals. The first measurement started 1 min after the drop-casting of pure cow milk (5 μL) onto the chip working surface, during which the injected milk was soaked by the PA-PANI/TiO_2 fabric. The subsequent measurements were conducted after 3, 5, 10 and 15 min of drop-casting the milk sample onto the working surface of the fabricated microfluidic chip. The phase angle shift of the sensor as a function of the time interval, at a frequency of 10 kHz, is presented in Fig. 9. The results indicated good robustness and thus reproducibility of the fabricated textile sensor with a steady and stable data after 5 min.

Table 1
Regression statistics of diluted cow milk with and without added salt at 10 kHz.

Diluted cow milk	Sensitivity (°/% dilatation)	LOD	LOQ
Without salt	0.06	21.55	65.31
With added salt	0.06	8.57	25.98

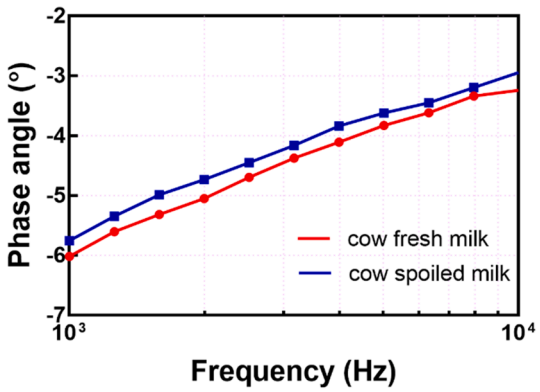

Fig. 8. Variation in phase angle of the sensor as a function of frequency for fresh cow milk and spoiled cow milk.

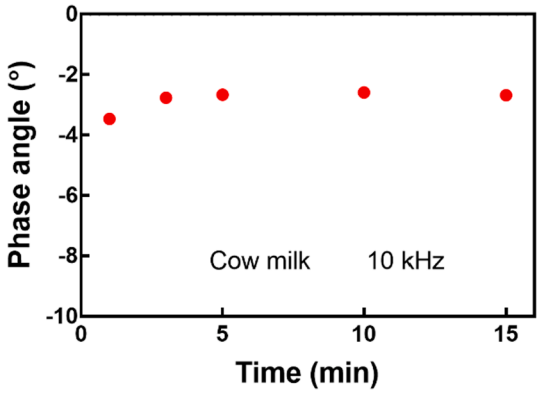

Fig. 9. Phase angle measurements of fabricated PA-PANI/TiO_2 textile sensor using pure cow milk at 10 kHz over different time intervals showing good robustness of the device.

The study justified the implication of prepared prototype for analytical sensing purposes enabling fast detection of milk adulteration by taking low volume of samples.

3.2.5. Repeatability measurements of PA-PANI/TiO_2 fabric-based sensor

To further justify the sensing ability and detection performance of the fabricated PA-PANI/TiO_2 textile sensor, the inter-day and intra-day repeatability studies were performed. The measurements were performed using the same 20% goat sample at different sensor prototypes at different times. For this purpose, 20% diluted goat milk samples were utilized to measure modulus impedance and impedance phase angle at 1 kHz over the span of 72 h using four independently prepared PA-PANI/TiO_2 sensors. The fabricated sensors showed high repeatability with acceptable relative standard deviation (% RSD) of 4.74% towards impedance measurements which is shown in Fig. 10a. Moreover, sensor exhibited a % RSD of about 0.46 % towards phase angle deviation as can be seen from Fig. 10b. Based on the above results, good accuracy and reliability of the developed methodology was justified with acceptable % RSD values for implementation of fabricated textile sensor towards detection of milk quality over a reasonable period of time. Already reported techniques for measuring of the admittance or conductivity of milk mostly required sensors which needed to be immersed in the milk (which demanded the washing of sensor in a diluted detergent). In contrast, our microfluidic fabric-based chip does not require contact with a high volume of taken milk sample. Therefore, the presented microfluidic platform represents a feasible and accurate rapid detection tool that can be applied to identify the freshness of raw milk.

The novelty of the presented sensor towards impedance analysis of milk adulteration is summarized in Table 2. The present paper utilizes a

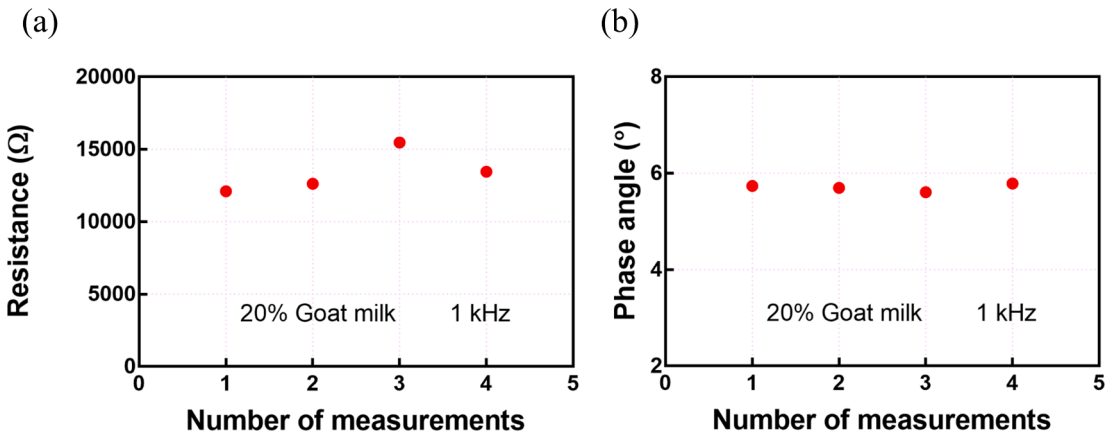

Fig. 10. Repeatability studies of PA-PANI/TiO_2 sensor towards (a) modulus impedance and (b) phase angle measurements using 20% diluted goat milk at 1 kHz.

Table 2
Different impedance sensors for milk quality detection.

Principle/Electrodes	Measuring parameter(s)	Detection Parameter	Reference
Integrated circuit (IC) AD5933	Resistance	Milk adulteration	(Durante et al., 2016)
Polymer coated Cu printed circuit board	Phase angle	Milk adulteration	(Das et al., 2011)
Al_2O_3 coated fluorine doped tin oxide	Phase angle	Milk adulteration	(Salmaz et al., 2019)
Interdigitated Au μelectrode on glass chip	Resistance, Phase angle	*E. coli* bacteria	(Liu et al., 2015)
Field-Programmable Gate Array circuit	Resistance	Milk fat	(Veiga and Filho, 2012)
Au electrode on glass substrate	Resistance	Milk adulteration	(Tripathy et al., 2017)
Textile-based microfluidic chip	Resistance, Phase angle	Milk adulteration	Present work

unique and innovative fabric-based microfluidic chip sensor for the detection of milk quality compared to other solid substrates reported in previous studies.

4. Conclusion

In this study, a microfluidic chip based on polyamide fabric impregnated with PANI/TiO_2 nanocomposite was presented, which was utilised as a variable resistor to detect milk adulterations. The change in the resistive part of the impedance of raw and diluted cow milk showed an increase in resistance with an increase in dilution. Milk dilution with tap water changed the salts concentration in the milk which had an impact on the electrical resistance of milk. The prepared textile-based microfluidic chip was further used to detect salt adulteration in diluted milk. It was measured on the basis of the observed shift in impedance phase angle as a function of dilution. A decrease in the phase angle was detected with an increase of added water into cow milk samples with and without subsequently added salt. Furthermore, by measuring the phase angle at various frequencies, the sensor was able to differentiate cow milk from goat milk or fresh milk from spoiled milk samples. This textile-based chip demonstrated a reliable and rapid sensing platform, with good reproducibility and low detection limit. With further modifications in the fabrication strategy of textile sensors, the present work extends its realm for exploitation of textile sensors in analytical sensing applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 854194. The authors would like to thank Milivoj Paroški for his assistance in testing the presented components.

References

Abdelkafi, A., Büschel, P., Fendri, A., Kanoun, O., 2015. Impedance investigation of milk dilution, Conference: AMA 2015, https://doi.org/10.5162/sensor2015/A7.2.
Agranovich, D., Renhart, I., Ishai, P.B., Katz, G., Bezman, D., Feldman, Y., 2016. A microwave sensor for the characterization of bovine milk. Food Control 63, 195–200.
Banach, J.K., Żywica, R., Szpendowski, J., Kiełczewska, K., 2012. Possibilities of using electrical parameters of milk for assessing its adulteration with water. Int. J. Food Properties 15, 274–280.
Bhadra, S., Thomson, D.J., Bridges, G.E., 2012. A wireless passive pH sensor for real-time in vivo milk quality monitoring. Sensors. https://doi.org/10.1109/ICSENS.2012.6411025.
Choudhary, S., Joshi, B., Pandey, G., Joshi, A., 2019. Application of single and dual fluorophore-based pH sensors for determination of milk quality and shelf life using a fibre optic spectrophotometer. Sens. Actuators, B 298, 126925.
Ćirić-Marjanović, G., Trchová, M., Stejskal, J., 2008. The chemical oxidative polymerization of aniline in water: Raman spectroscopy. J. Raman Spectrosc. 39, 1375–1387.
Das, S., Sivaramakrishna, M., Biswas, K., Goswami, B., 2011. Performance study of a constant phase angle based impedance sensor to detect milk adulteration. Sens. Actuators, A 167, 273–278.
Dias, L.A., Peres, A.M., Veloso, A.C.A., Reis, F.S., Vilas-Boas, M., Machado, A.A.S.C., 2009. An electronic tongue taste evaluation: Identification of goat milk adulteration with bovine milk. Sens. Actuators, B 136, 209–217.
Díaz-Alejo, L.A., Carmina Menchaca-Campos, E., Uruchurtu Chavarín, J., Sosa-Fonseca, R., García-Sánchez, M.A., 2013. Effects of the addition of ortho- and para-NH_2 substituted tetraphenylporphyrins on the structure of Nylon 66. Int. J. Polym. Sci. 2013, 323854.
Durante, G., Becari, W., Lima, Felipe, A.S., Peres, Henrique, E.M., 2016. Electrical impedance sensor for real-time detection of bovine milk adulteration. IEEE Sensors J. 16, 861–865.
Ferrero, F., Napoli, L., Tonin, C., Varesano, A., 2006. Pyrrole chemical polymerization on textiles: Kinetics and operating conditions. J. Appl. Polym. Sci. 102, 4121–4126.
Ghasemi-Varnamkhasti, M., Ghatreh-Samani, N., Naderi-Boldaji, M., Forina, M., Bonyadian, M., 2017. Development of two dielectric sensors coupled with computational techniques for detecting milk adulteration. Comput. Electron. Agric. 140, 266–278.
Govindarajalu, A.K., Ponnuchamy, M., Sivasamy, B., Venkatesh Prabhu, M., Kapoor, A., 2019. A cellulosic paper-based sensor for detection of starch contamination in milk. Bull. Mater. Sci. 42, 255.
Handford, C.E., Campbell, K., Elliot, C.T., 2016. Impacts of milk fraud on food safety and nutrition with special emphasis on developing countries. Compr. Rev. Food Sci. Food Saf. 15, 130–142.
Henningsson, M., Östergren, K., Dejmek, P., 2005. The electrical conductivity of milk-the effect of dilution and temperature. Int. J. Food Prop. 8, 15–22.
Ismar, E., Bahadir, S.K., Kalaoglu, F., Koncar, V., 2020. Futuristic clothes: electronic textiles and wearable technologies. Global Challenges. 4, 1900092.

Kaur, G., Adhikari, R., Cass, P., Bown, M., Gunatillake, P., 2015. Electrically conductive polymers and composites for biomedical applications. RSC Adv. 5, 37553–37567.
Lima, R.S., Danielski, G.C., Pires, A.C.S., 2018. Mastitis detection and prediction of milk composition using gas sensor and electrical conductivity. Food Bioprocess Technol. 11, 551–560.
Liu, J.-T., Settu, K., Tsai, J.-Z., Chen, C.-J., 2015. Impedance sensor for rapid enumeration of *E. coli* in milk samples. Electrochim. Acta 182, 89–95.
Mabrook, M.F., Darbyshire, A.M., Petty, M.C., 2006. Quality control of dairy products using single frequency admittance measurements. Meas. Sci. Technol. 17, 275–280.
Mabrook, M.F., Petty, M.C., 2003. A novel technique for the detection of added water to full fat milk using single frequency admittance measurements. Sens. Actuators, B 96 (1-2), 215–218.
Malon, R.S.P., Heng, L.Y., Córcoles, E.P., 2017. Recent developments in microflluidic paper cloth and thread-based electrochemical devices for analytical chemistry. Rev. Anal. Chem. 20160018.
Nascimento, W.W.G., de Oliveira, M.A.L., Furtado, M.A.M., de Carvahlo dos Anjos, V., Bell, M.J.V., 2013. Development and optimization of an alternative methodology for detection of milk adulteration by water. J. Food Eng. 3, 363–370.
Phimphisan, S., Sangiamvibool, W., 2015. Determination of water added in raw milk using interdigital capacitor sensor. PRZEGLĄD ELEKTROTECHNICZNY, ISSN 0033-2097, R. 91 NR 9/2015, 265-267, https://doi.org/10.15199/48.2015.09.65.
Poghossian, A., Geissler, H., Schoning, M.J., 2019. Rapid methods and sensors for milk quality monitoring and spoilage detection. Biosens. Bioelectron. 140, 111272.
Radoičić, M., 2013. Nanocomposites based on polyaniline and titan (IV) oxide: synthesis, characterization and application in photocatalysis. Ph.D. thesis. University of Belgrade, Beograd.
Radoičić, M., Ćirić-Marjanović, G., Šaponjić, Z., Mitrić, M., Konstantinović, Z., Stoiljković, M., Nedeljković, J., 2013. Structural and magnetic properties of nanocomposites based on nanostructured polyaniline and titania nanotubes. J. Mater. Sci. 48, 5776–5787.
Radoičić, M., Milošević, M., Miličević, D., Suljovrujić, E., Ćirić-Marjanović, G., Radetić, M., Šaponjić, Z., 2015. Influence of TiO_2 nanoparticles on formation mechanism of PANI/TiO_2 nanocomposite coating on PET fabric and its structural and electrical properties. Surf. Coat. Technol. 278, 38–47.
Radoičić, M., Ćirić-Marjanović, G., Miličević, D., Suljovrujić, E., Milošević, M., Kuljanin-Jakovljević, J., Šaponjić, Z., 2020. Fine-tuning of conductive and dielectric propertiesof polypyrrole/TiO2 nanocomposite-coatedpolyamide fabric. Compos. Interfaces. 27, 1–14.
Sadat, A., Mustajab, P., Khan, I.A., 2006. Determining the adulteration of natural milk with synthetic milk using ac conductance measurement. J. Food Eng. 77, 472–477.
Salmaz, U., Salamat, A., Islam, T., 2019. Fractional order sensor for measuring the quality of milk. Mater. Today:. Proc. 18, 1077–1085.
Stojanović, G.M., Radetić, M.M., Šaponjić, Z.V., Radoičić, M.B., Radovanović, M.R., Popović, Ž.V., Vukmirović, S.N., 2020. A textile-based microfluidic platform for the detection of cytostatic drug concentration in sweat samples. Appl. Sci. 10, 4392.
Tompson, R.C., 1984. Oxidation of peroxotitanium (IV) by chlorine and cerium (IV) in acidic perchlorate solution. Inorg. Chem. 23, 1794–1798.
Trchová, M., Konyushenko, E.N., Stejskal, J., Sedenková, I., Holler, P., Ćirić-Marjanović, G., 2006. Evolution of polyaniline nanotubes: the oxidation of aniline in water. J. Phys. Chem. B 110, 9461–9468.
Tripathy, S., Ghole, A.R., Deep, K., Vanjari, S.R.K., Singh, S.G., 2017. A comprehensive approach for milk adulteration detection using inherent bio-physical properties as 'Universal Markers': Towards a miniaturized adulteration detection platform. Food Chem. 217, 756–765.
Veiga, E.A., Filho, P.B., 2012. Bio-electrical impedance analysis of bovine milk fat. J. Phys. Conf. Ser. 407, 012009.