THE RELEVANCE OF A DIGITAL SYSTEM BASED ON NANOPARTICLES BIOSENSORS IN ASSESSING THE ANTIOXIDANT POTENTIAL OF SOME FOODS

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Abstract

Image analysis is a highly topical and perspective method in biomedical research and practice that underlies the development of portable tests for the autonomous detection of external energy sources outside the laboratory. The purpose of this paper is to implement a new method of detecting antioxidants in food, based on digital image acquisition and processing, using an experimental device with a paper-based biochemical sensor, marked with silver nanoparticles. The research consisted of evaluating the antioxidant potential of some teas (no = 5) and commercial red wines (no = 5), using the LabVIEW device and the LabVIEW graphical programming environment for data processing in linear 3D regression, referencing the RGB spatial vector. The results obtained in the testing of tea and red wine varieties had the following hierarchy regarding their antioxidant concentration (expressed in µg / mL gallic equivalents): Twinings traditional afternoon black tea (10.945); Lipton Green Tea (20.450); Lipton forest fruit tea (20.450); Fares forest fruit tea (29.956); Green Tea Earl Gray (34.708) or Recaş wine (15.698); Pinot Noir Folonari wine (15.698); Chateau Malpere wine (20.450); Cabernet Souvignon wine and Fetească Neagră wine (25.203). In conclusion, we appreciate the great outlook of this fast and cheap method, which can be accessed through software installed on your laptop or smartphone, allowing you to read the result with one click, including by non-experts.

Keywords: *antioxidants, paper biosensor, nanoparticles, teas, wines*

Introduction

The benefits of digital measurement systems are fully found in the field of biomedical research (Alam et al., 2013, Andrei et al., 2014). They are in the process of being enriched with new biochemical methods of digital measurement of the antioxidant potential of food products. In this respect, it is worth noting the recent advances of experimental analytical devices based on paper-biosensors, which are expedient and inexpensive enough in order to impose themselves as perspective tests (Yetisen et al., 2014).

A basic feature of digital systems is the storage capacity. Similar to animal memory and digital memory, there are three forms depending of its duration, including short-term storage for processing needs, online storage for relatively fast access to images, and archive storage, the access of which is still limited regarding the image (Magalhaes et al., 2008). As a rule, short-term storage is based on the use of computer memory and online storage on the use of different types of media (Munteanu and Todoran, 1997).

Problems in digital image processing communication can provide local communication between processing systems and long distance communication (Holonec et al., 2010). Although the hard drive and the software have great availability for local communications, and the computer network facilities are extremely generous, the image transmission rate is not yet satisfied (Munteanu and Todoran, 1997). It is estimated that it far exceeds other types of data transmissions. Solving long distance communication requires therefore sustained efforts from research and practice (Holonec, 2003).

Antioxidant investigations have intensified over the last 20 years, mainly due to the discovery of beneficial effects on human health. In the body, the excess of reactive oxygen species, generated by pollution, unhealthy food or smoking, can cause cardiovascular diseases, cancer, diabetes, inflammatory syndromes and other conditions (Pisoschi and Negulescu, 2011; Seifried, 2007). Several studies in this field show that the incidence of these diseases decreases with increased consumption of antioxidant-rich foods (Seifried, 2007). Regarding the use of antioxidant dietary supplements, there are different opinions, including that some excessive antioxidant consumption may cause various disorders, mainly disorders of the immune system (Karadag et al., 2009, Seifried, 2007).

Foods of vegetable origin, mainly fruits, have a rich content of antioxidant principles, their concentration being correlated with the color of the mature fruit. Most of the vitamins and nutrients in the plants also exert an important antioxidant role, vitamin A, beta-carotene and other carotenoids, flavonoids, vitamin C, vitamin E, zinc and selenium having a major impact. Among the hundreds of currently known antioxidants, the following five are more common and can be used at the level of public consumption: vitamins C and E, lipoic acid, glutathione and coenzyme Q10 (Andrei et al., 2014). At the same time, vitamins C and E are not produced in the body and must be fed through food, and lipoic acid, glutathione and coenzyme Q10 are synthesized in the body, but their level decreases with age, requiring supplementation (Andrei et al., 2004; Karadag et al., 2009).

Based on these considerations, in our study we chose to test the antioxidant activity of commercially available teas and bottled red wines using a colorimetric biosensor marked with Ag nanoparticles on paper, integrated into an experimental device. It is envisaged that this stand-alone system, based on acquisition and digital image processing, will allow the determination of the concentrations of certain substances of major interest in the food sector.

Materials and methods

The experimental device consisted of an original color-processing system, taken from a paper-based biosensor, marked with silver nanoparticles to reduce the antioxidant compounds in aqueous samples (Figure 1). The principle of this biosensor is based on the ability of antioxidants to reduce Ag ions present in AgNP species, emerging chromatic transitions with intensity dependent on the structure and concentration of antioxidants.

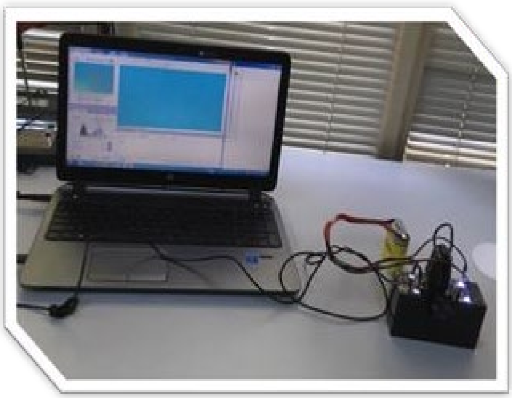


Figure 1. Overview of the experimental device

Digital image processing has sequentially traced the following hard and soft processing steps: image acquisition, with imaging and digitization sensor of the output signal; image processing and segmentation; recognition and interpretation of data. The hardware components were the sample, the lighting system, a webcam (Logitech model B910) and a personal computer. The antioxidant capacity of the analyte is detected based on the nanoparticle content of the paper support and the reduction of the Ag to Ag (0) ions, which gives a yellow color to the support, forming a "digital print" (Figure 2). The color intensity is dependent on the sample reduction power, being proportional to the antioxidant concentration. Silver ions have proven to be relevant colorimetric probes because they produce significant staining.

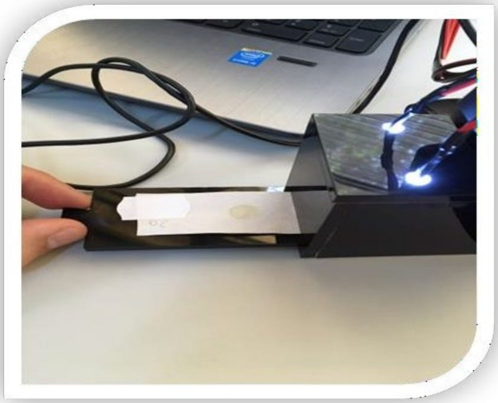


Figure 2. Sliding the loaded stand into the center unit of the device

The processing of the acquired images was performed using the LabVIEW graphics programming environment. In designing the software we used a 3D linear regression program, which was the basis for obtaining a spatial RGB vector as a reference. The operation of this program consists in placing a 3D spatial point corresponding to the lowest Euclidean distance, in relation to the vector values, which corresponds to the equivalent value of gallic acid (µg) (Figure 3).

Sample preparation consisted of preparing the impregnated biosensor on a paper support with gallic acid standard solutions marked with silver nanoparticles, with antioxidants contained in the test product reducing Ag + 1 to Ag0 (metallic).

Antioxidant extraction was done with 5 mL of methanol solution in water (20-80 g) starting from 0.2 g of tea, ultrasonically dried on the ultrasound bath followed by the filtration of the obtained extract by a 0.45 µm filter**.**

The deposition of the spots on the filter paper consisted in dipping it into the working solution of silver nanoparticles and drying it for an hour. Next, we added 10 µL of the standard solutions (gallic acid) or the solutions to be analyzed and leave to stand for 20 minutes. The Whatman filter paper of 3 mm and VWR 600 was used as comparision.

The calibration was based on the analysis of the correlations between the color intensity and the antioxidant concentration of the tested samples, in the determination of the calibration curve on gallic acid (3OH, 1.0). Thus, the gallic acid equivalent expression of the level of hydroxyl groups in the molecule, as well as its antioxidant resistance, was achieved.

The tested products included several teas (no = 5) and red wines (no = 5), frequently marketed, which we grouped into two samples. In the first sample, we introduced the following teas (prepared as infusions, according to the attached instructions): 1 - Twinings traditional afternoon black tea; 2 - Earl Gray Green Tea; 3 - Fares Berries Tea; 4 - Lipton Berries Tea; 5 - Lipton Green Tea. The second sample was composed of the following bottled wines: 1 - Recaş red wine; 2 - Chateau de la Soujeole de Malpere red wine; 3 - Cabernet Souvignon red wine; 4 - Fetească Neagră red wine; 5 - Pinot Noir Folonari red wine.

Results and discussions

The quality of the paper used significantly influenced the signal strength and implicitly the relevance of the biosensors. In this context, it should be noted that the biosensor paper support must ensure a clear colorimetric signal transduction, a good compatibility with the reagents used, a minimization of nonspecific absorption of the reagents and analytes, and a good degree of homogeneity in order to avoid dispersion of the fluid. Based on these considerations, researchers in the field recommend the use of chromatography paper that meets these requirements, ensures good accessibility of antioxidants to its surface and it is available at a low cost on the market (Varvari et al., 2008; Zhao et al., 2008).

The results obtained in determining the calibration curve at 6 concentrations of gallic acid (5, 10, 20, 30, 40 and 50 µg / mL) revealed positive correlations between color intensity and 5 concentrations. We also noted that after the 50 µg / mL threshold has been reached, the color intensity was maintained on plateau.

By looking at the increase in antioxidant concentration, expressed in µg / mL gallic acid equivalent, in the test tea sample we found the following hierarchy (Table 1): Twinings traditional afternoon black tea (10.945), Lipton green tea (20.450), Lipton berries tea (20.450), Fares berries tea (29.956) and Earl Gray Green Tea (34.708) (Figure 3).

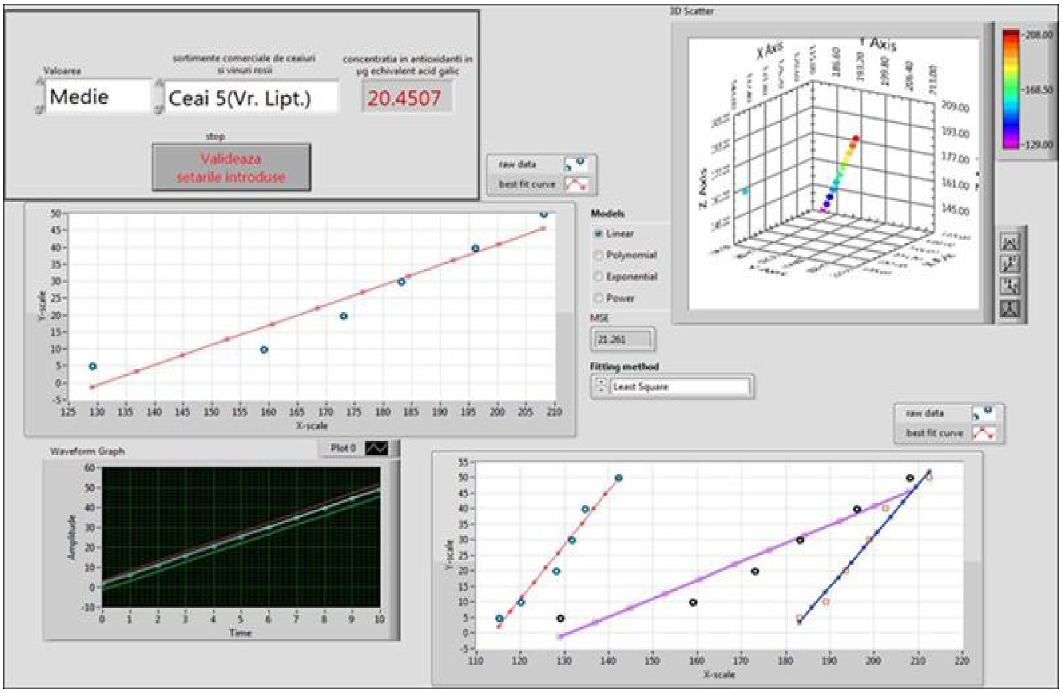


Figure 3. Viewing the content of antioxidants in tea 5

In the case of the sample composed of the 5 red wine varieties, the results obtained indicated the following ranking according to their concentration in antioxidants: Recaş red wine

(15.698), Pinot Noir Folonari red wine (15.698), Chateau Malpere red wine (20.450), Cabernet Souvignon and Fetească Neagră red wine (25.203) (Table 1).

Table 1.

Concentrations in antioxidants (µg gallic equivalents) of some teas and red wines, determined with biosensors and digital image processing

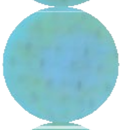
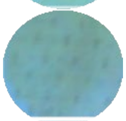
Products (n=10)	**Conc. (echiv.AG-µg)**	**Acquired images**	**Red (level)**			**Green (level)**			**Blue (level)**		
		Paper spot	*Min.*	*Max.*	*Media*	*Min.*	*Max.*	*Media*	*Min.*	*Max.*	*Media*
Tea 1 (Black)	10.945		124	144	130	172	188	180	128	162	145
Tea 2 (Earl Grey)	34.708		107	129	118	174	194	184	168	220	194
Tea 3 (F.p. Fares)	29.956		144	132	138	182	198	190	168	194	181
Tea 4 (Lipt. berry)	20.450		106	131	118.5	178	195	186.5	151	190	170.5
Tea 5 (Lipt. Green)	20.450		126	152	139	174	186	180	143	175	159
Wine 1 (Recaş)	15.698		119	145	132	165	182	173.5	134	181	157.5
Wine 2 (Chateau Malpere)	20.450		121	139	130	186	206	196	147	180	163.5
Wine 3 (Cabernet Souvignon)	25.203		112	139	125.5	173	197	185	161	191	176
Wine 4 (Fetească Neagră)	25.203		96	127	111.5	142	177	160	156	212	184
Wine 5 (Pinot Noir Folonari)	15.698		133	153	143	171	186	178.5	140	168	154

Image analysis is a highly current and prospective method that underlies the development of portable external energy source detection platforms with autonomous operation outside of laboratory conditions (Jayaprakasha et al., 2002). Such independent platforms are an alternative to laboratory analyzes and have the advantage of providing qualitative and quantitative information, even to non-expert users. Digital image processing goes through a series of hard and soft processing steps that end with the development of image processing solutions. This category also includes analytical devices with paper-based biosensors, which are becoming more and more attractive.

In biomedical testing, it is now preferred to use complex digital devices to allow more complex interpretations based on a single determined parameter (Dechakhamphu et al., 2014). With the increase of the health benefits from the consumption of dietary products rich in antioxidants, the need for the implementation of easily usable and effective tests to identify the antioxidant action of food products has been expanded (Ozyurek et al., 2012). In this context, there is also a growing interest in the use of nanoparticles sensors in the detection of antioxidants in food. Moreover, these tests can explore the ability of antioxidants to reduce noble metals, with the formation of nanoparticles or the use of antioxidants as reducing agents.

Conclusions

The research in this paper was completed with the implementation of an original digital color acquisition and processing device using a nanoparticle biosensor impressed by the antioxidants contained in the food. The relevance of digital image analysis was ensured by the processing of data in the LabVIEW graphics programming environment, through 3D linear regression, as reference to the RGB spatial vector. Our results on the evaluation of the antioxidant activity of some teas and red wines with this device provided a particular insight into such tests, accessible through software installed on a laptop or smartphone, with one-click reading.

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