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A Simple One-Pot Determination of both Total Phenolic Content and Antioxidant Activity of Honey by Polymer Chemosensors †

Lara González-Ceballos [1], Maria del Mar Cavia [2], Miguel A. Fernández-Muiño [2], Sandra M. Osés [2], M. Teresa Sancho [2,*], Saturnino Ibeas [1], Félix C. García [1], José M. García [1] and Saúl Vallejos [1,*]

[1] Departamento de Química, Facultad de Ciencias, Universidad de Burgos, Plaza de Misael Bañuelos s/n, 09001 Burgos, Spain; lgceballos@ubu.es (L.G.-C.); sibeas@ubu.es (S.I.); fegarcia@ubu.es (F.C.G.); jmiguel@ubu.es (J.M.G.)

[2] Department of Biotechnology and Food Science, Universidad de Burgos, Plaza de Misael Bañuelos s/n, 09001 Burgos, Spain; mmcavia@ubu.es (M.d.M.C.); mafernan@ubu.es (M.A.F.M.); smoses@ubu.es (S.M.O.)

* Correspondence: mtsancho@ubu.es (M.T.S.); svallejos@ubu.es (S.V.)

† Presented at the title, place, and date.

Abstract: We have developed a new method for the rapid (2 h) and inexpensive (< 0.02 €/sample) "2 in 1" determination of the total phenolic content and the antioxidant activity in honey samples. The method is based on sensory colorimetric films with pedant diazonium groups, which react with phenolic compounds rendering highly colored azo groups. The total phenolic concentration of the sample is closely correlated to its trolox equivalent antioxidant capacity (TEAC). Therefore, this sensor can be used to determine the antioxidant capacity of honey samples as well. Based on this, the intensity of the color allows us to determine both the content of phenolic compounds and antioxidant capacity of the sample by the analysis of a picture taken with a smartphone that is analyzed by the use of the color definition parameters (RGB). Thus, it is a simple method carried out by non-specialized personnel and it involves much lower money and time investment compared to traditional measurement methods.

Keywords: Honey; sensor; total phenolic content; TPC; ABTS; AOX; TEAC.

1. Introduction

Honey is a widely consumed product globally, so it is interesting to develop rapid and inexpensive methods for its authentication and quality control. On this line, two of the most studied parameters are total phenolic content (TPC) and antioxidant activity (AOX) [1].

The most common methods to analyze both parameters are spectroscopic assays using the Folin-Ciocalteu reagent (TPC) and 2,2′-azino-bis(3-ethylbenzothiazoline-6 sulfonic acid) diammonium salt (ABTS) as a radical source (TEAC) [2]. These methods require a large expenditure of money and time and specialized personnel.

This study's main objective is to develop a suitable method that allows us to quantify the total phenolic content and determine the antioxidant activity in a faster and cheaper way than the conventional methods. To achieve this objective, a chromogenic sensor has been developed for the rapid and low-cost determination of the both parameters mentioned above in a single measurement. In addition to being a faster and cheaper method, as it is a polymeric sensor, it has advantages of lack of migration of the sensor subunits, manageability, and possibility of working in solid-state.

2. Methods

2.1. Preparation of the Sensory Polymeric Films

The starting film was prepared by bulk radical polymerization of three commercial monomers: VP, MMA, and SNH_2 in a molar feed ratio of 49.5/49.5/1 (VP/MMA/SNH_2) (Figure 1) using 1% mol of AIBN as radical thermal initiator. The polymerization was carried out at 60 ºC, overnight, in a mold comprised between two silanized glasses (100 µm thick), in an oxygen-free atmosphere. The film was removed from the mold and 8 mm diameter discs were cut with a punch and dipped into an acid solution of $NaNO_2$ (10 mL of water, 1 mL of HCl 37 %, and 40 mg of $NaNO_2$) at RT for 90 min. In this way, sensory films with pendant benzenediazonium salt motifs were easily prepared [3].

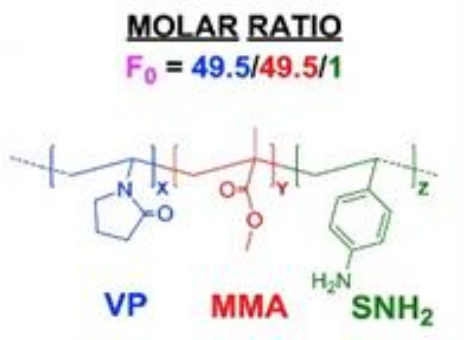


Figure 1. Chemical structure and molar ratio of the starting material.

2.2. Polymeric Film Method

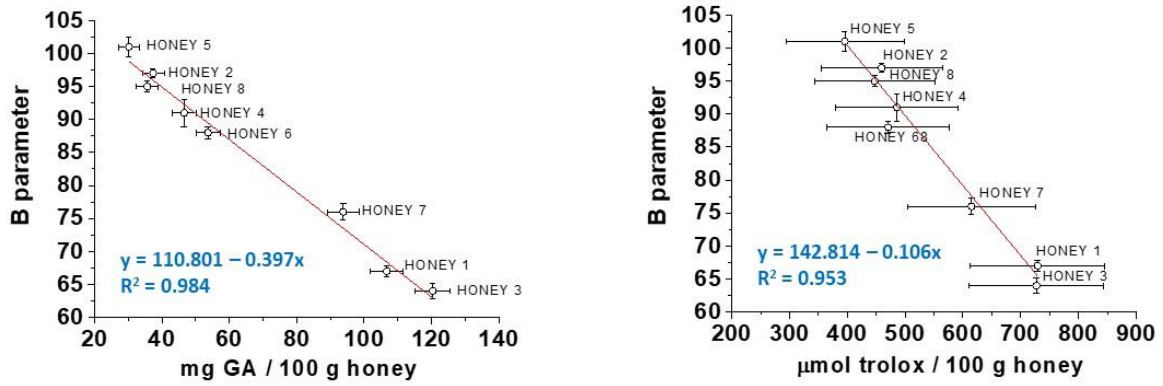
For the analysis with the sensory colorimetric films, 8 mm diameter discs were directly dipped for 2 hours in 10 ml of honey solution at room temperature, without a further experimental procedure. The discs were removed from the solution and washed 3 times with NaOH 0.1M for 15 minutes for finally taking the photographs in triplicate using a smartphone and a lightbox, essential to always reproduce the same light conditions. The digital color parameters (RGB) of the pictures were analyzed using generic image software, and it was found that the B (blue) parameter is the only significant variable, the only one that brings relevant information.

3. Results and Discussion

3.1. Correlation Study between Folin-Ciocalteu and ABTS Methods with the Sensory Colorimetric Films.

Fistly, the total phenolic content and the antioxidant activity were measured by Folin-Ciocalteu method [4] and TEAC assay respectively [5]. The method of the sensory colorimetric films is based on the color change produced by the formed highly colored azo groups between a sample´s phenols and the diazonium salt motifs of the discs. The measured experimental variable is the blue parameter of the RGB digital color parameters and is represented *vs* the obtained data from *Folin-Ciocalteu* and *TEAC* methods. The correlation between methods is observed in Figure 2 and Figure 3, and the initial proposal to determine both the total phenolic content and the antioxidant activity with a single analysis is confirmed, just by dipping the sensory colorimetric films for 2 hours into a honey solutions solutions at room temperature.

Figure 2. Sensory colorimetric films after dipping in 10 ml of honey solutions for 2 hours at room temperature.



(**a**) (**b**)

Figure 3. (a) Correlation between total phenolic content obtained by ***Folin-Ciocalteu*** method and B parameter of the sensory colorimetric films (B parameter); (**b)** Correlation between the antioxidant activity obtained by ***ABTS*** assay and B parameter of the sensory colorimetric films.

3.2. Proof of Concept. Determination of TPC and AOX with Sensory Colorimetric Films.

Once demonstrated the correlation between the reference methods and the proposed method, we made a proof of concept in wich we able to calculate the TPC and the AOX of all honeys only by substituting B parameter in the fitted equations showed in Figure 3. Table 1 shows the obtained results of TPC and AOX, both by reference methods and the proposed one.

Table 1. Total phenolic content and antioxidant activity of honeys measured both the reference methods (Folin-Ciocalteu and ABTS) and the proposed one (sensory colorimetric films).

	Total phenolic content (mg GA / 100 g honey)		**Antioxidant activity (µmol trolox / 100 g honey)**	
	Folin-Ciocalteu method	**Sensory colorimetric films**	**ABTS assay**	**Sensory colorimetric films**
Honey 1	106.71	114.78	729.33	722.96
Honey 2	37.44	33.11	459.53	428.56
Honey 3	120.90	122.95	727.39	752.40
Honey 4	46.85	49.44	485.84	487.44
Honey 5	30.16	22.22	395.83	389.30
Honey 6	53.91	57.61	470.66	516.88
Honey 7	93.68	90.28	615.07	634.64
Honey 8	35.72	38.55	447.48	448.18

4. Conclusions

We have developed a new method based on chemical sensors, or chemosensors to quantify the total polyphenol content and determine the antioxidant activity with a single analysis in all honey samples studied. This method is based on a sensor with diazonium moieties pendant to the main acrylic chains that can be used as a colorimetric chemosensor for the quantification of total phenolic content and the determination of the antioxidant activity on honey samples. The color of the sensors changes according to the samples' polyphenols concentration This method reduces the time and the cost of the analysis and does not require trained personnel, so it has great potential in the quality control of honey samples. By this way, Chemical sensors, or chemosensors, have great potential in the field of *in-situ*, fast, and low-cost analysis. Among chemical sensors, polymeric sensors have advantages of lack of migration of the sensor subunits, manageability, and possibility of working in solid-state.

Conflicts of interest: The authors declare that they have no conflict of interest.

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