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Optical Measurement System in food quality. Use of FoodLab method for analysis of extra virgin olive oil and olive fruit extracts

Ieri Francesca[1], Pinelli Patrizia[1], Pucci Simone[2], Curradi Giampiero[2], D'Andrea Mariano[3], Romani Annalisa[1]

[1]*Department of Pharmaceutical Science, University of Florence, Via Ugo Schiff 6, 50019 Florence, Italy.* annalisa.romani@unifi.it
[2]*CDR S.r.l., via degli Artigiani 6,50055, Ginestra F.na, Florence, Italy*
[3]*Olivicoltori Toscani associati s.c.a.p.a., via Empolese 20A, Cerbaia V.P. 50018 Scandicci (FI)*

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Abstract- In food quality optical measurement methods are time- and solvent-consuming and require specialized personnel and expensive instruments. CDR has created an innovative system (the FoodLab method) which permits determination of the total polyphenol value of an oil in a few minutes, using a micro quantity of sample. This innovative system was employed to evaluate polyphenols in extra virgin olive oil obtained from different geographical areas and olive cultivars and having a wide range of these compounds and in olive extracts, for a rapid and simple assessment of fruit ripening degree.

Introduction

The antioxidant power of minor polar compounds (MPCs), in part polyphenols, is important for extra virgin olive oil (EVOO) stability since there is a correlation between total polyphenol values and oxidation resistance time. Polyphenols have an important role in protecting not only oil quality but also cells of human body because they fight against the negative effects of free radicals. Since sensory properties are largely affected by MPCs, their quantitative and qualitative composition according to agronomic and technological conditions have been widely investigated [1,2]. The literature reports modifications of the phenolic concentrations in EVOO due to many agronomic parameters such as cultivar [3,4] and fruit ripening. Among technological variables, the MPC content seems particularly affected by malaxation and centrifugation [5,6,7,8,9]. A polyphenol test could permit verification of the quality of EVOO but also intervention in the extraction process, modifying the parameters that weigh negatively on the final content of these substances. Only a few laboratories determine olive oil polyphenol concentrations by HPLC analysis, while most evaluate the total polyphenol content by a spectrophotometric method, calibrating polyphenol content as gallic acid (Folin Ciocalteau method).
The present work aims to validate a method to determine the total polyphenol content of EVOO obtained from different geographical areas and olive cultivars and having a wide range of these compounds; a second important aim is to optimize the method for polyphenol determination in fruits using an innovative optical system (FoodLab method) which permits determination of the total polyphenol values in a few minutes, using a micro quantity of virgin oil and olive fruit extracts.

Material and Method

FoodLab method: the polyphenols, when in contact with a coloured complex in an alcohol solution, become oxidized and decolorate the violet colour, measured at 505 nm. The decoloration is directly proportional to the concentration of polyphenols in the sample. The concentration of total polyphenols in EVOO is expressed as mg Kg^{-1} of gallic acid. A quantity of 10 μL of olive oil is analyzed without manipulation. A quantity of 5 gr of olives is first crushed in a press (Ø 48 mm, volume 67 mL) and then extracted with 20 mL of ethanol. It is necessary, with the help of a spatula, to collect all the

material in a test tube and then centrifuge it (4000 revolutions/minute), in order to separate the extraction solvent from the residue.

Chromatographic analysis: Extra virgin olive oils and olive extracts are analysed by HPLC using a HP-1100 liquid chromatograph equipped with a DAD detector and a HP 1100 MSD API-electrospray (Agilent Technologies, Palo Alto, USA) according to Romani et al [10].

Folin-Ciocalteau method: The total phenolic content is determined according to Singleton et al. [11] and slightly modified on the basis of the analytical procedures used by *Camera di Commercio* Laboratories of Florence and other UNI EN 45000 certified laboratories. The concentration of total polyphenols in oil is expressed as mg Kg^{-1} of gallic acid, using calibration curves from 1 to 15 μg mL^{-1} (R^2 0.9985).

Result and Discussion

The FoodLab method is based on a redox reaction in which total polyphenols react with a chromogen modifying its colour intensity. The optical density of the colour is measured at 505 nm and it is proportional to the concentration of total polyphenols in oil, expressed as mg Kg^{-1} of gallic acid.
With this method it is possible to evaluate the antioxidant power of the polyphenols because it reproduces, in the test tube, the actions that take place, spontaneously in oil and in the human body, between polyphenols and free radicals.

In addition, the FoodLab method simplifies and speeds up the classical procedure (spectrophotometric method of Folin Ciocalteau) and allows direct use of olive oil without any manipulation. As reported in Figure 1, the correlation between the Folin Ciocalteau test and FoodLab is significant and very good (R^2=0.89).

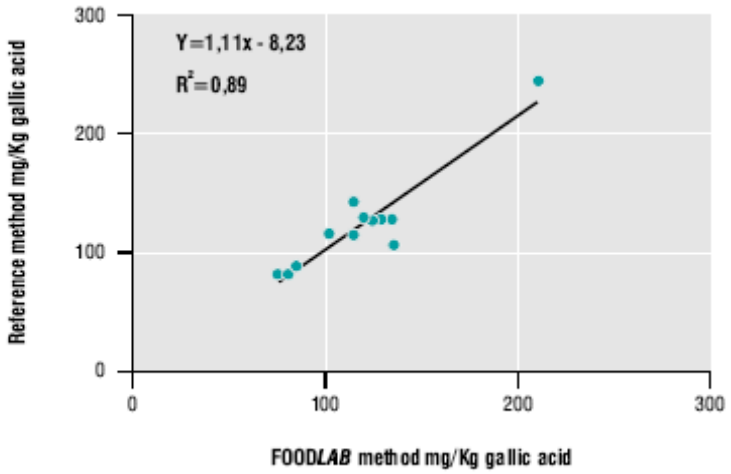

Figure1: Comparative tests between the Folin Ciocalteau reference method and the FOOD*LAB* method.

The first results, obtained for 100 commercial extra virgin olive oil samples, determined three different classes of olive oils with low (50-100 mg Kg^{-1}), medium (100-150 mg Kg^{-1}) and high polyphenol content (>150 mg Kg^{-1}), referring to extra virgin olive oil from the province of Florence. In this first stage, virgin olive oils with higher polyphenol contents (350-600 mg Kg^{-1}, data obtained both from Folin Ciocalteau method and/or as a sum of single compounds evaluated by HPLC/DAD) were incorrectly measured because were all calculated as 150-200 mg Kg^{-1} with the Foodlab method
The analysis of a greater number of extra virgin olive oils coming from different Italian regions, i.e. Liguria, Puglia and Tuscany, made it possible to increase the linearity range of the method, to assure a

correct evaluation of the polyphenol content of oils with high qualitative characteristics and antioxidant properties.
The reaction is linear up to 900 mg Kg^{-1} of gallic acid, and the sensitivity is 10 mg Kg^{-1} of gallic acid (see Table 1).

Linearity	**Accuracy**	**Repeatability**	**Correlation coefficient**	**Sensitivity**	**Total testing time**	**Test/hour**	**Unit of measurement**
900 mg Kg^{-1}	+/- 5%	CV<3%	R = 0.89	10 mg Kg^{-1}	5 min	70	mg Kg^{-1} gallic acid

Table 1: characteristics and parameters of the FoodLab apparatus.

The second aim of this work is the optimization of the method for polyphenol determination in olive fruits, to asses the antioxidant content in the olives and to correlate these values with fruit ripening. The polyphenol concentration in olives can be used to monitor the maturation process, and it plays an important role in indicating the correct harvest time.
During the optimization of the method some modifications, with respect to virgin olive oil quantifications, were necessary: in particular, the reagent concentration and absorption wavelength (630 nm) in order to better evaluate the total polyphenol content in a different medium (alcoholic solution). For this study olives with polyphenol concentration in the range 3-9 mg g^{-1} of fresh fruits were considered. The data were obtained by HPLC/DAD analysis, then compared with data from the spectrophotometric method and, finally, with data using the FoodLab method (data not shown).

	Abs 630 nm	mg g^{-1} gallic acid (FC method)	mg g^{-1} total polyphenols (HPLC method)
Sample 1	0.101	3.61	0.58
Sample 2	0.401	6.35	3.31
Sample 3	0.593	10.20	8.25
Sample 4	0.745	12.36	10.69

Table 2: The olive samples represent a different ripening degree of the olive fruit. Data are the mean of three determinations, CV<3%.

Conclusion

In food quality, these optical measurement methods are time- and solvent-consuming and require specialized personnel and expensive instruments. CDR has created an innovative system (the FoodLab method) which permits determination of the total polyphenol value of an oil in a few minutes, using a micro quantity of sample. The sample is used as it is, without any treatment for extra virgin olive oils, and in addition sample manipulation is very rapid for olive extracts. The system is composed of a portable analyzer and some specific reagents, it employs pre-filled cuvettes (single use) and does not need specialized personnel. The reagents are made exclusively of easily disposable substances with low toxicity that permit execution of the test in numerous conditions. This innovative system can be employed to evaluate polyphenols in olive extracts, for a rapid and simple assessment of fruit ripening degree, and it can be a useful tool for optimizing the transformation processes in the oil-mill.

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