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Quick spectrophotometric methods for antioxidant capacity and phenolic compounds determination: Different physico-chemical treatments of *Chemlali* olive paste

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In this research, different olive paste treatments, including physical treatments such as ultrasounds or microwaves as well as chemical treatments with addition of sodium citrate, were proposed. Therefore, the main objective of this work was to compare different treatments and optimize various classical and original spectrophotometric methods in order to evaluate the most appropriate and reproducible one for the determination of the phenolic content as well as the antioxidant capacity of olive oil. In addition, the combination of microwaves and sodium citrate tested at two concentrations (150 and 300 g/L) followed by a malaxation step for 20 min yielded good results in terms of polyphenols content compared with the conventional treatment. However, the shortest time of sonication treatment (10 min) led to the highest phenolic content determined by both Folin and gold nanoparticles methods (7.5 and 14.7 mg of gallic acid equivalent/kg of oil, respectively). This study provides assistance to the Virgin Olive Oil (VOO) sector by optimizing extraction procedures and introducing these different techniques with affecting the oil extraction yield.

Keywords: Olive oil, spectrophotometric methods, tyrosol, antioxidant capacity, phenolic compound.

Introduction

Olive oil is the only juice produced directly from a fruit; it retains many of its substances, antioxidants, vitamins and aromas that endow it a real nutritional value. The antioxidant content of olive oil varies according to the variety[1], the climate, the type of harvest, the degree of olives maturity[2], and production techniques[3]. Among the antioxidants, the phenolic compounds are present the most important groups of plant metabolite[4]. It is important to add value to olive oil production by combining olive oil organoleptic properties and richness in antioxidant compounds, mainly in phenolic compounds associated to health properties[5,6].

In recent years, the scientific community and the olive-growing sector have become increasingly unified by the main objective of improving the production and the quality of Virgin Olive Oil (VOO). Indeed, various technological innovations in the mechanical extraction process have been studied to improve the work efficiency and increase the health and sensory properties of VOO[7,8]. The proposed olive paste treatments based on the use of a heat exchanger (microwaves by electromagnetic waves) or the use of other techniques related to cavitation (as ultrasonic waves by mechanical waves) generally lead to a significant increase in phenols for all the treated pastes, by finally getting better quality products, bioactive and beneficial to health. The traditional techniques for extracting from olive oil often have constraints such as low yields, time-consuming extraction times and the use of large amounts of solvents. In recent years, numerous al-

ternative techniques to overcome these problems have been developed. Among them, there are emerging microwaves[9,10], supercritical fluids[11], ultrasound[10,12,13] or the addition of a co-adjuvant[14,15,16]. Several studies have shown that these physicochemical techniques can reduce the addition of water during the mixing process and subsequently avoid industrial discharges that are not degradable (waste water) into the environment.

The first objective will therefore be carried out with the aim of identifying the innovative extraction techniques used to achieve enrichment before extraction of olive oil in terms of quality and quantity. Olive paste preparation techniques are capable of changing the antioxidant content of different oils. In this context, due to the complexity of the oxidation processes and the diverse nature of antioxidants, with both hydrophilic and hydrophobic components, there was a lack of a highly sensitive method to measure the antioxidant capacity. Therefore, the second objective will be studying the evaluation of the antioxidant capacity and contents of the major compound (tyrosol) in olive oil and next the different oil extract obtained by different treatments. A comparative study was conducted using published analytical protocols to evaluate the effect of free radical scavenging ($ABTS^{°+}$, DPPH° and $O_2^{°-}$). The aim of this study was to propose a quick and reproducible method in order to evaluate the antioxidant capacity, as well as the quantification of phenolic compounds in olive oils. This work will allow choosing the most suitable conditions during the various stages of the raw material valorization: extraction, purification and concentration according to an optimal quality criterion: the concentration of phenolic compounds (Folin-Ciocalteu and gold nanoparticles formation methods) will be studied in parallel with their antioxidant activity.

Results and discussion

Spectrophotometric methods:

Several spectrophotometric methods were applied during this study using microplate spectrophotometer (Multiskan, Thermo Life Sciences, France) containing different filters 750, 620, 560, 490 and 405 nm: some of them allow the determination of the antioxidant capacity and others the quantification of phenolic compounds. All these methods require the use of standard compounds for calibration: tyrosol was selected because it is one of the main antioxidants contained in olive oil; trolox, ascorbic acid and gallic acid as there are usually used as references in the reported methods. Standard solutions of antioxidants were freshly prepared in ethanol. Contrary to what is generally done, colorimetric measurements were miniaturized and performed with a microplate spectrophotometer using 96 micro-well plates (PolySorp® flat bottom plates (Nunc, Roskilde, Denmark)).

General principle of the determination of antioxidant capacity in vitro:

Antioxidant deficiency *in vivo* destabilizes the antioxidants/free radicals balance and induces a severe increase of reactive oxygen species (ROS) in the human body, resulting in a deleterious process called "oxidative stress". Consequently, in human health, antioxidants are components that are protecting cells against free radicals attacks. By analogy, the antioxidant capacity should be determined *in vitro* thanks to the ability of antioxidants to scavenge free radicals. In the agri-food field, two colorimetric methods, named DPPH and ABTS, are commonly used to determine antioxidant capacities of vegetable extracts. These methods are based on synthetic and rather stable radicals, DPPH° and $ABTS^{°+}$ respectively. More recently[17], an original method proposed to determine the antioxidant capacity using radicals existing in living organisms. In this case, the spectrophotometric NBT method is based on superoxide radicals $O_2^{°-}$ generated during the enzymatic oxidation of hypoxanthine.

For all the performed methods, the scavenging effect of AOX on radicals was estimated by the following equation[18]:

$$\text{Scavenging ratio} = (A_0 - A_1) \times 100/A_0$$

A_0 is the maximum absorbance in absence of AOX

A_1 is absorbance after addition of AOX

The color intensity generally decreases with increasing the concentration of AOX until maximum radicals scavenging. Calibration curves representing the scavenging ratio versus the AOX concentration were plotted in order to calculate EC_{50} of tested antioxidants. By definition, EC_{50} is the concentration of AOX needed to trap 50% free radicals from its initial quantity. Therefore, the lower value of EC_{50} correspond the most efficient antioxidant.

Selection of the most appropriate method for olive oil antioxidant capacity determination:

Due to the complexity of the oxidation processes in foods, a single method cannot fully reflect the antioxidant profile of a sample[19]. Therefore, the objective of this part is to minia-

turize and optimize various classical and original spectrophotometric methods (DPPH, NBT, FRAP and ABTS assays) in order to evaluate the most suitable for studying the antioxidant capacity of olive oil. In this context, several AOX have been tested and only the results obtained with two of them, namely tyrosol, which is one of the major compounds present in olive oil and gallic acid, which is used as reference in most of spectrophotometric methods, are presented. For each method, the conventional reference AOX is also tested at different concentrations, allowing to plot a calibration curve. Table 2 mentioned the different spectrophotometric methods tested to measure the antioxidant capacity of each AOX and subsequently the calculated EC_{50} values. According to this table, DPPH, NBT and FRAP (data not shown) methods were unable to evaluate the antioxidant capacity of tyrosol and consequently it is clear that only ABTS method was capable of pertinently evaluating the antioxidant capacity of olive oils. These results are in accordance with the researches of Cioffi *et al.*[20] who also proved that tyrosol had no antioxidant capacity to scavenge DPPH° and $O_2^{\circ-}$ radicals. Actually, several studies concluded that some AOX cannot react with such radicals due to the absence of a hydroxyl group in the *ortho* position[20–22]. Therefore, ABTS method was chosen to analyze the antioxidant capacity of olive oil extracts.

Antioxidant capacity of different olive oil extracts using ABTS method:

Once the method has been selected, it is necessary to choose a reference antioxidant among the three tested compounds in order to express the results in equivalent of appropriate AOX per weight of olive oil. As shown in Table 2, the EC_{50} of trolox is really higher (41.5 μM) than gallic acid and tyrosol (10.75 and 11.5 μM, respectively). From these values, it seems that gallic acid and tyrosol have approximately the same capacity to trap $ABTS^{\circ+}$ radicals. Therefore, the antioxidant capacity for olive oil will be expressed in equivalent of gallic acid in order to be comparable to the results for total phenol content.

Table 3 displayed the equivalent content of gallic acid in mg/kg of oil after and without paste treatments, as well as the results of the statistical analysis. According to ABTS method, the equivalent contents were relatively low when treatments on olive paste were not carried out even if a higher malaxation time led to a better extraction of the antioxidant compounds (5.6 and 4.8 mg/kg of oil respectively for C (40 min) and C (20 min)). These results are in good agreement with Lukic *et al.*[23] who found that the effect of malaxation time was generally relatively weak, but significant for particular compounds such as tyrosol (9.52 and 9.84 mg/kg at respectively 30 and 60 min of malaxation from *Oblica* olive oil). By comparison with the two controls (C (40 min) and C (20 min)), it can be noticed that all olive paste treatments were able to improve the oil quality by significantly increasing the antioxidant capacity and the oil extraction yields (data not shown) were also improved.

All these values are between 4.8 ±0.7 and 7.0 ±0.4 mg/kg of oil and the combination of two treatments (M.W and S.C) has no significant effect on the phenolic content when comparing with the treatments applied individually.

By comparing the physical (U.S, M.W) and chemical (S.C) treatments, it was possible to evaluate their individual effects. The chemical treatments have been given up as no significant beneficial effect was observed despite the addition of an exogenous substance. The use of ultrasounds beyond 40 min and 60 min led to the highest antioxidant capacity (7.0 mg eq. gallic acid/kg of oil). However, these observations are in contradiction with Bejaoui *et al.*[8] who mentioned that the use of ultrasounds has no influence on phenol content. Even if the microwave treatment (M.W) is a little bit less efficient (6.8 mg eq. gallic acid/kg of oil), this technique will be preferred as the time saving in the paste preparation is considerable and the results confirm the previous conclusions of Leone *et al.*[24] and Tamborrino *et al.*[25].

Total of phenolic compounds:

F-C and AuNPs methods allowed the global quantification of phenols measuring their reduction capacity. As previously, two antioxidants (gallic acid and tyrosol) were used as reference. Since tyrosol could not lead to the formation of AuNPs, the phenol contents have been expressed in mg equivalent of gallic acid per kg of oil for both methods. The results obtained for oils resulting from different paste treatments are also presented in Table 3. As expected, all olive paste treatments are able to increase the phenol content. These results are in good agreement with the antioxidant capacity determined by ABTS method.

For the two controls (C (40 min) and C (20 min)), the F-C method led to similar results in terms of gallic acid equivalent content (2.6 and 2.5 mg/kg of oil respectively) while the control (C (40 min) showed a more important content than C

(20 min)) when using the AuNPs assay (11.5 and 9.8 mg/kg of oil respectively). This last result confirms the major role of mixing process to extract phenolic compounds during olive oil preparation. Accordingly, different studies have been realized to improve malaxation efficiency on olive oil when decreasing the mixing time[26,27]. In addition, the increase in malaxation time results in the decrease of some nutritional characteristics of VOO if the atmosphere in the headspace of the malaxer contains oxygen, which doesn't prevent to obtain good olive oil quality without compromising the yields[28].

The shorter duration for ultrasonic treatments (10 min) gave the best results as well as with the F-C assay (7.4 ±0.8 mg/kg of oil) than the AuNPs one (14.7 ±0.6 mg/kg of oil). This observation is in accordance with Aydar *et al.*[29] who showed that the most important phenol content corresponds to the minimum duration of ultrasounds. Moreover, whatever the analytical method, the combination of both treatments (M.W and S.C) has no beneficial effect on the phenol contents compared with individual treatment.

On the other hand, the results obtained were plotted (gallic acid equivalent content obtained by AuNPs versus of gallic acid equivalent content obtained by F-C). Briefly, Fig. 4 showed a positive correlation between the two proposed methods. Moreover, it seems that the results obtained by AuNPs were more sensitive than those obtained by F-C assays. This study showed the relevance of new method in order to quantify phenolic compounds in VOO samples.

Materials and methods

Chemical reagents:

All the chemicals used were of analytical quality. The standard antioxidants (tyrosol, gallic acid, ascorbic acid, trolox), acetyltrimethyl ammonium chloride (CTAC; 25% in water), hydrogen tetrachloroaurate ($HAuCl_4.2H_2O$; 99.9%), Folin-Ciocalteu reagent, sodium carbonate, 2,2-diphenyl-1-picrilhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), sodium chloride, potassium chloride, dibasic sodium phosphate (Na_2HPO_4), monobasic potassium phosphate (H_2KO_4P), dibasic potassium phosphate (HK_2O_4P), hypoxanthine (HX), xanthine oxidase (XOD), nitro blue tetrazolium (NBT), sodium acetate, potassium persulfate ($K_2S_2O_8$) and sodium citrate were provided by Sigma-Aldrich (France). Hexane and methanol were purchased from CARLO ERBA chemicals and 96% ethanol was

Table 1. Different olive pastes treatments

	Treatments										
	Controls		Microwaves(s)	Sodium citrate (g/L)		Microwaves 72 s + sodium citrate (g/L)		Sonication (min)			
Treatment conditions	–	–	72	150	300	150	300	10	20	40	60
Malaxation time (min)	40	20	20	20	20	20	20	20	20	20	20
Number of repetitions	3	3	3	3	3	3	3	3	3	3	3
Sample abbreviation	C (40 min)	C (20 min)	M.W	S.C (150)	S.C (300)	M.W + S.C (150)	M.W + S.C (300)	U.S (10 min)	U.S (20 min)	U.S (40 min)	U.S (60 min)
Paste T°(C) after treatment	17.6 ±0.5	15.2 ±0.6	20.8 ±0.2	15.3 ±0.5	15.9 ±0.1	16.1 ±0.1	16.2 ±0.1	16.0 ±0.3	15.5 ±0.2	17.2 ±0.2	17.1 ±0.1

(–): without treatment.
C (40 min): control 1: traditional malaxation, C (20 min): control 2: shorter malaxation duration, M.W: microwaves treatment during 72 s, S.C (150): sodium citrate treatment with 150 g/L, S.C (300): sodium citrate treatment with 300 g/L, M.W + S.C (150): microwaves treatment during 72 s and addition sodium citrate at 150 g/L, M.W + S.C (300): microwaves treatment during 72 s and addition sodium citrate at 300 g/L, U.S (10): ultrasonic treatment during 10 min, U.S (20 min): ultrasonic treatment during 20 min, U.S (40 min): ultrasonic treatment during 40 min and U.S (60 min): ultrasonic treatment during 60 min.

Table 2. Antioxidant capacities of standards measured by different spectrophotometric methods expressed as EC_{50}

DPPH				NBT				ABTS			
AOX standards	Gallic acid	Tyrosol	Ref. AOX[a]	AOX standards	Gallic acid	Tyrosol	Ref. AOX	AOX standards	Gallic acid	Tyrosol	Ref. AOX
EC_{50} (μM)	192	nd[b]	529	EC_{50} (μM)	4100	nd	39700	EC_{50} (μM)	10.8	11.5	41.5

(a) each method has a conventional reference AOX: for DPPH and ABTS: trolox, for NBT: ascorbic acid.

(b) nd: not detected.

supplied by Millipore Sigma.

Olive fruit variety:

Chemlali olive fruits were harvested from the chaal field (Sfax south Tunisia) during the crop season 2017-2018. The maturity index was determined according to the method developed by the Agronomic Station of Jaén[30] as a function of fruit color (epicarp and mesocarp). The maturity index was determined with olives selected randomly (homogeneous maturity) and was equal to 6. One day after harvesting, the olives were transported to the French laboratory for the paste preparation.

Samples preparation:

(i) Preparation of olive oil:

33 kg olives were crushed using a mechanical grinder (Santos, Type N°9 1984, 380 W, 50 Hz, 220 V, 1500 tr/min, made in France) in order to have a single homogenous lot. The obtained paste was then divided in batches of 700 g. Afterwards, the different paste treatments were realized as mentioned in Table 1. All these treatments required different apparatus and products: ultrasonic treatments (Ultrasonic stainless-steel case type Prolabo Transsonic T 700/H, frequency: 35 kHz, power: 560 W, made in Germany) during different times (0, 10, 20, 40 and 60 min); microwaves treatment (Proline Micro Chef, Voltage: 230 V ~ 50 Hz, rated power: 1200 W and power restored: 800 W, frequency: 2450 MHz, Model No.: SM12 WH, made in France) during 72 s[14,31] and finally chemical treatment with addition of a new co-adjuvant, sodium citrate, at two concentrations (150 or 300 g/L). After each treatment, the paste was malaxed for 20 min and the temperature of the olive paste was measured immediately to ensure that the oil obtained at “cold extraction” (Table 1). Two control pastes, without treatment, were prepared as followed (all guide the literature): one of them with a malaxation time of 40 min corresponding to a traditional preparation and another one with a reduced malaxation time of 20 min (half traditional duration) as carried out after each treatment[24,32]. The malaxation step is an important process of oil extraction facilitating coalescence of the oil droplets. Then, the oil was extracted with a manual press (250 mL of water were approximately added to extract the maximum quantity of oil) and the liquid was centrifuged to separate the oil from the aqueous phase at 3500 rpm for 15 min. Finally, oils were stored in black glass bottles and kept in a cold room at 4 to 5°C until further use.

(ii) Extraction of phenolic compounds from olive oil:

The method of phenolic compounds extraction, previously described by Rigane *et al.*[33], has been improved to improve the extraction solvent evaporation step. Therefore, 4 g of oil were added to 2 mL of *n*-hexane and 4 mL of a methanol/water (60:40; v/v). Then, the mixture was centrifuged (3500 rpm for 5 min, 25°C). The hydro-alcoholic phase was collected, and the organic phase was re-extracted twice with 4 mL of methanol/water (60:40; v/v) using a separatory funnel. Finally, the three hydro-alcoholic fractions were gathered, washed with 4 mL of *n*-hexane to eliminate the residual oil, and then dried by evaporative centrifugation under vacuum at 35°C using Genevac system (EZ-2, HT Series 3, High Speed Evaporators – Rocket, made in Germany). Each dried extract was then dissolved in 2 mL of ethanol before analyses.

Antioxidant capacity micro-assays:

(i) DPPH micro-assay:

The effect of antioxidants on the scavenging of DPPH° was estimated by miniaturizing the protocol initially described by Sánchez-Moreno *et al.*[34]. Briefly, 25 μL of DPPH° at 1.75 mM in ethanol was added to 200 μL ethanol and 25 μL of the antioxidant to be tested. After 10 min at 25°C, absorbance was measured at 490 nm (nearest wavelength to 515 nm corresponding to the maximum absorbance of DPPH°) and compared to a control sample without antioxidant com-

Table 3. Phenolic content measured by ABTS, F-C and AuNPs methods expressed as mg equivalent gallic acid/kg of oil

Treatments	C (40)	C (20)	U.S (60)	U.S (40)	U.S (20)	U.S (10)	M.W	S.C (150)	S.C (300)	M.W+S.C (150)	M.W+S.C (300)
GAEC* by ABTS	5.6±0.7^{a}	4.8±0.7^{a}	7.0±0.4^{b}	7.0±0.3^{b}	6.0±0.9^{b}	6.1±0.1^{c}	6.8±0.6^{b}	6.9±0.6^{b}	6.6±0.4^{b}	6.7±0.1^{d}	6.0±0.8^{e}
GAEC by F-C	2.6±0.3^{a}	2.5±0.2^{a}	4.2±0.7^{b}	5.8±0.9^{c}	5.6±0.5^{c}	7.5±0.8^{d}	5.4±0.3^{c}	3.6±0.3^{e}	5.0±0.3^{b}	3.3±0.2^{e}	3.8±0.5^{e}
GAEC by AuNPs	11.5±0.6^{a}	9.8±0,2b	12.3±0.2^{a}	13.6±0.1^{c}	14.0±0.4^{d}	14.7±0.6^{d}	14.0±0.4^{d}	13.4±0.3^{c}	13.7±0.5^{c}	12.3±0.1^{a}	13.1±1.6^{a,c,d}

Means with different letters in the same line were significantly different at $p < 0.05$. Results are expressed as mean ± standard deviation of 9 determinations.
GAEC*: Gallic acid equivalent content in mg/kg of oil.

pound. Standard solutions of antioxidants were ranging from 31.3 to 2000 μM in ethanol.

(ii) ABTS micro-assay:

The evaluation of the antioxidant capacity by the trapping of ABTS radical is determined according to the principle described by Re *et al.*[35].

ABTS$^{\circ+}$ is a radical-cation generated thanks to the monoelectronic oxidation of the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) by mixing 50% of potassium persulfate $K_2S_2O_8$ (2.5 mM) with 50% of a stock solution of ABTS at 7 mM[36]. The solution of ABTS$^{\circ+}$ and the antioxidant stock solutions were prepared in phosphate buffer (pH 7.4; 0.1 *M*). 150 μL of 1.7 10^{-4} *M* ABTS$^{\circ+}$ solution was added to 100 μL of antioxidants to be tested at different concentrations from 31.25 to 500 μM in phosphate buffer. The absorbance was carried out at 405 nm after 3 min incubation (400 rpm at 25°C).

(iii) NBT micro-assay:

According to previously published work[37], a simple and rapid colorimetric method has been optimized and miniaturized to determine the antioxidant capacity (Fig. 1). This test is based on the ability of antioxidants to scavenge superoxide radicals ($O_2^{\circ-}$). Due to their high reactivity, these radicals are not stable and not commercially available. For this reason, they were enzymatically generated *in vitro* during the catalytic oxidation of hypoxanthine by xanthine oxidase. Superoxide radicals reduced a water-soluble tetrazolium salt (NBT) into a dark blue colored compound, nitro blue tetrazolium formazan. After optimization, microplate assays were

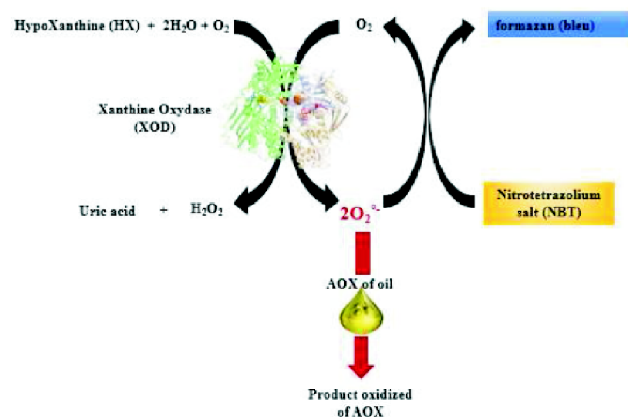

Fig. 1. Principle of the NBT method.

realized as follows. A reaction mixture was prepared with 1.5 mU of XOD (except for the blank), 25 µL of HX at 0.5 mM, 25 µL of NBT at 0.75 mM and 25 µL of antioxidant sample varying from 31.3 to 2000 µM in 50 mM phosphate buffer (pH 7.5) containing 0.1 mM EDTA (up to 250 µL). The blank was realized without enzyme and the control, corresponding to the maximum production of NBT formazan, was performed without antioxidant. After 15 min incubation at 350 rpm and 25°C, the absorbance was read at 560 nm using a microplate spectrophotometer.

Total phenols content determination micro-assays:

Two spectrophotometric methods were performed in order to determine the total phenolic content in olive oils: a classical method, Folin-Ciocalteu (F-C), and a recent one, based on the formation of gold nanoparticles. In contrast with the methods previously described to determine the antioxidant capacity, the color intensity increases with the phenol content.

(i) Folin-Ciocalteu (F-C) micro-assay:

The F-C method relies on the transfer of electrons in alkaline medium from phenolic compounds to form a blue chromophore constituted by a phosphotungstic/phosphomolybdenum complex, where the maximum absorption depends on the concentration of phenolic compounds. Even if the reduced F-C reagent is spectrophotometrically detectable between 690 and 760 nm according to Muñoz-Bernal *et al.*[38], the experiments were conducted at the optimal absorbance of 750 nm. Briefly, 125 µL of the commercial F-C solution diluted 10 times in water, 25 µL of standard solution of antioxidants at different concentrations varying from 85 to 880 µM were added in the reported order. Incubation was carried out for 8 min with agitation 400 rpm at 37°C and protected from light. Afterwards, 100 µL of sodium carbonate (Na_2CO_3: 7.8% equal to 3.9 g of sodium carbonate diluted in 50 mL in water) was added. Finally, incubation for one hour with agitation 400 rpm at 37°C was achieved.

(ii) Gold nanoparticles (AuNPs) micro-assay:

AuNPs were formed following the protocol described by Pelle *et al.*[39] based on a controlled reduction of $HAuCl_4$ (Fig. 2). In presence of CTAC (surfactant), this method consists to reduce gold(III) in gold(0) thanks to antioxidants such as phenolic compounds in olive oil. The protocol is as follows: 20 µL of CTAC at 0.19 *M*, 25 µL of $HAuCl_4$ solution at 10^{-2} *M*, and 25 µL of standards solutions of antioxidant varying from

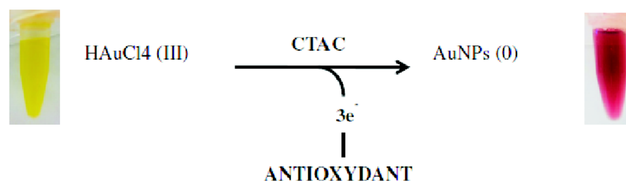

Fig. 2. Formation of gold nanoparticles AuNPs in the presence of AOX.

290 to 2500 µM were added in the reported order in phosphate buffer solution (pH 8.0; 10^{-2} *M*), up to final volume of 250 µL. Afterwards, vigorous agitation was carried out before heating for 10 min at 45°C in an incubator at 400 rpm. Finally, the reaction was then blocked in ice for 25 min. Spectrophotometric reading was conducted at 560 nm, wavelength corresponding to the maximum of absorbance. A sigmoidal curve reporting the absorbance at 560 nm versus the gallic acid concentration was performed with an OriginPro 8.6 software (Fig. 3). In contrary, the tyrosol (mono-phenol) has no effect to form the gold nanoparticles (AuNPs). Indeed, AuNPs data demonstrated that compounds containing *ortho*-diphenols are the most active in reducing gold(III) to gold(0). Intermediate activity was recorded for gallic acid, while tyrosol had significantly lower activity compared to other antioxidant compounds according to Pelle *et al.* (2015).

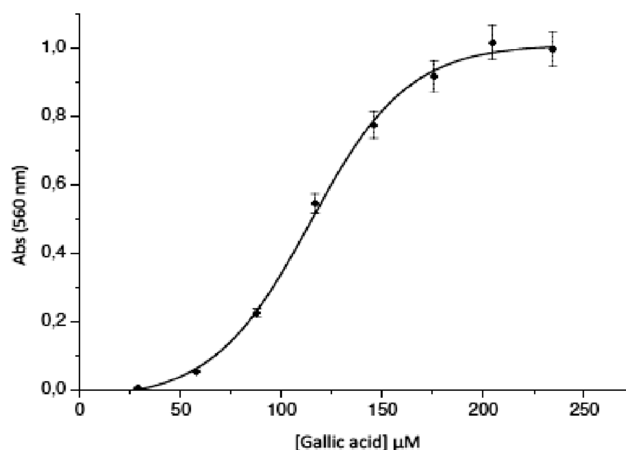

Fig. 3. Calibration curve of gallic acid by gold nanoparticles.

Statistical analysis:

Each treatment was performed on 3 different paste samples and each phenolic compound extract was then analyzed in triplicate. Finally, we obtained 9 spectrophotometric

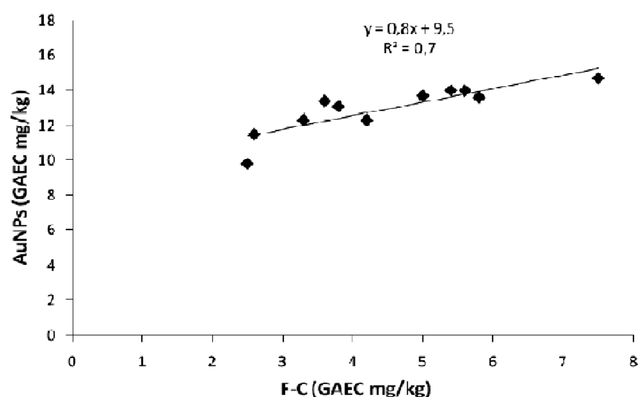

Fig. 4. Correlation curve between F-C and AuNPs methods.

measurements for one paste treatment characterization. Therefore, the results of the analytical determinations were expressed as mean ± standard deviation (SD) of nine measurements.

Conclusion

In this study, the experiment highlighted the importance of using a set of physicochemical treatments, performed before the malaxation step, while reducing by half the conditioning time (20 min instead of 40 min for the conventional treatment). In terms of oil quality, we demonstrated that the use of alternative conditioning technologies, realized individually or in combination, allowed increasing the antioxidant capacity. For the first time, antioxidant capacity of tyrosol was studied using ABTS method (EC_{50} of tyrosol equal to 0.63 mg/L). Moreover, a simple colorimetric test based on gold nanoparticles formation (AuNPs) for the quantification of the phenolic compounds of the VOO was optimized and miniaturized. This method was increasingly rapid and sensitive compared to the classical Folin-Ciocalteu one (F-C). Consequently, in the future, AuNPs formation could be used for further analytical assays in the agri-food, replacing the traditional tedious methods.

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Abbreviations

AOX: Antioxidant, VOO: virgin olive oil; CTAC: cetyltrimethylammonium, $HAuCl_4$: hydrogen tetrachlo-roaurate, DPPH: 2,2-diphenyl-1-picrilhydrazyl, ABTS: 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), HX: Hypoxanthine, XOD: xanthine oxidase, NBT: nitro blue tetrazolium , F-C: Folin-Ciocalteu, AuNPs: gold nanoparticles, M.W: microwaves, S.C: sodium citrate, C: control and U.S: sonication or ultrasound.

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