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Olive biophenol integral extraction at a two-phase olive mill

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ABSTRACT

The biophenol integral extraction protocol from vegetation water developed in the early 2000s for the three-phase olive mill was adapted to a large two-phase mill operating in Sicily during the 2016/2017 season. The new set-up allows extensive recovery of olive phenolics, affording after nine months natural extracts with hydroxytyrosol concentration exceeding 1500 ppm. Previous toxic waste is transformed into a source of additional revenues for the milling company, and of valued bioproducts for the bioeconomy partner, eliminating altogether a source of potential pollution.

1. Introduction

The production of large amounts (10–30 million m^3) of wastewater at olive mills in the short period of time of olive harvesting and milling is a well known environmental and economic hurdle in Mediterranean countries where most (about 75%) olive oil production takes place every year (Galanakis and Kotsiou, 2017). Called also vegetation water, olive mill wastewater (OMWW) originates from water contained in the olive fruits plus added water (0.3–0.8 m^3 per 1000 kg of olives, depending on the process) to the milled paste in order to extract the highest quantity of oil from the paste via centrifugation in the three-phase milling process. For decades, the latter method has been the process of choice to produce extra virgin olive oil (EVOO). The oil yield increases proportionally to the amount of added water, but external processing water further increases dissolution in the aqueous phase of valued biophenols showing strong antioxidant, anti-inflammatory, nutrigenomic, chemoprotective and anti-atherosclerotic activities (Parkinson and Cicerale, 2016), widely associated to health benefits of EVOO (Valls et al., 2015).

In general, OMWW cannot be used for irrigation and need to be disposed of as a pollutant at significant economic cost for olive mills (Tsagaraki et al., 2007). Several treatment methods have been proposed to improve the quality of OMWW and reduce its phytotoxicity prior to disposal (Galanakis and Kotsiou, 2017). The simplest disposal practices are storage/evaporation in lagoons and controlled dispersion in soil. Emission limits, however, are generally surpassed and soil and water contamination easily occurs with serious environmental consequences (and closure of olive mills not conforming to environmental laws. (Golia, 2015)).

Starting in the early 1990s, a new continuous centrifuge two-phase milling process in which very little water is added (maximum 5 wt%), started to be commercially adopted (Roig et al., 2006), first in Spain and then in Greece and in Italy. In place of OMWW, the new milling process affords high quality EVOO along with a wet pomace consisting of olive pulp (ca. 35%) and seed residues dispersed in water released by the olive fruit and about 2.5% of processing water. Composted with agricultural wastes, this two-phase olive mill waste (TPOMW) becomes an excellent fertilizer ideally recycled in land growing olive trees (Fernández-Hernández et al., 2014), though its significant phytotoxicity still requires its disposal as an industrial waste, whereas the large amount of solid matter can lead to fermentative phenomena

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complicating its processing.

We argued elsewhere (Ciriminna et al., 2016) that olive biophenols extracted from OMWW will shortly find large scale utilization, in a variety of nutraceutical (Rigacci and Stefani, 2016), food, beverage (Ciriminna et al., 2017), and cosmetic formulations (Lueder, 2011), in which they exert multiple functions and provide health benefits including powerful anti-inflammatory, antioxidant, antimicrobial, anti carcinogenic, skin and bone regenerative action. Furthermore, recent research suggests that olive phenolics could play a significant role in prevention and treatment of inflammation and of free-radicals mediated chronic diseases (Casamenti and Stefani, 2017). In the following, we show how the biophenol integral extraction protocol from vegetation water developed by Crea in the early 2000s for the three-phase olive mill was successfully adapted to a large two-phase mill operating in Sicily during the 2016/2017 season.

2. Integral biophenol extraction from wet pomace

Among several biophenol extraction techniques from olive mill waste developed in the last two decades (Galanakis and Kotsiou, 2017), the process invented by Crea in 2001 allows to obtain an integral *bouquet* of olive biophenols particularly rich in hydroxytyrosol (3,4-dihydroxyphenylethanol) starting from OMWW obtained in the three-phase milling process (Crea, 2001). In the latter process, the vegetation waters are centrifuged using a three-phase spin-dryer in order to remove the solid residuals and oil traces. The resulting aqueous phase, called “olive juice”, is chemically stabilized by the addition of citric acid, and then left to age in closed vessels for several months in order to allow for the full hydrolysis of the main glycosides (oleuropein and verbascoside).

Once aging is complete (full hydrolysis of oleuropein), the resulting biophenol extract possessing negligible COD and BOD_5 values (tradenamed *Hidrox*) (CreAgri Inc) can be processed in different ways to obtain nutraceutical or cosmetic formulations. This process has been in use in California now for more than a decade, resulting in manifold applications of the aqueous extract. Aiming at adapting the Crea's protocol to the two-phase milling process, we focused on the use of TPOMW as a source of biophenols, as well as trying to recover the emulsified olive oil usually lost in the wet pomace. The technology was developed at a large olive mill based in Chiaramonte Gulfi, Sicily, a renown Italian production centre of high quality extra virgin olive oil (EVOO) during the 2016 olive milling season.

The simple processing stages for the recovery and concentration of olive biophenol are briefly described below. No fractionation such as partitioning with an organic solvent, chromatographic methods, supercritical fluids, or membranes are employed. Two different modified industrial centrifuges were instead placed downstream the EVOO production line. In the first step, the TPOMW was forced in a two-phase horizontal spin-dryer in order to separate the solid particles from the liquid (oil + water) phase. The emulsified liquid phase thus obtained underwent centrifugation in a vertical three-phase centrifuge, namely a 3-phase separating decanter in which two liquids of different densities are separated from each other, and from the residual solid particles (d > 1 mm).

The water phase output of the vertical centrifugation, containing approximately 1–2% of micrometer solid residue comprised of olive pulp, peel and wax (Fig. 1), was clarified using an industrial

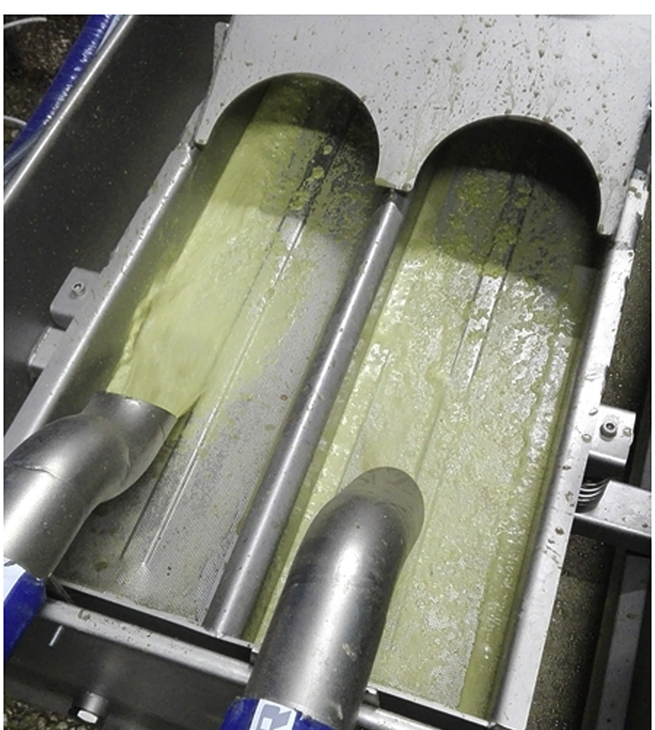

Fig. 1. Water phase output of the three-phase vertical centrifugation.

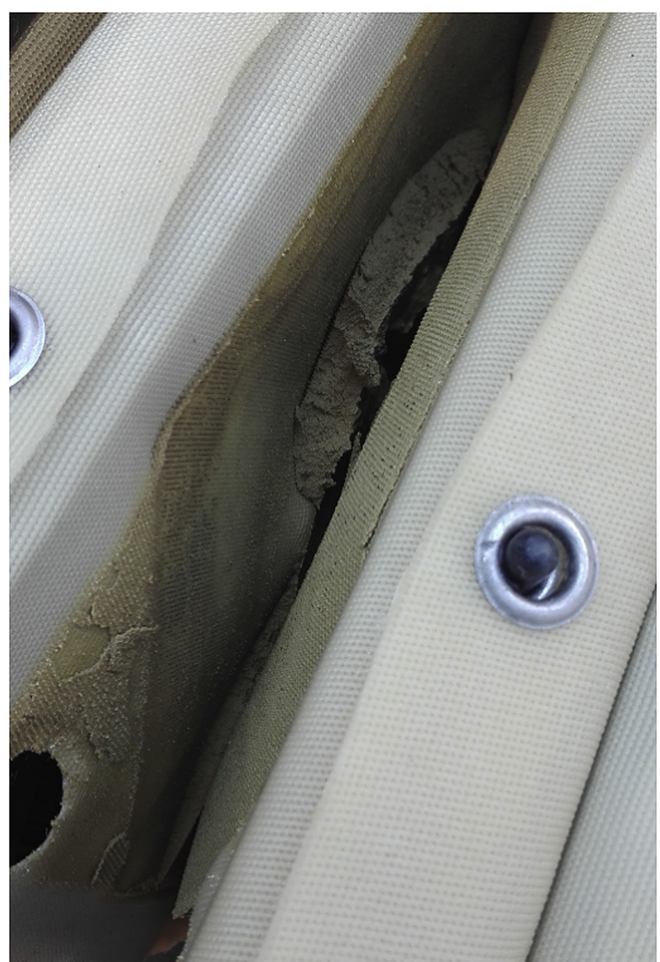

Fig. 2. Solid-cake resulting from the three-phase vertical centrifugation removed using a press filter.

press filter (Fig. 2). Following the Crea's protocol, the resulting olive juice was stabilized with citric acid. In this olive juice biophenols are more concentrated than in the corresponding juice obtained from the three-phase OMWW since very little water is added during the two-phase milling process.

3. Enhanced EVOO production

Along with the olive juice, the new process recovers from the wet pomace a significant yield of extra virgin olive oil, namely 90% of the oil not extracted during the conventional milling, without the need to use *n*-hexane. The EVOO obtained from this second round of extraction had significant economic value, 4–5 times higher than the value of hexane-extracted olive oil. It is important to notice, here, that the latter EVOO never comes in contact with citric acid, as the acid is only used in a subsequent step only to stabilize the olive juice and promote the hydrolysis of its glycoside components.

No oxidative or other degenerative phenomena of this EVOO fraction could take place, as the new extractor was placed directly downstream the mill's wet pomace output, with the newly recovered oil retaining excellent flavour with a greenish colour which was more eye-catching than first extraction oil, due to additional chlorophyll extraction from the TPOMW.

4. Additional pomace

Another valued output of the new extraction process was a dried pomace with 60% less humidity compared to that of conventional wet pomace which could indeed be sold to a local factory which further commercializes the dried ‘nocciolino’ (seed) as solid biofuel to power domestic and commercial biomass heaters. No waste was discharged into the environment, whereas the milling company supplying the wet pomace not only got rid of previous significant costs associated to processing the TPOMW as industrial waste, but converted it in a triple source of extra revenues obtained from selling: *i*) the TPOMW used as phenolics source to the extraction company; *ii*) high quality second-extraction EVOO; and *iii*) the dried pomace to a ‘nocciolino’ company.

5. High quality biophenol extracts

Three lots of stabilized biophenol extracts obtained according to the harvesting and milling time (late September-early October; late October; early November) were stored in Sicily in stainless steel silos (Fig. 3). Every lot is composed of olive juice coming from different organic Sicilian *Cultivar* (mainly tonda Iblea and Nocellara del Belice but also Bioancolilla, Cerasuola, Moresca and Nocellara Etnea). Regardless of the poor season (year-to-year decrease in production of olives approaching 70%) about 800 tonnes of olive juice could be obtained (see Fig. 4).

The juice from different lots was left to undergo hydrolysis according to Crea's original protocol for several months at buffered pH. The HPLC analyses aimed to assess the total biophenol, hydroxytyrosol (HT) and tyrosol (T; 4-hydroxyphenethyl alcohol) concentration were carried out in Italy by an accredited laboratory

Fig. 3. Silos stocking the olive juice obtained in Sicily in 2016 from a two-phase olive mill.

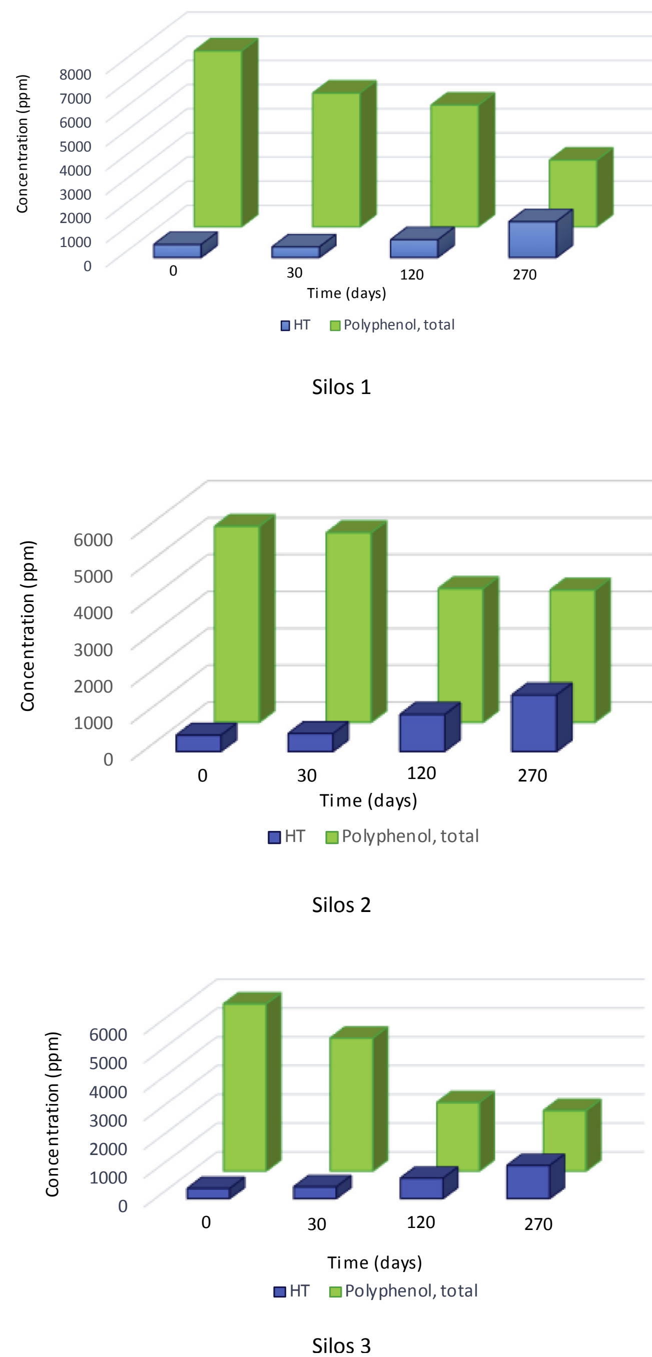

Fig. 4. Evolution of hydroxytyrosol and polyphenol total concentration in the silos of Fig. 3.

(Centro Analisi Biochimiche, Rizziconi) further registered for olive oil analysis by Italy's Agriculture Ministry.

All juice samples contain a large amount of total phenols, given in ppm (mg kg^{-1}) in Table 1, and in the following text and Tables. The first lot, coming from the early harvested drupes, contained the highest biophenol amount, in agreement with the known higher phenolics content in greener olive drupes.

Data in Table 2 show that after one month of aging, the pH buffered at 3.2 and the activity of the olives yeast (Ciafardini and Zullo, 2002), promoted the hydrolysis of the biophenol glycosides leading to increasing concentration of both HT and T.

Remarkably, the trend observed in Sicily with olive juice originating from the two-phase mill was considerably slower when compared with the hydrolytic profile observed in California for juice coming from a three-phase mill, likely due to the higher phenol concentration, as well as to the initial lower outdoor temperature during December 2017 and January 2017 in the area hosting the silos (Caltagirone, 610 m above sea level), both responsible for partial inhibition of the *Saccharomyces* yeast strain. This hypothesis was confirmed by the data obtained after four months aging (Table 3).

The considerably higher temperatures recorded in February and March 2017 (+2.69 °C for February and +2.52 °C for March, in Italy, in comparison to the 1971–2000 mean) (ISAC, 2017) promoted the glycoside hydrolysis to such an extent that in the aqueous extract from Lot 2, hydroxytyrosol represented 51.26 wt. % of the olive polyphenol composition, namely in the very high concentration range (40–90 wt. %) required for advanced medical applications such as the treatment of early-stage Parkinson disease (Crea, 2016). In 2001, Crea was the first scholar to associate a powerful anti-inflammatory activity to hydroxytyrosol (Crea, 2002).

Finally, data in Table 4 show that after nine months of aging with the temperature further raising due to Sicily's hot climate, the hydrolysis of the biophenol glycosides led to record concentration of both HT and T: 53.9% for hydroxytyrosol in the first lot and, in general, concentration approaching or even exceeding 1500 ppm which is, to the best of our knowledge, the highest concentration of hydroxytyrosol in an olive biophenol extract ever reported.

6. Conclusions

We have adapted to the two-phase olive mill the integral biophenol extraction protocol originally developed by Crea for the three-phase olive mill in 2001. The extraction, now, is from the wet pomace ("alperujo" in Spanish), and not from the vegetation waters. The new set-up allowed extensive recovery of olive phenolics, transforming previous toxic waste into a source of revenues for the milling company, and of valued bioproducts for its bioeconomy partner.

The olives were specific cultivars grown in southern Sicily where the unique climate and geographical conditions afford particularly high amounts of biophenols (Delisi et al., 2017). This translates into significant higher amounts of total biophenols in the aqueous extract, eventually affording an extract with hydroxytyrosol concentration exceeding 1500 ppm and 53.0 wt.% of the olive polyphenol composition.

Natural extracts with high concentration of hydroxytyrosol, the oleuropein metabolite, possessing potent pharmacological

Table 1
Total phenolics, tyrosol and hydroxytyrosol in three lots of olive juice as obtained from early to late harvesting.[a]

Entry	Total biophenol (ppm)	Hydroxytyrosol (ppm)	Tyrosol (ppm)	Hydroxytyrosol (wt%)	Tyrosol (wt%)
Lot 1	7288	545.1	79	7.48	1.42
Lot 2	5318	444	68.8	8.40	1.41
Lot 3	5810	355.3	53.7	6.11	0.98

[a] HPLC analytical method 0178–00 with syringic acid as internal standard; data from the accredited Laboratory Centro Analisi Biochimiche (Rizziconi, Italy).

Table 2
Total phenolics, tyrosol and hydroxytyrosol in three lots of olive juice obtained from early to late harvesting, after one month.[a]

Entry	Total biophenol (ppm)	Hydroxytyrosol (ppm)	Tyrosol (ppm)	Hydroxytyrosol (wt%)	Tyrosol (wt%)
Lot 1	5542	448	102.9	8.08	1.4
Lot 2	5144	486	74.7	9.45	1.4
Lot 3	4623	412.6	57	8.93	1.2

[a] HPLC analytical method 0178–00 with syringic acid as internal standard; data from the accredited Laboratory Centro Analisi Biochimiche (Rizziconi, Italy).

Table 3
Total phenolics, tyrosol and hydroxytyrosol in three lots of olive juice obtained from early to late harvesting, after 4 months.[a]

Entry	Total biophenol (ppm)	Hydroxytyrosol (ppm)	Tyrosol (ppm)	Hydroxytyrosol (wt%)	Tyrosol (wt%)
Lot 1	5045	743	126	14.73	2.5
Lot 2	3530	1004	127	28.44	3.6
Lot 3	1387	711	79	51.26	5.7

[a] HPLC analytical method 0178–00 with syringic acid as internal standard; data from the accredited Laboratory Centro Analisi Biochimiche (Rizziconi, Italy).

Table 4
Total phenolics, tyrosol and hydroxytyrosol in three lots of olive juice obtained from early to late harvesting, after 9 months.[a]

Entry	*Total biophenol (ppm)*	*Hydroxytyrosol (ppm)*	*Tyrosol (ppm)*	*Hydroxytyrosol (wt%)*	*Tyrosol (wt%)*
Lot 1	2766	1491	252	53.90	9.11
Lot 2	3690	1531	221	41.49	5.98
Lot 3	2107	1153	190	51.77	8.53

[a] HPLC analytical method 0178–00 with syringic acid as internal standard; data from the accredited Laboratory Centro Analisi Biochimiche (Rizziconi, Italy).

activities *in vivo*, are now required for human intervention studies (Parkinson and Cicerale, 2016) that could lead to the first biophenol-based drugs, ending what Quideau has called the century-long neglect of plant polyphenols in the development of new drugs (Quideau et al., 2011).

In brief, the integral extraction process originally developed in 2001 for the three-phase olive mill, has kept its promises to make the olive bioeconomy possible at affordable costs. The olive mill company transformed waste formerly processed at high cost in a triple source of revenues (waste disposal costs eliminated, extra EVOO, extra dried pomace); the bioeconomy company obtained a biophenol-rich extract ready for multiple health beneficial applications; and the environment benefited from not receiving pollutants. On November 18, 2016, Italy's Labour Minister and the State Secretary for Research visited the new Sicily-based biophenol extraction plant (Guccione, 2016), as a prominent example of the circular bioeconomy now actively supported in Europe as a tool to promote economic growth and to address environmental and societal challenges (Peters, 2016).

Conflict of interest disclosure

Renovo Bioindustry, a company, has an interest in the biophenol market. M.P., F.M. and R.C., affiliate with Italy's Research Council, have no competing financial interest.

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List of abbreviations

EVOO	Extra Virgin Olive Oil
HT	Hydroxytyrosol
T	Tyrosol
OMWW	Olive Mill Waste Water
TPOMW	Two-Phase Olive Mill Waste
COD	Chemical Oxygen Demand
BOD_5	Biological Oxygen Demand

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