Targeted Composting of Olive Mil Wastes.
Comparison Between Three-Phase and Two-Phase Waste Composting.

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Introduction.

Three-phase extraction systems for obtaining olive oil produce a liquid residue known as "olive mil wastewater" (OMWw) and a solid residue called "olive mil waste" (OMW), while the two-phase olive mil waste (TPOMW) systems produce a viscous residue called "two-phase olive mil waste" (TPOMW). Both are characterized by undesirable odour and colour, acid pH, high salt concentrations, high content of polyphenolic compounds, high chemical oxygen demand; and high amount of organic matter and low water activity. Therefore, they are potential contaminants of water and soil, inhibiting seed germination, plant growth and affecting the microbial diversity of the soil.

The microbiota of this waste is basically determined by the soil where the olive tree has been cultivated and the water used for irrigation.

As a general rule, it can be established that, bacteriologically speaking, olive milling residues are characterized by fermentative lactic acid bacteria (*Lactobacillus* and *Oenococcus*), acetic acid (*Lactobacillus* and *Oenococcus*), acetic acid (*Acetobacter* and *Gluconoacetobacter*), as well as style fecal bacteria *Ruminococcus* - *Eubacterium* - *Clostridium* (REC) (Kavroulakis 2011); followed by Actinobacteria, Firmicutes and Acidobacteria.[(1)]

However, if we refer to the diversity of fungi and yeasts present in these residues, the populations are very numerous, predominantly *Geotrichum*, *Candida*, *Picnia*, *Rhodotorula*, *Saccharomyces*, *Aspergillus* and *Penicillium* [(2)]; with high cellulase, β-glucanase, β-glucosidase, peroxidase and polygalacturonase activities; which gives them the capacity to degrade complex compounds, including polyphenols and to use them as a source of carbon and energy.[(1)]

Biotechnological interest of the microbiota in olive milling wastes is found in: **(A)** Biodegradation of olive milling wastes using indigenous microbiota and specially selected strains (generally used as degraders of in-vitro polyphenols); **(B)** Bioconversion of recalcitrant products into useful products: a biotechnological application of the microbiota present in olive milling residues is the biotransformation of recalcitrant compounds into other useful compounds[(3, 4,5)]; **(C)** Suppressive properties of plant pathogens (inhibit the sporulation, suppress phytopathogenic effect, compete for nutrients and ecological niches).[(6, 7, 8, 9)]

Objectives.

In order to achieve the beneficial effects that derive from the use of this type of compost, we aim to achieve a homogeneous product in all its volume, so that it was constant in any area of the windrow, without significant fluctuations in microbiota present in all volume of material.

In the same way, the objective is to target the selection of microbial populations that provide greater added value to the final product due to their beneficial value, either as biofertilizer or as biocontrol.

Methods & Materials.

The formation of two composting windrow is proposed, due to the different origin of the starting materials: TPOMW and OMV (Table 1).

	TPOMW (Tons)	OMW (Tons)
	140,00	135,00
Inoculum (Mature Compost)	14,00	0,00

Table 1: Initial composition of each compost windrow

The use of mature compost in the windrow from TPOMW allows to provide structure to conform the pile as bulking agent to the material, in addition to acting as starter of the composting process, with selected microbial populations. In the case of the waste from OMW, inoculum is not used because the initial humidity level of the material allows the formation of windrow, and the material is inoculated at origin.

The following parameters have been taken into account for monitoring and controlling the composting process: Average temperature of windrow (T^a), pH, Moisture (%H), Total Organic Matter (%TOM), Total Nitrogen (TN), Organic Carbon (OC), Cations (Mg, Fe, Mn) and Microbiological Populations Count (MPC).

The most common populations of the composted material have been isolated and identified by molecular biology (sequence matching DNAr ITS16).

Results and conclusions.

Tables 2 and 3 show the values of the parameters monitored throughout the composting process. It is observed that the C/N ratio is more favourable to an adequate fermentation process in TPOMW than in OMW, while in this second material this same parameter causes the selection of nitrogen-fixing bacteria, which is one of the effects sought with the controlled process in order to obtain a final product of high added value (10^8 vs. 10^6, at the end of the composting process).

On the other hand, OMW's high C/N ratio does not appear to be a reason for excessive CO_2 loss as described in the literature, as the final TOM values are 5% higher in OMW than in TPOMW. This may be because the composting process has been targeted, albeit through the selection of native populations from the same material, because in this case no external inoculation has been added.

Date	Tª (ºC)	%H	%TOM	pH	OC (g/100g)	TN (g/100g)	C/N	M.B.	T.B.	N_2 FIX.	M & Y	Mg (mg/Kg)	Fe (mg/Kg)	Mn (mg/Kg)
15/2	44,10	47,70	51,30	6,90	28,50	1,08	27,47	$6{,}10*10^7$	$4{,}00*10^4$	$7{,}90*10^6$	$1{,}55*10^5$	500	92	10
15/3	48,90	35,49	57,83	6,82	32,13	1,27	26,38	$2{,}00*10^8$	$4{,}00*10^8$	$4{,}70*10^7$	$2{,}00*10^6$	800	613	21
24/5	55,50	18,21	73,73	6,74	40,96	0,95	44,20	$1{,}50*10^{10}$	$7{,}00*10^7$	$2{,}30*10^8$	$1{,}30*10^8$	1200	1142	36
24/7	36,90	15,40	79,50	7,39	44,17	0,90	49,07	$1{,}50*10^{10}$	$7{,}00*10^7$	$2{,}30*10^8$	$1{,}30*10^8$	ND	ND	ND

Table 2: Parameter control of OMW compost windrow. **M.B.**: Mesophilic Bacteria; **T.B.**: Thermophilic Bacteria; **N_2 Fix.**: Nitrogen-fixing bacteria; **M&Y**: Moulds and Yeasts. **ND:** Not Determined.

Date	Tª (ºC)	%H	%TOM	pH	OC (g/100g)	TN (g/100g)	C/N	M.B.	T.B.	N_2 FIX.	M & L	Mg (mg/Kg)	Fe (mg/Kg)	Mn (mg/Kg)
15/2	17,90	60,30	36,90	5,26	20,50	1,49	14,84	$2{,}30*10^{10}$	$2{,}60*10^9$	$3{,}40*10^6$	$1{,}10*10^6$	1200	373	20
15/3	40,30	54,89	41,19	6,63	22,88	1,74	14,23	$8{,}00*10^8$	$1{,}00*10^5$	$8{,}70*10^6$	$2{,}50*10^4$	1200	378	34
24/5	56,30	37,63	56,22	6,42	36,46	1,70	22,53	$2{,}80*10^{10}$	$1{,}07*10^{10}$	$2{,}30*10^7$	$1{,}10*10^8$	1500	343	39
24/7	37,30	17,57	74,59	7,12	41,44	1,68	24,67	$4{,}10*10^{10}$	$7{,}50*10^9$	$2{,}00*10^6$	$1{,}97*10^7$	ND	ND	ND

Table 3: Parameter control of TPOMW compost windrow. **M.B.**: Mesophilic Bacteria; **T.B.**: Thermophilic Bacteria; **N_2 Fix.**: Nitrogen-fixing bacteria; **M&Y**: Moulds and Yeasts. **ND:** Not Determined.

The implementation of targeted composting processes is beneficial in both cases, not only for the selection of microbial populations, but also in the enrichment of macro and micro nutrients that is observed in both cases with different starting materials. There are no significant differences in terms of the effect of the addition of the starter compost, showing in both compost that the majority of the identified microbial populations are the same in both cases, regardless of the initial addition of inoculum, which in the case of TPOMW would have served only as bulking agent.

Among the microbial populations identified, there are nitrogen fixatives (*Rhizobium pusense*), degradation of recalcitrants (*Nocardiopsis alba*, *Penicillium*, *Aspergillus*), solubilized products of Fe (*Sinorhodobacter ferrireducens*), producers of Indolacetic Acid (*Ochrobactrum pseudogrignonense*), phosphate solubilizers (*Meyerozyma guilliermondii*); which makes the product a complete fertilizer and biofertilizer, not so much because of its macro and micro nutrient values, but because the added value of the achieved microbial populations.

Bibliography.

1. Vivas, A., Moreno, B., Garcia-Rodriguez, S. & Benitez, E. Assessing the impact of composting and vermicomposting on bacterial community size and structure, and microbial functional diversity of an olive-mill waste. *Bioresour. Technol.* **100,** 1319–1326 (2009).
2. Romo-Sánchez, S., Alves-Baffi, M., Arévalo-Villena, M., Úbeda-Iranzo, J. & Briones-Pérez, A. Yeast biodiversity from oleic ecosystems: Study of their biotechnological properties. *Food Microbiol.* **27,** 487–492 (2010).
3. Liebgott, P. P., Labat, M., Casalot, L., Amouric, A. & Lorquin, J. Bioconversion of tyrosol into hydroxytyrosol and 3,4-dihydroxyphenylacetic acid under hypersaline conditions by the new Halomonas sp. strain HTB24. *FEMS Microbiol. Lett.* **276,** 26–33 (2007).
4. Liebgott, P. P., Labat, M., Amouric, A., Tholozan, J. L. & Lorquin, J. Tyrosol degradation via the homogentisic acid pathway in a newly isolated Halomonas strain from olive processing effluents. *J. Appl. Microbiol.* **105,** 2084–2095 (2008).
5. Santos, M. M., Piccirillo, C., Castro, P. M. L., Kalogerakis, N. & Pintado, M. E. Bioconversion of oleuropein to hydroxytyrosol by lactic acid bacteria. *World J. Microbiol. Biotechnol.* **28,** 2435–2440 (2012).
6. Yangui, T., Rhouma, A., Triki, M. A., Gargouri, K. & Bouzid, J. Control of damping-off caused by Rhizoctonia solani and Fusarium solani using olive mill waste water and some of its indigenous bacterial strains. *Crop Prot.* **27,** 189–197 (2008).
7. Hoitink, H. & Boehm, M. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. *Annu. Rev. Phytopathol.* **37,** 427–446 (1999).
8. Kavroulakis, N. *et al.* Role of ethylene in the protection of tomato plants against soil-borne fungal pathogens conferred by an endophytic Fusarium solani strain. *J. Exp. Bot.* **58,** 3853–3864 (2007).
9. Kavroulakis, N. & Ntougias, S. Bacterial and β-proteobacterial diversity in Olea europaea var. mastoidis- and O. europaea var. koroneiki-generated olive mill wastewaters: Influence of cultivation and harvesting practice on bacterial community structure. *World J. Microbiol. Biotechnol.* **27,** 57–66 (2011).