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Evolution of physical-chemical parameters, microbial diversity and VOC emissions of olive oil mill waste exposed to ambient conditions in open reservoirs

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ABSTRACT

In the olive oil extraction process, 20% olive oil is obtained. About 80% corresponds to waste, mainly alperujo and orujo. When these residues are stored in open reservoirs for later stabilization or potential reuse, odorous Volatile Organic Compounds (VOCs) are generated as products of waste decomposition. In this work, these emissions were studied by means of TD-GC/MS in relation to the changes in the physical-chemical (ashes, moisture, total phenols, pH, proteins, fibers, oils, fats) and biological parameters (bacterial and fungal diversity in Illumina platform) of waste for 6 months. The dynamics of these parameters were statistically related to the evolution of environmental variables (temperature, relative humidity, precipitation) and their effects on the most relevant physical-chemical parameters in order to evaluate their incidence in odorant VOCs emissions over time. The results showed a progressive increase in the diversity of both fungi and bacteria that were related, mainly, to a progressive decrease in the concentration of fatty acid methyl esters and the concentration of alkenes in the emissions; and to an increase of odorous compounds, mainly aldehydes, ketones and carboxylic acids, which were responsible for the unpleasant odors of waste. No significant differences were observed between the evolution of orujo characteristics compared to those of alperujo.

1. Introduction

Olive oil production has increased considerably worldwide in recent years. 3.2 million tons were produced in the 2015–2016 season according to data from the International Olive Council (2017), where Chile represents 0.8% of world production. In the olive oil extraction process, 20% olive oil is obtained while about 80% corresponds to waste, mainly alperujo and orujo (Hernández et al., 2014). Olive oil extraction can be carried out in three different ways in relation to the types and characteristics of the waste generated and the costs of the process: (a) traditional pressing, which generates oil, water and olive cake; (b) two phases, in which oil and alperujo are obtained; (c) three phases, in which oil, alpechin and orujo are obtained (Kapellakis et al., 2008). Currently, the two phases process is the most used in Chile and worldwide because less water is consumed, costs are reduced by 18% and no alpechin is generated, thus waste production is reduced (Espionola, 1997).

Alperujo and orujo are mixtures of water, oils, cellulose, lignin, proteins, carbohydrates, nitrogen, organic acids, pectins, tannins, polyalcohols and a small fraction of active phenolic compounds and other derivatives (Alburquerque et al., 2004). The alperujo is the main waste of the olive-growing process in the world and, due to its composition, its richness in organic matter can be even usable for the cogeneration of electrical energy, composting, biodiesel production, among others (Alburquerque et al., 2006; Caputo et al., 2003).

In Chile, the alperujo and orujo have no specific final destination, transforming them into a waste without use that accumulates in the same agricultural land where it is produced (oil mills) or stored in specially constructed reservoirs. Studies show that alperujo cannot be applied directly to the soil due to the derived phytotoxic effects that come mainly from the phenolic substances it has (Martín et al., 2002). Its direct use in the soil produces structural instability of the soil and in its microbial population (Sampedro et al., 2005). Therefore, alternatives such as pellets

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production have been proposed to convert such wastes into valuable products (Christoforou et al., 2016). However, storage of waste from winter to spring to reduce their moisture and volume before further processing generates an important environmental problem, that is the generation of undesirable odors that affect the surrounding populations. Such odor emissions from open reservoirs have not been characterized. In fact, there is a lack of scientific literature specifying which odorant molecules are generated from alperujo and orujo when they are stored in open reservoirs for long periods of time. As in other countries, air quality standards in Chile are set for compounds and emissions other than odor, thus no odor emissions nor immission limits must be accomplished currently. Emissions impact is clearly shown by the complaints of affected neighboring population, which are the driving force for stablishing measures to avoid emissions impact. Rappert and Müller (2005) showed that nuisance odors can have detrimental effects on aesthetics, property values, and the quality of life in communities and that air pollution control authorities dealing with odorous emissions from industrial, municipal and agricultural activities are often faced with many complaints from the public.

In terms of emissions characteristics, Nasini et al. (2016) reported emissions of Volatile Organic Compounds (VOCs) from olive mill waste as a consequence of olive oil production. As an example, Brenes et al. (2004) identified among others 4-ethylphenol in concentrations of 476 mg kg^{-1} as one of the compounds responsible for unpleasant odors in the olive paste stored for 8 months under uncontrolled conditions. The formation of 4-ethylphenol during the storage of alperujo has been related to the growth of lactic acid bacteria, particularly *Lactobacillus pentosus* (Castro et al., 2015). It has been described that, under certain conditions, alperujo adapted as a substrate allows the growth of microorganisms such as *Saccharomyces* sp., *Candida boidinii* and *Geotrichum candidum* (Giannoutsou et al., 2004). Middelhoven (2002) describes that the growth of certain species in olive brine, alpechin, and other olive products, are unique because of their chemical composition. In addition, growth of certain microorganisms in different olive oils stored in high moisture conditions has been reported. Fungi such as *Aspergillus* and *Penicillium* and others belonging to the genus *Candida*, *Pichia* and *Saccharomyces* found in olive oils are capable of oxidizing free fatty acid chains, reducing carbonyls and esterifying alkyl derivatives, producing fatty acids, aldehydes, alcohols, ketones. Such short chain are volatile compounds that lead to the generation of unpleasant odors and aromas of these products (Morales et al., 2005; Pérez-Camino et al., 2002).

Both, orujo and alperujo are waste products of the olive oil production process. The difference relies in the way that olive oil is extracted. Alperujo comes from a two-phase process, while orujo is the residual of a three-phase process. Nowadays, the worldwide trend is to use a two-phase process, thus generating alperujo. However, the three-phase process is still relevant in many countries. Consequently, analysis of the behavior of both wastes in an independent manner when they are stored in open reservoirs as well as to observe the differences in the physical-chemical and biological compositions as time proceeds is warranted. Literature reveals that the mechanisms of orujo and alperujo decomposition in relation to emissions and the microbial diversity in such residues has not been described. Therefore, the present research aims at studying the evolution of the physical-chemical and microbiological characteristics as well as the generation of VOCs from alperujo and orujo when they naturally degrade in storage reservoirs under uncontrolled ambient conditions. The influence of ambient conditions on the evolution of the characteristics of such residues and their microbial diversity in a period of 6 months from winter to spring were evaluated in order to correlate environmental and physical-chemical parameters.

2. Materials and methods

2.1. Samples

Three samples of each waste were obtained from two different oil mills: (a) Orujo, namely O, was obtained from the Almazara del Pacifico located in the Alto Pangue area, Talca, Chile; (b) Alperujo, namely A, was obtained from Agrícola y Forestal Don Rafael oil mill, Molina, Chile. Each oil mill processed the olive varieties named Arbequina, Leccino and Picual. Samples were extracted directly from the oil mills to fill a total of six 200 L plastic containers that simulated the waste storage in oil mill reservoirs. Each sample was identified and standardized to a mass of 150 kg and moved and stored under uncontrolled ambient conditions at the Universidad de Talca, Curicó, Chile. Since the olives processing period is between May and June, all containers were kept open and outdoors between June and November (winter to spring in the southern hemisphere), that is the period in which the oil mills store these residues.

In order to estimate the degradation of A and O over time, samples of each container were taken monthly for a period of 6 months using a 250 cm2 dredger sediment sampler (Van Veen, 12.110 model, Sidmar). For the initial samples of fresh wastes from oil mills, a homogeneous sample of 1 kg was taken from each container and mixed to form a single sample per type of fresh waste. The samples were analyzed in triplicate to determine the initial characteristics of each residue. For monthly samples, eight subsamples of 250 g each were obtained from the upper part of each container, namely AT and OT for A and O, respectively. Samples were extracted with the dredge and mixed to obtain a single representative sample. Similarly, eight subsamples of the intermediate part (half of the height and diameter of each reservoir) were mixed to obtain a single representative sample for further analyses, namely AM and OM for A and O, respectively. Then, a total of 4 kg per container and per sampling event were withdrawn from each container. Since the upper part of the container was in contact with air and the intermediate part was not, different heights of the containers were sampled to check for potential gradients of properties. However, fatty acids and volatile organic compounds identified over time and Illumina sequencing were performed with a 1:1 mixture of samples representative of the upper and the intermediate part of the containers.

2.2. Analytical methods

For samples of A and O, the following physical-chemical analyzes were carried out over time in triplicates using standardized methods according to DIN (Deutsches Institut für Normung, 2017) and AOAC norms (Official Methods of Analysis of AOAC Internatioanl, 20th Edition, 2017): Moisture content (oven method – AOAC 945.15), crude protein (Kjeldahl method – AOAC 979.09), measuring heating value (Norm DIN 51.900 Series), ashes (muffle method – AOAC 940.26), crude fiber (gravimetric method- AOAC 920.169), fats (Soxhlet method – AOAC 963.15), total phenolic (Folin-Ciocalteu method as gallic acid equivalents) and pH. In all cases, except pH and total phenolic compounds, samples were dried at 103 ± 2 °C in an oven (Memmert, model W02WVU).

For the fatty acids methyl esters (FAMEs) analysis, 1 g of each wet sample humidity of A and O was taken. Extraction was carried out at 20 °C in 20 mL of hexane (Sigma-Aldrich) in a 100 mL separating funnel. The 20 mL of the liquid extract were methylated with 10 mL of KOH in 2 M methanol (Merck) as described by Cert et al. (2000). The organic phase was extracted and brought to dryness using a rotavapor (Steroglass strike, model 300) to be finally reconstituted in 1 mL of hexane and analyzed in a Gas