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ScienceDirect
Journal of Hazardous Materials 145 (2007) 339–343
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Short communication

Chemical and physicochemical profile of wastewaters produced from the different stages of Spanish-style green olives processing

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Received 31 August 2006; received in revised form 21 November 2006; accepted 29 December 2006
Available online 3 January 2007

Abstract

The main purpose of the processing of table olives is the removal, at least partially, of the natural bitterness of the fruit in order to render it edible. The preparation of Spanish-style green olives after harvesting involves cleaning followed by debittering using NaOH solution, washing with water, a lactic acid fermentation step and finally canning. Wastewaters originating from table olives processing industries pose an important environmental threat, as they are characterized by a very high organic load and high concentration of phenolic compounds, which are toxic to living organisms. In this communication, the chemical and physicochemical profile of wastewaters produced from the different stages of Spanish-Style green olives processing was investigated. Phenolic compounds, organic acids, amino acids and total sugars along with common physicochemical parameters were determined in order to appraise the specific features of each individually produced wastewater.

Keywords: Debittering; Spanish-style green olives; Table olive wastewater; Brines; Composition

1. Introduction

There are two types of commercial table olives: green olives often referred to as Spanish-style and black olives. Spanish-style green olives account for ca. 40–50% of the world production. The world production of table olives, mainly concentrated in the Mediterranean region, is highly important for the economies of Spain, Italy, Greece, Turkey, Tunisia and Morocco, constituting a major agro-industrial activity [1,2]. Table olive's processing aims at the removal, at least partially, of the natural bitterness of the fruit mainly owing to oleuropein, a polyphenol existing only in the olive fruit, in order to render it edible.

The preparation of Spanish-style green olives initially involves harvesting and cleaning. Afterwards, olives are placed into tanks and soaked in a lye solution (1–2%, w/v, sodium hydroxide solution) for about 8–12 h to debitter. During this stage hydrolysis of oleuropein, which is labile under alkaline conditions, takes place [3]. Lye is allowed to penetrate through three-quarters of the flesh, leaving a small volume around the stone unaffected. This part of the flesh provides the necessary sugars for subsequent fermentation and confers to the olives a slight bitter taste. Next, the lye solution is drained off and olives are washed with water twice in order to remove excess lye. Today, due to environmental issues, in most cases, washing takes place only once and lasts about 12–14 h in order to reduce the overall volume of wastewaters produced throughout the process. Finally, tanks are drained off the washing water and olives are soaked in brine (4–8%, w/v, sodium chloride solution with lactic acid added for pH control). The remaining lye from the previous stage plays a crucial role in the subsequent fermentation stage as it forms a regulating solution with lactic acid. This solution ensures the regulating faculty of the brine, which is essential as it improves the organoleptic attributes of olives. At the same time, it promotes the growth of lactobacter *Lactobacillus* in the brine that realize the fermentation. A lactic fermentation step proceeds for about two months after which olives are ready for commercial use [4,5].

Throughout all stages of treatment, large quantities of clean water are used and wastewaters of about 3.9–7.5 m^3/t of olives, depending on the olive variety, are produced [1]. Table olive's processing wastewater (TOPW) constitutes an important environmental concern in Mediterranean countries as it is usually

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doi:10.1016/j.jhazmat.2006.12.061

discharged untreated to streams, creeks or directly to the sea [6,7]. In other cases, it is transported to evaporation ponds, where malodours are a common nuisance, while the risk of polluting surface or ground waters is not always ruled out [1,8,9].

By its nature, TOPW is a turbid, dark effluent that carries a high organic burden and polyphenol compounds, which confer a sharp characteristic odor. It displays antibacterial properties, inhibits seed germination, it is phytotoxic and is characterized by a very high COD value. The organic fraction contains a complex consortium of sugars and phenolic compounds, particularly catechol, tyrosol, 4-methylcatechol and hydroxytyrosol, some nitrogenous compounds (especially amino acids), organic acids, tannins, pectins, carotenoids and oil residues. The inorganic fraction contains chloride, sulfate and phosphoric salts of potassium as well as calcium, iron, magnesium, sodium, copper and other trace elements in various chemical forms. TOPW's phytotoxicity is due to the phenolic substances and some organic acids, such as acetic and formic acid, which are often produced along with other microbial metabolites during storage.

Over the last years several methods have been proposed for the treatment of this type of wastewaters. Biological treatment methods have been recognized as overall economical and effective processes [9,10]. Also, chemical treatment methods using a strong oxidative agent, such as ozone, Fenton's reagent, a mixture of hydrogen peroxide and ferrous or ferric iron, a combination of UV radiation and hydrogen peroxide as well as photo-Fenton [11–13] together with combined methods (i.e. chemical and biological) have been put forward as alternatives [7,14].

The purpose of this study is to obtain, for the first time, an estimate of the profile of the wastewaters produced from the different stages of the processing of Spanish-style green olives in order to appraise their specific features, physicochemical and chemical characteristics. Wastewater's parameters, such as pH, COD, BOD_5, electrical conductivity, colour, total phenols and sugars were measured along with the chromatographic determination of 15 phenolic acids, 10 organic acids and 3 amino acids and useful conclusions were drawn.

2. Materials and methods

2.1. Wastewater and chemicals used

Fresh debittering, washing and brine wastewaters (TOPW) were obtained from the plant of the Agro-industrial Cooperation of Stylida (Lamia, Central Greece) – processing capacity: 5.000 t/year – during the olive harvesting period of 2004–2005. They were stored immediately at −20 °C to avoid the auto-oxidation and subsequent polymerisation of the phenolic compounds and tannins. Wastewaters were taken from one tank, from the same batch of olives, which were debittered for about 8 h, washed once with water for about 12 h and then fermented for approximately 2 months. For the laboratory experiments, all chemicals were obtained from Sigma–Aldrich (Sigma–Aldrich Hellas). Hydroxytyrosol was synthesised according to the method proposed by Balardi et al. [15].

2.2. Apparatus

Conductivity and pH were measured using a conductivity/TDS meter and a pH-meter both from Hach (HACH Co, Loveland, CO, USA). For BOD analysis, the manometric method was employed with the aid of Hach BOD Trak model 2173B. A single-beam Hach spectrophotometer DR/2010 was used throughout the study for the analysis of colour and total phenols. The same spectrophotometer was used for the COD analysis using the sulfuric acid-potassium dichromate method after incubation in a Hach COD reactor.

Gas chromatographic analyses were performed using a Shimadzu GC-17A (Shimadzu Corp., Kyoto, Japan) chromatograph equipped with a flame ionization detector and a SPB-5 capillary column, 30 m × 0.32 mm, thickness 0.45 μm, operating in the on-column injection mode. Peak identification was feasible by way of gas chromatography–mass-spectrometry (Shimadzu GC-17A gas chromatograph interfaced with a QP 5000 mass spectrometer). Finally, HPLC measurements were performed using a Shimadzu LC-10AD liquid chromatograph equipped with an MZ Analyzentechnik C_{18} column (30 cm × 3 mm, particle diameter 5 μm) and a UV detector.

2.3. Analytical methods

The method used for the extraction of organic components is outlined in the schematic of Fig. 1 and constitutes a modification of the analytical scheme proposed by Piperidou et al. [6]. Total phenols (simple phenolic and polyphenolic compounds) were measured according to the Folin–Ciocalteau method directly in the wastewater of each stage. Samples were diluted accordingly, so as the absorbances to be within the equipment's range of measurement. Results were expressed as ppm equivalent of gallic acid [16].

Electrical conductivity, pH, COD, BOD_5 were determined according to standard protocols [17]. The colour of the three wastewaters was determined by the difference of absorbances at 440 and 700 nm in 1-cm pathlength cells [18]. Finally, hexozes, pentozes and uronic acid were determined using the sulfuric acid–phenol method and expressed as ppm equivalent of glucose [19]. Analyses were performed in triplicate, and the results are given as mean values. Relative standard deviations for inorganic analyses do not exceed 2.5%, while for organic analyses it lies around 5%.

2.4. Chromatography

All phenolic and organic acids (except formic, acetic and butyric acid) were determined by GC/FID after derivatization with BSTFA. The operating parameters of the gas chromatograph were as follows: detector temperature, 280 °C; injector temperature, 240 °C; oven temperature, 50 °C (hold 2 min), 7 °C/min to 280 °C (hold-up time: 10 min). Helium was used as a carrier gas regulated at 1.0 ml/min [6].

Amino acids were determined after derivatization with PITC reagent by HPLC-UV at 254 nm with the chromatographic column thermostated at 43 °C. The mobile phase consisted of

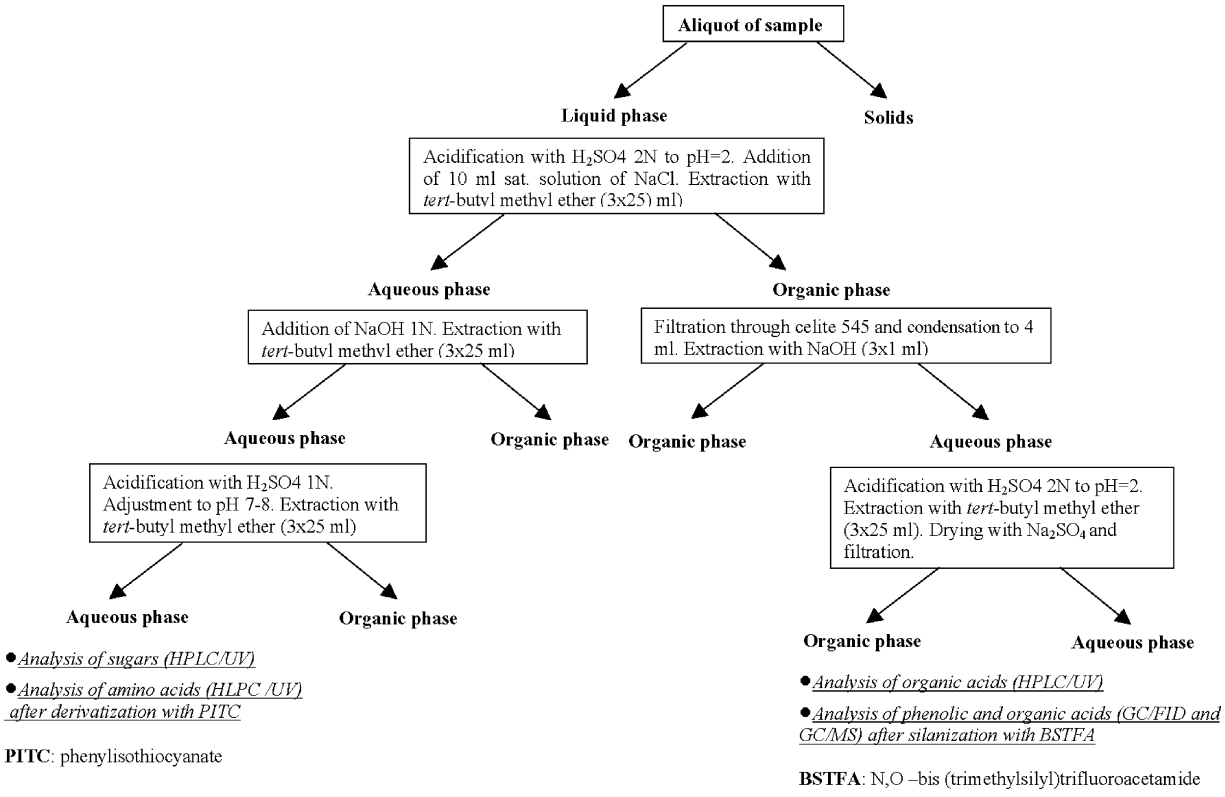

Fig. 1. Outline of the employed pre-treatment method.

solvent A: 0.1 M ammonium acetate (pH 6.5) and solvent B: 0.1 M ammonium acetate (pH 6.5)/acetonitrile/methanol at a ratio of 44:46:10. The flow rate was maintained at 1 ml/min and the separation was achieved following a gradient of 100% A to 100% B, in 25 min [20].

Finally, formic, acetic and butyric acid were determined by HPLC-UV at 210 nm with the column thermostated at 25 °C. The mobile phase was maintained at a nominal flow rate of 0.5 ml/min and consisted of 0.02 M monosodium dihydrogen phosphate-phosphoric acid solution (pH 2.5) [21].

3. Results and discussion

Although the basic characteristics of typical Spanish-Style green olives processing wastewaters, such as COD, BOD, suspended and dissolved solids values have been studied previously [1] no information exists which delineates their chemical composition. Table 1 shows the results from the analysis of common physicochemical parameters and chemical analysis of the typical wastewaters from the three stages of Spanish-Style green olives processing. The wastewater from the fermentation bears the highest COD value albeit colour is fader and its concentration in total phenols slightly lower. This is mainly accounted for by the elevated content in organic acids, as compared to the rest of the olive processing wastewaters. After the debittering and washing steps, the olive fruit loose the major part of its phenolic content. The remainder is transferred to the brine and transformed into nutrients for the development of lactobacters in a subsequent step. Formic and acetic acid are present at high concentrations during lactic fermentation being the main products of the action of the lactobacter genus *Lactobacillus* [22]. Lactic acid is the major product of the fermentation stage due to the action of lactobacters, which transform olive's sugars into lactic acid. Likewise, the elevated content in D,L-malic acid is attributed to the lactobacter *Leuconostoc* developed at the first stages of lactic fermentation before the final prevalence of *Lactobacillus* which virtually realizes the fermentation.

It is worth noting that tyrosol and hydroxytyrosol are present at comparable levels in all three wastewaters of the process. Hydroxytyrosol is the main product of the hydrolysis of oleuropein, which occurs during the debittering stage and it is principally removed during the washing stage. That explains its presence in larger amounts in the waste of the washing stage. The concentration of tyrosol increases sharply after brining, indicating that it is formed during alkaline processing.

Washing step is performed essentially with the aim to remove the NaOH excess. Results from this study show that total phenols are higher in the wastewaters of the washing step than those of debittering ones. This point is of great interest as it reveals that hydrolysis of complex phenols continues to occur in the olives during the washing step. Predictably, the composition of washing wastewaters suggests that the washing step is active in the green olive processing as regards the biochemical alterations.

Table 1
Common physicochemical parameters and chemical analysis of wastewaters from the three stages of Spanish-Style green olives processing

		Debittering process wastewater	Washing process wastewater	Fermentation process wastewater
1	COD (mg/l)	9,390	13,630	18,910
2	BOD_5 (mg/l)	3,115	4,640	6,050
3	pH	12.99	11.52	4.30
4	Electrical conductivity (ms/cm)	11.13	10.17	53.10
5	Colour	1.96	1.44	0.49
6	Total phenols (mg/l)	211.2	446.1	182.1
7	Phenolic compounds (mg/l)			
	Benzoic acid	0.93	1.50	0.80
	2-Phenoxyethanol	1.39	2.76	0.27
	trans-Cinnamic acid	1.67	1.23	N.D
	4-Hydroxyphenyl ethanol (tyrosol)	16.33	47.40	16.83
	D-3-Phenylacetic acid	8.09	1.79	1.69
	3,4-Dimethoxybenzoic acid	1.86	10.91	3.96
	Vanillic acid	5.55	5.43	2.69
	3,4-Hydroxyphenyl ethanol (hydroxytyrosol)	9.69	16.21	6.09
	3,4-Dihydroxybenzoic acid	10.26	1.52	0.26
	Syringic acid	N.D	10.25	4.59
	4-Hydroxycinnamic acid	4.18	6.00	1.33
	Dibutyl phthalate	7.03	9.71	32.82
	Gallic acid	5.09	0.96	3.89
	Ferullic acid	2.00	1.56	1.00
	Caffeic acid	6.32	N.D	0.19
	Total	80.39	117.23	76.41
8	Organic acids (mg/l)			
	Oxalic acid	4.21	6.19	0.98
	Cyclohexane carboxylic acid	5.00	N.D	N.D
	D,L-lactic acid	N.D	6.64	63.66
	D,L-malic acid	1.21	2.18	44.34
	Citric acid	2.48	2.86	2.40
	Palmitic acid	4.28	5.02	0.25
	Oleic acid	2.53	N.D	0.29
	Formic acid	3.77	4.31	25.31
	Acetic acid	N.D	N.D	20.00
	Butyric acid	3.90	N.D	N.D
	Total	27.38	27.20	157.23
9	Amino acids (mg/l)			
	Aspartic acid	14.52	21.36	7.20
	Glutamic acid	9.10	13.39	4.11
	Arginine	8.20	11.56	2.43
	Total	31.82	46.31	13.74
10	Total sugars (mg/l)			
	Hexozes	72.44	94.86	32.12
	Pentozes and uronic acid	76.89	98.54	31.87

N.D: Not detected. Detection limits: benzoic acid = 0.005 mg/l, 2-phenoxyethanol = 0.006 mg/l, *trans*-cinnamic acid = 0.006 mg/l, 4-hydroxyphenyl ethanol = 0.004 mg/l, D-3-phenylacetic acid = 0.007 mg/l, 3,4-dimethoxybenzoic acid = 0.005 mg/l, vannilic acid = 0.006 mg/l, 3,4-hydroxyphenyl ethanol = 0.004 mg/l, 3,4-dihydroxybenzoic acid = 0.005 mg/l, syringic acid = 0.007 mg/l, 4-hydroxycinnamic acid = 0.006 mg/l, dibutyl phtalate = 0.008 mg/l, gallic acid = 0.004 mg/l, ferullic acid = 0.004 mg/l, caffeic acid = 0.004 mg/l, oxalic acid = 0.011 mg/l, cyclohexane carboxylic acid = 0.006 mg/l, D,L-lactic acid = 0.005 mg/l, D,L-malic acid = 0.005 mg/l, citric acid = 0.008 mg/l, palmitic acid = 0.006 mg/l, oleic acid = 0.007 mg/l, formic acid = 0.011 mg/l, acetic acid = 0.015 mg/l, butyric acid = 0.011 mg/l, aspatric acid = 0.009 mg/l, glutamic acid = 0.006 mg/l, arginine = 0.008 mg/l.

Although sugar composition in olive tissues has been studied previously [23] no information exists on the changes of sugars during the olive processing. It is clearly seen that during fermentation sugar levels diminish after a slight increase during the washing process. The diminution of sugar concentration is attributed to the action of lactobacters deployed in the brine, which transform them to lactic acid. In a similar manner, amino acids are consumed during fermentation, as they constitute nutrient for lactobacters. During fermentation, lactobacters deployed in the brine transform fruit's sugars into lactic acid until its concentration reaches 0.8–1% (w/v) and pH decreases to 3.8–4.0, thus terminating the fermentation.

o-Dibutyl phthalate is observed to be at high concentrations throughout in common throughout all three stages. This is due to the construction material of polyester tanks and has nothing to do with the processing itself. Lower pH and higher residence

time during lactic fermentation step give rise to significantly higher amounts of the aforementioned ester at the end of the overall process.

There is a dearth of information in the literature focused on the composition of such wastewaters from the three processing stages. Taking into consideration that:

(1) The basic characteristics of typical Spanish-Style green olives processing wastewaters, such as COD, BOD vary within a limited range [1].
(2) Total polyphenol and phenolic compounds content of industrially fermented table olives as well as their chemical composition scarcely depends on the industrial process chosen to be followed, on the degree of ripeness of the olives and the environment of the olive cultivars [22,24], we can conclude that the composition of the wastewaters produced from Spanish-Style green olives processing also slightly depends on the factors abovementioned and their basic chemical characteristics do not significantly vary. The wastewater that results from edible olive production process is similar in nature to the olive-mill wastewater, albeit somewhat weaker in organic strength [6]. Nonetheless, it is characterized by a high organic content and is generated in large quantities at a specific time period of the year.

4. Conclusions

As indicated by the results obtained, washing step is an active step of green table olive's processing where further bio-chemical events occur. This could be of particular interest from food-technological and environmental point of view.

The knowledge of chemical and physicochemical profile of the wastewaters produced from the different stages of Spanish-style green olives processing can be a guide for the integrated management of the overall wastewaters produced from table olives industries. By properly mixing wastes from the individual olive processing steps, wastewaters of known characteristics can be generated. This practice can constitute a useful tool for designing and optimizing ways for treatment, as the action of micro-organisms during biological treatment and/or chemical oxidation is performed optimally under constant wastewater composition, giving higher remediation yields.

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