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ORIGINAL PAPER

Protein increase and lysine production by a *Paecilomyces variotii* strain grown on two-phase olive mill waste

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Abstract Two-phase olive-mill waste, the so-called "ecological", has been treated with a *Paecilomyces variotii* isolate in solid state fermentation experiments. The growth of the microorganism was estimated by measuring the production of carbon dioxide, using gas chromatography. A 46% increase of the protein content was achieved at the fermented product, after molasses addition at the initial mixture. The amino acid profile of the produced protein, as far as the essential amino acids are concerned, was significantly improved, resulting in a product that has the potential to be used as animal feed. Furthermore, it contains lysine, one of the essential amino acids that did not exist at the original product and is produced during fermentation. This is the first report on solid state fermentation of the two-phase olive mill waste (TPOMW) as a substrate, using a *Paecilomyces variotii* strain.

Keywords Two-phase olive mill waste · Microbial treatments · Solid state fermentation · *Paecilomyces* sp

Introduction

Olive mill wastes have been, and unfortunately continue to be, the most difficult agricultural waste to handle throughout the Mediterranean countries. Since the early 1990s, the two-phase extraction system was introduced as a new methodology and resulted in the production of a new semi-solid waste, called two-phase olive mill waste (TPOMW). This new extraction system has several advantages over the traditional press and the three phase system: reduction of the produced waste since water consumption decreases considerably, higher oil yield and less energy consumption. The main disadvantage of the two-phase system waste is its high moisture which causes difficulties in the transportation, drying and further treatment at oil refineries (Arjona et al. 1999). Besides this, the higher temperatures applied for the drying of this waste has led to the release of carcinogenic substances in Spain, called benzopyrenes (Roig et al. 2006).

The management of the two phase olive mill waste requires specific facilities, such as storage tanks with special valves, mass pumps and tank trucks (Roig et al. 2006). The valorization alternatives that have been applied for the treatment of this new waste include several methodologies. The main physicochemical treatments is drying and second extraction of the remaining oil, while the exhausted olive cake is used as a fuel for the production of electric or thermal energy (Caputo et al. 2003; Krokida et al. 2002). In addition, its high C/N ratio causes problems for its use as soil amendment, since it interferes with the nitrogen cycle in soil (Thompson and Nogales 1999). The anaerobic biodegradation, which was largely used for the treatment of the three-phase olive mill waste, is another alternative (Morillo et al. 2009; Rincon et al. 2008). The high level of phenolic compounds of this type of waste limits the possibility of the application of the above method (Borja et al. 2003). The extraction of valuable products from the waste (p.e. pectins, biophenols) has been studied by several workers (Boucid et al. 2005; Morillo et al. 2009). The most feasible treatment method of this waste seems to be the composting and its subsequent soil application. Although the composted material shows a complete detoxification of phenols, a good degree of humification and many mineral nutrients, there are

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certain problems that need to be surpassed in order to use it as soil amendment. One of these problems is the high pH reached after composting, which can be controlled with the addition of elementary sulphur (Roig et al. 2004).

Aloui and his co-workers (2007) investigated the possibility of using TPOMW as a substrate on solid state fermentation technologies. Until today, there are few reports on solid state fermentation experiments using the press cake as substrate. Most of them have used strains of *Phanerochaete chrysosporium*, *Geotrichum candidum* or *Pleurotus* spp. as inoculum, focusing on the delignification and the decolourisation processes (Ayed et al. 2005; Haddadin et al. 1999; Sampedro et al. 2007; Tsioulpas et al. 2002). Haddadin et al. (1999) have focused on the production of microbial protein after fermentation of waste pomace from the press system with various fungal strains. Molina Alcaide et al. (2003) have mentioned the role of lysine in the final product. Lysine, as an essential aminoacid, cannot be synthesized by animals and has to be included in their feed. These workers have stressed out that the deficiency of the protein produced on this aminoacid leads to the supplementation with other protein substrates. In our work, a *Paecilomyces variotii* isolate was used as inoculum in solid state fermentation experiments where TPOMW was the substrate. Our aim was to have a treatment that would lead in the release of a more friendly waste at the environment. The protein content of the final product was significantly increased and in general was accompanied by an improvement in the aminoacid profile.

Materials and methods

Isolation and identification of the strain

The *Paecilomyces variotii* ALPF1 strain was isolated from TPOMW (supplied by Dr S. Hrushka, Westfalia Co., Oelde, Germany), as described by Giannoutsou et al. (2004). The isolate was grown on Malt Extract Agar (MEA) (Merck, Darmstadt, Germany) and Czapek Agar (CzA) (Merck, Darmstadt, Germany) in order to study the colony colour and texture. Cells, from a pure culture of 48 h, were examined microscopically (magnification × 800) for the examination of micro morphological characteristics such as the shape of phialides and spores. Chromosomal DNA was extracted by the method of Diallinas and Scazzocchio (1989) and the 18S rRNA gene was amplified by PCR using the primers NS1 and NS8 as described by White et al. (1990). The PCR product was purified using a Nucleospin Extract II kit (Macherey–Nagel) according to the manufacturer's instruction. Direct sequencing of the purified PCR product was performed by Macrogen (Korea). The sequences were aligned, by using the BLAST program, with complete or nearly complete 18S rDNA gene sequences retrieved from the NCBI nucleotide sequence data libraries.

Physiological growth tests

In order to investigate the temperature and the pH range of the growth of *Paecilomyces variotii* strain, it was grown in Malt Extract broth (Merck, Darmstadt, Germany) liquid cultures for 48 h. In order to study the growth at different temperatures, a wide range from 20 to 55°C has been applied (pH 5). The ability of the microorganism to grow under various pH values was examined at 35°C.

In order to investigate the ability of the strain to grow on molasses, this waste was diluted in water giving various sucrose concentrations (1, 2.5, 5, 7.5, 10, 12.5, 15, 20, 25 w/v). The strain was grown on the diluted molasses at 35°C and pH 5.4 for 48 h.

TPOMW treatment

The TPOMW samples derived from two-phase decanters for olive oil production (supplied by Westfalia Separator, Oelde, Germany). Dried TPOMW (A5) derived from fresh TPOMW (A1) after the following procedure: (1) extraction of pits (2) removal of residual oil and (3) heating at 400°C and subsequent drying. This dried TPOMW was used as substrate on SSF experiments that followed.

Observation using scanning electron microscopy

In order to study *Paecilomyces variotii* isolate under S.E.M., the microorganism was grown on MEA. Before the complete solidification of the plates, a round coverslip (diameter 8 mm) was submerged into the agar plate, forming a 45° angle with the plate surface. Plates were inoculated with 30 μl of spore suspension and were incubated at 35°C for 48 h. All coverslips were carefully removed and then placed in a special case using Electrodag 915 (Acheson Colloids Company, Prince Rock). The samples were dried for 12–36 h in a silica gel drier, and then they were covered with a gold–palladium layer under vacuum, using E5200 Auto Sputter Coater (1200 V, 240 s). Finally, the samples were observed using a JSM-T33OA Scanning Microscope (JEOL).

Solid state fermentation system and automatic CO_2 measurement

A solid state system has been developed in our lab to be used in the examination of growth and activity of selected strains of yeasts and fungi under controlled conditions. This system which consists of 16 fermentation columns was designed to

allow temperature regulation by submersion of the column in a water bath, precise regulation of air flux and possibility of continuous analysis of gaseous effluent. Air, obtained from a compressor was filtered through a submicronic filter. The pressure was set by a pressure regulator and was distributed to feed 16 independent bioreactors of 250 ml each. On the bottom section, the bioreactor was composed by a glass humidifier with an air nozzle and water feeding, while the glass fermentation column was on the top part. The level of water in the humidifier was maintained at a constant value and temperature. Each fermentation column outlet was connected to a silica gel dessicator, than to an air flowmeter, and finally to a chilled water condenser. The condenser and dessicator remove the excess humidity, allowing the analysis of gaseous effluents by a gas analyser. In order to study the metabolic activity of the microorganism, the CO_2 production has been measured using a gas chromatograph connected to the solid state fermentation system. A 16 valve switch allowed the continuous automatic CO_2 measurement of all 16 fermentation columns consecutively on a 24 h basis (Saucedo-Castaneda and Trejo-Hernandez 1994).

Analytical methods

Sugar and phenol extraction was performed as described by Lambraki et al. (1994). Determination of total sugars was made according to the method of Dubois et al. (1956), while total phenols were determined with the Folin–Ciocalteau's method (Makkar et al. 1988). Total nitrogen (TN) and protein nitrogen (PN) was estimated by the Kjeldhal method (Giannoutsou et al. 2004). Total lipids were determined by the method of Bligh and Dyer (1959), while pH was measured as described by Ohlinger (1996).

Detection of aminoacids in TPOMW samples

Preparation of reagents and solvents as well as protein hydrolysates were performed as described by the protocol of Heems et al. (1998). Aminoacids derivatisation and detection has been performed as described by Heems et al. (1998), using an Hypersil ODS column (5 μm; 250 × 4.0 mm) (Life Sciences International). The two internal standards used, norvaline (I.S.1) and thioproline (I.S.2) were prepared as described by Heems et al. (1998). A simple binary gradient elution was performed from 100% mobile phase A (10 mM phosphate buffer was adjusted to pH 7.5 with phosphoric acid and completed with 0.8% of tetrahydrofuran) to 70% of mobile phase B [mobile phase A–methanol–acetonitrile (20:50:30, v/v)] over 24 min, at 1.2 ml/min, the column was then regenerated with 100% mobile phase B during 5 min and before returning to initial conditions.

Statistical analysis

All the points on graphs and tables are the means of three replicate samples. Statistical analyses were performed using MINITAB statistical package (Minitab Statistical Software. State College. Pa.) Minimum significant differences (MSD) were calculated from analysis of variance using the Tukey–Kramer method (Fry 1989).

Results and discussion

Strain characterization

Paecilomyces variotii ALPF1 colonies were flat and velvety in texture. The color was yellow brown (Code 4D6) on MEA and MA media and olive yellow (Code 3D6) on PDA medium (Kornerup and Wanscher 1978). Colony diameter reached 60 mm after 4 days of growth on the above media.

Septate hyaline hyphae of the strain ended in conidiophores that were arranged in branches. Conidiophores carried the phialides at their tips (Fig. 1a). The phialides were swollen at their bases (10–27 × 3.0–5.0 μm) and taper towards their apices (1.0–2.0 μm). Conidia were unicellular, hyaline, smooth and formed long chains. Chlamydospores were not observed under the specific growth conditions.

18S rRNA gene sequence data showed that the strain was closely related to *Paecilomyces variotii, Talaromyces spectabilis, Byssochlamys zollerniae* and *Byssochlamys fulva*. Since sexual reproduction (formation of ascospores) was not observed, the strain should be closely affiliated to *Paecilomyces variotii*. Sequence retrieved from this study has the GenBank Accession Number EU878301.1 and the results of its comparison with the existing databases gave a 99% sequence similarity with the *Paecilomyces variotii* strain CBS 102.74 (GenBank Accession Number AY526477.2).

SSF of fresh and dried TPOMW without molasses using *Paecilomyces variotii* strain

The specific *Paecilomyces variotii* isolate showed a remarkable capacity to grow on a wide range of pH and temperature (Fig. 2). As it can be seen in Fig. 2, the strain seems to grow better at around 35°C. Since the strain grows well at acidic pH values (Fig. 2), there was no correction of the initial pH of the culture which was equal to the value of 5.4.

In Table 1, the results of the chemical analyses of the unfermented and the fermented wastes without any addition of mollases during the fermentation are shown. Two different kinds of TPOMW wastes have been used as substrates: fresh TPOMW (A1) and dried (A5). In both wastes tested, the fermented product exhibited a decrease in the lipid and sugar content and a considerable increase in

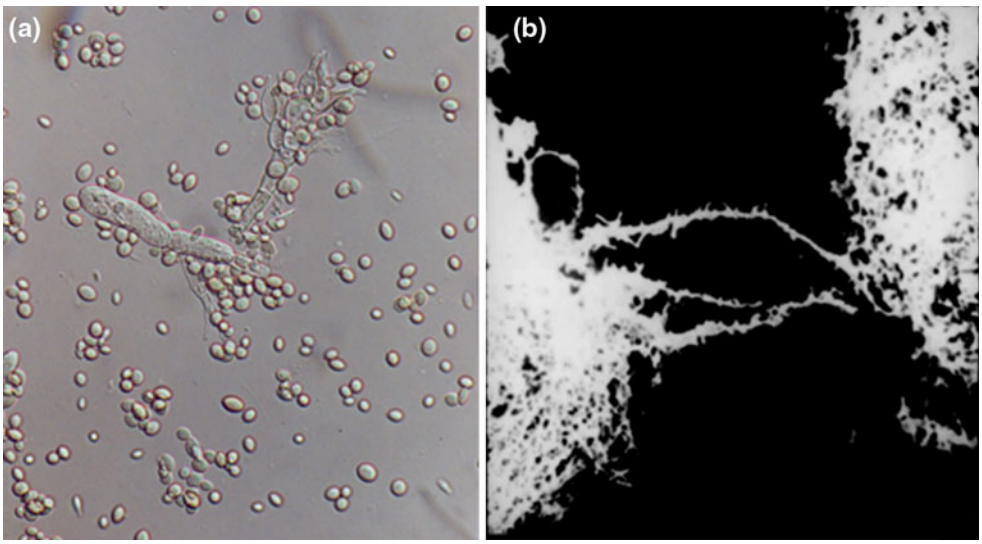

Fig. 1 **a** *Paecilomyces variotii* conidiophores carrying the phialides at their tips. Unicellular, hyaline, smooth conidiospores (magnitude × 800). **b** Scanning Electron Microscopy photograph of *Paecilomyces variotii* conidiospores and hyphae connecting two parts of fermented waste (magnitude × 120)

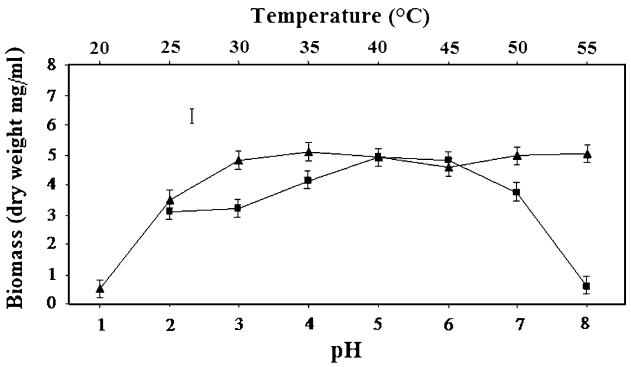

Fig. 2 The influence of pH (*filled rectangle*) and temperature (*filed triangle*) on the growth of *Paecilomyces variotii* ALPF1 in liquid cultures

the protein content (Table 1). Results showed that the dried waste can be used as substrate for SSF experiments as well as the fresh TPOMW, and the final fermented product has increased its protein content by 20%, without any addition of sugar beet molasses (Table 1; Fig. 1b). There are many advantages in the use of the dried TPOMW as substrate for SSF experiments: Firstly, stones (pits) have already been removed and can be used for energy production. Secondly, residual oil of low quality can be recovered and last, there is a significant reduction of the waste volume: 1,000 kg of fresh TPOMW gives 210 kg of dried (Westfalia Separator). It is known that one of the problems of the two phase extraction technique is the large volume of the resulting waste. With this procedure there is a significant decrease of the waste volume that is finally released at the environment.

Survival and metabolic activity of *Paecilomyces variotii* strain in TPOMW SSF systems

In order to improve its nutritional value, TPOMW was enriched with diluted sugar beet molasses. Molasses is a cheap, renewable industrial by-product with a very high sugar concentration, which is mainly used in animal feeding (about 60% of total molasses) as feed ingredient, pelleting aid or ensiling agent (http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/env-jm-mono (2002). Studies in liquid cultures of different sucrose concentrations of diluted sugar beet molasses showed higher biomass yield of *Paecilomyces variotii* at a 12.5% (w/v) sugar beet concentration (Fig. 3).

Solid state fermentation experiments were performed using TPOMW as substrate and adjusting the Water Holding Capacity (W.H.C) to 55%, by addition of diluted sugar beet molasses with sucrose concentration 12.5% (w/v). In order to study the metabolic activity of the microorganism, the CO_2 production has been measured using a gas chromatograph connected to the solid fermentation system. All the columns were submerged in a water bath, whose temperature was set up at 35°C and kept constant throughout the fermentation. The study of the respiration of *Paecilomyces variotii* strain growing on TPOMW–molasses medium showed that the metabolic activity was intense between 5 and 10 h of fermentation, when spore germination and extended mycelial growth occured (Fig. 4). From the 10th h up to the 20th h, there was a progressive decrease of the metabolic activity which implied a decline of the mycelial growth rate. The metabolic activity could be detected throughout the fermentation process indicating

Table 1 Chemical analysis on the unfermented and fermented samples of fresh and dried TPOMW

Fermentation duration: 4 days, W.H.C: 55% and Temperature: 35°C. Standard errors were calculated by common numerical analysis

Chemical analysis	Fresh TPOMW		Dried TPOMW	
	Unfermented	Fermented	Unfermented	Fermented
Total sugars (% w/w)	1.29 ± 0.3	1.01 ± 0.2	1.87 ± 0.3	1.28 ± 0.3
Phenols (% w/w)	1.25 ± 0.2	1.27 ± 0.2	1.33 ± 0.2	1.72 ± 0.2
Total lipids (% w/w)	7.46 ± 0.5	5.95 ± 0.5	12.48 ± 0.8	6.03 ± 0.5
Proteins (% w/w)	14.7 ± 0.9	17.28 ± 0.8	15.96 ± 0.7	19.12 ± 0.9

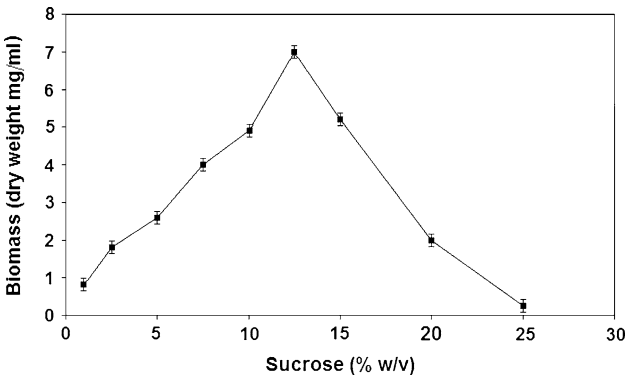

Fig. 3 The influence of different sucrose concentrations of diluted sugar beet molasses on the growth of *Paecilomyces variotii* ALPF1 in liquid cultures

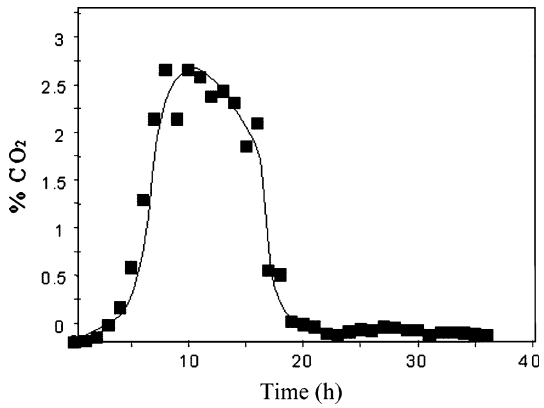

Fig. 4 Metabolic activity of *Paecilomyces variotii* ALPF1 expressed as % of CO_2 production grown on TPOMW–molasses medium at 35°C and W.H.C. 55%

that the microorganism remained active until the end of the experiment. Similar CO_2 curves have been described by Valmaseda et al. (1991b) in solid state fermentation experiments of wheat straw by *Trametes versicolor* and *Pleurotus ostreatus* strains. Two phases could be defined during fermentation of TPOMW, as well as wheat straw. The "colonization phase" was characterized by a strong increase in respiratory activity and decrease in free sugars and its duration was estimated to be 10 h for *Paecilomyces variotii* strain. The "degradation phase", which was generally characterised by fungal attack on polysaccharides, was accompanied by a decrease in growth rate and an increase in protein content (Fig. 4).

Changes in chemical analysis of TPOMW enriched with molasses after SSF fermentation, protein production and improvement of aminoacid profile of TPOMW

Samples were taken every 2 days and were chemically tested in order to define the differences that occurred in the waste during the fermentation procedure. All fermentation products were chemically tested and the results were compared with those of the original unfermented TPOMW (Table 2). Sugar concentration decreased rapidly after 2 days of fermentation, which means that *Paecilomyces variotii* used firstly all sugars that were available for its growth. Total lipid concentration decreased in the fermented products about 40%, while phenol concentration seemed to increase slightly. This slight increase in the phenolic content may be the result of the degradation of polyphenols to smaller phenolic compounds. This may really be an indication that the strain was actually active and could use the specific substrate for its growth.

The protein concentration in the fermented product increased from 14.75% in the initial dried TPOMW sample to 21.65% within the 10 days of fermentation. This represented a 46% increase in the protein content, which was a very promising result for the potential of the substrate to be used as an animal feed. It is obvious that, although the protein content of the waste increased by 20% after fermentation with the specific strain without any addition of molasses, they seem to offer the nutritional sources needed in order to increase the final protein content from 20 to 46%.

It is interesting to examine the initial aminoacid composition of the waste and the changes that occurred during fermentation. Apart from the protein increase, there was a change in the profile of the protein content after the fermentation showing that the product has a different aminoacid composition from that of the unfermented TPOMW. In previous similar work, Almeida e Silva et al. (1995) have used a *Paecilomyces variotii* strain for the fermentation of lignocellulosic material and concluded that the fermented product contained a really high quality protein,

Table 2 Chemical analysis on the fermented samples of TPOMW and comparison with the unfermented

Chemical analysis	Unfermented TPOMW sample		Fermented TPOMW sample				
	0 days (without molasses)	0 days (with molasses)	2 days	4 days	6 days	8 days	10 days
Total sugars (% w/w)	0.90 ± 0.67	7.54 ± 1.5	1.54 ± 0.35	1.68 ± 0.65	1.90 ± 0.42	1.67 ± 0.57	1.63 ± 0.52
Phenols (% w/w)	1.56 ± 0.08	1.50 ± 0.08	1.57 ± 0.1	1.54 ± 0.07	1.61 ± 0.11	1.71 ± 0.05	1.86 ± 0.1
Total lipids (% w/w)	10.20 ± 0.8	10.90 ± 1.0	8.92 ± 0.8	9.45 ± 0.9	7.72 ± 0.6	7.27 ± 0.5	6.07 ± 0.7
Proteins (% w/w)	14.75 ± 0.5	16.37 ± 0.5	18.15 ± 0.4	19.68 ± 0.6	19.46 ± 0.3	21.21 ± 0.6	21.65 ± 0.5

Duration of the fermentation: 2,4,6,8 and 10 days, W.H.C: 55% and Temperature: 35°C. Standard errors were calculated by common numerical analysis

regarding the aminoacid profile required for animal feeding.

Increase in quantities and change in profile of the present aminoacids of TPOMW has been observed in the fermented product due to the growth of the fungus (Fig. 5). The change in the content of aminoacids in untreated TPOMW and in the fermented product is shown in Table 3. As far as the FAO listed aminoacids are concerned, the higher increase appeared in methionine, whose amount was quadrupled in the protein of the fermented product, while threonine increased by 70% and valine by 51%. A lower increase appeared in isoleucine (45%) and aspartic acid (44%). It can also be observed that in the fermented product, we have the appearance of lysine (2.7 mg/g dry matter) which was absent in the unfermented waste. These results are in accordance with the work of Laconi et al. (2007) who used various fungal strains (4 *Pleurotus* sp., *Saccharomyces cerevisiae* and *Kluyveromyces lactis*) to produce microbial biomass on Olive Mill WasteWater (OMWW). In the dehydrated microbial biomass from OMWW of the final product, threonine and valine were present in high concentrations (0.64 and 0.79%, respectively), whilst lysine seemed to be produced too (0.19%). Their product may be considered a potential animal food integrator, having a nutritional value of average quality.

Two-phase olive mill waste has already been proposed as livestock feed (Molina Alcaide and Nefzaoui 1996), but due to its low concentration of proteins, especially lysine, it is recommended to use protein supplements (Molina Alcaide et al. 2003). The fermented product of the SSF experiments with the specific *Paecilomyces variotii* isolate

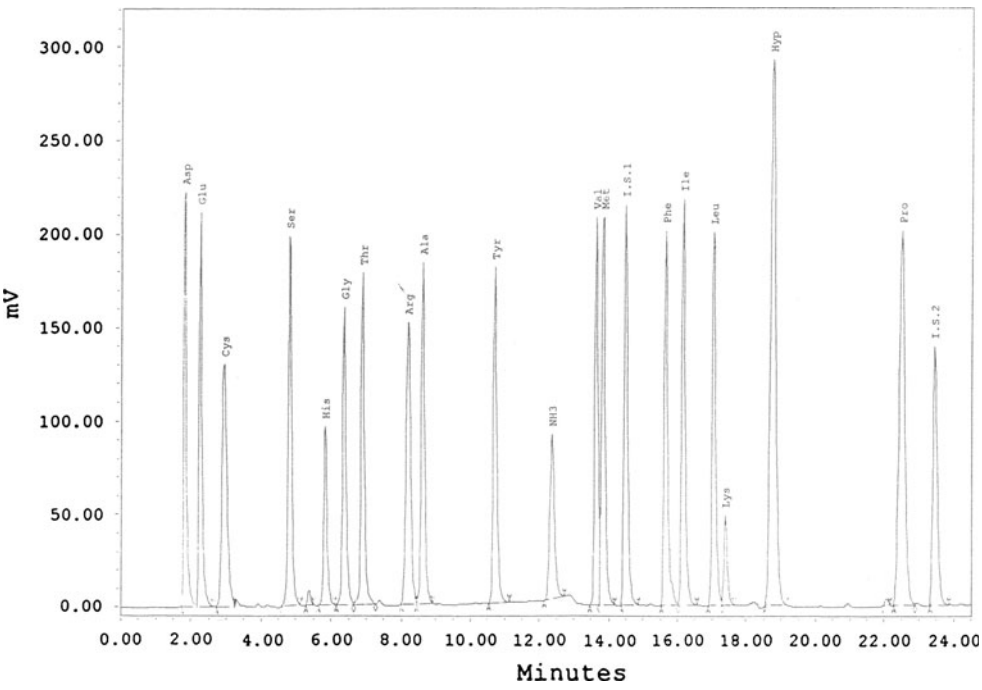

Fig. 5 HPLC chromatography showing aminoacids profile of the fermented product

Table 3 Aminoacid composition (mg/g dry matter) of molasses (Column 2), of the unfermented TPOMW (Column 3: no molasses and Column 4: with molasses addition) and the fermented (Column 5) with *Paecilomyces variotii* TPOMW

Aminoacid	Molasses	TPOMW Unfermented	TPOMW +molasses Unfermented	TPOMW +molasses Fermented
Aspartic acid	–	8.1 ± 0.3	8.4 ± 0.3	12.1 ± 0.4
Glutamic acid	–	16.0 ± 0.5	15.6 ± 0.6	12.1 ± 0.5
Cysteine	–	68.7 ± 1.2	69.2 ± 1.2	70.1 ± 2.7
Serine	–	6.0 ± 0.3	6.2 ± 0.3	17.8 ± 0.6
Histidine	0. 12 ± 0.01	0.6 ± 0.1	0.8 ± 0.1	4.2 ± 0.4
Glycine	–	6.5 ± 0.3	6.1 ± 0.3	11.8 ± 0.3
Threonine	1.11 ± 0.02	2.8 ± 0.2	3.4 ± 0.2	5.9 ± 0.3
Arginine	0.21 ± 0.02	3.1 ± 0.1	3.2 ± 0.2	5.5 ± 0.2
Alanine	–	5.2 ± 0.2	5.0 ± 0.3	8.2 ± 0.5
Tyrosine	–	0.4 ± 0.1	0.4 ± 0.1	1.5 ± 0.2
Valine	1.8 ± 0.4	5.2 ± 0.3	5.4 ± 0.4	8.2 ± 0.4
Methionine	1.1 ± 0.3	1.1 ± 0.2	1.1 ± 0.2	4.1 ± 0.3
Phenylalanine	0.41 ± 0.02	3.9 ± 0.4	4.1 ± 0.4	5.7 ± 0.4
Isoleucine	1.53 ± 0.05	3.5 ± 0.3	4.2 ± 0.4	6.1 ± 0.3
Leucine	1.8 ± 0.2	5.5 ± 0.3	6.4 ± 0.3	8.4 ± 0.6
Lysine	0.3 ± 0.1	–	0.2 ± 0.1	2.7 ± 0.2
Hydroxyproline	–	9.0 ± 0.5	9.0 ± 0.5	0.8 ± 0.1
Proline	–	4.7 ± 0.3	4.7 ± 0.3	6.9 ± 0.2

had a higher concentration of proteins and an aminoacid profile that meets the needs of FAO for animal feed. The presence of lysine, an aminoacid that cannot be synthesized by mammals, was a very promising result leading to the improvement of the nutritional properties of the waste. Lysine is part of the aspartate family and the pathway for its synthesis from aspartate has 10 steps. It is possible that the reduction in glutamic acid in the fermented TPOMW was connected to the production of aspartic acid and to the subsequent lysine production. This is only a first assumption on the fate of the amino acids involved, since comprehension of the metabolic pathways is a rather difficult process and needs thorough investigation. De novo synthesis of lysine has already been reported by the ruminal anaerobic fungi *Pyromyces communis* and *Neocallimastix frontalis* (Atasoglu and Wallace 2002). Valmaseda et al. (1991a) have already managed, after fermentation of wheat straw with *Pleurotus* sp. and *Trametes versicolor,* to increase significantly the percentage of lysine from 1% to 5-9% at the fermented product, in solid state fermentation experiments.

As phenols seem to be the major toxicity factor of olive mill wastes, phenol degradation is crucial in order to get a product that could be used as animal feed. This could be achieved by a pre-treatment step where yeast strains (*Candida boidinii*, *Saccharomyces* sp. and *Geotrichum candidum*) could be used as already described by Giannoutsou et al. (2004), or with an alkaline-oxidative pretreatment of the waste with $Ca(OH)_2$ and H_20_2 (Laconi et al. 2007). Furthermore, preliminary experiments where the fermented with *Paecilomyces variotii* product was given as feed in 20 mice for 15 days have been performed with very promising results concerning the palatability and digestibility of the product. The product was easily consumed and there were no signs of discomfort from the animals. Of course, further experiments have to follow in order to define the best way to obtain high nutritional value biomass from TPOMW and to further examine its safety for the nutrition of animals.

Conclusion

From the analysis of the fermented samples of TPOMW waste and their comparison with the original unfermented waste, it could be concluded that the fermentation of this waste with *P. variotii* isolate enriched it with microbial protein and changed its amino acid profile to an improved one in order to be used as animal feed. Furthermore, the synthesis and presence of lysine in the fermented product, one of the essential and the first limiting aminoacid in feed, is a really promising result which may offer a new alternative on the disposal of this specific waste.

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