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Refining of wine lees and cheese whey for the production of microbial oil, polyphenol-rich extracts and value-added co-products

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Abstract

BACKGROUND: Refining of renewable resources for the production of various end-products should be applied in order to develop sustainable processes and ensure the transition to the bio-economy era. With this principle in mind, a novel integrated biorefinery has been developed based on cheese whey and wine lees valorization.

RESULTS: Polyphenols, tartrate salts and ethanol were extracted from wine lees, while the remaining solids enriched in yeast cells were converted into nutrient-rich fermentation supplements. The composition of phenolics varied between solid and liquid fractions of wine lees. Protein concentrate was separated from cheese whey via ultrafiltration, while the concentrated lactose-rich permeate stream was supplemented with wine lees derived hydrolysates to form fermentation media for microbial oil production by *Cryptococcus curvatus* and *Mortierella ramanniana*. Functional oil containing 4.5% (w/w) of the omega-6 fatty acid γ-linolenic acid was produced in shake flask cultures by *Mortierella ramanniana* with total dry weight of 25.8 g L^{-1} and 30.6% (w/w) lipid content. Fed-batch bioreactor cultures with *Cryptococcus curvatus* using only crude resources led to one of the highest lipid concentrations (33.1 g L^{-1}) reported in literature-cited publications using cheese whey.

CONCLUSION: This is the first study proposing the integrated refining of cheese whey and wine lees for the production of both commodity and speciality products, namely whey protein concentrate, antioxidants, ethanol, tartrate salts and microbial oil.
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Keywords: wine lees; cheese whey; *Cryptococcus curvatus*; *Mortierella ramanniana*; microbial oil; biorefinery

ABBREVIATIONS

DCW	deproteinized cheese whey
FAN	free amino nitrogen
GA	gallic acid
PDA	potato dextrose agar
TDW	total dry weight
TKN	total Kjeldahl nitrogen
WPC	whey protein concentrate
YPDA	Yeast extract, peptone, dextrose, agar

INTRODUCTION

Wine making is one of the most important agricultural activities resulting in wine production capacities around 259 million hectoliters in 2016[1] also generating significant quantities of waste streams (e.g. grape stalk, grape marc and wine lees). Wine lees represent 2–6% of wine production, containing mostly ethanol, tartrate salts and yeast cells. Many studies reported the utilization of wine lees for the production of various commercial products, such as ethanol, tartaric acid and phenolic compounds.[2–5] Wine lees have also been proposed as nutrient supplements in several fermentation processes, including lactic acid, xylitol and PHAs production.[6–9]

The worldwide annual production capacity of cheese whey could be estimated at about 160 million t.[10] Cheese whey retains most of the milk lactose (39–60 g L^{-1}) as well as proteins (1.4–33.5 g L^{-1}), fats (0.99–10.58 g L^{-1}), and mineral salts (0.46–10%) among others.[11] Many studies have focused on the recovery of value-added components from cheese whey

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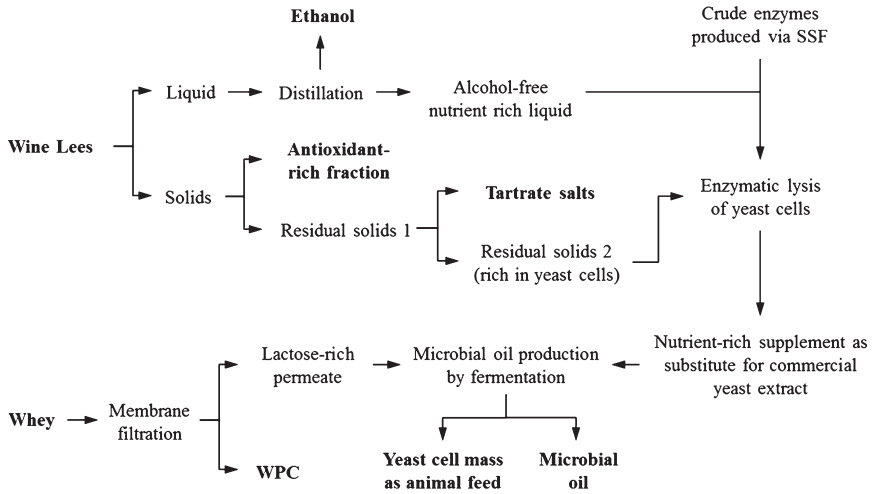

Figure 1. Integrated biorefinery concept based on wine lees and cheese whey valorization for the production or microbial oil and other value-added co-products.

(e.g. protein concentrates and isolates, lactose).[11] Cheese whey has also been used for the production of various fermentation products including microbial oil using oleaginous yeast and fungi,[12] such as *Cryptococcus curvatus* and *Mortierella isabellina*.[13,14] The microbial lipids produced by oleaginous yeasts and fungi have similar composition to vegetable oils and are mainly composed of triacylglycerols with a diversified fatty acid composition with predominantly C16 and C18 fatty acids depending on the fermentation conditions and the selected oleaginous strain. Besides, palmitic, stearic, oleic and linoleic acids, the oleaginous fungal strain *Mortierella ramanniana* can also produce γ-linolenic acid,[15] an omega-6 fatty acid that is of great importance because of its potential anticancer properties and its effectiveness against many other human diseases.[16] Although *Mortierella* species have proved promising fungal strains for microbial lipids and γ-linolenic acid production on lactose or cheese whey based substrates,[17] *M. ramanniana* has not been studied in the case of cheese whey.

Although microbial lipids could be used as food supplements, biodiesel, hybrid fuels and oleochemical production, the high production cost prevents their implementation in large scale processes. Koutinas *et al.* reported that the cost of manufacture of microbial oil using glucose as carbon source and the oleaginous yeast *Rhodosporidium toruloides* would be 5.5 US$ kg^{-1} at a glucose price of 0.4 US$ kg^{-1}.[18] The production cost could be reduced by 8% when considering the revenues derived from selling the remaining yeast biomass as animal feed at a moderate market price of 1 US$ kg^{-1}. An additional 8% reduction of the microbial oil production cost could be achieved by replacing the commercial yeast extract employed in the fermentation stage by a zero cost nitrogen source. However, further reduction of the microbial oil production cost would depend on the improvement of fermentation efficiency, the use of a low-cost carbon source and the development of a biorefinery concept using crude renewable resources leading to the production of various end-products with diversified market outlets. The principle of refining of raw materials has created mature industrial processes (e.g. petroleum and corn refineries) and should be applied to renewable resources in order to ensure the sustainable transition to the bio-economy era.

The cost of microbial oil production from cheese whey has been previously evaluated where the production of 1000 t of refined oil produced via continuous fermentation and 1800 t of yeast/whey protein dry mix as co-product using a whole whey flow rate of 20 m^3 h^{-1} and 250 days per year plant operation would cost US$2.8 million.[19] At a microbial oil market price of 0.7 US$ kg^{-1}, the internal rate of return was reported as 14% (after tax).[19] This study presents a potential biorefinery concept for the production of microbial oil by integrating the refining of cheese whey and wine lees (Fig. 1). The major innovation of this process is the fractionation of wine lees for the production of potable ethanol, antioxidant-rich extract, tartrate salts and a nutrient-rich fermentation supplement, which is derived via yeast cell lysis using crude enzymes produced via solid state fermentation. Dimou *et al.* presented the optimization of the production of the nutrient-rich fermentation supplement leading to a maximum free amino nitrogen concentration of around 1200 mg L^{-1} corresponding to an equivalent 24 g L^{-1} of yeast extract concentration.[8] This nutrient-rich supplement was subsequently combined with crude glycerol for the production of poly(3-hydroxybutyrate) at final concentration of 30.1 g L^{-1}.[8] The cost-competitiveness of the wine lees refining process alone is dependent on the market price of the antioxidant-rich fraction (Fig. 2). The development of profitable refining of wine lees was assessed at different wine lees processing capacities (500–5000 kg h^{-1} at 120 days per year plant operation) demonstrating that minimum selling prices of the antioxidant-rich extract in the range 122–11.06 US$ kg^{-1} should be achieved.[20] Dimou *et al.* presented the material and energy balances of the wine lees refining process (Fig. 2).[20] However, the market price of the antioxidant-rich extract is dependent on the composition of phenolic compounds, their purity, the antioxidant capacity of the extract and the final application of the extract. Therefore, before the evaluation of the cost-competitiveness of the process presented in Fig. 1, the composition of the polyphenol-rich extract and the fermentation efficiency of microbial oil production using cheese whey derived lactose and wine lees derived nutrient supplements should be evaluated. Furthermore, the separation of liquid and solid phases from the original wine lees could lead to the isolation of polyphenol-rich products with different polyphenol composition and thus different end-uses.[5]

This study presents the representative composition of polyphenols in the liquid phase of four wine lees and the extract from the solid phase derived from Merlot lees. The differences in the composition of the two phases demonstrate that the wine lees should

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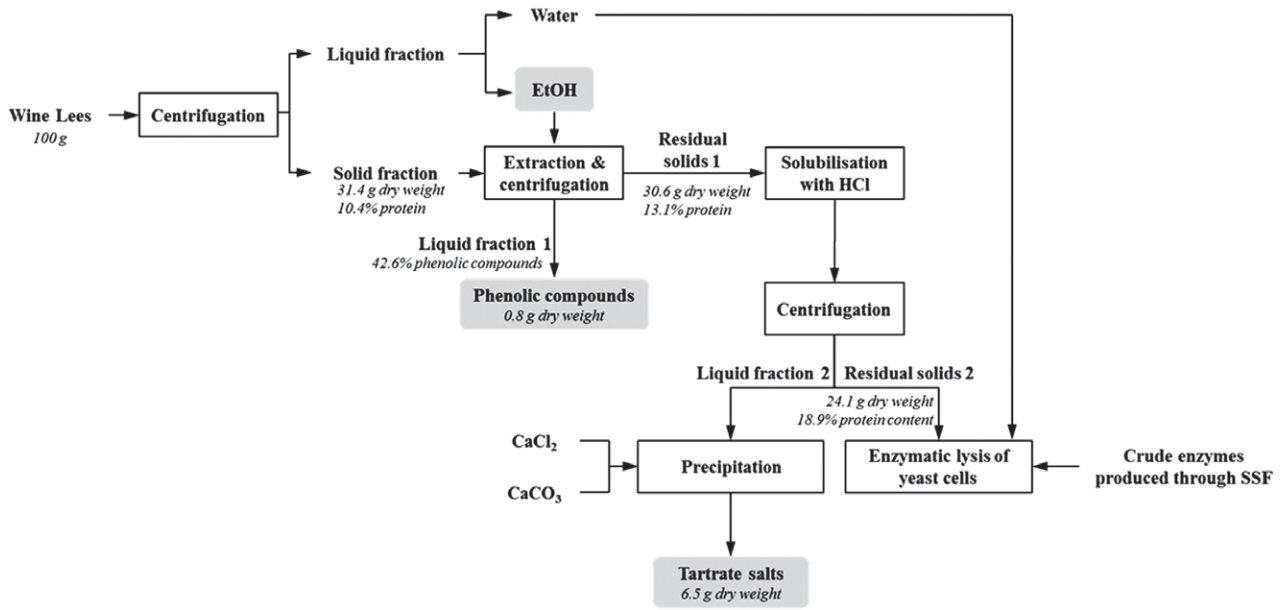

Figure 2. Material balances and process flow diagram of the wine lees refining concept.

not be used as a whole in the proposed biorefinery concept, but it should initially be divided into two phases. Subsequently, the nutrient-rich supplement derived from wine lees is combined with deproteinized cheese whey in order to evaluate the fermentation efficiency of microbial oil production by *C. curvatus* ATCC 20509 and *M. ramanniana* MUCL 9235. The two oleaginous microorganisms were chosen in order to assess the production of microbial oils with either food or non-food applications. To the best of our knowledge, this is the first study that proposes an integrated biorefinery concept using cheese whey and wine lees (Fig. 1) for the production of both commodity and speciality products, namely whey protein concentrate (WPC), antioxidants, potable ethanol, tartrate salts and microbial oil.

EXPERIMENTAL

Microorganisms

Fermentations for microbial oil production were performed using the oleaginous yeast strain *C. curvatus* ATCC 20509 and the oleaginous fungal strain *M. ramanniana* MUCL 9235, which were maintained on slants at 4 °C that contained (g L^{-1}): yeast extract, 10; peptone, 10; dextrose, 10; and agar, 20 (YPDA) and potato dextrose agar (PDA, LabM), respectively.

Solid state fermentations were carried out for the production of crude enzyme consortia using an industrial strain of *Aspergillus oryzae* (kindly provided by Professor Colin Webb, University of Manchester, UK).

Raw materials

Wine lees originated from the wine making process of four different grape varieties, two red (Merlot and Xinomavro) and two white (Assyrtiko and Malagousia), were kindly provided by the winery Ampelou Techni-Theodoros Stavrakis (Tyrnavos, Greece). The phenolic compounds were characterized for all varieties of wine lees. Subsequently, the Merlot variety was selected for fractionation and production of nutrient supplement used in the experiments on microbial oil production.

Wine lees fractionation

The fractionation of Merlot wine lees was carried out for the isolation of ethanol, phenolic compounds, tartrate salts and remaining solids enriched in yeast cells. The material balances are presented in Fig. 2 as were originally described by Dimou *et al.*[20] The Merlot wine lees contained water (62.9%, w/w), ethanol (5.7%, w/w) and solids (31.4%, w/w, on a dry basis).The wine lees is initially fractionated via centrifugation (12 000 × g, 15 min, 10 °C) into liquid and solid fractions (31.4 g dry solids per 100 g of the initial wine lees). The dry solid fraction of wine lees contained 5.8% (w/w) ash, 1.2% (w/w) lipids, 1.8% (w/w) total Kjeldahl nitrogen (TKN), and 10.4% (w/w) protein content (TKN × 5.8). The liquid fraction is used for the separation of ethanol via distillation producing an ethanol-free liquid stream that is subsequently used as process water in the enzymatic hydrolysis of the yeast cell enriched solids (remaining solids 2 in Fig. 2). The ethanol can be used for the extraction of phenolic compounds reducing the operating cost of the process. The solid fraction is used for the separation of a phenolic-rich extract using an ethanol:water solution (70:30, v/v) followed by centrifugation (12 000 × g, 15 min, 10 °C).

The content of tartrate salts was 6.5 g per 100 g of initial wine lees. The residual solid fraction (residual solids 1) that remained after the extraction of antioxidants (30.6 g dry weight) was acidified with HCl in order to solubilize the tartrate salts that were subsequently separated via treatment with calcium carbonate ($CaCO_3$) and calcium chloride ($CaCl_2$) targeting the precipitation of calcium tartrate. In particular, the residual solids 1 fraction is initially dissolved in 3.15 L water per kg dry weight and treated with 0.361 L HCl per kg dry weight for 10 min to solubilize tartaric acid, as described by Salgado *et al.*[21] Precipitation of tartrate salts was carried out using $CaCO_3$ (7.9 g per L of liquid fraction 2) and $CaCl_2$ (8.05 g per L of liquid fraction 2).[6] The precipitate was separated by centrifugation (12 000 × g, 15 min, 10 °C).

The residual solids (24.1 g dry weight) that remained after the extraction of antioxidants and tartrate salts contained 18.9% protein. Assuming a 40% protein content in yeast cells,[22] then 8.2 g (dry weight) of yeast cells was contained in this solid stream. The remaining solids (residual solids 2) were separated via

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centrifugation and subsequently used for the enzymatic hydrolysis of yeast cells leading to the production of a nutrient-rich solution.

Production of wine lees derived hydrolysate

Crude enzyme consortia were produced via solid state fermentation by *A. oryzae* cultivated on sunflower meal as was described by Kachrimanidou *et al.*[23] The enzymatic hydrolysis for the production of nutrient supplements was carried out at 45 °C at an initial pH value of 5.5.[8] After the end of hydrolysis, remaining solids were removed by vacuum filtration and wine lees hydrolysates were filter-sterilized using a 0.2 μm filter unit (Polycap ™ AS, Whatman Ltd).

Cheese whey treatment

Ultrafiltration of cheese whey was carried out with a Millipore Amicon Stirred Cell apparatus using 30 kDA molecular weight cut-off regenerated cellulose membranes. The pressure was about 3.5 bar, using continuous sparging of air. The cheese whey permeate (deproteinized cheese whey, DCW) was collected and stored at −20 °C. The DCW was concentrated via evaporation to a final lactose concentration of 300 g L^{-1}. Before fermentation, the DCW was filter sterilized as described above. The concentrated DCW stream was supplemented with the crude hydrolysate derived from wine lees and utilized for fermentative production of microbial oil.

Batch fermentations in shake flasks

Experiments were conducted in Erlenmeyer flasks of 250 mL containing 50 mL of varying amounts of wine lees derived hydrolysate and DCW, under aseptic conditions, in order to achieve different initial free amino nitrogen (FAN) and lactose concentrations. The FAN concentrations used in all shake flask fermentations were 150, 250 and 350 mg L^{-1}. The fermentation media used in shake flask cultures were supplemented with the following composition of minerals (g L^{-1}): KH_2PO_4, 7.0; Na_2HPO_4, 2.5; $MgSO_4 \cdot 7H_2O$, 1.5; $FeCl_3 \cdot 6H_2O$, 0.15; $ZnSO_4 \cdot 7H_2O$, 0.02; $MnSO_4 \cdot H_2O$, 0.06 and $CaCl_2 \cdot 2H_2O$, 0.15. The pH of the culture was adjusted to the optimum pH range (5.8–6.0) for *C. curvatus* and 6.0–6.2 for *M. ramanniana* growth. Inoculation was performed by adding 2% (v/v) of 24 h preculture (YPD) of *C. curvatus* or aqueous spore suspension of *M. ramanniana* (10^6 spores mL^{-1}). Incubation was carried out in an orbital shaker at 28 °C with an agitation rate of 180 rpm. The pH value was kept at the optimum level during the fermentation for each microorganism by using 5 mol L^{-1} NaOH and 10% (v/v) H_2SO_4. Fermentations were carried out in duplicate and the respective analyses in triplicate. Data presented are the mean values of duplicate experiments.

Fed-batch fermentations

Fed-batch bioreactor cultures with *C. curvatus* were performed in a 1 L bioreactor (New Brunswick Scientific Co., USA) with a working volume of 0.7 L using wine lees hydrolysate and DCW as the sole source of nutrients. An inoculum of 10% (v/v) of a 24 h preculture (YPD) of *C. curvatus* was used. Fermentation temperature was set at 28 °C, the aeration rate was maintained at a flow rate of 1 vvm and the pH was regulated using 5 mol L^{-1} NaOH and 10% (v/v) H_2SO_4. The agitation speed was controlled in the range 150–500 rpm in order to control the dissolved oxygen level in the bioreactor, which was set at 20% of saturation. Concentrated DCW was used as the sole feeding solution during fed-batch cultures.

ANALYTICAL METHODS

Wine lees fractions

Total Kjeldahl nitrogen (TKN) was determined by the KjeltekTM 8100 distillation Unit (Foss, Denmark). Total polyphenol concentration was determined with the Folin–Ciocalteu assay using the microscale protocol previously reported by Arnous *et al.*[24] In more detail, in a 1.5 mL Eppendorf tube, 0.78 mL of distilled water, 0.02 mL of sample and 0.05 mL of Folin–Ciocalteu reagent were added and vortexed. After exactly 1 min, 0.15 mL of aqueous sodium carbonate (Na_2CO_3) 20% (w/v) was added, and the mixture was vortexed and allowed to stand at room temperature in the dark, for 60 min. The absorbance was read at 750 nm, using a Rayleigh 7220G spectrophotometer (Beijing, P. R. China), and the total polyphenol concentration was calculated from a calibration curve (50–700 mg L^{-1}), using gallic acid (GA) as standard. Results were expressed as GA equivalents (g L^{-1} and mg g^{-1} for the liquid and solid fractions, respectively). All measurements were carried out in triplicate for each sample, and values obtained were averaged.

Tannins were precipitated using a protein solution prepared by dissolving bovine serum albumin in a buffer (200 mmol L^{-1} acetic acid, 170 mmol L^{-1} sodium chloride (NaCl), pH 4.9) to give a final protein concentration of 1 g L^{-1}. After centrifugation, the precipitate was redissolved in an alkaline buffer (5% triethanolamine v/v, 5% sodium dodecyl sulfate (SDS) w/v, pH adjusted to 9.4 with HCl), and the absorbance was measured at 510 nm. Tannin absorbance was measured after the addition of iron trichloride ($FeCl_3$) (0.01 N HCl, 10 mmol L^{-1} $FeCl_3$) solution using the TEA buffer as a blank. Tannin quantification was carried out using a (+)-catechin standard curve and expressed as catechin equivalents (mg L^{-1} and mg g^{-1} for the liquid and solid fractions, respectively).[25]

For the determination of the antioxidant activity the protocol presented by Makris *et al.* was used.[26] Each sample was appropriately diluted with methanol immediately before the analysis. Sample (0.025 mL) was added to 0.975 mL di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH)· solution (73 μmol L^{-1} in methanol), and the absorbance at 515 nm was recorded at t = 0 and t = 30 min. Results were expressed as Trolox equivalents (mmol L^{-1} TRE and μmol g^{-1} for the liquid and solid fractions, respectively).

The monomeric anthocyanins were determined by HPLC. The equipment used consisted of a Jasco AS-1555 Intelligent Sampler, a Jasco PU 2089 Plus Quaternary Gradient Pump, a Jasco MD-910 Multiwavelength Detector and a Jasco LC-Net II / ADC. The column was a Restek pinnacle II C18, 250 × 4.6, 5 μm, 250 × 4 mm, 5 μm. Eluent (A) was 10% aqueous formic acid and eluent B methanol and the flow rate 1 mL min^{-1}. The elution was as follows: 90% eluent A for 1 min, then from 90% to 50% in 22 min, from 50% to 5% in 10 min, which was kept isocratic for a further 2 min. Identification was based on comparing retention times and UV spectra of the peaks detected with those of original compounds or on previous observations.[27] Anthocyanin concentration was expressed as mg L^{-1} malvidin-3-O-glucoside equivalents. All analyses were performed in triplicate.

The individual polyphenolic constituents were determined by HPLC according to Kallithraka *et al.*[28] The chromatography apparatus used was an HP 1090, coupled with an Agilent 1100 diode array detector, and controlled by Agilent ChemStation software. The column was a Spherisorb ODS 2 (AnalyzenTechnik, MZ, Germany), 250 × 4 mm, 5 μm, protected by a guard column packed with the same material. Both columns were thermostatically controlled at 20 °C. Eluent (A) was 9 mmol L^{-1} aqueous orthophosphoric acid

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(pH 2.5) and (B) acetonitrile:water (4:6), containing 9 mmol L^{-1} orthophosphoric acid, and the flow rate 1 mL min^{-1}. The elution was as follows: 100% A for 20 min, then from 100% A to 60% A in 80 min, isocratic for 10 min, then from 60% A to 30% A in 20 min, and then isocratic for another 10 min (total run time 140 min). Identification was based on comparing the retention times of the peaks detected with those of original compounds, and on UV–vis spectral data. Quantification was performed by establishing calibration curves for each compound determined, using standards. Results were expressed as mg L^{-1}. All analyses were performed in triplicate.

All chemical determinations were run in triplicate and values were averaged. The standard deviation (SD) was also calculated. Data were subjected to one-way analysis of variance (ANOVA), using Statistica V.7 301 software (Statsoft Inc., Tulsa, OK). Comparison of mean values was performed using 302 Tukey's HSD test when samples were significantly different by ANOVA ($P < 0.05$).

Microbial fermentations

Duplicates from cultures were obtained at regular intervals and biomass was collected by centrifugation (16 000 × g, 15 min, 5 °C) (in the case of *C. curvatus*) or through a 106 μm stainless steel sieve (in the case of *M. ramanniana*), followed by at least two washes with distilled water. Total dry weight (TDW) was determined by drying at 105 °C for 24 h. Lactose concentration was quantified using high performance liquid chromatography (HPLC) analysis (Waters 600E) with an Aminex HPX-87H (300 mm × 7.8 mm, Bio-Rad, USA) column coupled to a differential refractometer (RI Waters 410) and a UV detector (Waters 486). Operating conditions were as follows: sample volume 20 μL; mobile phase 0.005 mol L^{-1} H_2SO_4; flow rate 0.6 mL min^{-1} and column temperature 60 °C. FAN analysis was performed as described previously by Kachrimanidou *et al.*[29] Total cellular lipid was extracted from dried biomass using a chloroform:methanol mixture of 2:1 (v/v) ratio. The fatty acid composition was determined by gas chromatography and fatty acid methyl esters were prepared according to Tsakona *et al.*[30]

RESULTS AND DISCUSSION

This study presents important aspects on the development of a biorefinery concept focusing on the fractionation of cheese whey and wine lees leading to the production of microbial oil as well as various value-added co-products with diversified end-uses (Fig. 1). As reported by Dimou *et al.*, the polyphenol-rich extract is vital in the overall process profitability[20] and, therefore, the antioxidant capacity and the polyphenol composition of wine lees derived extracts is presented in this study and compared with other literature-cited extraction protocols using wine lees as raw material. Furthermore, the second aspect evaluated in this study is the efficiency of microbial oil production using concentrated DCW and wine lees derived nutrient-rich supplements as the sole fermentation feedstocks.

Total phenolic content, total tannin content and antioxidant activity of four wine lees

The liquid and solid fractions of the four wine lees (Assyrtiko, Malagousia, Xinomavro, Merlot) were separated via centrifugation and the solid fractions were further treated with a 70% (v/v) ethanol solution in water for the extraction of a polyphenol-rich fraction. Table 1 presents the total phenolic content, the total tannin content and the antioxidant activity of the liquid and solid fractions derived from four wine lees. The antioxidant content of the lees was dependent on the specific grape variety used. In the case of the liquid supernatant, the highest total phenolic content (g L^{-1} GA equivalents) was measured in Merlot lees followed by Assyrtiko and Xinomavro. Malagousia lees contained significantly lower amounts of total phenolic content. A similar trend was observed for total tannin content. Merlot lees were the richest, whereas Malagouzia the poorest. Regarding total antioxidant activity, Merlot lees exhibited the highest values followed by Xinomavro, Assyrtiko and Malagouzia. In the case of the solid fractions obtained from the lees used, Merlot was also the grape variety that resulted in the richest lees in total phenolic and tannin contents and the highest antioxidant activity values. The second richest solid fraction was the one obtained from Xinomavro wine lees. The lees obtained from the two white wines studied (Assyriko and Malagouzia) contained significantly less total phenolic content and total tannin content and consequently exhibited lower antioxidant activity values.

Table 1. Total phenolic content, total tannin content and antioxidant activity in liquid and solid fractions of four wine lees

Variety	Total phenolic content (g L^{-1} GA)[1]	Total tannin content (mg L^{-1} catechin)[1]	Antioxidant activity (mmol L^{-1} trolox)[2]
liquid fraction			
Assyrtiko	1.04 [b] ± 0.02	3.66 [b] ± 0.11	1.03 [c] ± 0.00
Malagouzia	0.51 [d] ± 0.01	1.56 [c] ± 0.09	0.87 [d] ± 0.00
Xinomavro	0.95 [c] ± 0.02	3.84 [b] ± 0.06	1.51 [b] ± 0.02
Merlot	1.50 [a] ± 0.01	11.96 [a] ± 0.27	3.11 [a] ± 0.05
solid fraction			
Assyrtiko	9.94 [c] ± 0.26	11.28 [c] ± 0.31	3.23 [c] ± 0.26
Malagouzia	8.95 [c] ± 0.32	7.42 [d] ± 0.39	3.59 [c] ± 0.10
Xinomavro	17.90 [b] ± 0.89	13.95 [b] ± 0.55	9.70 [b] ± 0.38
Merlot	26.10 [a] ± 1.46	19.88 [a] ± 0.86	13.80 [a] ± 0.78

[1] mg g^{-1} for the solid fractions.
[2] μmol g^{-1} for the solid fractions.
[a,b,c,d] Values with different letters are significantly different ($P < 0.05$).

In the case of Merlot wine lees, Fig. 2 shows that around 0.8 g dry weight of phenolic-rich extract was separated from 31.4 g (extraction yield 2.6%, w/w) of the solid fraction in Merlot wine lees. The total phenol content in this extract was 26.1 mg of GA equivalents per g of dry wine lees as determined by the Folin–Ciocalteu assay,[20] whereas the antioxidant activity was 13.8 μmol of Trolox equivalents per g of dry wine lees as determined by the DPPH method. This fraction contained 42.6% (w/w) of phenolic compounds as was determined by the estimation of GA equivalents according to the Folin–Ciocalteu assay that was carried out in the antioxidant-rich extract. The antioxidant activity values presented above are similar to or even higher than the values reported for various fruits and vegetables.[31,32] Tao *et al.* reported a total phenolic content of 59 mg of GA equivalents per g of dry red wine lees, derived from a mixed variety of red wines (containing 30% Merlot), using an ultrasound-assisted extraction with an aqueous ethanol solution (44%, v/v).[33] The dried phenolic-rich extract obtained via microwave-assisted extraction from the dried solid phase of wine lees using an acidified 75% (v/v) ethanol in water mixture had a total phenolic content of 364 mg of GA equivalents per g of wine lees extract powder and an antioxidant activity of 3930 μmol

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Table 2. Phenolic compounds in liquid fraction of wine lees

	Phenolic compounds (mg L^{-1})*						
Variety	C	EC	Procyanidin B1	procyanidin B2	M	Q	
Assyrtiko	n.d. ^{c}	188.1 ^{b} ± 2.3	n.d. ^{c}	n.d. ^{c}	n.d. ^{c}	n.d. ^{b}	
Malagouzia	50.1 ^{a} ± 1.3	23.3 ^{c} ± 0.9	15.3 ^{b} ± 0.2	27.6 ^{b} ± 1.1	n.d. ^{c}	n.d. ^{b}	
Xinomavro	n.d. ^{c}	517.1 ^{a} ± 5.6	n.d. ^{c}	19.4 ^{a} ± 1.6	1.3 ^{b} ± 0.01	n.d. ^{b}	
Merlot	43.1 ^{b} ± 0.9	7.7 ^{d} ± 0.7	46.8 ^{a} ± 0.9	29.7 ^{b} ± 0.8	1.8 ^{a} ± 0.01	4.2 ^{a} ± 0.03	
	GA	CFA	CTA	CA	CmA	FA	
Assyrtiko	n.d. ^{d}	n.d. ^{b}	9.6 ^{a} ± 0.6	0.7 ^{a} ± 0.01	0.6 ^{c} ± 0.01	0.2 ^{c} ± 0.01	
Malagouzia	8.1 ^{c} ± 0.1	n.d. ^{b}	5.7 ^{b} ± 0.1	0.7 ^{a} ± 0.01	0.6 ^{c} ± 0.01	0.2 ^{c} ± 0.01	
Xinomavro	35.9 ^{a} ± 0.9	23.3 ^{a} ± 0.9	1.3 ^{c} ± 0.1	n.d. ^{b}	0.9 ^{a} ± 0.02	0.9 ^{a} ± 0.02	
Merlot	14.2 ^{b} ± 0.1	21.2 ^{a} ± 1.1	5.4 ^{b} ± 0.1	n.d. ^{b}	0.7 ^{b} ± 0.01	0.4 ^{b} ± 0.01	
	Dlp	Cy	Pt	Pn	Mlv	MlvAc	MlvCoum
Xinomavro	n.d ^{b}	n.d	3.0 ^{a} ± 0.06	n.d ^{b}	91.9 ^{a} ± 4.6	5.9 ^{a} ± 0.1	3.4 ^{a} ± 0.06
Merlot	2.9 ^{a} ± 0.01	n.d	5.9 ^{a} ± 0.03	4.4 ^{a} ± 0.04	69.1 ^{b} ± 3.9	5.4 ^{a} ± 0.9	1.9 ^{b} ± 0.01

*(+)-catechin (C), (-)-epicatechin (EC), procyanidins B1 and B2, myricetin (M), quercetin (Q), gallic acid (GA), trans-caftaric acid (CFA), trans-coutaric acid (CTA), caffeic acid (CA), p-coumaric acid (CmA) and ferulic acid(FA), delphinidin-3-O-monoglucoside (Dlp), cyanidin-3-O-monoglucoside (Cy), petunidin-3-O-monoglucoside (Pt), peonidin-3-O-monoglucoside, (Pn), malvidin-3-O-monoglucoside (Mlv), malvidin-3-*O*-acetylmonoglucoside (MlvAc) and malvidin-3-(6-*O*-p-coumaroyl) monoglucoside (MlvCoum).
$^{a, b, c, d}$ Values with different letters are significantly different ($P < 0.05$).

of Trolox equivalents per g of wine lees extract powder.[3] A 95% ethanol in water mixture was used by Wu *et al.* for the separation of 24.1% of total polyphenols using a soxhlet extraction system.[34]

The varying total phenolic content and antioxidant activity presented above in different studies depends on the efficiency of the extraction technologies (e.g. microwave, ultrasound) used and the optimization of process conditions. The main aim of this study was to evaluate the antioxidant capacity and composition of the extract obtained from the solid fraction of wine lees using the ethanol separated from wine lees as extraction solvent, which can be used as both extraction solvent and a final co-product as potable ethanol improving process profitability. Future studies should focus on the enhancement of total phenolic content, antioxidant activity and extraction yield of phenolic compounds. Phenolic compounds could also be separated from the liquid fraction prior to distillation using ion exchange resins.[35]

Determination of polyphenol composition in solid and liquid phases of wine lees

The analytical polyphenolic composition of the liquid fraction studied is illustrated in Table 2. The following phenolic compounds were identified: gallic acid, the flavanols: (+)-catechin, (-)-epicatechin, procyanidins B1 and B2, the flavonols: myricetin and quercetin, and the hydroxycinnamic acids: trans-caftaric, trans-coutaric, caffeic, p-coumaric and ferulic. The most abundant polyphenol detected in all samples examined was (-)-epicatechin with the highest content measured in Xinomavro lees and it was almost 67 times higher than the corresponding content of Merlot lees. Interestingly, Assyrtiko lees were the second richest in (-)-epicatechin. By contrast, the flavonols myricetin and quercetin were absent from the lees of the white wines and only minor constituents in the lees of the red ones. Barcia *et al.* also detected myricetin and quercetin in the lees of Cabernet Franc and Cabernet Sauvignon wines and they attributed their presence to the hydrolysis of flavonol 3-glucosides during winemaking.[36] The accumulation of flavonol aglycones in lees could be explained by considering the low solubility of these compounds in hydroalcoholic mixtures of low ethanol content such as wine.

Procyanidins B2 and B1 were absent from Assyrtiko, whereas procyanidin B1 was not detected in Xinomavro samples. Regarding gallic acid, Xinomavro samples were the richest whereas this compound was absent from Assyriko samples. Hydroxycinnamic acid derivatives were also identified in the samples and their presence and content were cultivar dependent in agreement with Barcia *et al.*[36] Trans-Caftaric acid was not detected in any of the lees obtained from the white wines in contrast to caffeic acid that was not detected in the lees originated from the red wines. Ferulic and p-coumaric acids were more abundant in red wine lees, whereas in the case of trans-coutaric acid the opposite trend was observed. The free hydroxycinnamic acids (ferulic, caffeic and p-coumaric acids) detected in the lees samples might have been released from the corresponding hydroxycinnamoyl-tartaric acids through hydrolysis during the winemaking process.

A total of seven anthocyanins were identified in the two red cultivar samples presented in Table 2. The major individual anthocyanins detected in both samples was malvidin-3-O-glucoside in contrast with cyanidin-3-O-glucoside, which was not detected in any of the samples in agreement with the findings of Barcia *et al.*[36] Petunidin-3-O-glucoside, malvidin-3-O-acetyl-glucoside and malvidin-3-(6-O-p-coumaroyl)-glucoside were also present in both samples examined. Delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, and peonidin-3-O-glucoside were only found in Merlot lees. According to Morata *et al.* these compounds are transferred from the grapes to wines during the winemaking process and can be partially absorbed by lees.[37]

Since Merlot was the variety that resulted in the highest antioxidant activity, total phenolic content and total tannin content in both the liquid phase and the extract from the solid phase (Table 1), it was used for the determination of the analytical phenolic composition of the solid wine lees fraction. A different trend was observed regarding the relative concentrations of the individual phenolic compounds, which could be attributed to

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Table 3. Cultivation of *Cryptococcus curvatus* and *Mortierella ramanniana* in batch shake flask fermentations using mixtures of wine lees derived hydrolysates and deproteinized cheese whey

Microorganism	FAN [a] (mg L^{-1})	Time (h)	L_o [b] (g L^{-1})	L_r [c] (g L^{-1})	TDW (g L^{-1})	Lipids (g L^{-1})	$Y_{L/X}$ [d] (%, w/w)
M. ramanniana	150	219	83.3 ± 1.3	3.8 ± 0.1	25.8 ± 1.2	7.9 ± 0.2	30.6 ± 0.2
	250	219	82.7 ± 3.0	4.2 ± 0.2	28.4 ± 1.1	8.2 ± 0.2	28.9 ± 0.1
	350	219	84.2 ± 2.7	4.8 ± 0.1	30.9 ± 1.4	8.0 ± 0.2	25.9 ± 0.1
C. curvatus	150	216	128 ± 2.5	13.8 ± 1.9	33.6 ± 0.9	6.7 ± 0.5	19.9 ± 0.6
	250	216	122.8 ± 2.2	26.0 ± 2.9	36.5 ± 2.4	3.5 ± 0.6	9.6 ± 0.3
	350	216	119.9 ± 1.9	51.7 ± 1.8	33.6 ± 2.3	2.9 ± 0.6	8.6 ± 0.3

[a] FAN: initial free amino nitrogen concentration of the fermentation medium.
[b] L_o: initial lactose concentration.
[c] L_r: remained lactose concentration.
[d] $Y_{L/X}$: intracellular lipid content based on g of lipid produced per g of TDW.

their different chemical structure and their different solubility in aqueous solutions. The most abundant compound identified was quercetin (218.4 μg g^{-1} dry weight) followed by (+)-catechin (86.48 μg g^{-1} dry weight). The other flavonols (rutin and myricetin) were the next most abundant compounds (75.58 and 57.04 μg g^{-1} dry weight, respectively). Considering their low solubility in aqueous media,[36] the solid fraction of wine lees would be a rich source of flavonols. Other flavanols present were (-)-epicatechin (34.48 μg g^{-1} dry weight) and procyanidin B1 (37.72 μg g^{-1} dry weight). The solid fraction of wine lees was poor in hydroxycinnamic acids, such as p-coumaric (7.23 μg g^{-1} dry weight) and caffeic acids (5.29 μg g^{-1} dry weight) and gallic acid (0.39 μg g^{-1} dry weight).

The ethanolic extracts obtained via microwave-assisted extraction from the solid phase of wine lees contain various compounds including primary amino acids, anthocyans, flavanols, flavonols, flavones and non-flavonoid phenolic compounds.[38] The spray dried extract obtained from the solid phase of wine lees via microwave-assisted extraction using 75% (v/v) ethanol in water mixture as extraction solvent contained malvidin-3-glucoside, p-coumaroyl derivatives, myricetin, quercetin, quercetin-3-*β*-glucoside, caffeic acid and p-coumaric acid.[3] Delgado de la Torre *et al.* separated the liquid from the solid phases of wine lees obtained from 11 different wineries in Spain and reported the differences of the two fractions regarding their composition in polyphenols and other compounds.[5] Quercetin, myricetin, and malvidin 3-galactoside were mainly identified in the extracts obtained from the solid fractions of wine lees, whereas the liquid fractions were rich in primary amino acids and phenolic compounds, especially flavonoids.[5] The phenolic compounds procyanidin B2 and pelargonidin 3-(6-p-coumaroylglucoside) were tentatively identified in the two samples from all wineries.[5]

The biorefinery concept presented in Figs 1 and 2 is based on the separation of liquid and solid phases from wine lees from which different co-products could be produced. As concluded by Delgado de la Torre *et al.*, the solid and liquid phases of wine lees could lead to the separation of extracts with different compositions and therefore these extracts could be used in different end-uses.[5] For instance, the liquid phases of the four wine lees evaluated in this study contain negligible quantities of quercetin and the flavonols rutin and myricetin (Table 1), while in the extract from the solid fraction of Merlot lees quercetin (218.4 μg g^{-1} dry weight), rutin (75.58 μg g^{-1} dry weight) and myricetin (57.04 μg g^{-1} dry weight) are major phenolic compounds. Furthermore, (+)-catechin (86.48 μg g^{-1} dry weight) is also a major phenolic compound in the extract of the solid phase of Merlot lees, but it is only contained in the liquid phases of Malagouzia and Merlot lees. Although the valorization of winery by-products for the separation of high-value compounds has mainly focused on grape seeds and skins, other streams such as wine lees and vine shoots[39] could be exploited for this purpose creating holistic utilization of winery by-products.

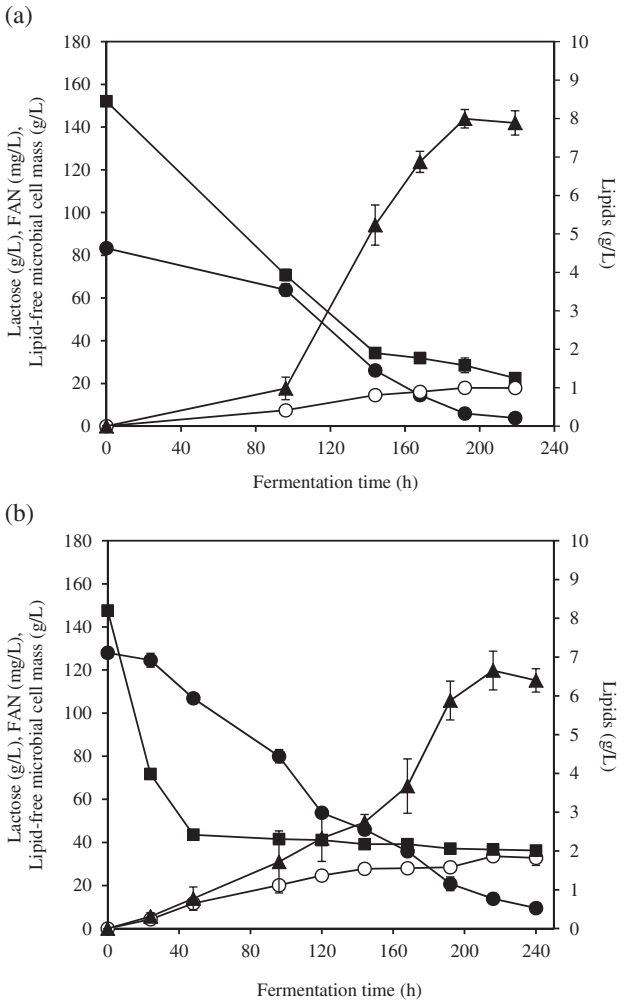

Figure 3. Consumption of lactose (●) and FAN (■) as well as production of lipid-free microbial cell mass (○) and lipids (▲) during shake flask cultures of: (a) *Mortierella ramanniana*; and (b) *Cryptococcus curvatus* on 150 mg L^{-1} initial FAN concentration using wine lees hydrolysate and concentrated deproteinized cheese whey.

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Table 4. Lipid production by various *Cryptococcus curvatus* strains and *Mortierella* species during submerged fermentation on different carbon sources

Microorganism	Substrate	Fermentation mode	TDW ($g\ L^{-1}$)	Lipid ($g\ L^{-1}$)	Lipid content [e] (% g g^{-1})	Yield [f] ($g\ g^{-1}$)	Productivity ($g\ L^{-1}\ h^{-1}$)	γ-linolenic acid [g] (% w/w)	Reference
M. ramanniana CBS 112.08	Glucose	Shake flasks	11.2	2.6	23.4	-	0.027	14.7	[40]
	Fructose	Shake flasks	12.0	3.0	24.9	-	0.031	15.1	
	Lactose	Shake flasks	8.7	1.1	13.2	-	0.012	19.4	
M. ramanniana MM15-1 (mutant)	Glucose	Turbine impeller bioreactor	67.7	37.4	55.2	-	0.173	14.6	[41]
M. ramanniana MUCL 9235	Glycerol	Shake flasks	7.0	3.7	53.1	-	0.017	-	[15]
	Glycerol	Bioreactor	9.7	3.2	32.7	-	0.015	-	
	DCW[b] and wine lees hydrolysate	Shake flasks	25.8	7.9	30.6	0.10	0.036	4.5	This study
M. isabellina ATHUM 2935	Lactose	Shake flasks	9.5	3.5	36.8	0.14	-	3.8-4.1	[17]
	Cheese whey[b] supplemented with lactose	Shake flasks	32.0	8.1	25.3	0.15	0.041	3.7	[14]
M. isabellina DSM 1414	Treated DWP[c]	Shake flasks	26.5	17.1	64.5	0.18	0.192	2.9	[42]
C. curvatus Y-1511	Lactose	Shake flasks	14.5	4.3	29.7	0.15	0.008	-	[43]
C. curvata D[a]	Lactose	Bioreactor-continuous	18.0	5.6	31.0	0.19	0.224	-	[44]
		Bioreactor-batch	12.5	4.9	39.2	0.16	0.054	-	
C. curvatus KCTC 27583	Cheese whey pretreated[d]	Shake flasks	7.2	4.7	65.0	-	0.195	-	[45]
A. curvatum ATCC 20509 [a]	Whey permeate	Bioreactor-batch	19.7	11.4	58.0	-	0.123	-	[13]
		Bioreactor-recycling	85.0	29.8	35.0	-	0.372	-	
		Bioreactor-continuous	20.0	7.2	36.0	-	0.382	-	
		Bioreactor-partial recycling	91.4	30.2	33.0	-	0.995	-	
C. curvatus ATCC 20509	DCW[a]	Bioreactor	56.0	29.7	53.0	-	0.251	-	[46]
	DCW[b] & wine lees hydrolysate	Bioreactor-fed batch	66.8	33.1	49.6	0.18	0.494	-	This study

[a] *Cryptococcus curvatus* was formerly known as *Candida curvata* and *Apiotrichum curvatum*.
[b] DCW: deproteinized cheese whey.
[c] Deproteinized whey permeate treated with lactase.
[d] Cheese whey pretreated with hydrodynamic cavitation.
[e] Lipid content: g oil per 100 g of dry biomass.
[f] Yield: g oil per g substrate consumed.
[g] Expressed as the percentage of totals lipids.

Microbial oil production from DCW and wine lees derived nutrient-rich supplement

Table 3 presents the TDW and microbial lipid production achieved in shake flask fermentations carried out by *M. ramanniana* and *C. curvatus* cultivated in mixtures of wine lees derived hydrolysates and concentrated DCW. In the case of *M. ramanniana*, the initial lactose concentration was around 83 g L^{-1} in all cases and the initial FAN concentration was 150 mg L^{-1}, 250 mg L^{-1} and 350 mg L^{-1} in the three shake flask fermentations. In the case of *M. ramanniana* (Fig. 3(a)), lactose was almost completely consumed at 219 h at all initial FAN concentrations. The consumption of FAN followed the same trend until 144 h, then it was reduced to 219 h (Fig. 3(a)). Increasing initial FAN concentrations led to increasing final microbial cell mass and reducing intracellular lipid content. The lipid concentration was similar in all cases (around 8 g L^{-1}). The highest intracellular lipid content (30.6%, w/w) was observed at the lowest initial FAN concentration (150 mg L^{-1}). This occurred because increasing initial FAN concentration favours fungal growth rather than lipid accumulation, which is only triggered under nutrient limiting conditions. The pH value during all shake flask fermentations of *M. ramanniana* varied between 5.5 and 5.8.

Table 4 presents microbial oil production using mainly lactose and cheese whey as well as some other carbon sources. To the

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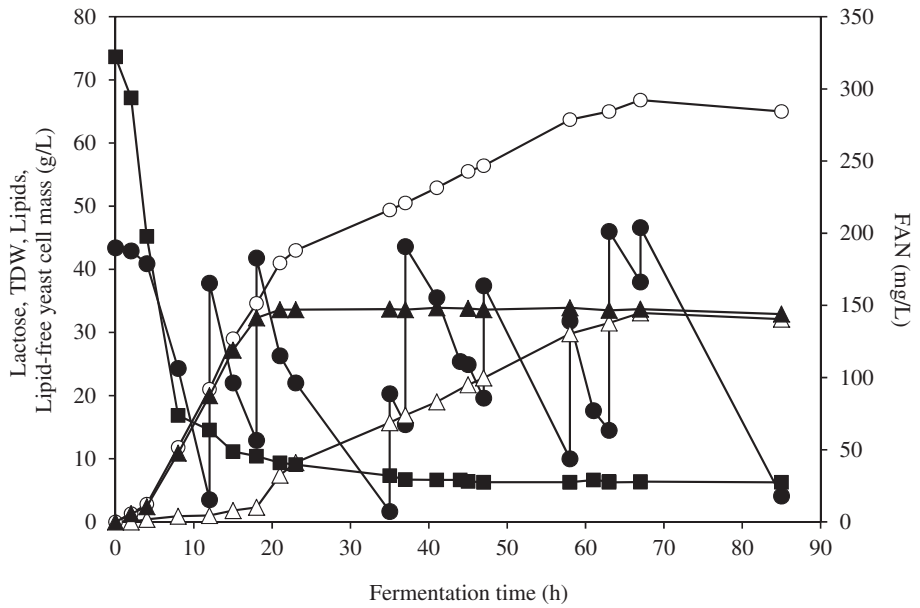

Figure 4. Consumption of lactose (●) and free amino nitrogen (FAN) (■) as well as production of TDW (○), lipid-free yeast cell mass (▲) and lipids (△) during fed-batch bioreactor culture of *Cryptococcus curvatus* on wine lees derived hydrolysate and concentrated deproteinized cheese whey without addition of minerals.

best of our knowledge efficient utilization of cheese whey for the production of lipids by *M. ramanniana* has not been previously reported. Most literature-cited publications on microbial oil production by *Mortierella* species focus on the utilization of pure carbon sources, such as glucose, fructose and glycerol (Table 4). This study presents the highest lipid production by *M. ramanniana* achieved in shake flask cultures. The fungal strain *M. isabellina* has been cultivated in shake flasks using cheese whey supplemented with lactose leading to the production of 32 g L^{-1} TDW and 8.1 g L^{-1} lipid concentration.[14] Demir *et al.* reported the production of 17.1 g L^{-1} of lipids and 26.5 g L^{-1} TDW when *M. isabellina* was cultivated in shake flasks in deproteinized whey permeate treated with lactase.[42]

In the case of *C. curvatus* cultivations in shake flasks (Table 3), the production of microbial cell mass and the lipid content were affected by the initial FAN concentration as in the case of *M. ramanniana*. The initial lactose concentration was 119.9–128 g L^{-1}, whereas the initial FAN concentration was 150 mg L^{-1}, 250 mg L^{-1} and 350 mg L^{-1} in the three shake flask fermentations. The highest lipid concentration (6.7 g L^{-1}) and lipid content (19.9%, w/w) were achieved when the initial FAN concentration was 150 mg L^{-1} (Table 3). At initial FAN concentrations of 250 mg L^{-1} and 350 mg L^{-1}, the lactose was not completely consumed even at a fermentation duration of more than 216 h. Figure 3(b) shows that FAN consumption almost stopped at 48 h and the FAN concentration remained constant until 216 h. Lactose concentration was reduced to 13.8 g L^{-1} at 216 h when 150 mg L^{-1} of initial FAN concentration was used (Fig. 3(b)), but at higher initial FAN concentrations the consumption of lactose followed a significantly slower trend (results not presented). The pH value varied in the range of 5.5–5.8 during all shake flask fermentations of *C. curvatus*. The initial lactose concentration in the cultures carried out with *C. curvatus* was higher than the shake flask cultures conducted with *M. ramanniana* because preliminary experiments with these two strains showed that *C. curvatus* is more efficient and tolerant than *M. ramanniana* in the consumption of lactose concentrations higher than 100 g L^{-1}.

Table 4 shows that the oleaginous yeast *C. curvatus* has been used extensively for lipid production from lactose and cheese whey, but in all cases synthetic mineral supplements and yeast extract were used. The use of wine lees derived hydrolysates and concentrated DCW in shake flask cultivations by *C. curvatus* led to efficient yeast cell growth and lipid accumulation, mainly at the lowest FAN concentration used. The production of lipids by *C. curvatus* was subsequently studied in fed-batch bioreactor cultures in order to assess its efficiency in lipid accumulation.

Figure 4 presents fed-batch bioreactor culture carried out with *C. curvatus* cultivated on wine lees derived hydrolysates and concentrated DCW with initial FAN and lactose concentrations of 322.2 mg L^{-1} and 43.4 g L^{-1}, respectively. Lipid accumulation started at around 20 h when the highest yeast cell concentration (33.6 g L^{-1}) was attained. The onset of lipid accumulation coincided with the reduction of FAN consumption. The FAN concentration was reduced to 40.9 mg L^{-1} at 20 h and it was only decreased to 27.5 mg L^{-1} until 47 h. The TDW (66.8 g L^{-1}), lipid concentration (33.1 g L^{-1}) and lipid content (49.6%, w/w) reached the maximum values at 67 h. Based on the results presented in Table 4, the highest lipid concentration has been achieved in this study. The productivity (0.494 g L^{-1} h^{-1}) achieved in this study could be further improved by employing cell recycling, as has been demonstrated by Ykema *et al.*[13] It should be stressed that fed-batch fermentations with *C. curvatus* were carried out with or without nutrient supplementation leading to similar results showing that the use of only wine lees and cheese whey derived media is sufficient for microbial lipids production. Fed-batch bioreactor cultures were also carried out with *M. rammaniana*, but in this case lipid accumulation was not observed because the fungal strain could consume the low protein concentration in the concentrated whey permeate that was used as feeding solution. In the case of *C. curvatus*, this problem did not occur because these proteins were not consumed by this strain.

The fatty acid composition of *C. curvatus* and *M. ramanniana* at different growth phases is presented in Table 5. The microbial oil produced by *C. curvatus* at the end of all fermentations was

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Table 5. Fatty acid composition of microbial oils produced by *Cryptococcus curvatus* and *Mortierella ramanniana* during shake flask and bioreactor cultures using wine lees derived hydrolysates and deproteinized cheese whey

Microorganism	Fermentation mode	FAN [a] (mg L^{-1})	Time (h)	C16:0	C18:0	$^{\Delta 9}$C18:1	$^{\Delta 9,12}$C18:2	$^{\Delta 9,12,15}$C18:3	$^{\Delta 6,9,12}$C18:3	Others
C. curvatus	Shake flask	150	96	27.6	13.5	50.4	6.1	-	-	2.4
			192	26.0	9.7	53.2	7.2	0.3	-	3.6
	Shake flask	250	96	26.9	17.8	47.4	5.7	-	-	2.2
			192	24.8	15.1	49.5	6.6	0.4	-	3.6
	Shake flask	350	96	26.3	14.4	51.7	7.6	-	-	-
			192	24.9	15.5	49.4	7.7	0.5	-	2.0
	Fed batch -bioreactor	322.2	23	28.0	8.6	50.0	10.5	-	-	2.9
			47	24.0	11.0	54.3	7.0	-	-	3.7
			85	19.7	13.7	54.4	8.2	-	-	4.0
M. ramanniana	Shake flask	150	96	28.0	9.3	47.1	15.3	-	-	0.3
			219	24.5	8.1	45.9	15.8	-	4.5	1.2
	Shake flask	250	96	25.4	11.5	48.3	14.7	-	-	0.1
			219	24.0	8.3	47.5	14.5	-	4.3	1.4

[a] FAN: initial free amino nitrogen concentration.

generally characterized as unsaturated, as oleic and linoleic acids represented more than 55% of the final fatty acid content in the case of shake flask fermentations and more than 60% in the case of fed batch fermentation. The production of palmitic acid ranged from 19.7% to 28% at any fermentation time. The fatty acid composition was similar in all fermentations. The fatty acid composition produced by *C. curvatus*, when it was cultured on lactose, is in agreement with the one presented by Evans and Ratledge.[44] In particular, Evans and Ratledge stated that oleic acid was the major fatty acid (47–49%), while palmitic acid was about 30% of the total fatty acid composition.[44] Similar findings have been presented by Seo *et al.*, who utilized pretreated cheese whey as substrate.[45]

The final fatty acid composition of the microbial lipids produced by *M. ramanniana* was also similar in all shake flask fermentations carried out at different initial FAN concentrations. The unsaturated fatty acids were also predominant as in the case of *C. curvatus*. Unsaturated fatty acids were more than 60%, while oleic acid was the major fatty acid. Up to 4.5% γ-linolenic acid was observed at the late phase of lipid production. The presence of γ-linolenic acid turns microbial oil into a high value product. The synthesis of γ-linolenic acid, a precursor molecule of prostaglandins, takes place after the dehydrogenation reaction of linoleic acid catalyzed by delta-6 desaturase. The seeds of *Oenothera biennis*, *Borago officinalis* and *Ribes nigrum* plants are the main sources of γ-linolenic acid with content in the range 8–25%.[47] Besides nutritional factor, γ-linolenic acid is also a therapeutic agent with wide applications in pharmaceuticals for eczema, premenstrual tension, diabetes, certain cancers and other pathological condition treatments.[16]

CONCLUSIONS

The results presented in this study showed that the biorefinery concept presented in Figs 1 and 2 should begin with separation of the liquid and solid phases of wine lees, because these two phases could lead to the separation of polyphenol-rich extracts of different composition that could be used in different applications. Furthermore, the ethanol separated from the liquid phase of wine lees could be used for the fractionation of a polyphenol-rich extract from the solid phase of wine lees reducing the operating cost of the whole process. However, screening of different extraction technologies (e.g. microwave, ultrasound) and optimization of process conditions is still required in order to increase the antioxidant activity, purity and extraction yield of polyphenolic compounds. Bustos *et al.* and Rivas *et al.* used distilled wine lees for the extraction of tartaric acid and subsequently as nutrient supplement for the production of lactic acid.[6,48,49] This study shows that besides ethanol and tartrate salts, wine lees could be used for the production of two different polyphenol-rich extracts and nutrient supplements that when combined with concentrated DCW could be used for the production of microbial lipids with food or non-food applications. This work complements the studies of Dimou *et al.* towards the development of a novel biorefinery concept based on the combined utilization of wine lees and cheese whey.[8,20]

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