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Oil production by *Mortierella isabellina* from whey treated with lactase

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HIGHLIGHTS

- De-proteinized whey can be utilized as a feed stock for production of microbial oil.
- DP-whey treated with lactase resulted in the highest microbial oil concentration.
- A higher biomass and oil were obtained with higher initial lactose concentrations.
- The best kinetic parameters were determined in DP-whey treated with lactase.
- The maximum γ-linolenic acid content of 5.48% was obtained at the highest lactose concentration in DP-whey without enzyme addition.

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ABSTRACT

Whey, a by-product of cheese manufacturing is rich in nutrients such as lactose, proteins, and mineral salts. The fungus *Mortierella isabellina* was used for production of oil containing γ-linoleic acid (GLA) during fermentation on deproteinized whey permeate (DP-WP) with and without lactase addition. The maximum oil concentration was 3.65 g/L in DP-whey (16.0% lactose) without enzyme treatment. Treatment of DP-WP with lactase resulted in an increase in oil content to 17.13 g/L. Palmitic (22.50–25.80%) and oleic acids (37.60–48.56%) were the major fatty acids along with GLA (2.18–5.48%), linoleic (16.21–22.43%) and stearic acid (3.20–10.08%). This study suggests that whey can be utilized as a feedstock for production of microbial oil.

1. Introduction

Microbial oil (single cell oil, SCO) is commercially produced as a source of specific lipids for dietary supplements (Fidler et al., 1999; Lewis et al., 2000; Kimura et al., 2004; Papanikolaou et al., 2004; Huang et al., 2009). Several microorganisms accumulate intracellular lipids at more than 50% of the dry cell weight (Ratledge, 1993; Kavadia et al., 2001; Kimura et al., 2004; Papanikolaou et al., 2007). Among these oleaginous microorganisms, certain fungi accumulate intracellular lipids, especially triacylglycerol (TG), depending on the culture conditions.

Oleaginous fungi from the genus *Mortierella* have been used for production of lipids rich in polyunsaturated fatty acids (PUFAs). The major PUFAs produced by fungi are γ-linolenic acid (GLA- C18:3 ω-6), dihomo-γ-linolenic acid (DHGLA- C20:3 ω-6), arachidonic acid (ARA- C20:4 ω-6), and docosahexaenoic acid (DHA- C22:3 ω-3). These PUFAs have medical and dietetic applications (Wibert et al., 1997; Kavadia et al., 2001; Huang et al., 2002; Kimura et al., 2004; Papanikolaou et al., 2004). Long chain polyunsaturated fatty acids (LCPUFA), also called vitamin F, are important nutrients (Boswell et al., 1996; Zhang and Ratledge, 2008). Although GLA production by filamentous *Mortierella* sp. has been reported (Hiruta et al. 1996a), evening primrose oil is currently the major commercial source of GLA (Ratledge 1993; Kavadia et al., 2001). In Japan, *Mortierella isabellina* is used as the SCO producer and GLA-containing SCO is used as food additives in beverages and candies and in the form of tablets as a functional food supplement.

Whey is a by-product of the cheese industry, which represents about 85–95% of the milk volume and contains nutrients, such as lactose, soluble proteins, lipids, minerals, vitamins, and organic acids. The disposal of cheese whey is a continuing and growing issue in the dairy industry. Cheese production, in particular, can cause significant environmental problems if it is not utilized or treated before disposal (Cristiani-Urbina et al., 2000; Athanasiadis et al., 2002; Paraskevopoulou et al., 2003). Due to the high carbohydrate content of whey, it has been utilized for production of value added products, especially by fermentation (Economou et al., 2011a; Vamvakaki et al., 2010).

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Therefore, this study was undertaken not only to evaluate the potential of reconstituted whey for microbial oil production with *M. isabellina*, but also to show the effects of initial sugar concentration and lactase treatment on microbial oil production and to determine the fatty acid profile of the microbial oil.

2. Methods

2.1. Microorganisms and medium

M. isabellina (DSM 1414) was purchased from the German Collection of Microorganisms and Cell Cultures (Braunschweig, GERMANY). The culture was grown on malt extract-peptone agar containing 30 g of malt extract, 3 g of soya peptone, and 15 g of agar per liter of deionized water at pH of 5.6 for 3 days at 27 °C. The culture was stored at 4 °C and sub-cultured bi-weekly in order to maintain viability. For long-term storage, stock cultures were maintained in 20% of glycerol at −80 °C.

2.2. Preparation of whey

Whey powder including (w/w) >65% lactose, 11% protein, 1.5% lipids and 4.5% moisture was supplied by the Izi Dairy Product Company (Konya, Turkey). Whey powder solutions were prepared with different initial lactose concentrations (4.5%, 8.0%, 12.0%, and 16.0% of lactose) using deionized water and deproteinized by heat treatment at 115 °C for 10 min in an autoclave. Precipitated materials were removed by centrifugation at 22,000*g* and at 4 °C for 10 min to produce deproteinized whey permeate (DP-WP) (Cristiani-Urbina et al., 2000).

2.3. Fermentation medium

M. isabellina was firstly inoculated into pre-culture medium containing 5 g of glucose, 1 g of $(NH_4)_2SO_4$, 7 g of KH_2PO_4, 2 g of Na_2HPO_4, 1.5 g of $MgSO_4{\cdot}7H_2O$, 0.1 g of $CaCl_2{\cdot}2H_2O$, 0.008 g of $FeCl_3{\cdot}6H_2O$, 0.001 g of $ZnSO_4{\cdot}7H_2O$, 0.0001 g of $CuSO_4{\cdot}5H_2O$, 0.0001 g of $Co(NO_3)_2{\cdot}H_2O$, 0.0001 g of $MnSO_4{\cdot}5H_2O$, and 0.5 g/L yeast extract per liter of deionized water and incubated at 28 °C, 180 rpm for 48 h. Before inoculation, the pH of the medium was adjusted to 6.0 with 4 N NaOH and the medium was sterilized at 121 °C for 15 min. For DP-WP studies, deproteinized whey was used without any supplementation. Pre-culture was added to the main culture at 5% for all fermentation trials.

2.4. Microbial oil fermentation

The fermentation was carried out in a 250-mL conical flask containing 100 mL of medium at 28 °C and 180 rpm. All fermentations were performed under the conditions described in Section 2.2 for 10 days. Lactose concentrations of 4.5%, 8.0%, 12.0%, and 16.0% were used to determine the effect of initial lactose concentration on microbial oil production in DP-WP medium.

Microbial oil fermentation was also performed in DP-WP containing 4.5%, 8.0%, 12.0%, and 16.0% of lactose after treatment with lactase. Before inoculation with 5% prepared inoculum, DP-WP was treated with 0.25% of lactase solution ($\geqslant$2600 U/g according to the manufacturer (Sigma–Aldrich Chemie, St. Louis, MO, USA) for 12 h at 37 °C, 180 rpm.

2.5. Analysis

Residual sugar content, fatty acid profiles and as well as the amount of microbial oil and biomass were determined in samples taken daily from shaker incubator throughout all fermentation period.

The biomass of the samples was determined by taking 100 mL of fermentation broth filtering through a pre-weighed Whatman No:1 filter paper, and drying at 70 °C for 24 h. Biomass (g/L) was calculated by subtracting the tare of Whatman No:1 paper from weight of the paper containing dried mycelia and dividing by the volume of the sample.

Lipids were determined in biomass harvested by filtration (Whatman No:1) and washed with distilled water. The wet biomass was dried at 70 °C overnight. The dried biomass was extracted in a centrifuge tube using 20 mL of hexane, homogenized with a basic ultraturrax (IKA LABORTECHNIK, Staufen, Germany) for 5 min, kept in a water bath at 55 °C for 10 min and centrifuged at 936*g* for 10 min. The supernatant was transferred to another centrifuge tube. The extraction was repeated twice using 10 mL of hexane, and the supernatant was collected and evaporated using a vacuum rotary evaporator at 45 °C for 10 min. The dried biomass and lipids were measured with an analytical balance (Dong and Walker, 2008).

The lipid profiles were determined by gas chromatographic analysis of the total fatty acids directly transmethylesterified from dried cells. Lipid extracted from dried cells (0.01 g) was added to 2 mL of 10% methanolic HCl and 1.0 mL of methylene chloride mixture and kept at 60 °C for 3 h (Kimura et al., 2004). The reaction was stopped by the addition of 4 mL of saturated NaCl solution and 2 mL of hexane. The resulting methyl esters recovered in the hexane layer were analyzed with a gas chromatograph (GC–MS-QP2010S, Shimadzu, Japan) equipped with a TRB-5MS capillary column (30 m × 0.25 mm × 0.25 μm) at 70 eV (*m*/*z* 40–400; source at 250 °C and quadruple at 100 °C) in the EI mode. Injector temperature was 250 °C with the detector at 250 °C (Kimura et al., 2004). The oven temperature was kept at 100 °C for 2 min and increased to 230 °C at a rate of 4 °C per min. and kept at 230 °C for 3 min. Helium was used as a carrier gas at a flow rate of 34 cm s^{-1}. The injection volume was 1 μL with a split ratio of 25:1. Calibration of the instrument was done using a 14-component FAMEs standard mixture (C8-C24) (Sigma–Aldrich Supelco 18919-1AMP) with margaric acid standard as an internal standard. The mass fragmentation patterns were compared with spectral data from the Wiley and NIST libraries. Library searches were performed using Wiley Registry 9th Edition and Mass Spectral Library 08 MS Search an AMDIS V.2.0 for NIST.

Sugars (lactose, glucose, and galactose) were determined by a Shimadzu LC-20AD HPLC solvent delivery system (Shimadzu, Tokyo, Japan). The HPLC system was equipped with a guard column (CARBOsep Coregel 87P, 4 × 20 mm, Transgenomic, Omaha, NE, USA) and connected to an analytical column (CARBOsep Coregel 87P, 7.8 × 300 mm, Transgenomic, NE) and a Shimadzu RID-10A refractive index detector (Shimadzu). The column was heated to 85 °C with a Varian Mistral column oven (Varian, Palo Alto, CA, USA). MilliQ water as the mobile phase was allowed to flow at a rate of 0.6 mL min^{-1}. The samples were injected with a 20-μL injection volume using a Shimadzu SIL-20A autosampler (Shimadzu, Tokyo, Japan) (Tetik et al. 2011).

2.6. Statistical analysis

All statistical calculations were performed using SAS Statistical Software (SAS Institute Inc., Cary, NC, USA). In order to evaluate the significance of the results, the Generalized Linear Model (GLM; with $p < 0.05$) and Duncan Multiple Test were used. All fermentation processes were replicated twice.

3. Results and discussion

This study was undertaken not only to evaluate the potential of reconstituted whey for microbial oil production, but also to show