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Influence of medium composition and structure on the biosynthesis of the natural flavour 1-octen-3-ol by *Penicillium camemberti*

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Abstract

Biomass production of *Penicillium camemberti* was investigated in selected media, including synthetic media supplemented with either linoleic acid or soybean oil and a dairy medium containing soybean oil and found to be 6.5, 8.3 and 61 g/l, respectively. The presence of soybean oil in the synthetic medium enhanced the production of 1-octen-3-ol, in vitro, by a factor of 8 compared to that obtained with the same synthetic medium containing linoleic acid. The use of the dairy medium in its liquid form or in its acid-gellified milk state as culture media enhanced the production, in vitro, of 1-octen-3-ol by 1.2- and three-fold, respectively, by the enzymic extracts obtained from the biomasses of *P. camemberti* compared to that found using the synthetic medium containing linoleic acid. However, the enzymatic production of 1-octen-3-ol by the *P. camemberti* extracts obtained using the dairy media was lower than that found using the synthetic medium supplemented with soybean oil.

Keywords: Bioconversion; *P. camemberti*; Dairy media; Lipoxygenase; Hydroperoxide lyase; 1-Octen-3-ol

1. Introduction

Penicillium camemberti is a fungus widely used as a starter culture in the food industry. Due to the presence of lipolytic and proteolytic activities in *P. camemberti*, volatile compounds are produced which contribute to the formation of the aroma and flavour of Brie and Camembert cheeses [1]. The production of certain natural flavour compounds, including C_5 to C_9 aliphatic alcohols and carbonyl compounds, results from a biosynthetic pathway involving several enzymic activities, in particular lipoxygenase (LOX) and hydroperoxide lyase (HPL) and polyunsaturated fatty acids (PUFAs) such as linoleic acid as substrates. This biosynthetic pathway is initiated by the oxidation of PUFAs, possessing a 1(*Z*),4(*Z*)-pentadiene moiety, into stereo- and regio-specific hydroperoxides (HPODs), which are in turn converted into C_5 to C_9 compounds, including volatile alcohols, aldehydes, alkanes and alkenes, as well as non-volatile oxoacids [2]. Among the C_8 volatile compounds, 1-octen-3-ol contributes to the typical mushroom-like flavour [3]. However, the presence of high concentrations of 1-octen-3-ol contributes to an important off-flavour [1].

1-Octen-3-ol is generated from linoleic acid by the LOX pathway in fungi [4] as well as in basidiomycetes [5–9]. LOX is a dioxygenase that catalyzes the conversion of PUFAs into 10-hydroperoxides, which are sequentially cleaved by HPL into 1-octen-3-ol and 10-oxo-8-decenoic acid [7–9].

The effect of linoleic acid induction on biomass production as well as changes in glucose consumption, pH and

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1-octen-3-ol profiles during the growth of *P. camemberti* were previously investigated by our group [10,11]. In addition, changes in the 1-octen-3-ol profiles during the growth of *P. camemberti* on synthetic medium containing free linoleic acid were also investigated by this group [12].

In the present work, the effect of the presence of soybean oil in synthetic and dairy media was investigated with respect to biomass production of *P. camemberti*, glucose and lactose consumption, pH values and the production of 1-octen-3-ol. In addition, a comparison of 1-octen-3-ol production by the enzymic extracts obtained from the *P. camemberti* biomasses cultivated on the dairy medium in its liquid or acid-gellified solid state was carried.

2. Materials and methods

2.1. Strain, media and culture conditions

Spores of *P. camemberti* Thom were obtained from Rhodia (Dangé Saint Romain, France) and conserved at −18 °C. The salts were purchased from Sigma (St Quentin Fallavier, France), glucono-δ-lactone (Lysactone®) from Roquette (France) and Tween 20 from ICN, Biomedicals Inc. (Costa Mesa, CA). An ultra low-heat processed powder containing 365 g total nitrogen matter, 80 g non protein nitrogen, 21 g non casein nitrogen, 79 g ash, and 950 g dry matter per kg powder, respectively, was prepared as described by Fairise et al. [13].

The culture media used in this study, included synthetic media and dairy media. The synthetic medium was made by mixing 1 l of sodium phosphate buffer solution (0.1 M, pH 6.5) containing 10 g glucose, 3 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g KCl and 10 mg $MgSO_4$ with either 0.1% (w/v) linoleic acid (synthetic medium 1) or 0.2% (w/v) refined soybean oil (synthetic medium 2). The mixtures were blended at room temperature and 10,000 rpm for 3 min with a 20 mm saw tooth bottom probe (Polytron PT3000, Kinematica, Switzerland) to disperse the oil. The dairy medium was made by dispersing the low-heat-processed powder (10%, w/v) in demineralised water and stirring for 2 h at room temperature and pH 6.5. The reconstituted skim milk was then mixed with 0.2% (w/v) refined soybean oil and an emulsion was made using a 20 mm saw tooth bottom probe (Polytron PT3000, Kinematica, Switzerland) at 10,000 rpm for 3 min and room temperature. The dairy emulsion was subsequently sterilized at 115 °C for 15 min [14]. The dairy emulsion then was divided into two portions where one portion was used in its liquid state (dairy medium 1), while the second portion (dairy medium 2) was treated with glucono-delta-lactone (2.2%, w/v), an acid precursor, according to a modified method described by Cayot et al. [15], in order to mimic the bacteria acidification of yoghurt or cheese and incubated for 12 h at 25 °C to reach a pH of 4 and produce an acid-gellified emulsion. In order to evaluate the stability of the emulsions, the average droplet diameter (d_{43})[1] of the emulsions in the synthetic media 1 and 2 and the dairy medium 1 was measured using a laser counter Mastersizer Hydro2000G (Malvern Instruments, Malvern, England).

The 500 ml flasks containing 200 ml of culture medium were directly inoculated with 1 ml of spore suspension (10^7 spores/ml) and incubated at 25 °C with continuous shaking (120 rpm) for 10 days in an orbital shaker New-Brunswick (Model G-25, Edison, NJ).

2.2. Preparation of mushroom homogenate

Freshly harvested pellets of the microbial biomass were obtained by filtration using a Büchner system and homogenized with a mortar for 5 min at −70 °C using liquid nitrogen. The homogenate was suspended (1:2, w/v) in sodium phosphate buffer solution (0.1 M, pH 6.5). For determination of dry biomass, freshly harvested pellets were placed in an oven until constant weight.

2.3. Protein determination

The enzymic fractions were analyzed for their protein content according to the Lowry method, using bovine serum albumin (Sigma Chemical, St.-Louis, MO) as a standard for calibration [16]

2.4. Bioconversion assay

Production of 1-octen-3-ol by enzymic extracts obtained from the *P. camemberti* biomass, grown on the four media, was determined daily during culture incubation. A stock solution (10 mM) of 65% linoleic acid (ICN, Biomedicals Inc., Costa Mesa, CA) was prepared in sodium phosphate buffer (0.1 M, pH 6.5) containing 0.5% (v/v) Tween 20 using an homogenizer (Ultraturrax, Janke and Kunkel, Staufen i. Br., Germany) at 12,200 for 15 s. The enzyme assay contained 1.6 ml of the phosphate buffer, 0.4 ml linoleic acid stock solution and 2 ml mushroom homogenate. A control assay, containing 2 ml of sodium phosphate buffer (0.1 M, pH6.5), saturated with O_2 for 1 min, and 2 ml of mushroom homogenate, was conducted in tandem with the enzyme assays. The bioconversion reaction was carried out at 25 °C for 15 min, with continuous stirring (500 rpm) and then stopped by adjusting the pH of the reaction medium to 3.0 with 2 M HCl solution. The production of 1-octen-3-ol was expressed as μg 1-octen-3-ol per mg enzymic protein.

2.5. Extraction and determination of 1-octen-3-ol

Each of the four reaction media (2 ml) was mixed with 100 μg of 2-decanone and 1 g NaCl and extracted with 2 ml of diethyl ether. The organic extract was centrifuged (15,000 × *g*, 2 min) and any traces of water in the upper organic

[1] $d_{43} = (n_i d_i^4 / n_i d_i^3)$ where n_i is the number of droplets of diameter d_i.