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Yeast flavour production by solid state fermentation of orange peel waste

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ABSTRACT

Consumer demand for natural products and the requirement for eco-friendly processes promote development of innovative processes for flavour synthesis *via* biotechnology. In this direction, cultivation of selected industrial yeast strain under solid state fermentation of orange peel (OP) was studied. For this purpose, autoclave sterile OP for the elimination of D-limonene and natural microflora was evaluated with regard to yeast viability, nutrient consumption and cell ability to produce flavour active compounds. Non-sterile OP was also used to follow pros and cons of the sterilization process. Yeast cells showed better growth performance under sterilized process conditions, than under non-sterilized ones. In the first case, the enhanced *de novo* synthesis of "fruity" esters was demonstrated (48.7, 25.2, 9.3, 6.3 and 4.5 mg/kg of fermented OP for isoamyl acetate, ethyl dodecanoate, decanoate, octanoate and phenyl ethyl acetate, respectively, after 72 h). Yeast cells exhibited accelerated synthesis of ethyl hexanoate (154.2 mg/kg OP at 48 h). Biotransformation of naturally occurring aroma compounds by yeast may be considered in this process. The proposed process, resulting in high yields of industrially important volatile aroma esters (total of ~250 mg/kg OP), could be applied to a sustainable biorefinery for the valorization of OP waste.

1. Introduction

Flavour-active compounds contribute significantly in the organoleptic properties of many food products. In terms of value, the flavour market accounts for more than 50% of the global market for flavours and fragrances, which is expected to grow from US$ 21.4 billion in 2013 to US$ 25 billion in 2018 [1]. Nowadays, most flavours are either extracted from plant sources or synthesized by chemical means. The rising concern of consumers about natural products and the requirement for eco-friendly processes definitely encourage research and development of alternative processes for flavour synthesis *via* biotechnology. This aspect has been strengthened by the latest EC legislation [2], according to which natural flavours include biotechnology-derived products based on microorganisms, plant cell cultures and enzymes [3–6].

Adoption of microbial technology for flavour production by industry is hindered by high manufacturing costs involved. To build a sustainable and an economically competitive microbial process, the use of expensive substrates, the low productivity and the high pre-treatment and downstream processing costs must be overcome. In this direction, the production of flavour compounds from agro-industrial wastes with negligible or even no-cost, such as orange peels, is an interesting approach. Orange peel (OP) is the major solid waste generated by the fruit processing industry. Its use as a potential substrate for the *de novo* synthesis of isoamyl acetate, phenylethyl acetate and ethyl esters (hexanoate, octanoate, decanoate and dodecanoate) by *Saccharomyces cerevisiae* in liquid fermentation has been recently determined [7,8]. Its notable activity was mainly due to its high level of fermentable carbohydrates, *i.e.* coming from naturally occurring simple sugars (glucose, fructose) and polysaccharides (cellulose, hemicellulose, pectin) after being hydrolysed, along with amino nitrogen. Amongst the various types of microbial processes to convert solid wastes into value-added compounds, the use of solid-state fermentation (SSF) as a means to improve cost effectiveness of these processes and its application for the production of aroma compounds has been recommended. As examples, cassava bagasse, sugarcane bagasse, apple pomace, soya bran and coffee husk have been evaluated for this purpose by cultivating different microorganisms (Table 1). SSF is a process carried out in a solid matrix with sufficient moisture content for microbial growth and metabolism requirements but almost no free water in the system [18]. Due to the limited amount of water, capital and operating costs are

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Table 1
Agro-industrial wastes used for the flavour-active compound production by solid state fermentation.

Microorgansims	Substrates	Flavour active compounds	References
Ceratocystis fimbriata	Cassava bagasse, wheat bran, sugarcane bagasse	Acetaldehyde, 3-methyl butanol, 3-methylbutyl acetate, ethyl acetate, ethyl propionate	[9]
Rhizopus oryzae	Cassava bagasse, soybean meal, apple pomace	Acetaldehyde, 3-methyl butanol, 1-propanol, ethyl acetate, ethyl propionate	[10]
Kluyveromyces marxianu	Cassava bagasse, giant palm bran	Isoamylic alcohol, ethyl acetate, propyl acetate, butyl acetate, ethyl propionate, ethyl isobutyrate, isoamyl acetate	[11]
Ceratocystis fimbriata	Coffee husk	Isopropanol, ethyl acetate, ethyl isobutyrate, isobutyl acetate, isoamyl acetate, ethyl-3-hexanoate	[12]
Moniliella suaveolens, *Trichoderma harzianum*, *Pityrosporum ovale*, *Ceratocytis oniliformis*	Linseed cake, castor oil cake, olive press cake, sunflower cake	δ-and γ-decalactone	[13]
Ceratocystis fimbriata	Mixture of citric pulp and soya bran, sugarcane molasses, soya molasses	Isoamyl acetate	[14]
Aspergillus niger, metabolically engineered *Escherichia coli*	Cereal or maize bran, sugar beet pulp	Vanillin	[15]
S. cerevisiae, *K. marxianus*, Kefir culture	Mixed solid and liquid food industry wastes (*i.e.* cheese whey, molasses, brewer's spent grains, malt spent rootlets, orange and potato pulp)	ϵ-pinene	[16]
Trichoderma viride	Sugarcane bagasse	6-pentyl-α-pyrone	[17]

reduced as a result of lower working volumes per product yield and process wastewater as well as lower energy costs for sterilization and stirring [18–22]. Moreover, SSF of agro-industrial wastes simulates the natural environment of many microorganisms offering high productivity rates, higher product stability and lower extent of catabolite repression [19]. Despite the fact that SSF remains a sustainable approach for the production of natural aroma compounds using various agro-industrial residues, only the work of Rossi et al. [23] has focused on the utilization of citric pulp for the production of aroma volatiles by *Ceratocystis fimbriata* in solid-state cultures. According to their results, citric pulp supplemented with soya bran, sugarcane molasses and mineral saline solution produced a strong fruity aroma.

Following our previous works, where the production of volatile bio-esters by a commercial wine yeast strain (Vitilevure MT) was studied in submerged fermentation (SmF) using OP complemented with a nutritive medium containing glucose, yeast extract and salts [8] or OP hydrolysate [7], in this work the potential of such a waste as a substrate for flavour-active compounds production by SSF using the same microorganism was investigated. In such a case, the SSF would be preferred to SmF if it could provide several advantages such as higher productivity and lower pre-treatment, downstream processing and waste disposal costs. Since elimination of autoclave sterilization is expected to reduce the overall cost of the final products, the effect of non-sterile conditions on process parameters such as cell viability and nutrient assimilation was also assessed. Gas chromatography (GC–MS, GC–FID) analysis was employed to monitor changes in the composition of flavour-active compounds during the fermentation process.

2. Materials and methods

Fresh Washington Navel oranges were purchased from the local market. The peel (white mesocarp and orange–yellow exocarp), remained after the extraction of the juice, was sliced into small pieces and grounded with an electric mill (Braun 4240, Germany).

2.1. Compositional analysis

Orange peel moisture content was determined by gravimetric analysis after drying at 105 °C to constant weight. Key nutrients in the different fractions of OP were quantified as described by Mantzouridou and Paraskevopoulou [8].

2.2. Yeast strain and inoculum preparation

A commercial wine yeast strain, Vitilevure MT (*S .cerevisiae*) that expresses and enhances varietal aromas very well was employed in this study. The selected yeast was provided in dry form by a Greek wine industry (Tsantalis S.A., Chalkidiki, Greece). Cells were activated by adding the appropriate quantity of distilled water (1:10, w/v), followed by periodical stirring inside a water bath (35–37 °C)

Table 2
Key nutrient composition analysis of raw and fermented orange peel (OP) in the sterile and non-sterile trials.

Nutrient	Value[b] (% on dry basis)			
	Raw OP		Fermented OP[c]	
	Non sterile	Sterile	Non sterile	Sterile
Alcohol-soluble carbohydrates[a]	26.70 ± 0.60^{C}	28.90 ± 2.10^{D}	20.09 ± 0.31^{B}	1.73 ± 0.05^{A}
Alcohol-insoluble solids	8.67 ± 0.11^{C}	7.35 ± 0.86^{B}	5.92 ± 0.08^{A}	7.88 ± 0.12^{B}
EDTA-soluble solids	18.46 ± 0.51^{B}	18.00 ± 2.33^{B}	11.58 ± 0.33^{A}	17.41 ± 0.62^{B}
Water unextractable polysaccharides[a]	15.83 ± 0.25^{B}	13.78 ± 1.65^{A}	14.00 ± 0.28^{A}	$14.96 \pm 0.32^{A,B}$
Crude protein	4.25 ± 0.56^{A}	$4.70 \pm 0.32^{A,B}$	5.23 ± 0.16^{B}	9.72 ± 0.55^{C}
Fat	1.22 ± 0.10^{A}	1.28 ± 0.08^{A}	1.30 ± 0.02^{A}	1.36 ± 0.05^{A}

[a] Expressed on a glucose equivalent basis.
[b] Mean value of three independent measurements ± standard deviation.
[c] After 5 days of the fermentation process; different capital letters were used to label significantly different values in the same row.