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BOOK OF ABSTRACTS

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22A-10

APICULATE WINE YEASTS: GROWTH KINETICS AND AROMA COMPOUNDS PRODUCTION

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The growth of non-*Saccharomyces* yeasts, mainly belonging to the genera *Hanseniaspora*, characterize the first stages of grape juice fermentation; these strains disappear during the later fermentation stages, when prevailing *Saccharomyces cerevisiae* strains complete the process. The combined action of these yeasts species determines the type and concentration of many compounds that may contribute to the aroma and flavor of wine. In this work, the growth kinetics of the yeasts *Hanseniaspora uvarum*, *Hanseniaspora guilliermondii* and *Saccharomyces cerevisiae* in commercial culture media and grape musts from the Douro region was followed. Pure and mixed yeast cultures were tested. The influence of the yeast species on the concentration of volatile compounds of the fermented media and wines, such as higher alcohols, esters and sulfur compounds, was also studied. The growth kinetics of the yeasts were followed using the viable cell count in Yeast Malt Agar medium. Volatile compounds were analyzed using Gas Chromatography. Growth of each yeast species during commercial media fermentation showed that apiculate yeasts present a stronger fermentative capacity than *Saccharomyces cerevisiae*, reaching highest cell concentrations. Using grape must, *Saccharomyces cerevisiae* keeps its activity until the end of fermentation, while apiculate wine yeasts dye off after a few days. Fermented media and wines elaborated with *Hanseniaspora uvarum* present a low content in higher alcohols and sulfur compounds. Furthermore, *Hanseniaspora guilliermondii* produces high amounts of esters (2-phenethyl acetate and isoamyl acetate) and 2-phenylethanol, when inoculated as pure or mixed cultures in commercial media and grape must. The analysis of sulfur-containing compounds showed that yeasts produce different amounts of these compounds being able to produce different compounds, according to the growth medium. By understanding the behavior of non-*Saccharomyces* yeasts we will be able to improve the control of alcoholic fermentation and therefore wine quality.

22A-11

OPTIMIZING GAMMA-LINOLENIC ACID PRODUCTION BY *CUNNINGHAMELLA ECHINULATA* IN SUBMERGED TANK

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Seed oil of evening primrose is rich in gamma-linolenic acid (GLA) and has therapeutic effects on premenstrual tension and diabetes. Productivity of GLA from seed oil is extremely low. The fungus *Cunninghamella echinulata* CCR 31840 has been demonstrated as a culture possessing great GLA production potentiality in shake-culture. A production strain, however, should be able to perform in a submerged tank culture, which is assumed to be more representative of an industrial system. The objective of this study was to examine and optimize the effects of temperature, pH, dissolved oxygen tension (DOT), agitation rate, and impeller type on GLA production by *C. echinulata* CCR 31840 in a stirred-tank fermentor. Each variable was independently examined and the optima of each variable were then combined in the final study. Yields and/or contents of biomass, lipid and GLA from each batch were determined and compared. As temperature was raised from 19 to 31 °C, the GLA content in lipid dropped from 17.6 to 7.7%. The increase in DOT from 2 to 7.5 ppm resulted in the increase of lipid yield from 7.1 to 9.0 g/L. The GLA yield at 600 rpm was 2.8 times of that at 200 rpm. The biomass and lipid yields from paddle-type impeller were 19.3 and 3.8 g/L, respectively. Those from propeller-type impeller were 27.4 and 8.9 g/L, respectively. Under the combined conditions of individual optima (temperature, 22 °C; pH, 7.0; DOT, 7.5 ppm; agitation, 600 rpm; impeller, propeller-type), the GLA yield after 5 days was 1,720 mg/L. Temperature was the major determinant of fatty acid composition, whereas DOT and agitation rate were critical to both lipid and biomass yields. To our knowledge, this is the first report of the changes in the microbial lipid production and fatty acid composition due to different flow patterns created by various impeller configurations.

22A-12

TRYPSIN IMMOBILIZATION ON DERIVATIZED CELLULOSE BEADS BY BIOSPECIFIC AVIDIN-BIOTIN INTERACTION AND CHARACTERIZATION OF THE IMMOBILIZED ACTIVITY

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Use of immobilized enzymes in bioprocesses offers many advantages, among which is greater productivity because the same enzyme molecules can be used over a long period of time. The cost of immobilized enzyme preparation has limited commercial application of enzyme bioreactor processes. Development of suitable, inexpensive support matrices such as cellulose beads for enzyme immobilization is therefore necessary for commercialization of immobilized enzyme technology. Our objectives are: (1) to immobilize trypsin on derivatized cellulose beads by using avidin-biotin technology and (2) to characterize the trypsin bioreactor and compare this with trypsin immobilized on controlled-pore glass (CPG) beads. Trypsin was immobilized on cellulose beads using the biospecific and high affinity avidin-biotin interaction. Trypsin and cellulose beads were biotinylated with sulfosuccinimidyl-6-(biotinamido)hexanoate (NHS-LC-biotin). Avidin and bioti-

nylated trypsin were sequentially adsorbed to the biotinylated cellulose beads. A similar procedure was carried out using CPG beads. The properties of the two trypsin bioreactors were examined and compared. The substrate used for the assay of trypsin activity was p-toluene-sulfonyl-L-arginine methyl ester (TAME) and the extent of biotinylation of biotinylated trypsin and of immobilized biotin on cellulose beads and on CPG beads were determined using the HABA dye-binding method. Biotinylated trypsin in solution retained about 82% of the specific activity of native trypsin. Cellulose beads contained 0.184 mol/ml (1.15 mol/g) biotin and CPG beads, 0.329 mol/ml (0.987 mol/g). After regeneration, the biotin contents became slightly lower, namely, 0.159 mol/ml for cellulose beads and 0.315 mol/ml for CPG beads. The specific activities of trypsin immobilized on cellulose beads and CPG beads were 32.32 U/ml (202.03 U/g) and 43.45 U/ml (130.35 U/g), respectively. These studies indicate that cellulose beads, already used commercially in the food industry, can be biotinylated for use as a bioselective support. For example, streptavidin-enzyme fusion proteins produced by fermentation of recombinant cells could be purified and immobilized in one-step to produce active immobilized enzyme bioreactors. Furthermore, bioreactor activity could be regenerated by desorption of inactive enzyme followed by reabsorption from the crude culture lysates.

22A-13

MUTANTS OF *LIPOMYCES TETRASPORUS* OVERPRODUCING AMYLASES AND RESISTANT TO CATABOLIC REPRESSION

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Of all the groups of microorganisms studied as source of microbial biomass, the yeast have the most favorable characteristics. Food processing wastewater can be an excellent feedstock for yeast production. In a previous work using waste starch, *Lipomyces tetrasporus* hydrolyzed the starch to simple sugars very efficiently. However the synthesis of amylases of this microorganism is repressed by glucose, the main product of the degradation of starch. The objective of this work was to isolate a strain of *Lipomyces tetrasporus* that overproduces amylases and is resistant to catabolic repression. Ethyl methane sulphonate was used as mutagenic agent and 2-deoxy-glucose (2-DG) was employed to get the resistance to repression by glucose. Isolation of mutants was using plaques with starch-agar-peptone (g/l: 10.23 and 3.5 respectively) and 2-DG (0.08%). Strain mutant *L. tetrasporus* L-000675/100 was obtained and showed good growth (4.7 mg/ml) with very high production of amylolytic activity (5.3 times more than the wild type). Genetic stability of the strain was showed and the mutant did not lose their resistance to catabolic repression. This strain could have a potential use in the production of biomass from starch wastes.

22A-14

PURIFICATION OF CARBOXYPEPTIDASES FROM SQUID HEPATOPANCREAS

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Carboxypeptidase has the potential to de-bitter protein hydrolysates. The bitter taste of peptides appears to be closely related to the content and sequence of hydrophobic amino acids and is the main hindrance to enzymatic hydrolysis of protein. The hepatopancreas from Atlantic short finned squid was shown to contain several carboxypeptidases. Furthermore, hepatopancreas is an abundant waste from the squid processing plants. Identification of carboxypeptidases with the ability to de-bitter protein hydrolysates would increase the value of squid viscera relative to its current use for animal feed. The objective of the study was to develop a chromatographic method for purifying squid hepatopancreas (SH) carboxypeptidases. CBZ-dipeptides were used as substrates to detect the presence of different carboxypeptidases. The released amino acid was determined by using the OPA method. After ammonium sulfate fractionation, a series of methods including DEAE-cellulose and gel filtration chromatography were used to purify individual carboxypeptidases. The purification of carboxypeptidases was also monitored by activity gel electrophoresis. Nine substrates, out of 33 CBZ-dipeptides, were hydrolyzed extensively by SH extracts. These were Phe-Leu, Ala-Leu, Val-Leu, Ile-Leu, Gly-Phe, Ala-Phe, Val-Phe, Leu-Tyr and Phe-Met. These substrates had Leu, Phe, Tyr, which are hydrophobic amino acids, at the C-terminal of CBZ-dipeptides. A purification of Phe-Leu carboxypeptidase up to 35 fold was achieved. The purified SH carboxypeptidase will be tested as Cheddar cheese ripening aids. It has promise as a de-bittering agent because of its ability to hydrolyze peptides containing hydrophobic amino acid residues.

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PURIFICATION OF PROTEASES FROM PRAWN HEAD WASTES

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World enzyme market has been growing during the last several decades, and proteases are used by 60% of the international market. The establishment of fast and easy methods for protease separation may help to understand better the activity mechanisms and applications. The objectives of this study was to purify proteases from prawn wastes using ultrafiltration and isoelectro focusing systems, as well as to characterize pH and temperature profiles. Prawn hepatopancreas were