Development of production technology and application forms of glutathione with high bioavailability

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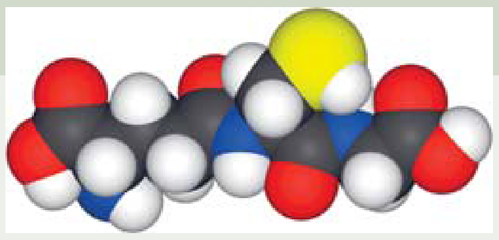

INTRODUCTION

Glutathione (GSH), a tripeptide (γ-glutamyl-cysteinylglycine), is the major free thiol in most living cells and is involved in many biological processes such as detoxification of xenobiotics, removal of hydroperoxides, or maintaince of normal function of the immune system. It is the key antioxidant in animal and human tissues. Oxidation of GSH leads to the formation of glutathione disulphide (GSSG). GSH can be regenerated from GSSG by the enzyme glutathione reductase.

Two yeast strains of different genus - a commercial bakery yeast *Saccharomyces cerevisiae* and *Candida ethanolica* (ATCC 1, RIFIS 417) produced by aerobic fermentation in synthetic medium containing ethanol as the only source of carbon have been examined as a potential source for GSH production. GSH yields after yeast cell disintegration by thermolysis were determined. Effects of different incubation conditions of the yeast biomass in the GSH biosynthesis and different isolation methods have also been tested.

Starch and mucoadhesive biopolymers, such as CM-chitosan, gelatine and hyaluronic acid were utilised to produce nanofibers as potential excipients for glutathione GSH peroral, sublingual and buccal delivery systems. Universal centrifugal system, based on a rotating bell enclosed in a tube, for the nanofibers formation has been developed and tested on laboratory scale.

MATERIAL AND METHODS

Yeast strains

1) *Sacharomyces cerevisiae*, compressed bakery yeast LINCO (F.X. Wieninger GmbH, Passau, Germany), 31,89 % of dry matter (DM)
2) *Candida ethanolica* , ATTC1, RIFIS417, centrifuged paste, 22 % of DM (Food Research Institute Prague, Czech Republic)

GSH determination during aerobic cultivation of Candida ethanolica

Aerobic fermentation was carried on a synthetic medium with ethanol as a carbon source, ammonia as a nitrogen source and mineral salts, temperature 33°C, pH 4,5, and aeration 1,3 dm3. h-1 per litre of the fermentation medium. Ethanol concentration in the medium was kept in the range 0,3 - 0,8 % v/v.

Bakery yeast incubation with addition of N-Acetyl-L-Cysteine

The yeast suspension in water containing 3,19 % (w/w) DM was supplemented with different concentrations of N-Acetyl-L-Cysteine (Wessex House, UK) in the range from 0,25 to 16 %, relative to the yeast DM. The suspension was incubated under semi-aerobic conditions in Erlenmeyer flaks using an orbital thermostatted shaker (100RPM) for 4 hours at 32°C. Values of pH in the suspensions changed in the range from 4,40 to 2,55 along with the increasing N-Acetyl-L-Cysteine concentration.

GSH extraction

All the samples of yeast biomass pretreated as described above were kept at 75°C for 30 min under continuous stirring and centrifuged (RCF 10620 x g, 30 min.). The supernatant was used for DM and GSH determination.

RP HPLC determination of GSH

GSH concentration were determined according to a method described by Chwatko and Bald (Chwatko G., Bald E. Determination of cysteine in human plasma by high-performance liquid chromatography and ultraviolet detection after pre-column derivatization with 2-chloro-1-methylpyridinium iodide. Talanta 2000; 52(3):509-15). RP-HPLC separations were conducted using the Agilent Technologies 1200 Series apparatus equipped with UV-VIS DAD detector, 20 µl sample loop, and Onyx Monolithic C18 column (Phenomenex) was used.

Preparation of nano / microfibers with GSH

Universal centrifugal system for nano / microfiber formation on the basis of a rotating bell has been developed and tested on laboratory scale. The nanofibers produced by the system, containing up to 33 % (w/w) GSH in a mixture with starch, gelatine and CM-chitosan in different combinations, were characterized by microscopic methods.

RESULTS AND DISCUSSION

GSH determination during aerobic cultivation of Candida ethanolica

Significant GSH accumulation was observed repeatedly in the last grow phase (death phase) after ethanol (carbon source) depletion during aeration of the yeast suspension (Fig. 1). Possible explanation of this effect can be an induction of biosynthesis of storage compounds after the nutrient depletion.

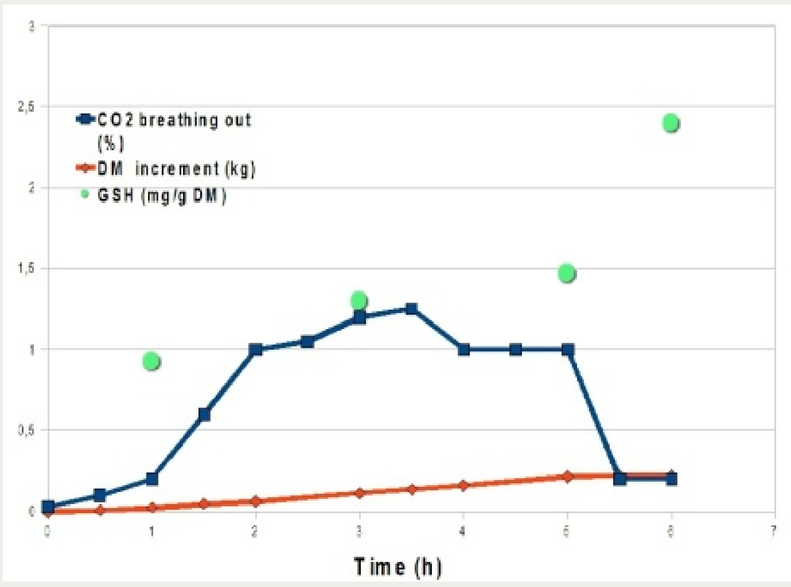

Fig.1. GSH determination during aerobic cultivation of Candida ethanolica

Bakery yeast incubation with addition of N-Acetyl-L-Cysteine

Intracellular GSH contents in the commercial bakery yeasts *Saccharomyces cerevisiae* range usually from 0,3 to 1 % of yeast DM. N-Acetyl-L-cysteine addition stimulated the GSH synthesis in a dose dependent manner up to the concentration of 1 %, relative to the yeast DM, when the yeast intracellular GSH concentration was increased by a factor 4,5. Higher N-Acetyl-L-cysteine than 1 %, relative to the yeast DM, has already decreased the intracellular GSH content, probably because of an inhibition by the acidic pH values.

Preparation of nano / microfibers with GSH

Fig. 2 shows microfibers containing GSH (33,3 % w/w) in a mixture with gelatine and CM-chitosan. Micro- and nanofibers containing GSH in combination with different starch content were also prepared.

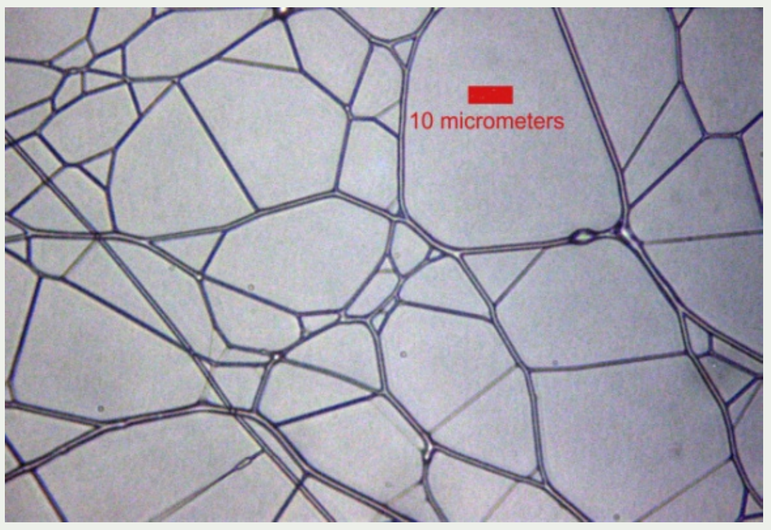

Fig. 2. CM-Chitosan (33,3%) - Gelatin (33,3%) - GSH red. (33,3%) nanofibers (prepared in 5% formic acid)

CONCLUSIONS

Industrial extraction of glutathione from yeast biomass appears as perspective, especially in the case of complex utilization of the yeast biomass with simultaneous production of other byproducts with commercial potential, such as glucomannan complex and food yeast extracts.

Nano / microfibers containing up to 33 % (w/w) GSH in a mixture with starch and mucoadhesive polymers are to be tested *in vivo*, especially for sublingual and buccal GSH administration with high bioavailability.

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