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Original Article

EVALUATION OF PROTEIN RELEASE RATE FROM MYCOPROTEIN - *FUSARIUM VENENATUM* BY CELL DISRUPTION METHOD

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ABSTRACT

Objective: Single cell protein based on mycoprotein is now extensively used as human and animal feed in various parts of the world. It is used because of the high nutrient content particularly protein and the supply of protein is an essential criteria of utilization of mycoprotein. The present study is about evaluation of protein release rate from mycoprotein - *Fusarium venenatum* by cell disruption method.

Methods: *Fusarium venenatum* was cultivated in Vogel's mineral medium and the separated biomass was subjected to lyophilization followed by grinding and sonication under different time periods to release the protein. Liberated protein was estimated by Lowry's method and the protein release rate was determined.

Results: Maximum protein release rate constant 0.680 min was recorded in grinding with sonication.

Conclusion: Protein release rate from mycoprotein – *Fusarium venenatum* by cell disruption method is the useful study to determine the optimal utilization of nutrient factors supplied by the mycoprotein to the consumers.Further studies will be helpful to determine the release profile with suitable animal model.

Keywords: Mycoprotein, *Fusarium venenatutum*, Release constant.

INTRODUCTION

A microbial cell contains various sources like proteins, enzymes, fatty acids, polymers and antibiotics. These sources can be extracted and used for various pharmacological and nutraceuticals applications. Generally microbial products are two types i.e intracellular and extracellular products. Extracellular products can be easily separated and easily purified, but the intracellular compounds are highly sensitive, so efficient methods have to be applied to liberate from the cells. In downstream processing, cell disruption is an important technique to release the intracellular products. However they have several disadvantages, the most important being high energy requirement leading to increased cost, degradation of biomolecules during the process due to high heat generation and development of very fine debris that may interfere during subsequent downstream processing[6&9].The cell disruption process can be generally classified as mechanical, non mechanical and biological methods. Among the above methods, grinding and sonication methods are widely used in all the biological laboratories to release the intracellular compounds. Grinding method is simplest method, and the shear forces break up the cell wall. Sonication method is effective method for bacteria and yeast. The principle behind on this the ultrasonic converter, equipped with an ultrasound oscillating system, transforms the electrical energy delivered by the generator into mechanical energy. Oscillations of the same frequency the disruption that occurs when the cells are irradiated with ultrasonic energy is due to the cavitation phenomenon. Doulah has suggested that cell disintegration is caused by shear stresses developed by viscous eddies arising from shock waves produced by imploding cavitation bubbles[5]. In the present study, protein release constant of *Fusarium venenatum* is discussed. *Fusarium venenatum* was chosen at the year of 1960 and after intensive testing the Mycoprotein for 12 years it was approved for sale as consumable by the Ministry of Agriculture[14]. The product is now available in Six European Countries only and the filaments of the fungi were used as mycoprotein which are rich in protein (44%) and less cholesterol. Pharmacological activities such as antioxidative and anti-tumor activities of *F.venenatum* has been recently reported[10]. Generally releasing of intracellular compounds from fungi is not an easy task since they have rigid cell wall. The cell wall is composed with chitin (Poly-N-acetylglucoamine) and β-glucans with β 1-3 and β 1-6glycosidic linkages[9].The aim of this study is to develop an efficient method of cell disruption for release rate of protein from *Fusarium venenatum*.

MATERIALS AND METHODS

Fungal strain

Fusarium venenatum was obtained from Fungal Biodiversity Centre Netherland in lyophilized form and the fungi was activated in oats meal medium. An activated fungal culture was maintained on the oats meal agar slant as monosporic culture at 4°C.

Preparation of medium

Fusarium venenatum inoculum was prepared in Vogel's minerals medium which consisted of 10 g glucose, 2.6 g $Na_3C_6H_5O_7 \cdot 2H_2O$, 2.52 g KNO_3, 2.88 g $(NH_4)H_2PO_4$, 1.6 g KH_2PO_4,0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g, $CaCl_2 \cdot 2H_2O$, 2.5 mL of biotin solution and 5 mL of trace elements per liter. The trace elements solution consisted of 0.1 g Citric acid, 0.1 g $ZnSO_4 \cdot 7H_2O$, 0.02 g $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$, 5 mg $CuSO_4 \cdot 5H_2O$, 1 mg $MnSO_4 \cdot H_2O$,1 mg H_3BO_3, 1 mg $Na_2MoO_4 \cdot 2H_2O$ per 100 mL. The pH of the medium was adjusted to 5.8[12].

Collection and Processing of Biomass

After the incubation period, the culture broth was subject to heat shock at 64-65°C for 20-30 min[14]. After the heat processing, RNA of the biomass was determined adopting Ahangi *et al* method[1]. Then the broth is filtered through Whatman No.1 filter paper, the filtrate was discarded. The collected mycelial biomass was washed with milipore water and the washed biomass transferred to sterile flask. It was incubated overnight at -20°C.Then the frozen biomass was subjected to lyophilization. The lyophilized biomass was stored in a sterile container for cell disruption studies.

Cell disruption

100 mg of lyophilized biomass was gently homogenized by morter and pestle, followed by 5ml of extraction buffer with 100mg of glass pieces. Grinding was attempted with different intervals such as 5,10,15,20,25 and 30 mins.At end of the experiment protein content

was determined by Lowry's method. In addition, sonication with grinding method was attempted with various time intervals such as 2,4,6,8, and 10 min sonication and 5,10,15,20 and 25 min grinding, the protein releasing content was determined at respective time periods.

Protein Estimation

The concentration of protein was determined by the Lowry method using bovine serum albumin as standard[7]. The total protein in the biomass suspension was measured after alkaline hydrolysis, followed by folins phenol reagent and read at 620 nm.

RESULTS AND DISCUSSION

Microbial sensitized products are usually located inside the cells. In order to release the compounds without denaturation by cell disruption method. Many methods are followed; they are physical, mechanical, and biological method. To find out the rate of intracellular protein release rate generally follows first order kinetics is generally adopted and in this study release of intracellular mycoprotein could be described by the following equation:

$$\ln (R_m/R_m - R) = Kt \text{ ---------- } (1)$$

Where Rm is the maximum protein available for release R is the amount of protein released and K is the release rate constant (/min). Anand *et al.* modified the equation (1) to determine the release of protein during permeabilization using pretreatment[3]. Similarly in the present study, two methods were attempted to release the mycoprotein from *Fusarium venenatum*, namely grinding with glass pieces and sonication followed by grinding.

Table 1: Effect of grinding on protein release (µg) of *F.venenatum*

Grinding time in mins	Released protein content µg (mean±SD)
5	50±10
10	110±14
20	200±20
25	280±25
30	340±30

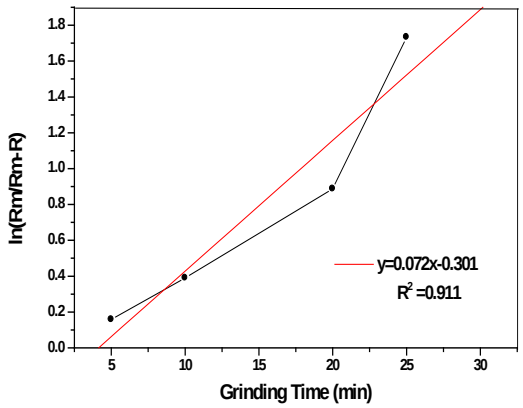

Fig. 1: Protein release kinetics from *Fusarium Venenatum* by grinding

Table 2: Effect of sonication with grinding method on protein release of *F.venenatum*

Sonication (mins)	Grinding time in mins	Released protein content µg (mean±SD)
2	5	150 ± 0.12
4	10	210 ± 0.14
6	20	320 ±0.23
8	25	400 ±0.39
10	30	580 ±0.33

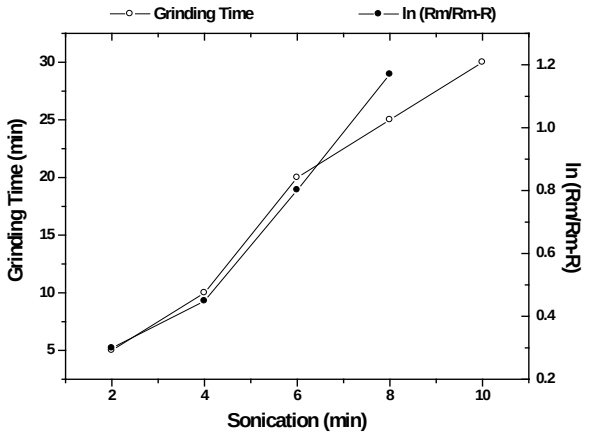

Fig. 2: Kinetics of protein release from *Fusarium Venenatum* by sonication with grinding

Maximum protein release was recorded at 10 min pretreatment of sonication with 30min grinding and rate of protein release constant K was 0.680 min^{-1} with 580 µg of protein content (Table 2, Fig.2). Anand *et al.*studied release of acid phosphatase in *Saccharomyces cerevisiae* pretreated with EDTA solution under high pressure homogenization[3]. Protein release kinetics study with grinding reveals 30min of grinding shows maximum protein release kinetics 0.792 min^{-1} with 340µg of protein content. Apar and Ombekstudied the protein release kinetics of *Saccharomyces cerevisiae* using ultrasonication method[4].

Their study demonstrated that increased protein release kinetics was recorded at pH 7 without the effect of cell disruption. In contrast, protein release kinetics was highly influenced by changes in pH and cell disruption. But on some cases, protein release was totally independent of cell concentration. In another experiment, Lipoic acid in *Saccharomyces cerevisiae* was influenced by cell disruption methods[11]. Wang *et al* also reported that the release of total protein was independent of cell concentration[13].

CONCLUSION

In the present study maximum protein release rate K was 0.680 min with 580 µg of protein. A further study in this area is essential to explore the principle of protein release kinetics of *F.venenatum* for the nutraceutical application.

CONFLICT OF INTERESTS

Declared None

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