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RESEARCH ARTICLE

STUDIES ON APPLICATIONS OF FUNGAL GLUCOSE-OXIDASE IN APPLIED INDUSTRY

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ABSTRACT

Glucose oxidase a thermotolerant enzyme was produced in our laboratory from screened strain of *Aspergillus niger* strain F-C405-2. This extracted enzyme was used to study its application in Food industry, such as stabilizer in wine and additive in bakery and other food products and Pharmaceutical Industry, such as antibacterial agent and diagnostic enzyme. During wine preservation the presence of glucose oxidase reduces the alcohol content in wine by 2.5% upto 3 months when compared to control. Glucose oxidase acts as a stabilizing agent for colour (White and Red Wine) and flavour (light, crisp, fruity). As an additive glucose oxidase improved the macro properties like weight, height to width ratio, water absorption property and tenacity. Over 12 days period comparison made between control (without glucose oxidase) and test (with 0.0075% glucose oxidase) it was observed that weight of bread loaf decreased from 46.94 to 33.15gms. However height to width ratio increased from 0.33% to 0.70%, water absorption property is increased as 1.11% to 3.52% and tenacity increased from 41mm to 88mm. Bactericidal activity was observed on Lab scale for common food contaminants like *E.coli, Salmonella typhi, Pseudomonas aeruginosa and Staphylococcus auerus.* Primarily the immobilized GO was used to measure blood sugar level from various patients to detect the diabetes. At lab scale gluconic acid was produced. The gluconic acid (4.11%) was estimated from fermentation liquor which has wide application in food and pharmacy. Cheap and economic production of fungal glucose oxidase will be widely applicable to food and pharmaceutical industry.

Key words: Fungal glucose oxidase, Food industries (Bread and Wine), stabilizing agent and additive, bactericidal agent and Pharmaceutical industry.

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INTRODUCTION

Glucose oxidase (E.C. 1.1.3.4) is a non-hydrolytic enzyme belonging to the oxidoreductase family. It is also called as glucose aerodehydrogenase. Glucose oxidase catalyses the oxidation of β-D glucose to D-glucono1,5-lactone and H_2O_2 and finally to gluconic acid. It is produced from fungi, yeast and few bacteria of various origin. Glucose-oxidase (GO) is important enzyme in Food and Pharmaceutical industry. Glucose oxidase has generally regarded as safe (GRAS) under FDA classification. It is used as food additive in liquid or powder and often classified with antioxidant, preservatives and stabilizer properties. As there are demands for reduced alcohol in wines and easiest ways to do this is to add glucose oxidase before fermentation. GO consumes some of the glucose present, making them unavailable for alcohol fermentation, there by resulting in wine with reduced alcohol. Glucose-oxidase (GO) is an important diagnostic enzyme and used extensively in detection of glucose from blood, food, and fermentation products. Glucose oxidase is also used as commercial source of gluconic acid, which is used as food additive to act as an acidity regulator or bleaching in food manufacturing. GO, as well as peroxidase can be used as anti-microbial agents in oral care products.

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MATERIALS AND METHODS

Immobilised Glucose-oxidase wine preservation: Role of Glucose-oxidase in wine preservation on Lab scale was studied for 3 months. Bulk (white and red) and finished wine were collected from wineries. A set of finished wine (white and red) was kept as control and bulk wine was added with immobilized glucose oxidase (5mg~Beads/250ml). All sets of wine were preserved for aging at 5^0C for 3months. During preservation samples (5ml quantity) were drawn from each set for every month and tested for any change in colour and flavour at same time for alcohol content by potassium dichromate method.

Glucose oxidase in food preservation: A basic bread formula based on flour weight was used. Dough was optimally mixed until dough development, divided into 315g pieces. Hand rounded pieces put into well greased tin pans for 90 minutes at 30^0C and 75% RH for maturation, and baked into an electric oven for 35 minutes at 200^0C. Loaves were removed from the pans, cooled for 2 hours at RT, then packed in plastic bags and

stored at 25^0C for aging about 12 days. Bread analysis was carried out by measuring weight, height to width ratio of central slice and water holding capacity (Porosity).

Glucose oxidase is found as a natural preservative: Bactericidal activity was observed on lab scale for common food contaminants like *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa and Staphylococcus aureus.* Antimicrobial activity was determined by disc diffusion method. Presterilized nutrient agar plates containing growth of food pathogens were layer with well containing 100µl of GO. Plates were incubated at 37^oc for 18-24hrs. Antimicrobial activity measured in terms of zone of inhibition around well.

Designing of Glucose-oxidase biosensor by using immobilized enzyme and monitoring of blood sugar: Primarily the immobilized Glucose-oxidase was used to measure blood sugar level from various patients to detect the diabetes. The next level was designing glucose oxidase biosensor by preparing glucose sensitive electrode.

Biosensor Preparation: In a typical procedure, Cellulose acetate was soluble in pyrrole with 14 wt % of Cellulose acetate in the Pyrrole solvent. The Py–CA viscous solution was stirred overnight at room temperature until a homogenous solution was obtained.

The CA film was then prepared using wet cast technique as follows: The resultant Py–CA viscous solution was spread on a glass plate (75 x 25 mm) with about 100 um thickness of the spread solution. The thickness of the film was controlled by using lumirror film (Toray, Japan) used as a spacer to be about 100 um. Then, the plate with the spread Py–CA solution layer was immersed immediately in aqueous solution containing 80mM concentrations of $FeCl_3$ at 25^0C. The CA coagulation was conducted in water (100 ml) containing 80mM concentration of $FeCl_3$, since the oxidation polymerization of Py was occurred. The PPy formed in the CA film matrix on the glass plate became black after a certain time. The resultant black film washed using distilled water. These membranes were then placed in glucose oxidase solution prepared in 0.1M phosphate buffer (pH 6.0) at 25^0C for 24 h and then washed thoroughly with de-ionized water in order to remove the unbound enzyme. The change in response potential of the active device is the parameter of interest for sensor application. The conductivity of PY-CA-GO electrode is depend on several factors, such as analyte pH , temperature, polymer film potential, substrate concentration and enzyme loading. The GO was immobilized on PY-CA film by cross-linking via glutaraldehyde. The potential-time relationship of PY-CA-GO electrode when applied current of enzyme was set 0.5 mA in phosphate buffer as shown in Plate No.14. It was found that the response potential of enzyme electrode easily reached steady state. It was found that, potential increases with increasing in glucose concentration in range from 500µg/ml-4000µg/ml. In present case assuming that the enzyme is uniformly distributed throughout the film, the reaction takes place predominantly on surface of the film. With the increasing concentration of glucose, the response potential also increases and finally reached steady state value. Standard graph of glucose was plotted and different concentration of glucose from normal, diabetic and saviour diabetic persons were determined. Efficiency of glucose oxidase biosensor was determined by correlating our outputs with the results obtained using commercial biosensor (Glucometer form) prepared using glucose oxidase synthesised chemically.

In production of gluconic acid as food preservatives: Gluconic acid produced by inoculating 8.0% v/v inoculums size in 50ml production media. Temperature of media was adjusted at 50^oc and incubated for 72hrs.After incubation the supernatant was separated from cell mass and used as crude source of gluconic acid. 5.0ml of supernatant was taken and then equal amount distilled water was added to it, boiling for 30 sec. after cooling 2-3drops of phenolphthalein was added as an indicator and titrated with 0.01N NaoH till light pink colour appeared.

RESULTS

Immobilised GO in wine preservation: GO acts as a stabilizing agent for colour (colourless and red) and flavour (light, crisp, sweet and fruity). GO also reduces alcohol content in wine during preservation by converting glucose to gluconic acid. No any significant changes were observed in colour and flavour (Table No.1) during preservation. GO slowly reduces alcohol content (2%) during preservation means our enzyme is playing important role in converting glucose to gluconic acid as pH reduces preservative efficacy goes on increasing. Thus GO can act as an agent for preventing spoilage of a wine.

GO in food preservation: GO is used as an additive due to its oxidizing effects. It promotes for stronger dough in bakery. Bread analysis was carried out by measuring weight, Height to width ratio, water absorption property and tenacity over 12 days period. It is observed that weight of bread loaf decreased (46.94, 44.52, 40.61, 39.59, 35.18 and 33.15 gm respectively). However height to width ratio (0.33, 0.42, 0.54, 0.58, 0.64 and 0.70 respectively) and water absorption property (1.11, 1.31, 1.89, 2.2, 2.45 and 3.52 % respectively) increased substantially (TableNo.2). During preservation GO efficiently producing CO_2 which is responsible for changing rheological properties of bread.

Glucose oxidase is found as a natural preservative: Go at the surface reduces atmospheric O_2 hydrogen peroxide (H_2O_2), which acts as an antimicrobial barrier. GO similarly acts as a bactericide specific to food pathogen. Bactericidal activity was observed on Lab scale for common food contaminants like *E.coli*, *Salmonella* typhi, *Pseudomonas aeruginosa* and *Staphylococcus auerus* after 48 hours of incubation. Glucose oxidase also had shown effective control against MDR *E.coli* and MRSA *Staphylococcus auerus* (Table No.3 and Plate No.3).

Designing of biosensor and its application- monitoring of blood sugar: The analytical accuracy of our own designed and prepared biosensor of glucose oxidasewas measured by estimating blood sugar. The results were presented in Table No.4. Blood sugar level was monitored from patient's blood sample using our own designed biosensor of glucose oxidase a product of our research. To prove the efficiency and accuracy of our designed biosensor our results were compared with that determined using commercially used glucometer in all pathological laboratories. While comparing it was observed that the deviations obtained were not statistically significant.

Table 1. Role of glucose oxidase in wine preservation

Characterization		Initial	15days	30days	60days	90 days
Colour:	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless
	Red	Red	Red	Red	Red	Red
Taste:	Colourless	Fruity, acidic and Bitter	Fruity, acidic and Bitter	Fruity, acidic and Bitter	Fruity, acidic and Bitter	Fruity, acidic and Bitter
	Red	Fruity, acidic and Bitter	Fruity, acidic and Bitter	Fruity, acidic and Bitter	Fruity, acidic and Bitter	Fruity, acidic and Bitter
Alcohol Content in mg/ml	Colourless	5.2	3.6	3.3	2.8	2.6
	Red	5.9	3.7	3.2	3	2.8

Table 2. Rheological properties of dough/bread quality of dough containing increase in concentration of GO

Glucose oxidase dosage (%)	Weight (Gm)	Water activity (%)	Height to width ratio (%)	Tenacity
0.000	46.94	1.11	0.33	41
0.001	44.52	1.31	0.42	47
0.002	40.61	1.89	0.54	57
0.003	39.59	2.20	0.58	65
0.005	35.18	2.45	0.64	72
0.0075	33.15	3.52	0.70	85

Table 3. Bactericidal activity of glucose oxidase against common food pathogens

Name of food Pathogen	Zone of Inhibition (mm)
S. aureus	30
P .aeruginosa	23
E.coli	13

Table 4. Blood sugar detection by GOD Biosensor

Person	Blood sugar estimated in mg/dl	
	Commercial Glucometer	Glucose Oxidase based designed biosensor
2F	77	74
75F	105	110
3F	124	130
5P	148	147
3P	171	169
4P	291	287
1F	339	335
6P	370	386
1P	495	490
SD	143.88	143.68
CV	61.05	60.07

When correlation studies were carried out it was observed that both data positively correlate with each other. Similar results were observed by Sudarat Manochiopinij in 1985 in proposed method of membrane bound biosensor offers same precision and accuracy of analysis as that of glucometer from pathological laboratories. Chua *et al.* (1978) and Sokol *et al.* (1980) showed similar pattern of findings and supports results obtained for the work carried out by us. The advantages of our biosensor is its thermo stability by which it will help to increase durability, long time use and economical. Statistical analysis revealed that there is no as such difference in coefficient of variation of blood sugar measurement by both glucometer and lab made biosensor. Hence the entire experiment performed using biosensor was statistically accepted with the same accuracy and precision as commercial glucometer.

In production of gluconic acid as food preservatives: The mutant strain *Aspergillus niger* F-C405-2 showed effective gluconic acid production with utilization of glucose in cheap carbohydrate source. At lab scale gluconic acid was estimated from fermentation liquor which has wide application in food and pharmacy. The production of gluconic acid by glucose oxidase from *Aspergillus niger* is a challenge in fermentation technology. % Gluconic acid = B.R. x Normality of NaOH x Molecular weight of gluconic acid (196.16 g/mole) x10 (factor or result expression in g/100ml)/ Volume (ml) of sample used for titration.

B.R. in ml = 4.2 (Mean),
Normality of NaOH= 0.01N
% Gluconic acid = 4.2 x 0.01x 196.16 x10/ 20
= 4.11%

Conclusion: In vitro utility of our enzyme was studied in various fields. It was found to be a novel stabiliser in Wine Industry. It not only reduces alcohol content (by 2%) in wine but also increases shelf life of wine. Similarly we have also used this enzyme in bread industry and found as food additive. GO is an effective oxidant to produce bread with improved macro properties of bread like improved texture, increased loaf volume, water holding capacity and tenacity. Glucose oxidase is found as natural preservatives in various food products. Gluconic acid produced by GO is used in food manufacturing process.

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