Minufiya J. Agric. Res. Vol.37 No. 4(1): 781 – 791 (2012) **"http://www.mujar.net"**

التحول الحيوي للجليسرهيزين إلي الحمض ١٨ بيتا جليسرهيتنك بإستخدام الخميرة تريكوسبورون جيروفيسياي بإستخدام تصميم بلاكت – بورمان

أشرف فرج الباز حواس[١] ، يسرية محمد حسن شتية[٢] ، محمود عبد الحميد السمان[١] ، محمد محمد عمار[٣] ، إبراهيم عبد المقصود إبراهيم[١]

[١] معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية – جامعة المنوفية – مدنية السادات.

[٢] قسم الميكروبيولوجي – كلية العلوم – جامعة عين شمس – القاهرة.

[٣] قسم النبات الزراعي – كلية الزراعة – جامعة المنوفية – شبين الكوم.

الملخص العربي

تحتوي جذور نبات العرق سوس على مادة صابونية تعرف باسم الجليسرهيزين ذات طعم حلو تفوق درجة حلاوتها أكثر من خمسين مرة من السكر الناتج من قصب السكر، وعندما تتحلل مائياً او انزيميا يتكون مركبا جلوكوني يعرف باسم حمض الجليسرهيتينك ذو التأثير العلاجي لكثير من الأمراض، مع جزئين من حمض الجلوكويورنيك.

في هذة الدراسة تم إستخدام الخميرة تريكوسبورون جيروفيسياي في إنتاج حمض الجليسرهيتنك من الجليسرهيزين المستخلص من جذور نبات العرق سوس، من خلال إستخدام تصميم بلاكت بورمان. تم تحديد تسع متغيرات مستقلة وهي درجة الحموضة، درجة الحرارة، تركيز الجليسرهيزين كبادىء وميعاد إضافتة، فترة التحضين، تركيز الجلوكوز، تركيز مستخلص الخميرة، مستخلص الذرة والتهوية. وكانت النتائج المتصل عليها هي أن أفضل تركيزمن مستخلص الخميرة كان ٠.٣ %، والجلوكوز ١.٠ %، ومستخلص الذرة ٠.٨ %، ودرجة حموضة ٦ وفترة تحضين ٧ أيام. والمتغيرات المستقلة السابقة كانت أكثر المتغيرات المؤثرة في عملية إنتاج حمض الجليسرهيتنك حيث كان أعلي تركيز متحصل علية هو ١١٤ ملجم لكل ٠.٦ جرام من المستخلص الخام للجليسرهيزين.

BIOTRANSFORMATION OF THE GLYCYRRHIZIN INTO18B-GLYCYRRHETINIC ACID BY *TRICHOSPORON JIROVECII* USING A PLACKETT-BURMAN DESIGN

A. F. El-Baz[(1)], Yousria M. Shetaia[(2)], M. A. Al-Saman[(1)], M. M. Ammar[(3)] and I. A. Ibrahim[(1)]

[(1)] Genetic Engineering and Biotechnology Research Institute (GEBRI), Minoufia University, Sadat City.
[(2)] Microbiology Department, Faculty of Science, Ain Shams University, Cairo.
[(3)] Plant Pathology, Faculty of Agriculture, Minoufia University, Shebin El-Kom.

(Received: Jun. 3, 2012)

ABSTRACT: *Glycyrrhizic acid is a molecule composed of a hydrophilic part, two molecules of glucuronic acid, and a hydrophobic fragment, glycyrrhetinic acid. Which is 50 times as sweet as sugar. GL is transformed into glycyrrhetinic acid, which has several advantages over GL. In the present study, yeast Trichosporon jirovecii was screened for 18β-glycyrrhetinic acid (GA) production using crude glycyrrhizin (GL) extracted from licorice roots. Nine independent factors including initial pH, temperature, concentration of GL as an inducible, addition time of GL, incubation time, glucose, yeast extract, corn steep liquor and aeration were surveyed and effective variables for GA production were determined using a Plackett-Burman design. Results analysis showed that incubation time (7 days), pH (6), yeast extract (0.3 %), glucose (1.0 %) and corn steep liquor (0.8 %) were the most effective variables for the highest level of GA production respectively.*

Key words: *Trichosporon jirovecii, glycyrrhizin, 18β-glycyrrhetinic acid, Plackett-Burman design.*

INTRODUCTION

Glycyrrhiza glabra belonging to family Leguminosae is commonly known as licorice (liquorice). They have been used medically since at least 500 BC and licorice has been described as 'the grandfather of herbs' Ody (2000).

Licorice root contains triterpenoid saponins (4-20%), mostly glycyrrhizin (GL) a mixture of potassium and calcium salts of glycyrrhizic acid (also known as glycyrrhizinic acid and a glycoside of glycyrrhetinic acid).

Glycyrrhizic acid is a molecule composed of a hydrophilic part, two molecules of glucuronic acid, and a hydrophobic fragment, glycyrrhetinic acid. Which is 50 times as sweet as sugar (Greek *glukos*, sweet, and *riza*, a root) Blumenthal *et al.* (2000).

Hydrolyzed one molecule of glucuronic acid, GL is transformed into glycyrrhetinic

acid mono-glucuronide (GAMG) (Fig. 1), which was several advantages over GL, as a sweetener with high sweetness and low calorie; sweetener is 941 times of the sucrose, and 5 times of the GL Akao and Hattori (1991).

18β-glycyrrhetinic (GA) acid has shown anti-inflammatory properties in different animal models Capasso *et al*. (1983); Amagaya *et al*. (1984); Inoue *et al*. (1989) and a better hepatoprotective drug than GL in *in vitro* study Nose *et al*. (1994). GA could also activate the proapoptotic pathway by inducing mitochondrial permeability transition and this property may be useful for inducing apoptosis of tumor cells Salvi *et al*. (2003); Fiore *et al*. (2004). GL-containing substances have been successful in healing peptic ulcers and Addison's disease, certain negative side effects have been revealed. These pharmacological properties are mainly due to the inhibitory effects of GA Baker (1994).

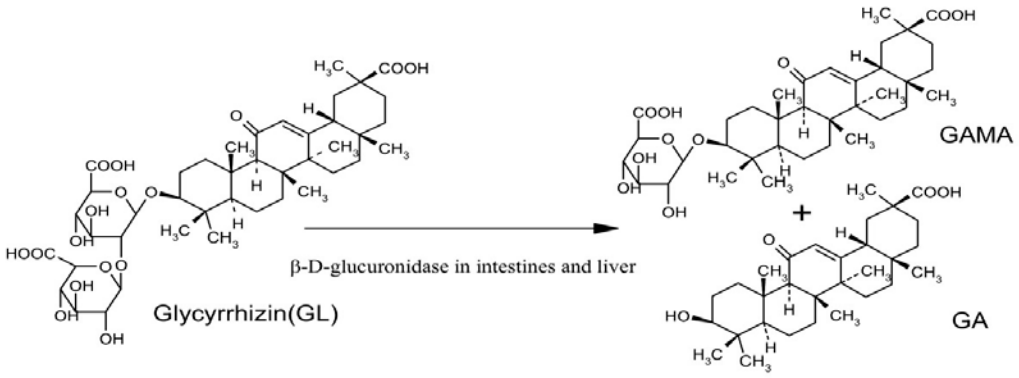

Figure (1): Metabolism of glycyhrrizin by lysosomal β-D-glucuronidase.

GA had an anti-hyperglycemic effect, which is evidenced by lowered plasma glucose with a simultaneous increase in the insulin secretion. 100 mg/kg/body weight showed promising anti-hyperglycemic effect, and is comparable to glibenclamide (Kalaiarasi and Pugalendi (2009).

GA is 200-1,000 times more potent as an inhibitor of 11-ß-hydroxysteroid dehydrogenase than glycyrrhizic acid; therefore, its pharmacokinetics after oral intake is more relevant. After oral dosing, GA is rapidly absorbed and transported via carrier molecules to the liver. In the liver it is metabolized to glucuronide and sulfate conjugates, which are subsequently rehydrolyzed to GA. GA is then reabsorbed, resulting in a significant delay in terminal clearance from plasma. Yamamura *et al*. (1992).

The main target of the present study is to contribute a model that can be applied for optimization of GA production from GL by yeast cells *Trichosporon jirovecii* using Plackett-Burman screening design.

Traditional optimization of fermentation factors is generally a time uncontrollable and labor-intensive process. On the contrary, statistically designed two level-factorial experiments were proved to be valuable tools for optimizing microbial culture conditions Hooijkaas *et al*. (1998).

MATERIALS AND METHODS:

1. Chemicals:

GL (glycyrrhizic acid ammonium salt) ≥ 95.0 % (*Biochemika*), GA 97.0 % (*Aldrich*) and ρ-nirtophenyl glucuronide (*Sigma*) were purchased from Sigma-Aldrich Chemical Co. All other chemicals were of analytical reagent grade.

2. Plant:

The used licorice roots were obtained from the Medicinal and Aromatic Plants Research department, Horticulture Research Institute (HRI), Agriculture Research Center, Ministry of Agriculture, Gize, Egypt; at the normal harvesting time (is harvested in the autumn).

The sample was washed, then dried immediately at 35˚C before grinding to a fine powder, or stored at -18˚C before drying and grinding. The ground sample was stored at 4˚C until extraction was carried out.

3. Microorganism:

The yeast used in this study is *Trichosporon jirovecii* which is previously isolated and identified Shetaia *et al*. (2005). This strain was selected to test its biotransformation ability for GL into GA.

4. Growth media:

4.1. Maintenance medium:

Yeast-malt agar (YMA) medium, which has the following composition expressed as a percentage (g/ 100 ml): yeast extract 0.3; malt extract 0.3; glucose anhydrous 1.0; agar 1.5, the final pH (at 25˚C) was adjusted to 6.2±0.2.

4.2. Yeast cultivation:

The inoculum was prepared by scratching with a sterile needle from YMA slant into 10 ml of Yeast Malt Broth (YM Borth). The suspension was used to inoculate 100 ml of sterile GL fermentation medium which consist of as a percentage (g/ 100 ml) glucose 1, 2.5; yeast extract 0.3, 0.5; C.S.L. 0.2, 0.8 and GL 0.6, 1 in addition to $NaNo_3$ 0.3; $MgSo_4.7H_2O$ 0.05 and K_2HPO_4 0.1 dispensed in a 250 ml Erlenmeyer flask as indicated in **table (1)**. Flasks were then incubated on a rotary shaker with 120 rpm for different periods of incubation and at certain temperature (according to experimental design).

After that, cultures were centrifuged at 6000 rpm for 15 min to separate the yeast cells from the culture filtrate. pH, yeast biomass, glucose content, protein content, enzyme activity and GA content were then determined.

5. Extraction of GL from Licorice Root (as the fermentation substance):

Ground licorice root was extracted with water at a 60˚C for 2 hrs, an aqueous extract was produced of the root, The aqueous extract was treated with sufficient acid (H_2SO_4 conc.) to acidify the extract to a pH ranging from about 1 to 2.5, an acid insoluble precipitate was formed of crude glycyrrhizic acid (brown sediment). Crude glycyrrhizic acid was separated by centrifugation at 6000 rpm/10 min. the glycyrrhizic acid was treated with a mixture of alkali having potassium hydroxide, magnesium hydroxide and calcium hydroxide, in an amount 2.5, 2, 1 %, respectively to solubilize and alkalify the glycyrrhizic acid to a pH of from about 5 to 6.5 Hartung and Harold (1979).

6. Analytical methods for Biotransformation of GL into GA:

6.1. Glucose determination:

It was carried out colorimetrically using enzyme colorimetric GOD-POD (glucose oxidase-peroxidase) kit (Diamond Diagnostics). Measurement was carried out at 37 ˚C after 10 min of mixing the samples with the reagent, and then the color intensities were measured versus a standard using a spectrophotometer (UV-200-RS LW Scientific). Procedures of measurement were carried out according to manufacture′s instructions Kapan *et al*. (1984).

6.2. Protein determination:

It was carried out colorimetrically using Biuret reagent kit (Spectrum Diagnostics total protein reagent). Measurement was carried out at room temperature after 10 min of mixing the samples with the reagent, and then the color intensities were measured versus a standard using a spectrophotometer Gornall *et al*. (1949).

6.3. Biomass determination:

The dry weight of cells was determined by centrifuging 10 ml of fermentation broth at 6000 rpm/15 min, washed three times with distilled water, recentrifuged, and kept for drying at 70˚C till constant weights.

6.4. Isolation and determination of GA:

The ethyl acetate solution (containing the transformation products) was concentrated to a small volume and GA was determined qualitative on TLC plate and quantitative using HPLC.

6.5. Condition of TLC:

Thin layer chromatography (TLC) analysis was carried out on silica gel plates (*Fluka*, silica gel 60F-254, layer thickness 0.2 mm). The plates were developed with the petroleum ether - ethyl acetate - acetic acid (10:6:1, by vol.) as running solvent for GA detection.

6.6. Chromatographic conditions:

The HPLC system consisted of a quaternary G1311A chromatographic pump, variable wave length G1314A detector and Zorbax 300SB C18 column (4.5mm×250mm).

For the GA the mobile phase consisted of acetonitrile-water-acetic acid (80:20:1, v/v); at the flow rate of 1.5 ml/min, and for the GL the mobile phase consisted of acetonitrile water-acetic acid (40:60:1, v/v); at the flow rate of 1.0 ml/min.

The column was maintained at room temperature and the chromatograms were monitored at a wavelength of 254 nm throughout the experiments. The sample injection volume was 20 μl. The concentration of the GA samples was calculated according to their peak areas Tanaka *et al*. (1990).

6.7. β-glucuronidase activity assay:

The change of β-glucuronidase activity of samples was measured by ρ-nirtophenyl glucuronide. A unit of β-glucuronidase activity was expressed as the amount of enzyme which liberated 1μmol of ρ-nirtophenyl/min Szasz (1967).

7. Experimental designs:

7.1. The Plackett-Burman design:

In this step, the Plackett-Burman experimental design, a fractional factorial design Yu *et al*. (1997), was used to reflect the relative importance of various environmental factors on GA production in liquid cultures. Nine independent variables (Table 1) were screened in twelve runs organized according to the Plackett-Burman design matrix (Table 2). Factors studied include initial pH, temperature, concentration of GL as an inducible, addition time of GL, incubation time, glucose, yeast extract, corn steep liquor and aeration.

For each variable, a high level (+) and low level (-) was tested. All trials were performed in duplicates in 250 ml Erlenmeyer flasks and Final data was the mean of the duplicate data. High and low levels of variables have been shown in Table (1). The averages of GA production results were treated as the responses.

Table (1): Factors examined as independent variables affecting GA production by *Trichosporon jirovecii* and their levels in the Plackett-Burman design experiment.

Independent variables	Units	Experimental values	
		Lower level (-)	Higher level (+)
Initial pH	pH	4	6
Temperature	°C	30	45
Inducible (%)	g/100 ml	0.6	1
Inducible addition time	hour	20	60

Incubation time	day	3	7
Glucose (%)	g/100 ml	1	2.5
Yeast extract (%)	g/100 ml	0.3	0.5
C.S.L. (%)	g/100 ml	0.2	0.8
Aeration	ml/ 250 ml flask	25	100

Table (2): The Plackett-Burman experimental design for 9 variables.

Trial	pH	Temp.	Inducible %	Inducible addition time	Incubation time	Glucose %	Yeast extract %	C.S.L. %	Aeration
1	4	30	1	20	7	2.5	0.5	0.2	25
2	4	30	1	20	3	2.5	0.3	0.8	100
3	6	45	1	60	7	2.5	0.5	0.8	100
4	4	30	0.6	60	3	1	0.5	0.2	100
5	6	30	1	60	7	1	0.3	0.2	100
6	6	30	0.6	60	3	2.5	0.5	0.8	25
7	4	45	1	60	3	1	0.3	0.8	25
8	6	30	0.6	20	7	1	0.3	0.8	25
9	6	45	1	20	3	1	0.5	0.2	25
10	4	45	0.6	20	7	1	0.5	0.8	100
11	6	45	0.6	20	3	2.5	0.3	0.2	100
12	4	45	0.6	60	7	2.5	0.3	0.2	25

RESULTS AND DISCUSSION:

1. Extraction of crude glycyrrhizin from licorice root:

Techniques for extraction of active components from the licorice root generally include initial comminution of the root and extraction with hot water and steam. H_2SO_4 conc. is added to the aqueous extract to increase the yield of glycyrrhizin from fresh root. Crude glycyrrhizic acid is dissolved in a solution consisting of potassium hydroxide, magnesium hydroxide and calcium hydroxide to resemble the natural material. Amount of crude glycyrrhizic acid (as the fermentation inducible substrate) was 5.38 g per 100g ground licorice roots, with the purity of 64.44% as estimated by using HPLC.

2. Characteristics of fermentation process:

The relative importance of various chemical and environmental factors involved in the process of GA production was explored using the Plackett- Burman design described in material and methods section.

Examined levels of 9 culture variables; the design was applied with 12 different fermentation conditions. All expermintals were performed in duplicated, and the averages of the observations, (fermentation kinetics) are presented in tables (3, 4, 5, 6 and 7). pH, protein content, glucose content, biomass, enzyme activity and GA concentration were determined after 1, 2, 3, 5, 6 and 7 days according to the proposed design.

Concerning the first stage parameter (pH), Table (3) showed change in values of pH during periods of incubation. ∆ pH increase in all experiments and ranged between 0.28-3.58 degrees.

Wang *et al*. (2010) demonstrated that during the death phase, cells autolyzed and released ammonia; in addition to GL acid with higher acidity than GA acid, which consumed by the microorganism and GA was accumulated. All these together made the pH value of fermentation mixture increase gradually.

Table (3): Effect of independent variables on pH and GA production at different incubation days by *Trichosporon jirovecii*.

Trial	pH zero	Day 1	2	3	5	6	7	Δ pH
1	4	5.85	6.03	6.26	6.30	6.83	7.08	3.08
2	4	5.74	5.64	5.92	-----	-----	-----	1.92
3	6	5.95	6.18	6.35	6.36	5.68	6.42	0.42
4	4	5.63	6.11	6.08	-----	-----	-----	2.08
5	6	6.72	6.93	6.67	6.68	7.81	8.01	2.01
6	6	6.54	7.26	8.00	-----	-----	-----	2.00
7	4	5.87	5.77	7.36	-----	-----	-----	3.36
8	6	6.95	7.70	8.63	8.57	8.78	9.12	3.12
9	6	6.19	6.53	6.60	-----	-----	-----	0.60
10	4	5.54	5.65	5.62	5.59	5.71	6.11	2.11
11	6	6.25	6.49	6.28	-----	-----	-----	0.28
12	4	6.01	6.09	6.49	6.50	7.62	7.58	3.58

As shown in Tables (4 and 5) protein and carbon sources decreased with yeast cells growth during incubation days in all experiments. It was observed in the some experiments a rapid decline in the first three days. Lu *et al*. (2006) demonstrated that in the first stage of the cultivation, glucose was consumed exclusively as carbon source without consumption of GL. Just when glucose disappeared in the medium, assimilation of GL started with concomitant increase of the hydrolysis activity.

Also this may be due to the cell growth reached the stationary phase and then the death phase, and the consumption of protein sources was slowed down.

The relationship between cell growth and the amount of the main nutrients was shown in Table (6). During yeast cells growth, β-glucuronidase, which hydrolyzes GL into GA, was produced. As shown in Table (7), the enzyme activity increased with cell growth, and reached the maximum value at 7 days. The amount of GA correlated directly and proportionally with increase of the enzyme activity and the content of GA was the highest (114 mg/100 ml) at seventh day of incubation and 0.6 % GL.

Kuramoto *et al*. (1994) demonstrated that β-glucuronidase activities were very sensitive to the medium pH. The optimum pH values for production GA were 6 and 5 respectively.

Based on the results obtained from the Plackett- Burman experiment, a formula of the following composition (%) is predicted to be near optimum for GA production: glucose, 1 %; yeast extract, 0.3 %; corn steep liquor, 0.8 %; GL, 0.6 %; $NaNo_3$, 0.3%; $MgSo_4.7H_2O$, 0.05%; K_2HPO_4, 0.1 % with inducible addition time, 20 hours; incubation time, 7 days; pH, 6; temperature, 30°C and aeration, ml/ 250 ml flask.

Based on statistical analyses of the data (*t* ratio), the results of this experiment suggested also the most effective variables, concerning GA production were the concentrations of yeast extract, glucose and corn steep liquor with incubation time and pH as shown in Fig. (2).

Table (4): Effect of independent variables on protein content and GA production at different incubation days by *Trichosporon jirovecii*.

Trial	Day 1	2	3	5	6	7
1	0.98*	0.60	0.57	0.53	0.46	0.36
2	0.65	0.61	0.56	-----	-----	-----
3	0.96	0.78	0.72	0.65	0.65	0.61
4	0.49	0.43	0.32	-----	-----	-----
5	0.46	0.37	0.31	0.27	0.26	0.22
6	1.81	0.86	0.37	-----	-----	-----
7	0.69	0.68	0.53	-----	-----	-----
8	1.44	1.06	0.53	0.48	0.48	0.30
9	0.80	0.46	0.30	-----	-----	-----
10	0.79	0.75	0.74	0.66	0.60	0.50
11	0.48	0.38	0.37	-----	-----	-----
12	0.62	0.61	0.55	0.45	0.43	0.28

*% of total protein (g/100 m l)

Table (5): Effect of independent variables on glucose content and GA production at different incubation days by *Trichosporon jirovecii*.

Trial	Day 1	2	3	5	6	7
1	0.926*	0.901	0.755	0.718	0.571	0.235
2	0.799	0.770	0.592	-----	-----	-----
3	0.986	0.942	0.719	0.717	0.669	0.631
4	1.016	0.735	0.535	-----	-----	-----
5	0.945	0.781	0.102	0.105	0.000	0.000

6	1.032	0.578	0.276	-----	-----	-----
7	1.094	0.880	0.201	-----	-----	-----
8	0.152	0.091	0.002	0.000	0.000	0.000
9	1.053	0.862	0.359	-----	-----	-----
10	1.048	1.019	0.764	0.718	0.424	0.367
11	0.707	0.538	0.498	-----	-----	-----
12	0.685	0.635	0.497	0.429	0.028	0.024

*% of glucose (g/100 ml)

Table (6): Effect of independent variables on biomass and GA production at different incubation days by *Trichosporon jirovecii*.

Trial	Day 1	2	3	5	6	7
1	0.40*	0.55	1.30	0.75	0.70	0.60
2	0.25	0.65	0.80	-----	-----	-----
3	0.25	0.50	0.50	1.15	1.25	1.65
4	0.40	0.55	0.61	-----	-----	-----
5	0.30	0.80	1.55	2.60	2.10	2.05
6	0.15	0.80	0.85	-----	-----	-----
7	0.30	1.60	1.20	-----	-----	-----
8	0.45	0.70	1.05	1.40	1.95	2.40
9	0.50	1.15	0.70	-----	-----	-----
10	0.50	0.65	1.10	1.00	0.95	0.90
11	0.25	0.35	0.65	-----	-----	-----
12	0.50	1.25	1.25	0.70	0.35	0.30

*dry weight (g/ 100 ml)

Table (7): Effect of independent variables on enzyme activity and concentration of GA at different incubation days by *Trichosporon jirovecii*.

Trial	GL % (g/100 ml)	Enzyme activity	GA % (mg/100 ml) *Days* 5	6	7
3	1	0.274*	4.0	18.5	47.0

5	1	0.358	---	22.5	59.5
8	0.6	0.591	---	47.5	114.0
10	0.6	0.198	4.5	10.5	32.5
12	0.6	0.182	---	12.5	33.0

****Unit of β-glucuronidase activity was expressed as the amount of enzyme which liberated 1μmol of ρ-nirtophenyl/min***

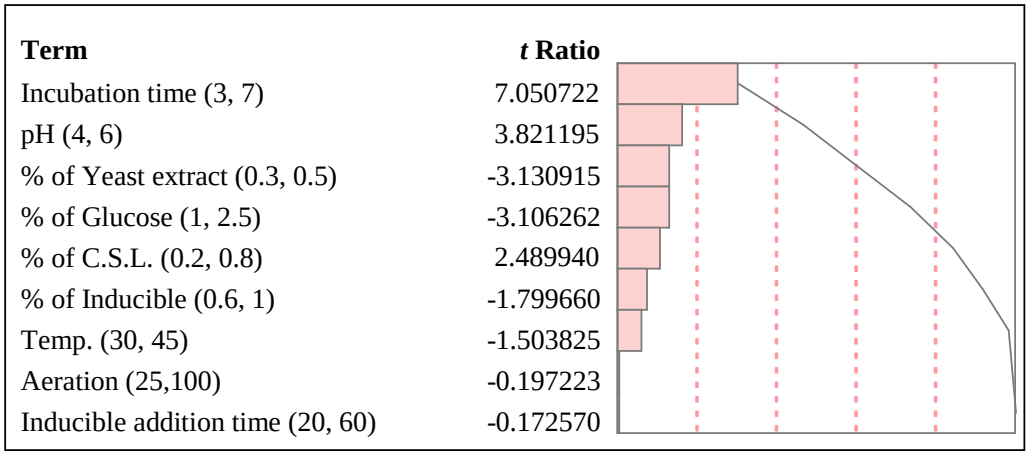

Figure (2): Pareto Plot of the effects for experimental variables.

CONCLUSION

The statistical design of experiment offers efficient methodology to identify the significant variables and to optimize the factors with minimum number of experiments for GA production by *Trichosporon Jirovecii*. These significant factors identified by Plackett-Burman design were considered for the next stage in the medium optimization by using response surface optimization technique and the studies in large scale in the next study.

REFERENCES

Akao, T. and M. Hattori (1991). Hydrolysis of glycyrrhizin to 18 beta-glycyrrhetyl monoglucuronide by lysosomal beta- D-glucuronidase of animal livers. *Biochem Pharmacol* 41, 1025-1029.

Amagaya, S., E. Sugishita, Y. Ogihara, S. Ogawa, K. Okada and T. Aizawa (1984). Comparative studies of the stereoisomers of glycyrrhetinic acid on anti-inflammatory activities. *J Pharmacobiodyn* 79, 923-928.

Baker, M. E. (1994). Licorice and enzymes other than 11 betahydroxysteroid dehydrogenase: an evolutionary perspective. *Steroids* 59, 136-141.

Blumenthal, M., A. Goldberg and J. Brinckmann (2000). Herbal Medicine: Expanded Commission E Monographs. *American Botanical Council: Newton*, 233-236.

Capasso, F., N. Mascolo, G. Autore and M. R. Duraccio (1983). Glycyrrhetinic acid, leucocytes and prostaglandins. *J Pharm Pharmacol* 35, 332-335.

Fiore, C., M. Salvi, M. Palermo, G. Sinigagliab, D. Armaninia and A. Toninello (2004). On the mechanism of mitochondrial permeability transition induction by glycyrrhetinic acid. *Biochim Biophys Acta* 1658, 195-201.

Gornall, A. G., C. J. Bardawill and M. M. David (1949). Determination of serum protein by means of the biuret reagent. *J Biol Chem* 177-751.

Hartung and A. Harold (1979). Potassium-magnesium-calcium glycyrrhizin, *U. S. Patent 4176228.*

Hooijkaas, L.P., E.C. Wilkinson, J. Tramper and Buitelaar. (1998). Medium optimization for spore production of Conithyrium minitans using statistically Based expereimental designs. *Biotechnol. Bioeng*. 64, 92-100.

Inoue, H., T. Mori, S. Shibata and Y. Koshihara (1989). Modulation by glycyrrhetinic acid derivatives of TPA-induced mouse ear oedema. *Br J Pharmacol* 96, 204-210.

Kalaiarasi, P. and K. V. Pugalendi (2009). Antihyperglycemic effect of 18β-glycyrrhetinic acid, aglycone of glycyrrhizin, on streptozotocin-diabetic rats. *European Journal of Pharmacology* 606, 269-273.

Kapan, L. A. Glucose. Kalpan A. (1984). *Clin Chem.* The C. V. Mosby Co. St Louis. Toronto. Princeton. 1032-1036.

Kuramoto, T., Y. Iko, M. Oda, Y. Tamura and S. Kitahata (1994). Microbial production of glycyrrhetic acid mono-glucuronide from glycyrrhizin by Cryptococus magnus MG-27. Biosci. Biotech. 58 (3), 544-458.

Lu, D., H., Y. Li and P. Quyang (2006). Biocatalytic properties of a novel crude glcyrrhizin hydrolase from the liver of the domestic duck. Journal of Molecular Catalysis B: Enzymatic 43, 148-152.

Nose, M., M. Ito, K. Kamimura, M. Shimizu and Y. Ogihara (1994). A comparison of the antihepatotoxic activity between glycyrrhizin and glycyrrhetinic acid. *Planta Med* 60, 136-139.

Ody, P. (2000). *The Complete Guide Medicinal Herbal*. Dorling Kindersley: London, 75.

Salvi, M., C. Fiore, D. Armanini and A. Toninello (2003). Glycyrrhetinic acid-induced permeability transition in rat liver mitochondria. *Biochem Pharmacol* 66, 2375-2379.

Shetaia, Y. M., A. F. El-Baz and H. A. El-Enshasy (2005). Production of yeast killer toxin by *Trichosporon jirovecii*. *Afr. J. Mycol. Biotechnol* 13, 73-89.

Szasz, G. (1967). Comparison between ρ-nirtophenyl glucuronide and phenolphthalein glucuronide as substrates in the assay of β-glucuronidase. Clinical Chemistry 13 (9), 752-759.

Tanaka, M., Y. Anzai, F. Kato and Y. Koyama (1990). Isolation of bacterial strains, which hydrolyze glycyrrhizin and produce glycyrrhezic acid, from soil. *J. Pharmacobia-Dyn*. 13, 361-366.

Wang, J., Q. Sun, P. Gao, J. F. Wang, C. Xu and Q. L. Sun (2010). Bioconversion of Glycyrrhizinic Acid in Liquorice into 18_β_Glycyrrhetinic Acid by *Aspergillus parasiticus* Speare BGB1. *Applied Biochemistry and Microbiology.*46 (4), 421-425.

Yamamura, Y., J. Kawakami and T. Santa (1992). Pharmacokinetic profile of glycerrhizin in healthy volunteers by a new high-performance liquid chromatographic method. *J Pharm Sci* 81, 1042-1046.

Yu, X., S. G. Hallett, J. Sheppard and A. K. Watson (1997). Application of the Plackett-Burman experimental design to evaluate nutritional requirements for the production of Colleterichum coccores spores. *Appl. Microbial. Biotechnol.* 47, 301-305.

التحول الحيوي للجليسرهيزين إلي الحمض ١٨ بيتا جليسرهيتنك بإستخدام الخميرة تريكوسبورون جيروفيسياي بإستخدام تصميم بلاكت – بورمان

أشرف فرج الباز حواس[١] ، يسرية محمد حسن شتية[٢] ، محمود عبد الحميد السمان[١] ، محمد محمد عمار[٣] ، إبراهيم عبد المقصود إبراهيم[١]

[١] معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية – جامعة المنوفية – مدنية السادات.

[٢] قسم الميكروبيولوجي – كلية العلوم – جامعة عين شمس – القاهرة.

[٣] قسم النبات الزراعي – كلية الزراعة – جامعة المنوفية – شبين الكوم.

الملخص العربي

تحتوي جذور نبات العرق سوس على مادة صابونية تعرف باسم الجليسرهيزين ذات طعم حلو تفوق درجة حلاوتها أكثر من خمسين مرة من السكر الناتج من قصب السكر، وعندما تتحلل مائياً او انزيميا يتكون مركبا جلوكوني يعرف باسم حمض الجليسرهيتينك ذو التأثير العلاجي لكثير من الأمراض، مع جزئين من حمض الجلوكويورنيك.

في هذة الدراسة تم إستخدام الخميرة تريكوسبورون جيروفيسياي في إنتاج حمض الجليسرهيتنك من الجليسرهيزين المستخلص من جذور نبات العرق سوس، من خلال إستخدام تصميم بلاكت بورمان. تم تحديد تسع متغيرات مستقلة وهي درجة الحموضة، درجة الحرارة، تركيز الجليسرهيزين كبادىء وميعاد إضافتة، فترة التحضين، تركيز الجلوكوز، تركيز مستخلص الخميرة، مستخلص الذرة والتهوية. وكانت النتائج المتصل عليها هي أن أفضل تركيزمن مستخلص الخميرة كان ٠.٣ %، والجلوكوز ١.٠ %، ومستخلص الذرة ٠.٨ %، ودرجة حموضة ٦ وفترة تحضين

٧ أيام. والمتغيرات المستقلة السابقة كانت أكثر المتغيرات المؤثرة في عملية إنتاج حمض الجليسرهيتنك حيث كان أعلي تركيز متحصل علية هو ١١٤ ملجم لكل ٠.٦ جرام من المستخلص الخام للجليسرهيزين.