**Inulinase Production Capability of a Promising Medicinal Plant: *Inula viscosa***

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**Abstract:** The present study was designed to examine the inulinase production capability of *Rhodotorula glutinis* SO-28 by using *Inula viscosa*, a promising medicinal plant, as sole carbon source in submerged fermentation. *Inula viscosa*, a perennial member of Asteraceae family, is a popular and widespread medicinal plant in the Mediterranean region. It is termed as "yapışkan andız otu" in Turkey and has been widely used in folk medicine since the ancient times. Taguchi design of experiment (DOE) technique was utilized for the inulinase production optimization process. An orthogonal array layout of L16 was utilized with four influential factors as following: *Inula viscosa* amount, agitation speed, incubation temperature, and incubation time at four levels. The obtained results showed that optimized inulinase production enhanced enzyme activity as 99.63 U/ml which was four fold higher than the unoptimized condition. In brief, *Inula viscosa* can be used effectively for inulinase production and use of statistical optimization techniques like Taguchi DOE significantly increases the enzyme yield.

**Keywords:** Dittrichia viscosa, enzyme, Rhodotorula glutinis, Taguchi DOE.

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1. Introduction

In ancient times, people used to cure their diseases by using medicinal plants as the chemicals were not manufactured in labs. There are many active ingredients derived from plants for production of drugs and medicines and these ingredients are less poisonous and have fewer side effects than the synthetic drugs (Seca et al., 2014). Asteraceae family includes many different kinds of aromatic plants in different habitats as tropics, cold arctic, or alpine countries (Aissa et al., 2019); besides, some of the members (*Inula racemosa*) of this family are used in Ayurveda in India (Kalachaveedu, Raghavan, Telapolu, Kuruviella, & Kedike, 2018; Mangathayar, Kuruviella, Balakrishna, & Venkatesh, 2009). An Asteraceae family member, the genus *Inula*, has almost 100 species worldwide and many of the species have important facilities such as bactericidal, antidiabetic, and anti-inflammatory effects (Gokbulut, 2013). *Inula viscosa* (L.) Aiton (syn. Dittrichia viscosa (L.) Greuter), a perennial member of Asteraceae, is a popular and widespread medicinal plant in the Mediterranean region, especially in Turkey, Middle East, Bulgaria, Portugal, and Spain (Al-Dissi, 2001; Al-Eisawi, 1998; Baytop, 1984). *Inula viscosa* has different names as ‘false yellowhead, sticky cleabane, Magramane (in Algeria), aromatic inula, yapışkan andız otu, and kanser otu (cancer weed) (in Turkey)’ (Gueribis et al., 2019; Ozkan, 2019). *Inula viscosa* contains many important bioactive compounds such as sesquiterpenes, sesquiterpene acids, flavonoids, azulenes, costic acids, and essential oils; besides, *Inula viscosa* has high amounts of phenolic compounds that contribute to the overall antioxidant potential of the plants. Therefore, this plant is frequently used to control or inhibit symptoms of health problems (Al-Dissi, 2001; Al-Qura’n, 2009; Benbacer, 2012; Danino, 2009; Gokbulut, 2013; Ozkan, 2019; Seca et al., 2014; Zeggwagh, Ouahidi, Lemhadri, & Eddouks, 2006). *Inula viscosa* has been widely used for therapeutic purposes in folk medicine because of its anti-inflammatory, antipyretic, antioxidant, antimicrobial, anti-septic, anti-phlogistic, anthelmintic, balsamic, expectorant, diuretic, anti-anemic, and muscle relaxant activities (Al-Dissi, 2001; Al-Qura’n, 2009; Al-Shtayeb, 2000; Barbetti, 1985; Baytop, 1984; Lauro, 1990; Ozkan, 2019; Zeggwagh et al., 2006). In Jordan, *Inula viscosa* is prescribed as a promoter agent in the induction of abortion and female sterility (Al-Dissi, 2001; Karim, 1990). *Inula viscosa* is used in drug production for the treatment of diabetes, cancer, hypertension, tuberculosis, infertility, lung and gastro-

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Inulinase is an inducible enzyme in the roots, and tubers of some plants such as onion, garlic, leek, and etc. Inulin consists of linear chains of β-2,1-linked D-fructofuranose residue through a sucrose-type linkage at the reducing end (Canli & Kurbanoglu, 2012; Chi, Chi, Zhang, Liu, & Yue, 2009; Tasar, Erdal, & Algur, 2015; Xiong, Jinhua, & Dongsheng, 2007). Inulinase (2,1-D-fructan fructohydrolase, EC 3.2.1.7) is an important enzyme that catalyzes the removal of the terminal fructose residues from the non-reducing end of the inulin molecule (Chi et al., 2009). Fructose plays a considerable role in food industry due to its higher sweetening capacity with low calories for diabetics, prevention of the colon cancer, increment property of iron absorption in children, and on the like (Chen, 2009; Chi et al., 2009; Singh, Dhaliwal, Puri, 2006; Tasar et al., 2015). Inulinase is an inducible enzyme and inulin-rich feed stocks as leek, onion, Jerusalem artichoke, dahlia, asparagus, etc. can be used for substrate and inducer for inulinase production (Canli & Kurbanoglu, 2012; Erdal, 2011; Singh, Chauhan, Kaur, & Pandey, 2020; Singh, Dhaliwal, Puri, 2006).

The demand for fermentation process statistical optimization techniques has been increasing day by day due to its higher performance for yield enhancement with lower human power and production cost. Many prior studies were made by using classical “one variable at a time” approach for optimization of the fermentation process (Aydogan, Taskin, Canli, Arslan, & Ortucu, 2014; Canli & Kurbanoglu, 2011, 2012; Taskin, Erdal, & Canli, 2010; Taskin et al., 2016). This method is usually used for specific requirements for growth and product formation by adding or subtracting some components from the medium with minimal complicated medium interactions. On the other hand, statistical optimization techniques such as response surface methodology, Box-Behnken design, Plackett-Burman design, and Taguchi design allow quick screening of large experimental domain and reflect the role of each of the components (Farid, Ghoneimy, El-Khawaga, Negm-Eldine, & Awad, 2013). The Taguchi-based optimization technique has produced a unique and powerful optimization discipline that differs from traditional practices and it is used for maximizing robustness of products and processes with high quality at low cost and less labor (Kvak, 2014; Phadke, 1989; Rao, 2008). In Turkey, also in many different countries in the world, people used to consume the Inula viscosa without any extraction method as they usually consume it as a herbal tea in folk medicine.

Autoclave-based extraction of the plants was used before (Deng et al., 2019; Passos et al., 2006). The autoclave was employed for the sterilization process of the medium and; thus, partial extraction of the Inula viscosa leaves was used in this study. Inulinase production was researched by using different microorganisms and substrates previously (Canli, Tasar, & Taskin, 2013; Chaturvedi, Bhattacharya, Nain, Prasanna, & Khare, 2019; Erdal, 2011; Tasar et al., 2015; Xiong et al., 2007) but, to the best of our knowledge, there is not any report about inulinase production by Rhodotorula glutinis with using the Inula viscosa as substrate. The aim of this study was to research the ability of Inula viscosa as a novel carbon source for inulinase production without any further extraction process.

2. Materials and Methods

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Inula viscosa was purchased from local markets as herbal tea (Black Natural, Fitofarma Corp., Istanbul, Turkey). Rhodotorula glutinis SO-28 (GenBank accession number KX017572) was obtained from our laboratory (YUTAM, Erzrum, Turkey) culture collection. The cultures were maintained on potato dextrose agar slants at 4°C and recultured bimonthly. To prepare the yeast starter, 250-ml Erlenmeyer flasks containing 100 ml of potato dextrose broth was inoculated with one loopful of a 24-hr-old culture of yeast grown on potato dextrose agar and then incubated at 30°C and 200 rpm for 48 hr on an orbital shaking incubator (Zhicheng ZHWY-200B, China). After growth, the yeast cells were collected by centrifugation at 5000 x g for 20 min and resuspended in sterile saline water. The final cell concentration was adjusted to 10⁶ cells/ml. The initial fermentation medium was composed as follows (g/l): 10 Inula viscosa, 1 KH₂PO₄, 3 bacterial peptone, 0.5 KCl, 0.5 MgSO₄.7H₂O, 1.5 NH₄NO₃, 0.2 CaCl₂, 0.2 NaCl. The unoptimized fermentation medium was run at 30°C at 100 rpm for 24 hr. All the experiments were run at 250 ml- Erlenmeyer flasks including 50 ml medium and initial pH was adjusted to 6.0 with 1 N NaOH or 1 N HCl and autoclaved at 121°C for 20 min. This step was for both of partially extraction of Inula viscosa and sterilization of the medium. The flasks were cooled to room temperature and 0.5 ml of yeast cell suspension was transferred as inoculum material into each flask.

2.1. Taguchi DOE methodology

Higher yield and less production cost are the main factors for industrial production process. For this purpose, researchers apply effective optimization techniques in process engineering. The fundamentals of these techniques are the full factorial design and fractional factorial design (Tan, 2005). The full factorial design needs all of the factors at each level but if the experiments do not include a few factors, this technique cannot be applied due to numerous runs. In this context, Dr. Genichi Taguchi developed a powerful optimization process, namely...
Taguchi design of experiment (DOE) method, that caused the recovery of the national economy in a short time in Japan after the World War II. This method has enabled the results to be standardized and easy to apply by the researchers. Taguchi method includes standard tables known as orthogonal arrays (OA) for the design of the experiments. Table 2 shows an OA that consists of four factors with four levels, which were effective for inulinase production by *Rhodotorula glutinis* SO-28, with the symbol of L₁₆. A traditional full factorial design needs 4⁴ (four levels with four factors) experiments that are impossible to run; however, Taguchi method suggests only 16 experiments (a fraction of the full factorial design) for optimization. Fewer experiments mean less human power and energy consumption and; therefore, Taguchi method enables a powerful optimization process (Ozden Canli Tasar, 2017). Taguchi DOE methodology also uses interactions between the parameters on some arrays (e.g. L₃) but not on L₁₆ orthogonal array due to the multiplicity of the factors. Recent papers indicated that Taguchi DOE does not account for interaction between the parameters in order to get cost-effective optimization process; consequently, these interactions can be neglected (Tan, 2005; Canli Tasar, 2017). Taguchi DOE methodology includes controllable and uncontrollable variables (Alhaddad, Cabibihan, Hayek, & Bonarini, 2019; Mori, 2011). This method uses the S/N ratio (signal-to-noise, unit: dB) for performance characteristics instead of the average value to interpret the results into a value for characteristics evaluation in the optimal setting analysis (Tan, 2005). This method is based on three different characteristic categories as the larger-the better, the nominal-the better and the smaller-the better. The aim of this study was to increase the inulinase production. Hence, the larger-the better criterion for the signal-to-noise ratios was used as the quality characteristic for the evaluation of the experimental results was used and the equation is shown below:

\[
S/N = -10 \log_{10} \left( \frac{1}{n} \sum_{i=1}^{n} \frac{1}{Y_i^2} \right)
\]

where S/N are performance statistics, defined as the S/N, n the number of repetitions for an experimental combination, and Yi is a performance value of the ith experiment. S/N ratio was formulated to obtain the selection of the highest result by the designer (Jean & Tzeng, 2013). Use of *Inula viscosa* as substrate for inulinase production was affected from *Inula viscosa* amount (g/l) and some environmental conditions like agitation speed (rpm), incubation temperature (°C), and incubation time (h) with four levels were researched in the current study (Table 1).

Table 1. Selected culture conditions and assigned levels.

| Serial no. | Factor     | Level | 1  | 2 | 3 | 4 |
|------------|------------|-------|----|---|---|---|
| 1          | *Inula viscosa* (g/l) | 10  | 20 | 30 | 40 |
| 2          | Agitation (rpm)    | 100 | 150| 200| 250|
| 3          | Temperature (°C)   | 30  | 32.5| 35 | 37.5|
| 4          | Time (h)          | 24  | 48 | 72 | 96 |

2.2. Enzyme assay

The extracellular inulinase activity was determined as the crude-enzyme activity in culture filtrate with some modification (Burkert et al., 2006). One exo-inulinase unit is defined as the amount of enzyme produced 1 µmole of glucose per minute under standard assay conditions. Culture medium was centrifuged at 5000 rpm for 15 min and supernatant was used as inulinase source. Reaction mixture consists of 0.1 ml enzyme extract and 0.9 ml of 0.1 M sodium acetate buffer (pH 5.5) containing 2% inulin (w/v) and this was placed in glass test tubes and incubated at 50°C for 15 min. The reaction mixture was then assayed for glucose as a reducing sugar using DNS method (Miller, 1959). Thus, 1 ml of DNS reagent was added to each test tube and then put in the boiling water bath for 10 min. After cooling to room temperature, the volume of the glass tubes was raised to 8 ml with distilled water. A spectrophotometer (Thermo MultiSk an Go, Finland) was used to determine the percentage of transmittance at 592 nm.

2.3. Analysis of variance

The analysis of variance (ANOVA) of the obtained experimental results was calculated to indicate the quality characteristics variation using the obtained parameters. The most effective factors had the greatest impact on the inulinase production and all of these parameters were controlled carefully. Minitab® 19.1.1 Statistical Software (United States) was employed both of Taguchi DOE and ANOVA analysis. All of the experiments were run three times with two parallels and the results were taken as averages.

3. Results and Discussion

Taguchi DOE uses mathematical and statistical tools for the formulation of the medium and the other environmental conditions. In the current study, Taguchi DOE method was utilized for inulinase production by *Rhodotorula glutinis* SO-28 using *Inula viscosa* as sole carbon source. *Inula viscosa* has a great potential for treating many disorders due to its effective compounds. The results showed that inulinase production and S/N ratios had a great variation correlated to each experimental design with the larger-the better quality character (Table 2).

\[
S/N = \frac{10 \log_{10} \left( \frac{1}{n} \sum_{i=1}^{n} Y_i^2 \right)}{\text{averages.}}
\]

S/N ratio determines the deviation of the quality characteristics from the obtained results (Sharma, Verma, Sidhu, & Pandey, 2005; 2006, Taskin et al., 2013). The minimization of the noise factor ensures higher enzyme production and; in addition, it decreases the undesirable effects. The lowest inulinase activity (1.10 U/ml) was obtained from 16th experimental design and; therefore, the lowest S/N ratio was obtained from the same experimental design. On the other hand, the highest enzyme activity (93.61 U/ml) was carried out using the 10th experimental design and; in accordance with this, the highest S/N ratio was obtained from the same experimental design.

Response data for S/N ratios and their comparison were given in Table 3. Delta value is the difference between the maximum and minimum average response (signal-to-noise ratio or standard deviation) for the factor and the rank is the rank of each Delta where Rank 1 is the largest Delta. The ranking in Table 3 demonstrates that incubation temperature and incubation time had relatively strong impacts on the inulinase production while *Inula viscosa* amount and agitation speed had relatively weak impacts. The analysis of variance (ANOVA) confirmed the ranking made on the basis of the amplitude of S/N ratio variation (Table 4).
The carbon source, in the order of their relative influences, were incubation time > agitation speed > *Inula viscosa* amount, respectively. The importance of the environmental conditions for enzyme production was reported before (Canlı & Kurbanoglu, 2011; Mandal, 2015; Tasar, 2017; Taskin et al., 2016). In a recent paper about utilization of different substrates before such as leek (Canlı & Kurbanoglu, 2011; Mandel, 2015; Tasar, 2017; Taskin et al., 2016), it was reported that leek powder, the most powerful factor among all the other factors (Tasar et al., 2015). However, in a different previous study about production of β-fructofuranosidase by *Rhodotorula glutinis*, time factor had the greatest effect among all other parameters and the sugar beet powder, the substrate which was used as carbon source in the study, had less effect than time factor while the percentage contribution of the temperature factor was found fewer than the sugar beet powder (Tasar, 2017). On the contrary of this result, it was reported that leek powder, the substrate that was selected as carbon source, was the most powerful factor while the time factor had the least effect among all the other factors for inulinase production by *Rhodotorula glutinis* (Tasar et al., 2015). These results might be originated from the structural difference of the carbon sources and the response of the microorganisms to the medium ingredients and other environmental conditions.

Inulinase production by the microorganisms were carried out using different substrates before such as leek (*Allium ampeloprasum* var. *porrum*) (Canlı & Kurbanoğlu, 2012; Tasar et al., 2015), Jerusalem artichoke (*Helianthus tuberosus*) (Erdal, 2011), *Asparagus racemosus* (Singh,

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**Table 2. Taguchi L16 orthogonal array, inulinase activity (U/ml) and S/N ratios.**

| Exp. no. | *Inula viscosa* | Agitation | Temperature | Time | Inulinase (U/ml)* | S/N ratio |
|---------|-----------------|-----------|-------------|------|------------------|-----------|
| 1       | 1               | 1         | 1           | 1    | 18.36±1.23       | 25.278    |
| 2       | 2               | 2         | 2           | 2    | 28.47±1.21       | 39.085    |
| 3       | 1               | 3         | 3           | 3    | 41.79±1.14       | 32.4213   |
| 4       | 1               | 4         | 4           | 4    | 79.23±1.71       | 37.9742   |
| 5       | 2               | 1         | 2           | 3    | 41.33±1.13       | 32.3253   |
| 6       | 2               | 2         | 1           | 4    | 2.68±0.17        | 8.4546    |
| 7       | 2               | 3         | 4           | 1    | 67.77±1.33       | 36.6212   |
| 8       | 2               | 4         | 3           | 2    | 88.07±2.65       | 38.8970   |
| 9       | 3               | 3         | 3           | 4    | 1.13±0.18        | 1.0588    |
| 10      | 3               | 2         | 4           | 3    | 93.61±2.45       | 39.4269   |
| 11      | 3               | 3         | 1           | 2    | 66.20±0.37       | 36.4169   |
| 12      | 3               | 4         | 2           | 1    | 1.44±0.15        | 3.1695    |
| 13      | 4               | 1         | 4           | 2    | 45.80±2.57       | 33.2166   |
| 14      | 4               | 2         | 3           | 1    | 36.61±1.12       | 31.3704   |
| 15      | 4               | 3         | 2           | 4    | 35.22±1.35       | 30.9325   |
| 16      | 4               | 4         | 1           | 3    | 1.10±0.12        | 0.8184    |

*Values mean ± standard deviation (SD).

**Table 3. Response table for S/N ratios and their comparison.*

| Level | Inula viscosa | Agitation | Temperature | Time |
|-------|--------------|-----------|-------------|------|
| 1     | 31.19        | 22.77     | 17.78       | 24.09|
| 2     | 29.10        | 27.09     | 23.89       | 34.41|
| 3     | 19.42        | 34.10     | 25.71       | 26.26|
| 4     | 24.07        | 20.23     | 36.81       | 19.44|
| Delta | 11.37        | 13.87     | 19.03       | 14.97|
| Rank  | 4            | 3         | 1           | 2    |

* Larger is better

**Table 4. Analysis of variance for means.**

| Source          | DF | Seq SS | Contribution (%) | Adj SS | Adj MS | F   | P    |
|-----------------|----|--------|------------------|--------|--------|-----|------|
| *Inula viscosa* | 3  | 835.0  | 5.66             | 835.0  | 278.3  | 0.18| 0.981|
| Agitation       | 3  | 1384.9 | 9.39             | 1384.9 | 461.6  | 0.31| 0.822|
| Temperature     | 3  | 6004.6 | 40.65            | 6004.6 | 2015.5 | 1.33| 0.411|
| Time            | 3  | 2008.0 | 13.65            | 2008.0 | 669.3  | 0.44| 0.739|
| Residual Error  | 3  | 4530.6 | 30.63            | 4530.6 | 1510.2 |     |      |
| Total           | 15 | 14763.1| 100              |        |        |     |      |

DF: Degree of freedom; Seq SS: Sequential sum of square; Adj SS: Adjusted sum of square; Adj MS: Adjusted mean of squares; F: F value; P: P value.

The percentage contribution indicates the individual contribution of each factor on the mean response and was calculated using sequential sum of square of a factor to the total sequential sum of square (Fig. 1). The highest contribution was provided by the temperature factor and the time factor, respectively. *Inula viscosa*, the carbon source of the medium, had the least effect and the incubation temperature factor was the most powerful factor among all of the other factors.
Dhaliwal, Puri, 2006), agro-industrial residues (Squirezi, 2009), and garlic (Allium sativum) (Sharma, Kainth, & Gill, 2006). However, to the best of our knowledge, there is not any report about inulinase production using any Inula sp. as carbon source. Inula viscosa flowers, leaves, and roots can be consumed as raw or cooked food in Turkey (Ertuğ, 2014). Recent studies showed that different parts of Inula viscosa had higher antioxidant activities when extracted with water and less antioxidant activity was carried out by methanol extract using both of the DPPH radical scavenging and ABTS assays (Gokbulut, 2013).

Table 5. Proposed optimal levels of each individual factors.

| Inula viscosa | Agitation | Temperature | Time |
|---------------|-----------|-------------|------|
| Level         | 1         | 3           | 4    | 2   |
| Optimal condition | 10       | 200         | 37.5 | 48  |

For prediction analysis of Taguchi DOE, the main effect plot was utilized (Fig. 2). Taguchi DOE proposed a new design for the factors using the optimal levels (Table 5). For validation analysis, the proposed experimental methodology, inulinase production was performed using the optimum level of each individual factor of Taguchi prediction. The obtained data from the proposed design (99.63±1.20 U/ml) was close to the predicted value (101.80±2.11 U/ml) and; thus, the statistical evaluation was validated. Besides, the experimental result was 5-fold higher than the unoptimized condition (18.36±1.23 U/ml).

4. Conclusions

Inula viscosa is an important and promising plant in folk medicine. It has many benefits on human health and treatment of many diseases such as, cancer, diabetes, infertility, and etc. Inulinase is an industrially important enzyme and has been widely used. Taguchi DOE method was successfully employed for inulinase production by Rhodotorula glutinis using Inula viscosa as sole carbon source in the current study. The experimental results showed that Inula viscosa has a great potential for inulinase production. This study evaluates that the substrate choice and environmental conditions may affect the enzyme production significantly and optimization methods could be effectively used for microbial enzyme production.

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References

Aissa, I., Nimbarte, V.D., Zardi-Bergaoui, A., Znati, M., Flamini, G., Ascrizzi, R., & Jannet, H.B. (2019). Isocostic Acid, a Promising Bioactive Agent from the Essential Oil of Inula viscosa (L.): Insights from Drug Likeness Properties, Molecular Docking and SAR Analysis. Chemistry Biodiversity, 16(4), e1800648. doi:10.1002/cbdv.201800648

Al-Dissi, N.M., Salhab, A.S., & Al-Hajj, A.H. (2001). Effects of Inula viscosa leaf extracts on abortion and implantation in rats. Journal of Ethnopharmacology, 77(1), 117-121.

Al-Easawi, D. (1998). Field Guide to Wild Flowers in Jordan and Neighboring Countries. Jordan Foundation Press, Amman, 97.

Al-Qura’n, S. (2009). Ethnopharmacological survey of wild medicinal plants in Showbak, Jordan. Journal of Ethnopharmacology, 123, 45-50.

Alhaddad, A.Y., Cabibihan, J.J., Hayek, A., & Bonarini, A. (2019). Influence of the shape and mass of a small robot when thrown to a dummy human head. SN Applied Sciences, 1(11). doi:10.1007/s42452-019-1447-7

Ali-Shtayeh, M.S., Yaniv, Z. & Mahajna, J. (2000). Ethnobotanical survey in the Palestinian area: a classification of the healing potential of medicinal plants. Journal of Ethnopharmacology, 73, 221-232.
alkaloids, A. & Atta, A.H. (1999). Pharmacological screening of the anti-ulcerogenic effects of some Jordani an medicinal plants in rats. Journal of Ethnopharmacology, 67, 341-345.

Amin, S., Kazuo, Z.A., Singh, S., & Altarf, T. (2013). Medicinal Importance of Genus Inula- A Review. International Journal of Current Research and Review, 05, 20-26.

Aydogan, M.N., Taskin, M., Canli, O., Arslan, N.P., & Ortucli, S. (2014). Tris-sucrose buffer system: a new specially designed medium for extracellular invertebrate production by immobilized cells of isolated yeast Cryptococcus laurentii MT-61. Folia Microbiologica (Prague), 59(1), 91-96. doi:10.1007/s12223-013-0258-2

Baytop, T. (1984). Therapy with Medicinal Plants in Turkey. Sanal Press, Istanbul, Turkey.

Benbacer, L., Merghoub, N., El Btaouri, H., Gmouch, S., Attaleb, M., Morjani, H., Amza, S., & El Mzibi, M. (2012). Antiproliferative effect and induction of apoptosis by Inula viscosa L. and Retama monosperma L. extracts in human cervical cancer cell lines, in: Rajamanickam, Topics on Cervical Cancer with an Advocacy for Prevention, InTech, Rijeka, Croatia, 267-284.

Beyranvand, F., Alizard, A., Rahimzadeh, S., Arzabanjani, K., Safarzadeh, A., Mohammad, M. & Sepahvand, A. (2018). A review of the most effective medicinal plants for dermatopathies in traditional medicine. Biomedical Research and Therapy, 5(6), 2378-2388. doi:10.15419/brmt.v5i6.450

Burkert, J.F.M., Kalil, S.J., Filho, F.M., & Rodrigues, M.I., 2006. Parameters optimization for enzymatic assays using experimental design. Brazilian Journal of Chemical Engineering, 23, 163–170.

Canli, O., & Kurbanoglu, E.B. (2011). Utilization of ram horn peptone in the production of glucose oxidase by a local isolate Aspergillus niger OC-3. Preparative Biochemistry and Biotechnology, 41(1), 73-83. doi:10.1080/10826068.2010.534223

Canli, O., & Kurbanoglu, E.B. (2012). Application of low magnetic field on the enzyme production by Geotrichum candidum under solid state fermentation using leek as substrate. Toxicology and Industrial Health, 28(10), 894-900. doi:10.1177/0748233711432127

Canli, O., Taras, G.E., & Taskin, M. (2013). Inulinase production by Geotrichum candidum OC-7 using migratory locusts as a new substrate and optimization process with Taguchi DOE. Toxicology and Industrial Health, 29(8), 704-710. doi:10.1177/0748233711432127

Chaturvedi, S., Bhattacharya, A., Nain, L., Prassana, R., & Khare, S.K. (2019). Valorization of agro-starchy wastes as substrates for oleaginous microbes. Bioresource and Biotechnology, 82(2), 211-220. doi:10.1007/s12258-018-1827-1

Chen, H.Q., Chen, X.M., Li, Y., Wang, J., Jin, Z.Y., Xu, X.M., Zhao, J.W., Chen, X.T., & Xie, Z.J. (2009). Purification and Characterisation of Exo- and Endo-Inulinase From Aspergillus ficuum JNSPS-06. Food Chemistry, 115, 1206-1212.

Chen, H.Q., Chen, X.M., Li, Y., Wang, J., Jin, Z.Y., Xu, X.M., Zhao, J.W., Chen, X.T., & Xie, Z.J. (2009). Purification and Characterisation of Exo- and Endo-Inulinase From Aspergillus ficuum JNSPS-06. Food Chemistry, 115, 1206-1212.

Chen, H.Q., Chen, X.M., Li, Y., Wang, J., Jin, Z.Y., Xu, X.M., Zhao, J.W., Chen, X.T., & Xie, Z.J. (2009). Purification and Characterisation of Exo- and Endo-Inulinase From Aspergillus ficuum JNSPS-06. Food Chemistry, 115, 1206-1212.

Chen, H.Q., Chen, X.M., Li, Y., Wang, J., Jin, Z.Y., Xu, X.M., Zhao, J.W., Chen, X.T., & Xie, Z.J. (2009). Purification and Characterisation of Exo- and Endo-Inulinase From Aspergillus ficuum JNSPS-06. Food Chemistry, 115, 1206-1212.

Chen, H.Q., Chen, X.M., Li, Y., Wang, J., Jin, Z.Y., Xu, X.M., Zhao, J.W., Chen, X.T., & Xie, Z.J. (2009). Purification and Characterisation of Exo- and Endo-Inulinase From Aspergillus ficuum JNSPS-06. Food Chemistry, 115, 1206-1212.

Chen, H.Q., Chen, X.M., Li, Y., Wang, J., Jin, Z.Y., Xu, X.M., Zhao, J.W., Chen, X.T., & Xie, Z.J. (2009). Purification and Characterisation of Exo- and Endo-Inulinase From Aspergillus ficuum JNSPS-06. Food Chemistry, 115, 1206-1212.

Chen, H.Q., Chen, X.M., Li, Y., Wang, J., Jin, Z.Y., Xu, X.M., Zhao, J.W., Chen, X.T., & Xie, Z.J. (2009). Purification and Characterisation of Exo- and Endo-Inulinase From Aspergillus ficuum JNSPS-06. Food Chemistry, 115, 1206-1212.

Chen, H.Q., Chen, X.M., Li, Y., Wang, J., Jin, Z.Y., Xu, X.M., Zhao, J.W., Chen, X.T., & Xie, Z.J. (2009). Purification and Characterisation of Exo- and Endo-Inulinase From Aspergillus ficuum JNSPS-06. Food Chemistry, 115, 1206-1212.

Chen, H.Q., Chen, X.M., Li, Y., Wang, J., Jin, Z.Y., Xu, X.M., Zhao, J.W., Chen, X.T., & Xie, Z.J. (2009). Purification and Characterisation of Exo- and Endo-Inulinase From Aspergillus ficuum JNSPS-06. Food Chemistry, 115, 1206-1212.

Chen, H.Q., Chen, X.M., Li, Y., Wang, J., Jin, Z.Y., Xu, X.M., Zhao, J.W., Chen, X.T., & Xie, Z.J. (2009). Purification and Characterisation of Exo- and Endo-Inulinase From Aspergillus ficuum JNSPS-06. Food Chemistry, 115, 1206-1212.

Chen, H.Q., Chen, X.M., Li, Y., Wang, J., Jin, Z.Y., Xu, X.M., Zhao, J.W., Chen, X.T., & Xie, Z.J. (2009). Purification and Characterisation of Exo- and Endo-Inulinase From Aspergillus ficuum JNSPS-06. Food Chemistry, 115, 1206-1212.
Sharma, P., Verma, A., Sidhu, R.K., & Pandey, O.P. (2006). Effects of processing parameters on the magnetic properties of strontium ferrite sintered magnets using Taguchi orthogonal array design. Journal of Magnetism and Magnetic Materials, 307, 157-164.

Singh, R.S., Chauhan, K., Kaur, K., & Pandey, A. (2020). Statistical optimization of solid-state fermentation for the production of fungal inulinase from apple pomace. Bioresource Technology Reports, 9. doi:10.1016/j.bitr.2019.100264

Singh, R.S., Dhaliwal, R., & Puri, M. (2006). Production of Inulinase from Kluyveromyces marxianus YS-1 Using Root Extract of Asparagus racemosus. Process Biochemistry, 41, 1703-1707.

Tan, O., Zaimoglu, A.S., Hinisioglu, S., & Altun, S. (2005). Taguchi approach for optimization of the belleding on cement-based grouts. Tunnelling and Underground Space Technology, 20, 167-173.

Tasar, O.C. (2017). Enhanced β-fructofuranosidase biosynthesis by Rhodotorula glutinis using Taguchi robust design method. Biocatalysis and Biotransformation, 35(3), 191-196. doi:10.1080/10242422.2017.1304386

Tasar, O.C., Erdal, S., & Algur, O.F. (2015). Utilization of Leek (Allium ampeloprasum var. porrum) for inulinase production. Preparative Biochemistry and Biotechnology, 45(6), 596-604. doi:10.1080/10826068.2014.940538

Taskin, M., Erdal, S., & Canli, O. (2010). Utilization of waste loquat (Eriobotrya Japonica Lindley) kernels as substrate for scleroglucan production by locally isolated Sclerotium rolfsii. Food Science and Biotechnology, 19(4), 1069-1075. doi:10.1007/s10068-010-0150-7

Taskin, M., Esim, N., Gencsel, M., Ortucu, S., Hasenkoglu, I., Canli, O., & Erdal, S. (2013). Enhancement of invertase production by Aspergillus niger OZ-3 using low-intensity static magnetic fields. Preparative Biochemistry and Biotechnology, 43, 177-188.

Taskin, M., Ortucu, S., Unver, Y., Tasar, O.C., Ozdemir, M., & Kaymak, H. C. (2016). Invertase production and molasses decolourization by cold-adapted filamentous fungus Cladosporium herbarum ER-25 in non-sterile molasses medium. Process Safety and Environmental Protection, 103, 136-143. doi:10.1016/j.psep.2016.07.006

Xiong, C., Jinhua, W., & Dongsheng, L. (2007). Optimization of solid-state medium for the production of inulinase by Kluyveromyces S120 using response surface methodology. Biochemical Engineering Journal, 34(2), 179-184. doi:10.1016/j.bej.2006.12.012

Zeggwagh, N.A., Ouahidi, M.L., Lemhadri, A., & Eddouks, M. (2006). Study of hypoglycaemic and hypolipidemic effects of Inula viscosa L. aqueous extract in normal and diabetic rats. Journal of Ethnopharmacology, 108(2), 223-227. doi:10.1016/j.jep.2006.05.008