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# Pullulan Production Using Molasses and Corn Steep Liquor as Agro-industrial Wastes: Physiochemical, Thermal and Rheological Properties

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## Abstract

**Background and Objective:** Pullulan is a microbial exopolysaccharide with wide uses in various industries. The aim of this study was to investigate pullulan production from agro-industrial wastes and study of pH, molasses concentration and corn steep liquor concentration as independent variables and yield of pullulan as response.

**Material and Methods:** Briefly, 5% (v $v^{-1}$) of the inoculation media (yeast extract 3 g, malt extract 3 g, peptone 5 g and sucrose 10 g per liter of distilled water), including *Aureobasidium pullulans* were added into media, containing 100 ml of molasses (100, 150 and 200 g $l^{-1}$) and various corn steep liquor concentrations (20, 40 and 60 ml $l^{-1}$) at adjusted pH (4.5, 5.5 and 6.5). After extraction and separation of the biomass using centrifuge, two folds of the supernatant volume of cold ethanol were added to the samples and stored at 4 °C for 24 h. After centrifuging, pullulan was dried and analyzed using Fourier transform infrared spectroscopy, X-ray diffraction, thermogravimetric analysis and rheological tests.

**Results and Conclusion:** Findings revealed that the maximum production yield (18.29 g $l^{-1}$ ±0.10) was achieved under optimum fermentation conditions (pH of 5.3, molasses concentration of 165 g $l^{-1}$ and corn steep liquor concentration of 43 ml $l^{-1}$). Then, physiochemical and thermal properties of the pullulan under the highlighted conditions were investigated. Pullulan included 78.8% solubility with no hygroscopicity. Furthermore, structural analysis using Fourier transform infrared and X-ray diffraction verified presence of pullulan with an amorphous structure in the supernatant. The exopolysaccharide included acceptable thermal stability and gel-like behavior; in which, the elastic component was predominant based on the results of thermogravimetric analysis and rheological properties, respectively.

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## 1. Introduction

Pullulan, as an exopolysaccharide produced by *Aureobasidium (A.) pullulans*, is composed of repeating maltotriose subunits (structured with α-D-glucan units) linked by α-1,4-glycosidic bonds [1-3]. Furthermore, the linear structure is interconnected via α-1,6-glycosidic linkages [2,4,5]. This water-soluble exopolysaccharide forms flexible, edible, transparent biodegradable films and can be used for coating purposes. Other specifications include oil resistant and impermeability to oxygen, which are accounted as important properties for the packing industries [1,6,7]. Additionally, pullulan can be used as starch replacer in low-calorie food formulations, texturizing material in cosmetic emulsions and material for drug delivery systems [6-9]. Despite these uses, pullulan production is limited due to costly substrates and media [8,10]. To solve this problem, studies have focused on using agricultural wastes as inexpensive substrates for the production of this product. Moreover, results have shown that these waste included acceptable production yields. Biotechnolgists are intersted in production of valuable products from biowastes using microorganisms [11,12]. For example, Goksungur et al. [6] produced pullulan from hydrolyzed potato starch wastes. In another study, An et al. [4] studied the pullulan production using mixtures of potato starch hydrolysates and sucrose and reported that production efficiency of pullulan in media was favorable. It is noteworthy that production of pullulan

from agrowastes is a biorefinery process; in which, economically valuable materials are produced through the fermentation process in addition to decreasing environmental pollution [6]. Therefore, finding novel low-cost sources for the production of this exopolysaccharide seems attractive.

Molasses derived from beetroot or sugarcane refining processes (sucrose purification) can significantly be considered for microbial carbon source due to its high sucrose contents, substrates with no pre-treatment needs prior to the fermentation [13,14]. However, molassess includes no sufficient nitrogen contents and; therefore, needs nitrogen sources. Corn steep liquor (CSL) is generated by associated amylase enzymes in starch processing factories. It is a nitrogen nutrient that can be incorporated into media and support production of pullulan [15]. Therefore, the aim of this study was to optimize pullulan production conditions from a novel inexpensive source and to investigate physic-chemical, structural, thermal and rheological properties of the product. Box-Behnken design with three independent variables of pH, molasses concen-tration and CSL concentration and a rsponse of pullulan production yield were used.

## 2. Materials and Methods

### 2.1 Materials

*Aureobasidium pullulans* (KY767024) was provided by Department of Food Science and Engineering, University of Tehran, Tehran, Iran. Beet molasses was purchased from the local sugar refinery (Buin Zahra, Qazvin, Iran). The CSL was supplied by Glucosan, Qazvin, Iran. Potato dextrose agar (PDA), malt extract, yeast extract, sulfuric acid and sodium hydroxide were purchased from Merck Chemical, Darmstadt, Germany and peptone from Sigma Chemical, St. Louis, MO, USA. All chemicals included analytical grades.

### 2.2 Chemical analysis of molasses and corn steep liquor

Beet molasses and CSL were analyzed for their moisture, nitrogen, sugar and ash contents (Table 1) based on methods described by Lee et al. [16] and Horwitz [17].

### 2.3 Pretreatment of molasses and addition of corn steep liquor

To decrease harmful compounds (coloring substances and heavy metals as growth inhibitors during fermentation) for the microorganisms, pretreatment of molasses was carried out based on a method by Lazaridou et al. [18] with mild modification. Molasses solution was adjusted to pH 3 with $H_2SO_4$ (2 N) and set for 24 h at ambient temperature. Then, solution was centrifuged at 5000 ×g for 15 min. To eliminate color of the solution, supernatant was treated three times with activated carbon (3% w $v^{-1}$) and stirring for 1 h at 25 °C) and then filtered through Whatman No. 1 filters. Subsequently, CSL was added to molasses solution and the medium was adjusted to a certain pH (with 1 N NaOH) as a favorable environment for the microorganism growth.

**Table 1**. Chemical compounds of the agricultural wastes of molasses and corn steep liquor

| Chemical compounds (%) | Molasses | Corn steep liquor |
|---|---|---|
| Moisture | 24 | 33.4 |
| Protein | 6.5 | 40 |
| Sugar | 54 | 4.5 |
| Ash | 9.7 | 17 |

### 2.4 Inoculum preparation

To prepare inoculum media, cultivated *A. pullulans* colonies were transferred into 100 ml of the sterilized inoculum liquid containing 3 g of yeast extract, 3 g of malt extract, 5 g of peptone and 10 g of sucrose per liter of distilled water and poured into 250-ml flasks with pH adjusted to 5.5. Flasks were transferred to a shaking incubator (Stuart Orbital Incubator S150, Staffordshire, UK) at 25 °C and sub-cultured every three days.

### 2.5 Fermentation and pullulan production

Briefly, 5% (v $v^{-1}$) of the inoculation media were added into a media containing 100 ml of molasses and CSL with adjusted pH based on Box-Behnken design. Mixture was transferred to shaking incubator (28 °C, 180 rpm) and stored for 4 d under constant conditions.

### 2.6 Pullulan extraction

After fermentation time, culture was centrifuged at 15000 ×g for 15 min. After separation of biomass from the supernatant, two folds of the supernatant volume of cold ethanol were added to the samples and stored at 4 °C for 24 h. To separate polysaccharides, samples were centrifuged at 15000 ×g for 15 min. Pullulan was dried at 50 °C until reaching a constant weight [19]. The pullulan production yield was reported as gram of the produced pullulan per liter of culture media (g $l^{-1}$).

### 2.7 Solubility of the produced pullulan

Dried pullulan (0.1 g) was suspended in 10 ml of distilled water and incubated at ambient temperature by constant agitation (100 rpm) for 90 min. Then, mixture was centrifuged at 7000 ×g for 15 min. Supernatant was dried at 55 °C using oven until a constant weight is achieved. The percentage of solubility was calculated using Eq. 1:

$$\text{Solubility } (\%) = (W_S/W_i) \times 100 \quad \text{(Eq. 1)}$$

Where, $W_i$ was the initial weight of pullulan powder and $W_s$ was the weight of dried supernatant.

### 2.8 Hygroscopicity

Pullulan powder sample (~1 g) was stored in a container with saturated NaCl solution (75.29% RH 25 °C). After one week sample was weighed and hygroscopicity was calculated as Eq. 2 [20]:

$$\text{Hygroscopicity } (\%) = [(w_1 - w_0)/w_0] \times 100 \quad \text{(Eq. 2)}$$

Where $w_0$ was the initial weight of powder and $w_1$ was the weight of powder after moisture adsorbing under humid air environment.

### 2.9 Fourier transform infrared spectroscopy analysis

The FTIR spectrometer (Perkin Elmer MA USA) was used to assess pullulan structure using KBr disk method in the range of 4000-500 $cm^{-1}$. Results were compared to those of commercial pullulan to verify presence of the pullulan structure.

### 2.10 Crystallinity analysis by X-ray diffraction

Briefly, X-ray diffractometer (Philips, Amsterdam, Netherland) with diffraction angle (2θ) of 5-80°, step size of 0.02° (2θ) and time per step of 1s/step was used to study crystallinity of the pullulan.

### 2.11 Thermo-gravimetric analysis of the produced pullulan

Briefly, Thermogravimetric analyzer analyzer (TA Q600, USA) with a heating range of 10-700 °C and a ramping rate of 10 °C $min^{-1}$ under argon flow of 100 ml $min^{-1}$ was used to analyze thermal properties of the pullulan [8].

### 2.12 Rheological behavior of the pullulan solution

Viscoelastic properties of the pullulan solution (2% w $v^{-1}$) were assessed using rotational rheometer (MCR301, Anton Paar, Austria). To analyze the linear viscoelastic region, oscillation strain sweep was carried out at a constant oscillation frequency of 1 Hz prior to dynamic experiments and then the storage modulus ($G'$) and loss modulus ($G''$) were calculated using oscillatory frequency sweep test at frequencies of 0.1-10 Hz, strain value of 0.01% (within the linear viscoelastic region) and temperature of 25 °C. The $G'$ and $G''$, which represented viscoelastic rheological behaveiors, could be expressed based on the Eqs. 3 and 4:

$$G' = \sigma_\circ/\gamma_\circ \cos(\delta) \quad \text{Eq. 3}$$

$$G'' = \sigma_\circ/\gamma_\circ \sin(\delta) \quad \text{Eq. 4}$$

The loss tangent was calculated based on the Eq. 5:

$$\tan\delta = G''/G' \quad \text{Eq. 5}$$

The complex modulus was quantitated by the Eq. 6:

$$G^* = \sigma_\circ/\gamma_\circ \quad \text{Eq. 6}$$

The complex viscosity was calculated using ratio of the complex modulus to frequency according to Eq. 7:

$$\eta^* = G^*/\omega \quad \text{Eq. 7}$$

### 2.13 Statistical analysis

In this study, Box-Behnken design with three variables of pH (4.5, 5.5 and 6.5), molasses concentration (MC) (100, 150 and 200 g $l^{-1}$) and CSL concentration (20, 40, and 60 ml $l^{-1}$) was used to achieve the maximum production yield of pullulan (response) (Table 2). Computations and graphics were reated using Design Expert 11 and Excel 2010.

## 3. Results and Discussion

### 3.1 Chemical composition of molasses and corn steep liquor

In the present study, pullulan production yield was optimized using response surface methodology procedure. Molasses and CSL were incorporated into the culture media as carbon and nitrogen sources, respectively. Specifications of the industrial wastes are presented in Table 1. Molasses and CSL included 48% sugar and 38% protein, respectively, as the dominant sections of the highlighted materials. Results were similar to results from other studies [14,15]

### 3.2 Pullulan production and statistical analysis

Box-Behnken design was used to optimize pullulan production yield considering three parameters of pH and molasses and CSL concentrations, as independent variables, at three levels with three replications at center point (for pure internal errors) (Table 2). As seen in the table, production yield varied from 2.30 (Run 2) to 18.30 g $l^{-1}$ (Run 14 as center point). Results of analysis of variance are shown in Table 3.

Fit summary results suggested that the quadratic model could be the most confidently for the calculation of the pullulan production yield. The quadratic polynomial regression was well-fitted to the experimental data since the lack-of-fit was insignificant ($p > 0.05$) and the respective regression model was extremely significant ($p < 0.01$) [1,6]. Furthermore, effects of linear and quadratic terms of pH and molasses and CSL concentrations on pullulan yield were significant ($p < 0.01$) while interactions term effects were less significant on pullulan yield ($p < 0.05$). Ass reported for the regression model, these data could be well linked to the high calculated coefficient ($R^2$ = ~0.99) [8]. Thereby, high significance of the polynomial quadratic model was validated and sufficient to predict variable effects on the response [21]. The coded quadratic equation was as Eq. 8:

$$Y\,(g\,l^{-1}) = 18.10 - 1.66A + 2.97B + 0.68C + 0.47AB - 0.40AC + 0.37BC - 6.05A^2 - 4.62B^2 - 2.35C^2 \quad \text{Eq.8}$$

Where, Y was the calculated pullulan production yield and A, B and C were the terms contributed to the linear effects of pH and molasses and CSL concentrations, respectively. AB, AC and BC were expressed as the terms showing interaction effects. $A^2$, $B^2$ and $C^2$ were linked to the terms showing quadratic effects on pullulan production yield.

**Table 2**. Levels of the variables (pH and carbon and nitrogen sources) and values from the calculated and predicted pullulan yields (g $l^{-1}$)

| Samples | pH | Molasses concentration (g $l^{-1}$) | Corn steep liquor concentration (ml $l^{-1}$) | Experimental pullulan yield (g $l^{-1}$) | Predicted pullulan yield (g $l^{-1}$) |
|---|---|---|---|---|---|
| 1 | 4.5 (-1) | 100 (-1) | 40 (0) | 6.40 | 6.50 |
| 2 | 6.5 (+1) | 100 (-1) | 40 (0) | 2.30 | 2.30 |
| 3 | 4.5 (-1) | 200 (+1) | 40 (0) | 11.60 | 11.50 |
| 4 | 6.5 (+1) | 200 (+1) | 40 (0) | 9.40 | 9.20 |
| 5 | 4.5 (-1) | 150 (0) | 20 (-1) | 10.30 | 10.20 |
| 6 | 6.5 (+1) | 150 (0) | 20 (-1) | 7.60 | 7.70 |
| 7 | 4.5 (-1) | 150 (0) | 60 (+1) | 12.60 | 12.40 |
| 8 | 6.5 (+1) | 150 (0) | 60 (+1) | 8.30 | 8.30 |
| 9 | 5.5 (0) | 100 (-1) | 20 (-1) | 8.00 | 7.80 |
| 10 | 5.5 (0) | 200 (+1) | 20 (-1) | 13.00 | 13.00 |
| 11 | 5.5 (0) | 100 (-1) | 60 (+1) | 8.50 | 8.40 |
| 12 | 5.5 (0) | 200 (+1) | 60 (+1) | 15.00 | 15.10 |
| 13 | 5.5 (0) | 150 (0) | 40 (0) | 18.00 | 18.10 |
| 14 | 5.5 (0) | 150 (0) | 40 (0) | 18.30 | 18.10 |
| 15 | 5.5 (0) | 150 (0) | 40 (0) | 18.00 | 18.10 |

**Table 3**. Analysis of variance for the quadratic model of pullulan yield

| Source | Sum of squares | df | Mean square | F-value | *p*-value |
|---|---|---|---|---|---|
| Model | 307.62 | 9 | 34.18 | 735.07 | <0.0001 |
| A-pH | 22.11 | 1 | 22.11 | 475.51 | <0.0001 |
| B-Molasses concentration | 70.80 | 1 | 70.80 | 1522.69 | <0.0001 |
| C-Corn steep liquor concentration | 3.78 | 1 | 3.78 | 81.32 | 0.0003 |
| AB | 0.9025 | 1 | 0.9025 | 19.41 | 0.0070 |
| AC | 0.6400 | 1 | 0.6400 | 13.76 | 0.0139 |
| BC | 0.5625 | 1 | 0.5625 | 12.10 | 0.0177 |
| $A^2$ | 135.15 | 1 | 135.15 | 2906.40 | <0.0001 |
| $B^2$ | 78.98 | 1 | 78.98 | 1698.51 | <0.0001 |
| $C^2$ | 20.39 | 1 | 20.39 | 438.51 | <0.0001 |
| Residual | 0.2325 | 5 | 0.0465 | | |
| Lack of fit | 0.1725 | 3 | 0.0575 | 1.92 | 0.3609 |
| Pure error | 0.0600 | 2 | 0.0300 | | |
| Total | 307.86 | 14 | | | |
| $R^2$ | | | 0.9992 | | |
| Adjusted $R^2$ | | | 0.9979 | | |
| Predicted $R^2$ | | | 0.9906 | | |
| C.V.% | | | 1.93 | | |

### 3.3 Effects of media conditions on pullulan production

#### 3.3.1 pH effects

As seen in Fig. 1, pH was an effective factor for the pullulan production by *A. pullulans*. Figure showed that the production yield first increased with increases in pH from 4.5 to 5.5 and then decreased. These observations are strongly linked to optimum conditions of the strain [22,23]. Therefore, it can be predicted that the best pH might vary for various strains in various media [7,24]. For example, Wu et al. [25] reported that the best pH for the pullulan production by *A. pullulans* JN207852 from synthetic medium was 6.0 while Sugumaran et al. [8] stated that this parameter for the pullulan production from Asian palm kernel by *A. pullulans* MTCC 2670 was 6.6

#### 3.3.2 Molasses concentration effects

Molasses was incorporated into the culture media as a sole carbon source. Molasses is a by-product, which is achieved during the sugar production. Majorly, molasses is composed of sucrose (approximately 55% of total solid content) and other nitrogen compounds are the residual ingredients. Sucrose is degraded by microbial enzymes and consequently bioconversion of simple sugars (fructose and glucose) occurs [26,27]. Based on Fig. 1, the modest quantities of carbon sources in media resulted in higher pullulan production yields. Indeed, higher molasses concentrations could concentrate media and create viscos fluids in submerging culture media. In this state, oxygen transition could be limited and subsequent cell metabolisms (growth and activity) might be disturbed [18]. Hence, it was concluded that carbon substrates should exist in sufficient quantities, reaching by optimization processes. The maxi-

mum pullulan production yield was achieved when the molasses concentration was ~150 g $l^{-1}$ (sugar concentration of ~81 g $l^{-1}$ based on Table 1). Lazaridou et al. [18] reported that the maximum pullulan production yield from beet molasses using *A. pullulans* and stirred tank reactor was achieved at a sugar concentration of 100 g $l^{-1}$. Researchers stated that the maximum pullulan yield from beet molasses in batch culture was achieved at a sugar concentration of 50 g $l^{-1}$ [28]. This difference in quantities of optimal molasses can be linked to difference in fermentation conditions as well as media compounds such as nitrogen sources.

### 3.3.3 Corn steep liquor concentration effects

Nitrogen source is an important factor in pullulan synthesis and several studies have shown that excessive increases in nitrogen source can decrease production yields [23,29]. As illustrated in Fig. 1, increases in CLS concentration to 40 g $l^{-1}$ included direct relationships with the response. However, further increases led to decreases in efficiency. Generally, large increases in nitrogen source lead to increases in biomass concentration and simultaneously decreases in product concentration [7]. These findings were similar to those reported by Singh et al. [30] and Mehta et al. [31].

### 3.4 Optimization process

In this study, response surface methodology was used to optimize pullulan production conditions to reach the maximum yield. Optimization was accompanied with the validation study. The optimum point (pH of 5.3, MC of 165 g $l^{-1}$ and CSL concentration of 43 ml $l^{-1}$) was validated with experimental calculation of the respective point with three replications and its comparison with the predicted point. Results of *t*-test showed that the experimental value (18.29 g $l^{-1}$ ±1.0) included no significant differences with the predicted one (18.75 g $l^{-1}$) ($p < 0.05$).

### 3.5 Pullulan powder properties

In this step, solubility and hygroscopicity of the experimental pullulan under optimum production conditions were assessed. In general, solubility of the biopolymer compounds depends on various parameters such as media acidity, pH, ionic strength, temperature and inter and intramolecular interactions [32]. The last parameter is important since further interactions of the biopolymer chains with water molecules can increase solubility due to increases in hydrogen bond formation. Thus, further hydrogen bond formation with water molecules rather than biopolymer-biopolymer bonds increases solubility. In the present study, solubility included 78.8%, which was significantly lower than that of the commercial pullulan (100% solubility). The difference could be attributed to drying methods. Indeed, commercial pullulans are mostly dried using lyophilizing procedures while the experimental pullulan was oven-dried. Freeze-drying due to sublimation process generates porous structures and, thereby, water conveniently penetrates powder particles [32]. Results of hygroscopicity showed no weight gain after a week storage in NaCl saturated solution. These findings were similar to findings by previous studies [33,34].

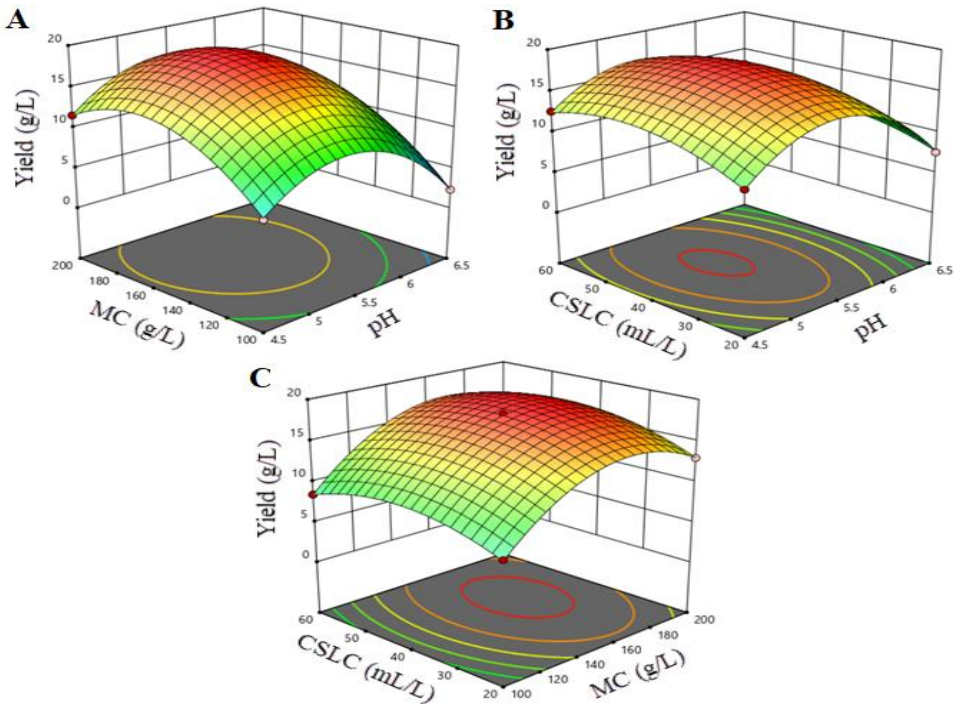


**Figure 1**. Three-dimensional surface plots of pullulan production yield affected by three independent variables of pH and molasses and corn steep liquor concentrations. MC: molasses concentration; CSLC: corn steep liquor concentration

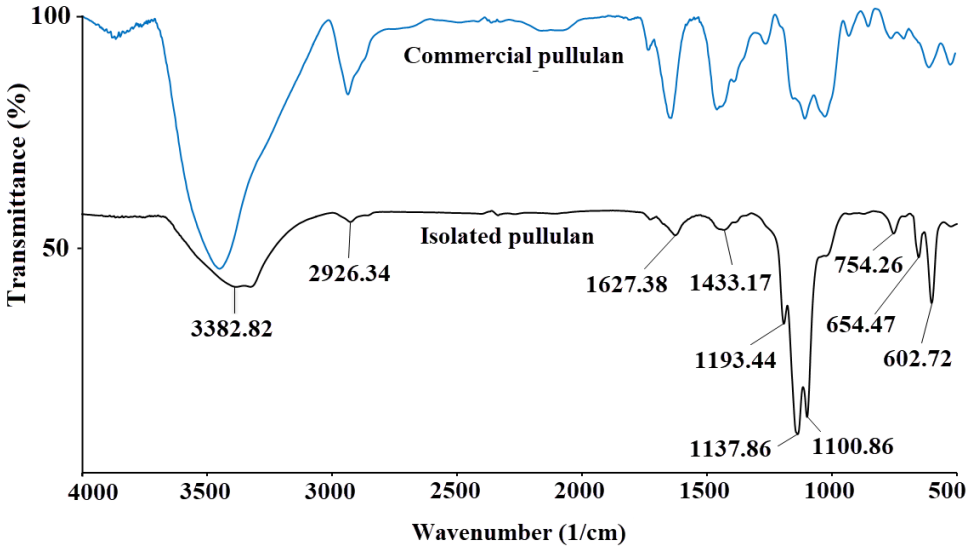


**Figure 2**. Fourier transform infrared spectra of the commercial and experimental pullulan

### 3.6 Structural analysis using Fourier transform infrared spectroscopy

In this study, FTIR spectroscopy was used to study chemical structures of the pullulan. Figure 2 shows FTIR spectra of the experimental pullulan under optimum conditions and the commercial pullulan. Each of detected peaks could be linked to specific functional groups. As shown in figure, a broadband was detected in range of 3300-3500 $cm^{-1}$, which was attributed to hydroxyl (-OH) stretching groups abundantly found in polysaccharide structures. The stretching peak at 2800-3000 $cm^{-1}$ was associated to symmetric and asymmetric -CH vibrations (methyl groups in alkane structures) [35,36]. The peak at 1627 $cm^{-1}$ could be linked to O-C-O group and the peak at 1433 $cm^{-1}$ to C-O-H group. Detected sharp stretch peaks at 1193 and 1137 $cm^{-1}$ were linked to C-O-C and C-O groups, respectively. Peaks at 1000-1100 $cm^{-1}$ were derived from glycosidic linkages. It has been found that peaks in range of 800-1200 $cm^{-1}$ are referred to as fingerprint region, which is unique for each compound. As stated for the pullulan structure, α-D-1,4-glucopyranoside and α-D-1,6-glucopyranoside are the main bonds, which include two peaks of 754 and 1100 $cm^{-1}$, respectively. Results showed that the supernatant was rich in linear glucan with maltotriose repeating units connected by (1→6) linkages. Data were similar to data reports by Hamidi et al. [23] and Wang et al. [5] for pullulans from synthetic media and rice hull hydrolysate, respectively.

### 3.7 X-ray diffraction analysis

Patterns of the produced pullulan are represented in Fig. 3. Generally, presence of sharp signals in XRD pattern reflects the crystalline structure of a polymer.

In contrast, their absence shows the amorphous structure of a polymer [24]. Thus, the achieved sample included an amorphous structure. Similar results were reported in previous studies by other researchers [8,37,24].

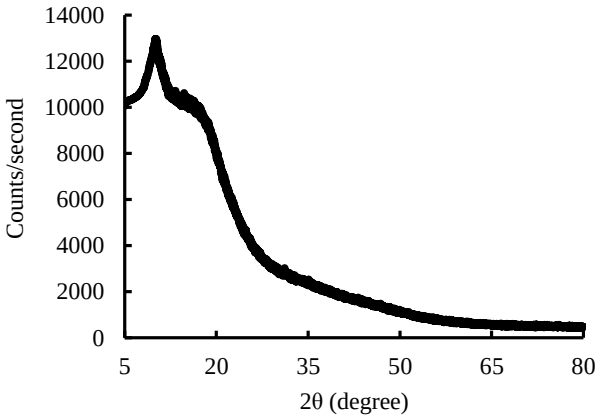


**Figure 3**. X-ray diffraction patterns of the experimental pullulan under optimum conditions

### 3.8 Thermogravimetric analysis

Thermostability is an important property in industrial pullulan scales. Thermal stability of the produced pullulan is illustrated in Fig. 4. As it can be observed in the figure, thermal decomposition of the pullulan initiated at 245.59 °C, demonstrating that the produced pullulan included an acceptable thermal stability according to the previously reported data by other researchers [2,8,23,38].

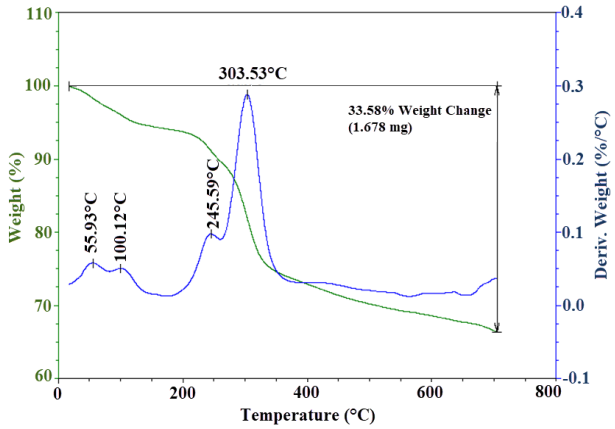


**Figure 4.** Thermogravimetric analysis of the extracted pullulan at the optimum point

### 3.9 Rheological attributes

Rheological properties of the pullulan solution (2% w $v^{-1}$) were investigated to assess viscoelastic characteristics of the produced exopolysaccharides. Hence, oscillatory strain sweep was carried out to calculate the viscoelastic region and then oscillatory frequency sweep test was carried out to assess storage, loss modulus values and complex viscosity [39]. Results are presented in Figs. 5 and 6. At lower and higher frequency values of the solution, $G'$ was greater than $G''$ (Fig. 5). It was shown that elastic behavior of the pullulan solution was dominant at lower or higher frequency values [40]. It could be estimated that loss tangent showed more elastic value, which could be attributed to higher storage module values, compared to loss module values. In terms of complex viscosity, crossovers were shown with storage modules at higher frequencies. Complex viscosity could be calculated using complex module and frequency. At lower frequencies, complex viscosity was lower due to further stresses on biopolymer solution. At higher frequencies, complex viscosity was higher due to use of less stresses, reaching storage module as the major component of elastic behavior. Thus, pullulan solution with the highlighted concentration included gel-like behaviors; in which, the elastic component was predominant [41].

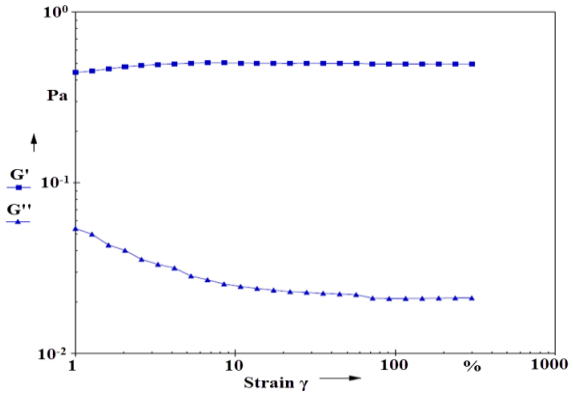


**Figure 5**. Strain sweeps at 1.0 Hz to calculate moduli G′and G′′for the extracted pullulan under optimum conditions

## 4. Conclusion

The present study was carried out to optimize pullulan production from agroindustrial wastes using *A. pullulans*. The pH, molasses and CSL concentrations were considered as independent variables while the pullulan production yield was considered as dependent parameter or response. The response surface methodology results showed that the maximum production yield (18.29 g $l^{-1}$ ±0.10) was achieved with pH of 5.3, molasses concentration of 165 g $l^{-1}$ and CSL concentration of 43 ml $l^{-1}$ as the optimal conditions. The FTIR analysis revealed presence of the chemical structure of pullulan in the supernatant.

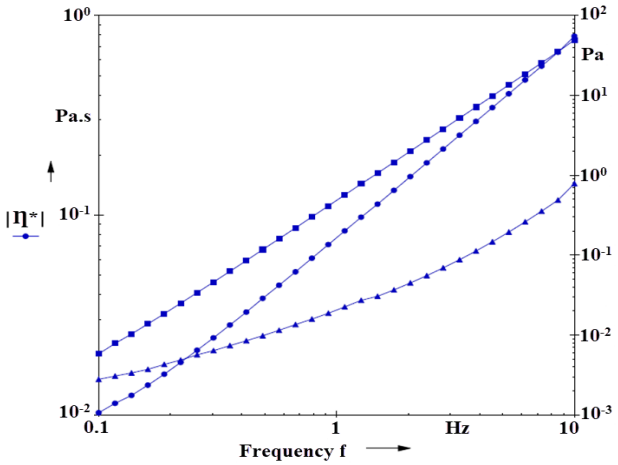


**Figure 6**. Frequency sweeps at 0.01% strain to calculate storage modulus (G′) and loss modulus (G′′) for the pullulan solution (2% w $v^{-1}$) in association with the complex viscosity (η)

Moreover, the produced polymer included an amorphous structure with high thermal stability and gel-like behavior. Eventually, it can be concluded that pullulan production from agro-wastes can be promising and molasses and CSL can be used as inexpensive but efficient sources for the production of this biopolymer.

## 5. Acknowledgements

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## 6. Conflict of Interest

The authors report no conflicts of interest.

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# تولید پولولان با استفاده از ملاس و شربت مایع ذرت به عنوان ضایعات کشت و صنعت: خواص فیزیکوشیمیایی، حرارتی و رئولوژیکی

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## چکیده

**سابقه و هدف:** پولولان پلی‌ساکاریدی برون‌سلولی میکروبی با کاربرد گسترده در صنایع گوناگون می‌باشد. هدف این مطالعه بررسی تولید پولولان از ضایعات کشت و صنعت و مطالعه pH، غلظت ملاس و شربت مایع ذرت به عنوان متغیرهای مستقل و راندمان پولولان به عنوان پاسخ بود.

**مواد و روش ها:** به طور خلاصه، ۵ درصد حجمی حجمی محیط کشت تلقیح (۳ گرم عصاره مخمر، ۳ گرم عصاره مالت، ۵ گرم پپتون و ۱۰ گرم سوکروز دریک لیتر آب مقطر) حاوی *آئروبازیدیوم پولولانس* به ۱۰۰ میلی‌لیتر محیط کشت متشکل از ملاس (۱۰۰، ۱۵۰، و ۲۰۰ گرم بر لیتر) و غلظت‌های گوناگون شربت مایع ذرت (۲۰، ۴۰، و ۶۰ میلی‌لیتر بر لیتر) با pH تنظیم شده (۴/۵، ۵/۵، و ۶/۵) اضافه شد. پس از استخراج و جداسازی زی‌توده[1] به کمک سانتریفوژ، اتانول سرد به میزان دو برابر روماند[2] اضافه و به مدت ۲۴ ساعت در درجه حرارت ۴ درجه سلسیوس نگهداری نگهداری شد. پس از سانتریفوژ، پولالان خشک و آزمون‌های طیف بینی مادون قرمز تبدیل فوریه[3]، پراکنش پرتو ایکس[4]، گرماوزن‌سنجی[5] و رئولوژی انجام شد.

**یافته‌ها و نتیجه‌گیری:** یافته‌ها نشان داد بیشینه راندمان تولید (۱۸/۲۹ گرم بر لیتر ±۰/۱۰) در شرایط بهینه تخمیر (pH برابر ۵/۳، غلظت ملاس ۱۶۵ گرم بر لیتر و غلظت شربت مایع ذرت ۴۳ میلی‌لیتر بر لیتر) به دست آمد. سپس خواص حرارتی و فیزیکوشیمیایی پولولان تحت شرایط مشخص‌شده مورد بررسی قرار گرفت. پولولان بدون نم‌گیرایی[6] حلالیتی برابر ۷۸/۸ درصد داشت. همچنین، آزمون‌های ساختاری طیف‌بینی مادون قرمز تبدیل فوریه و پراکنش پرتو ایکس وجود پولولان با ساختاری غیرمتبلور در روماند را تایید کرد. پلی‌ساکارید برون‌سلولی، بر اساس نتایج آزمون گرماوزن‌سنجی و خواص رئولوژی به ترتیب پایداری حرارتی قابل قبول و رفتار شبه ژل داشت که در آن ترکیب الاستیک غالب بود.

**تعارض منافع:** نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

**تاریخچه مقاله**

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**واژگان کلیدی**

- شربت مایع ذرت
- ملاس
- پولولان
- ویژگی‌های رئولوژیکی
- آنالیز حرارتی

***نویسنده مسئول**

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1 Biomass
2 Supernatant
3 Fourier transform infrared spectroscopy or FTIR
4 X-ray diffractometry or XRD
5 Thermogravimetric analysis or TGA
6 Hygroscopicity