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Extraction of lycopene from tomato waste using solid state fermentation

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Abstract

Lycopene is a well-known carotenoid, causing red color of fresh tomatoes. The significance of lycopene as antioxidant agent and coloring in the cosmetics, and its use in pharmaceutical and food industries has expanded in the recent years. Extraction of lycopene was improved effectively under solid state fermentation process; whereby, cellulase produced from the fermentation process was employed to degrade the cell-wall constituents, which facilitated the release of intracellular contents. The optimum conditions for the fermentation process were determined using Response Surface Methodology (RSM). The Facecentered Central Composite Design (FCCD) was employed to investigate the effects of three independent factors: moisture content in the range of 60 to 80 %, inoculum size ranging between 5 to 15% while the incubation time was set at 2, 3 and 4 days. Twenty runs of experiment were conducted and each one was repeated three times. The obtained data was analyzed using the Design Expert software v.6.0.8. Regression analysis showed that 94.56% of the variation was explained by the software. Under the optimized conditions, the highest lycopene yield was 307.2 µg/g when the moisture content was 80%, the inoculum size was 15% in 4 incubation days. The experimental values agreed with the predicted values, thus proving stability of the model used and the success of RSM. This study showed as to how fermentation can improve the extraction process by comparing the result with the control (extraction without fermentation) which was 0.8 µg/g.

Introduction

Plant and plant by products contain many natural pigments which impart red, orange and yellow color in a wide variety of plants and fruits are known as carotenoids and comprised of various phytochemicals usually found in the food matrix such as β-carotene (orange), lycopene (red), lutein (yellowish-green), chlorophyll (green) and anthocyanin (blue-purple) (Krinsky and Johnson, 2005; Mortensen, 2006; Konwarh *et al*., 2012). Carotenoids, have proven to be excellent antioxidants and have been applied in many medical as well as nutraceutical industries. There has been an exponential demand for lycopene which has directed researchers to produce lycopene with a more cost effective method at a large scale to meet this need.

Amongst these carotenoids, tomato and its products are considered as one of the richest sources of lycopene, as the total lycopene content in tomato varied between 90 and 190 µg/g fresh weight.The waste during tomato processing is obtained in the form of seeds and skin residues, which can be a rich source of lycopene (Choudhari and Ananthanarayan, 2007). Production of several thousand tons of wastes from tomato juice factories itself was reported in Japan (Suzuki *et al*., 2002). The major part of the waste, which comes from the pulper, is tomato pomace constitutes where the wet pomace consists of 33% seed, 27% skin and 40% pulp, while the dried pomace contains 44% seed and 56% pulp and skin (Basuny *et al*., 2009).

Papaya, pink guava, watermelon, pink grapefruit, and apricots can be considered as other significant sources of lycopene. Lycopene is the reason behind the red color of fresh tomatoes. The importance of this natural carotenoid as a coloring and antioxidant agent in the food, cosmetics and pharmaceutical industries, has increased rapidly (Choudhari and Ananthanarayan, 2007; Nobre *et al*., 2009). Lycopene has antioxidant properties which have various useful health benefits. In fact, extensive studies show that the antioxidant properties of lycopene have potential protective agent against different types of cancers such as colon, prostate, breast, cervix, and some other chronic diseases (Giovannucci *et al*., 1995).

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While the concentration of lycopene in tomatoes is limited due to the location of this compound within the intracellular components such as the chromoplasts or the choloroplasts, therefore, to enhance the purity and yield of the extracted compound, the extraction of lycopene involves its isolation from the intracellular components (Jamal *et al*., 2015). Thermal processing is better known to yield a higher level of lycopene from the tomatoes or other sources of lycopene due to better release as it liberates this carotenoid from complexes with proteins, and thus increases its bioaccessibilty (Jamal *et al.*, 2016). Lin and Chen (2003) showed that with an increased temperature favors the lycopene bioavailability from the tomatoes. Similarly, a study conducted by van den Berg *et al.* (2000) stated that heat processing can breakdown cellular walls, rupture chromoplast membranes and decrease cellular integrity leading to an increased bio accessibility of various carotenoids especially lycopene. Solid state fermentation which involves the extraction of lycopene by its isolation from the cellular walls and is followed by the digestion of said organelles with hydrolytic enzymes of proteins such as pectins and/or proteases. This process makes it possible to release the lycopene bound to various structural proteins and is a favored method to increase the availability of lycopene from the cellular matrix.

Enzyme aided extraction is one of the most efficient methods of lycopene extraction in which cellulase is added to the tomato as the plant cell-wall consist of cellulose. This method is used to enhance the recovery of lycopene by degrading the plant cell-wall, thus assisting in the release of the intracellular content (Choudhari and Ananthanarayan, 2007; Ranveer *et al*., 2013). But the major problem with this method is the high cost of the cellulase enzymes (Dhillon *et al*., 2012). On the other hand, solid state fermentation processes (SSF), which is the microbial transformation of biological materials in their natural state has been proven to be extremely effective and inexpensive method for the production of enzymes such as cellulase by different types of fungi with a high activity. While the production of cellulose has been reported from many bacteria and fungi, almost all fungi of genus *Aspergillus* synthesize cellulase, therefore this genus has the potential to dominate the enzyme industry These enzymes are excreted as extracellular enzymes; and *Aspergillus niger (A.niger)* has shown the ability to produce large amount of cellulolytic enzymes (Dhillon *et al*., 2012; Delabona *et al*., 2013).

The production of tomato juice has increased recently due to the interest in health promoting bioactive compounds present in it, which at the same time resulted in the waste generation. Thus, this study is important due to the environmental concern to minimize waste production and use it as a potential substrate for the fermentation. Moreover, economically, enzyme aided extraction of lycopene with *A.niger* is cost efficient and hence minimizes the cost of lycopene production.

In solid state fermentation process, an enzyme is produced by *A.niger*, which is cellulase under a set pH and temperature as expalined by Suhail *et al.* (2009) in their cellulose production from *A.niger*. It acts on cellulose, which is present in the primary wall beneath the first layer of the middle lamella of the plant cell wall. In this study, solid state fermentation using *A.niger* was employed to enhance the production of lycopene from the tomato waste; cellulase was produced during the growth of *A.niger* on the tomato waste, which helped in the release of lycopene by degrading the cell wall of the tomato, then normal extraction was conducted according to Fish *et al.* (2002) method. Response Surface Methodology (RSM) was adapted (followed) to optimize the process conditions for the solid state fermentation. A Face centered Central Composite Design (FCCD) was used to investigate the effects of three independent factors which are moisture content (60 to 80%), inoculum size (5 to 15 %) and incubation time (ranging between 2 to 4 days) on the yield of lycopene from the tomato pomace.

Materials and methods

Microorganisms, culture media and mineral solution

Tomato pomace was dried in an oven at 60°C and stored till further use. Culture of A.niger was maintained on potato dextrose agar (PDA) plates and incubated at 32°C for four days. After that, the cultured plates (4 plates) were rinsed with 100 mL of sterilized distilled water. Inoculum was prepared by gently rubbing the surface with a sterilized small glass-hockey stick and the mycelia suspension was transferred into a sterilized 250 mL conical flask by filtration. The inoculum was then stored in the refrigerator at 4°C. Mineral solution was prepared to contain the following quantities of nutrients salts per liter (w/v) %: $CaCl_2.2H_2O$, 0.0074; $MgSO_4.7H_2O$, 0.05; $MnSO_4.2H_2O$, 0.0005; $ZnSO_4.7H_2O$, 0.0006; $CuSO_4.5H_2O$, 0.001.

Solid state fermentation process

The fermentation process was carried out by using 250 mL Erlenmeyer flaks for a fermentation period of 2, 3 and 4 days. Total weight of fermentation medium

was 20 g. A 250 ml Erlenmeyer flask was filled with 18% w/w (3.6 g) or 28% w/w (5.6 g) or 38% w/w (7.6 g) tomato waste (depending on the moisture content required) followed by 2% w/w (0.4 g) nutrients (peptone (0.125 g), cellulose (0.225 g) and potassium hydrogen phosphate (0.05 g). Thereafter, different amounts of distilled water (50-70% v/w) and 5% v/w (1 mL) of mineral solution was added accordingly. The well-mixed medium was cotton plugged, and autoclaved to sterilize at 121°C for 20 minutes. The inoculum concentration varied from 5% v/w (1 mL), 10% v/w (2 mL) and 15% v/w (3 mL), then it was poured on the medium after it was left to cool down to the room temperature. The cotton-plugged flasks were then incubated at 32°C for 4 days.

Extraction process

Extraction was performed according to Fish *et al.* (2002) method and the Total lycopene content was determined using a method called the reduced volume lycopene assay as per the following Equation (1):

$$Lycopene\left(\frac{mg}{Kg\,tissue}\right)=\left(\frac{A_{503}}{17.2X10^{4}/M\,cm}\right)X\left(536.9\frac{g}{mole}\right)X\left(\frac{1L}{10^{3}\,mL}\right)X\left(\frac{10^{3}mg}{1\,g}\right)X\left(10.0\frac{mL}{Kg\,tissue}\right)=$$

$$\frac{A_{503}\,X\,0.0312}{Kg\,tissue}=\frac{A_{503}\,X\,31.2}{g\,tissue} \qquad (1)$$

Where A_{503} is the absorbance measured at 503 nm.

Response surface optimization

FCCD) under (SRM) using Design Expert v.6.0.8 (State-East Inc. Minneapolis, USA) was selected to determine the maximum production of lycopene. The selected experimental factors were moisture content (A), inoculum size (B) and incubation time (C). The boundary levels for each parameter were as follows: Moisture content (60 to 80%), inoculum size (ranging between 5 to 15%), incubation time of 2, 3 and 4 days. The concentration of lycopene to be produced was treated as the dependent response (Y).

Results and discussion

Factors such as moisture content, incubation time, carbon and nitrogen sources and inoculum size and some other factors may significantly influence the fermentation process. There are some other factors such as enzyme concentration and incubation time, which have significant effects on the extraction process (Choudhari and Ananthanarayan, 2007; Ruqayyah *et al.*, 2014; Idris *et al*., 2015).

Twenty experiments were conducted with three replicates each. The highest lycopene content obtained was 307.2 µg/g at 80% (v/w) moisture content 15% (v/w) inoculums size for 4 days of incubation(not all data are shown). Meanwhile, the observed predicted value for lycopene content was 300.19 µg/g, according to the software. Whereas, for the control (unfermented tomato waste) in this experiment tomato waste was subjected to direct extraction as stated in the method without any fermentation, the amount of total lycopene content was very low (0.8 µg/g) as compared to the total lycopene content obtained after fermentation (307.2 µg/g). The fermentation-aided extraction showed an increase in lycopene yield by 383%. The quadratic model demonstrated the significance of the model, as evidenced by the Fisher's F-test with F-value of 19.31 and relatively low probability value, (Pmodel> F) = 0.0001. The determination of coefficient R^2= 0.9456 which indicated a very high degree of correlation between the experimentally observed and predicted values, and indicated the degree of precision with which the total lycopene content is attributed to the independent variables, moisture content, inoculum size and the incubation time for fermentation to take place.

Table 1.Observed and total lycopene yield response

Run	Factors			Lycopene µg/g	
	Moisture content %(v/w)	Inoculum Size %(v/w)	Incubation Time (day)	Experimental	Predicted
1	60	5	4	95.8	88.63
2	80	15	2	151.6	166.59
3	60	15	2	66.7	79.98
4	70	10	3	89.5	92.54
5	80	10	3	265.6	258.47
6	80	15	4	307.2	300.19
7	70	10	3	87.9	92.54
8	70	15	3	109.2	84.69
9	80	5	2	124.2	128.79
10	70	10	4	84.4	100.79
11	60	15	4	91	94.23
12	70	10	3	77.9	92.54
13	80	5	4	261	255.54
14	70	10	3	78.1	92.54
15	70	10	3	75	92.54
16	70	10	3	84.2	92.54
17	70	10	2	78	30.29
18	70	5	3	69.8	62.99
19	60	5	2	66.4	81.23
20	60	10	3	155.9	131.71

The corresponding analysis of variance (ANOVA) is presented in Table 2. The ANOVA for the response surface quadratic model demonstrates the significance of the model, as evidenced by the

Table 2. ANOVA for Response Surface Quadratic Model

Source	Sum of Squares	Degree of Freedom	Mean of Square	F-Value	Probe > F
Model	94573.06	9	10508.12	19.3	< 0.0001
A	40170.24	1	40170.24	73.83	< 0.0001
B	1177.22	1	1177.22	2.16	0.1721
C	12425.62	1	12425.62	22.84	0.0007
A2	28922.95	1	28922.95	53.16	< 0.0001
B2	961.18	1	961.18	1.77	0.2133
C2	2004.08	1	2004.08	3.68	0.0839
AB	762.45	1	762.45	1.4	0.2639
AC	7122.21	1	7122.21	13.09	0.0047
BC	23.46	1	23.46	0.043	0.8397

Fisher's F-test with F-value of 19.31 and relatively low probability value, (Pmodel> F) = 0.0001. The value of "Prob> F" less than 0.05 indicates that the model terms are significant. The variables with low P values contribute to the model, whereas those with high P values can be neglected and eliminated from the model (Alam *et al.*, 2009).

ANOVA suggested the model to be significant at $P < 0.05$. The P-values were used as a tool to check the significance of each coefficient, which in turn indicated the pattern of the interactions between the variables.

The coefficient of interactive effect and significance of moisture content, inoculums size and incubation time can be seen from Table 2. It shows that the F-values for independent variables suggest that the linear effect of moisture content (A) was significant since the value was 0.0001; also, the other linear effect of incubation time (C) was significant since the value was 0.0001. The interaction between moisture content and incubation time (AC) was also significant since the value was 0.0047.

Tomatoes and various tomato products derived from the processed tomatoes are a potent source of carotenoids, especially lycopene. Davis *et al.* (2003) conducted a study on 13 tomatoes (four different cultivators) and 38 tomato products available in the market, determined the lycopene content to be in the range of 6.6 to 490 mg/kg in the original tissue. Another study conducted by Baranska *et al*. (2006) on various tomato fruit breeds and products fell into the range of 2.62 to 629 mg/100 gm fresh weight basis. Brandt et al. (2003) reported an average lycopene content of 64.9 mg/kg fresh weight basis. However, most of the extraction processes of lycopene include a simple solvent extraction which may not be the most optimal method of getting a higher yield of lycopene.

The extraction of lycopene has improved effectively under the fermentation process. Cellulase produced from the fermentation process is employed to degrade the cell wall constituents, thus assisting in the release of intracellular contents (Ranveer *et al.*, 2013) and thereby a higher yield of lycopene is achieved at 307.2 µg/g on fresh weight basis.

Interactions of variables can be determined effectively by the orientation of the principle axes of the contour plots as shown in Figures 1 and 2 From the contour plots, the optimal values of the independent variables could be observed, and the interaction between each independent variable pair can be described. The maximum predicted value at 105.514 µg/g is indicated by the surface confined in the smallest ellipse in the contour plot. Elliptical contour is obtained when there is a perfect interaction between the independent variables.The contour plots based on independent variable was obtained and the isoresponse contour plots of RSM as a function of two factors at one time, holding all other factors at fixed levels, are helpful for understanding both the main and interaction effects of these two factors. The effect of varying levels of inoculum size (B) and incubation time (C) on the total lycopene content while moisture content was fixed at 70% are shown in Figures 1 and 2.

Figure 1 shows that when the moisture content was kept constant, the interaction between the two variables (inoculum size and incubation time) showed that the total lycopene content was sensitive even when inoculum size and incubation time were subjected to small alterations. Under certain conditions, a maximal contour (total lycopene content of 105.514 µg/g) could be determined (Figure 2),

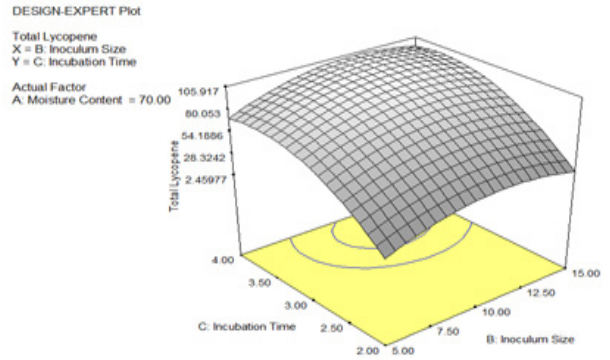

Figure 1. 3D surface plot showing the effect of inoculum size and incubation time on the highest lycopene yield

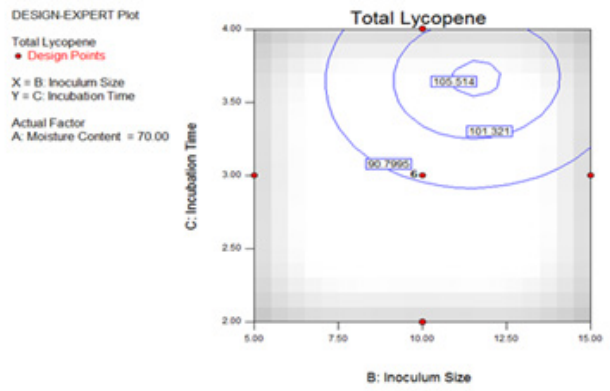

Figure 2. Contour plot showing the effect of inoculum size and incubation time on the highest lycopene yield

meaning that further change in amount of inoculum size and incubation time would not increase the total lycopene content, but might have a negative effect on the yield of lycopene. Figure 2 showed that highest lycopene content was obtained at Inoculum size of 11.6% and incubation time of 3.65 days while moisture content was held constant at 70% while the process conditions for media and culture growth were maintained as stated by Idris *et al.* (2015).

Conclusion

Concentration of lycopene was improved effectively by solid state fermentation process suggesting that the cellulase produced from the fermentation process was able to degrade the cell wall constituents thus assisting in the release of intracellular contents. A lycopene yield of 307.2 µg/g was achieved when the moisture content was 80%, the inoculums size was 15% in 4 incubation days which is a much greater improvement upon normal extraction.The lycopene content can further be increased by improving several other factors such as strain improvement, change of the fermentation type such as using liquid state fermentation, and improvement the fermentation medium.

Conflict of Interest

There is no potential conflict of interest.

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