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Increasing Protein Content of Tomato Pomace using Solid-State Fermentation with Industrial Bakery Yeasts

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Abstract

Background and Objective: Tomato pomace as the major waste of tomato paste can be used in food formulation due to its nutritional and technological characteristics. The aim of this study was to create a cheap simple method to increase the protein content of tomato pomace, which could be used as a cheap and more efficient food source for livestock and poultry.

Material and Methods: The study was carried out in three stages: 1) selection of further appropriate yeasts by assessing effects of two types of avaiable *Saccharomyces cerevisiae* and *Saccharomyces bulardi* industrial yeasts and fermentation time in the moisture content of 5% tomato pomace with particle size of less than 500 µm on the protein content of tomato pomace, 2) asessment of optimum conditions for increasing the protein content of tomato pomace by assessing effects of four parameters of the quantity of the yeasts, initial moisture content, substrate particle size and cultivation time in three levels, based on the Taguchi method in nine experiments in laboratory scale, 3) increase of the protein content of tomato pomace in bench scale tray bioreactor by investigation of three factor substrate depth, distance between trays and substrate particle size in three levels and one aeration factor in two levels based on the Taguchi method.

Results and Conclusion: *Saccharomyces cerevisiae* and 5-d cultivation time were chosen to continue the study. Under optimal conditions in laboratory scale, 0.03 g dry yeast/g tomato pomace of yeast, moisture content of 70% (w w^{-1}), particle size of less than 150–250 µm (mesh 100) and process time of 5 d, protein content of 24.72% with fat content of 3.29%, ash of 16.45% and carbohydrate of 55.52% (w w^{-1}) were achieved. Under optimal conditions, including bed depth of 1.2 cm, tray distance of 4 cm, particle size of 250-500 µm (mesh 60) without aeration, the maximum protein content of 25.82% were achieved, which were more than 80%, compared to the primary tomato pomace protein content (14.21% w w^{-1}). This is the highest protein content already reported for tomato pomace, using the simplest technology at the lowest cost.

Conflict of interest: The authors declare no conflict of interest

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1. Introduction

Disposal of agricultural substrates and wastes from food factories is one of the major challenges for the producers because it leads to increased costs and pollution of factory area and the environment. However, most of these wastes are rich in dietary fibers, antioxidants, essential fatty acids, antimicrobial compounds, proteins, vitamins and minerals that can be used in food formulations due to their nutritional and technological characteristics [1]. Tomato pomace is the major in waste of tomato paste and other tomato products [2], which depends on the processing method and characteristics of the raw tomatoes, including various proportions of skin (27%), seeds (33%), pulp (40%) and 5–30% (w w^{-1}) of

tomatoes. Tomato pomace is primarily used as animal feed, soil fertilizer and more recently for the production of high value-added commercial products such as lycopene and other carotenoids and more recently for the production of bio-lacquer for indoor and outdoor coatings of metal food packages [3,4].

Currently, large quantities of this valuable waste are composted or dumped in landfills, roadsides and rivers, leading to environmental polution. An appropriate alternative to such disposal methods can include their processing using solid-state fermentation (SSF) technology to develop low-cost feed supplements for livestock and poultry [5]. In SSF, microorganisms are mixed with wet solid substrates and high concentrations of the substrate are provided for the fermentation [6]. Several factors, including solid particle size, solid substrate humidity, fermentation process temperature, initial pH, aeration, height and depth of the solid substrate, type of the microorganism and bioreactor, agitation, quantity and age of inoculum and specific additives, affect the efficiency of SSF. Hence, these factors should be optimized for the SSF of each solid substrate by each microorganism [7,8]. Despite disadvantages such as long cultivation time and difficulty in monitoring and controlling SSF parameters, SSF is an attractive and alternative process for the solid waste processing in agricultural and food industries [6-8]. Various types of bioreactors have been developed for SSF based on the type of aeration and agitation, including tray bioreactors, packed-bed and air-solid fluidized bed bioreactors stirred and rotating drum bioreactors [6,9,10]. Tray fermenters are usually the most used solid-state fermentation systems because of their simple design. A tray bioreactor includes a chamber with several trays in its rows and humid air with controlled temperature circulates between the trays [10].

Saccharomyces cerevisiae (*S. cerevisiae*) is the yeast with the most associations to humans, one of the most economical microorganisms used in industries with several uses in foods and beverages [11]. It has been shown that *S. cerevisiae* increases dry matter, neutral detergent fiber (NDF), nutrient digestibility, initial rate of fiber digestion and milk production in dairy cattle and improves ruminal fermentation and various ruminal bacteria [5,11]. Therefore, solid-state fermentation of tomato pomace by *S. cerevisiae* is expected to increase its digestibility in addition to increasing the protein content. Various factors affect SSF performance in tray bioreactors, including dimensions of the bioreactor and trays (length, width and height), bioreactor internal chamber temperature, bioreactor internal humidity, type of aeration or air circulation inside the bioreactor, bed depth of the substrate on the trays and substrate particle size and their initial moisture, as well as the type of substrate enrichment with carbon and nitrogen sources [12,13].

Various solid-state fermentation processes for tomato pomace have been reported, including production of neutral protease enzymes by *Aspergillus oryzae* [14], xylanase and laccase by *Pleurotus ostreatus* and *Trametes Versicolor* [15], *Aspergillus awamori* [16] and enzymatic extraction of lycopene using *Fusarium solani* [17], improving quality and nutritional values using lactic acid bacteria (LAB) and various yeasts, especially *S. cerevisiae* [5]. In none of the studies, affecting parameters on the performance of the tomato pomace solid-culture process have been investigated. However, the only study presented to increase the nutritional value of tomato pomace has investigated effects of various microorganisms on the protein content of tomato pomace in laboratory scale and has increased its value from 14 to 17.89% in the best case. In the current study, the aim was to increase its protein content significantly; hence, it could be used as a further effective food source, using SSF of tomato pomace and cheap industrial yeasts and optimizing conditions in laboratory and bench scales.

Statistical design methods, especially response surface and Taguchi, provide powerful efficient ways to optimize bioprocesses, using decreased number of experiments and simultaneous examination of affecting factors. [18,19]. Taguchi approach can investigate further factors and qualitative parameters. Generally where the cost and time limitations make it difficult to carry out further experiments to process optimization, Taguchi design is preferred in optimization studies in bench-scale bioreactors [20]. Therefore due to the long fermentation time for increasing protein content of tomato pomace in this study, Taguchi method was used to optimize the solid-state fermentation process of tomato pomace with industrial baker yeasts. By analyzing variance of the results via Qulitek-4 Software, effects of parameters and optimum conditions were assessed to achieve further protein contents.

2. Materials and Methods

Substrate preparation

Industrial tomato pomace was provided by ETKA, Tehran, Iran. Before using the substrate in solid-state fermentation, dried tomato pomace was crushed using ordinary electric grain mill (Model ML-320P, Pars Khazar, Rasht, Iran, and then granulated using sieves (Atlas Sieve, Isfahan, Iran) with specific meshes. Sufficient quantities of water or additives were added to the substrate to regulate moisture or enrich tomato pomace. Then, pH of the wet bed was adjusted by the addition of 1 N NaOH (Merck, Germany) and sterilized at 121 °C for 15 min to increase the availability of substrate nutrients.

Microorganisms

Type and method of inoculum preparation depended on the microorganisms. In this study, microorganisms were

industrial yeast strains of *S. boulardii* [DailYeast® capsule containing 250 mg active *S. boulardi*, equivalent to 10^{10} colony-forming unit (CFU) purchased from Zist Takhmir Company, Tehran, Iran] and *Saccaromyces cerevisiae* PTCC 5269 (Razavi Yeast Company, Mashhad, Iran). After counting the yeast cells, yeasts were diluted with distilled water (DW) to achieve 10^5 cells per ml of inoculum. This was equal to 0.01 g per gram of dried tomato pomace. Based on the necessary yeast number of each experiment, a certain volume of inoculum was added to the culture media.

Solid-state cultivation in flask

Briefly, 5 g of the dried and granulated tomato pomace were transferred into a 250-ml Erlenmeyer flask. Then, moisture and pH were adjusted and the sample was sterilized using autoclave. After reaching the optimum temperature, the necessary inoculum was added to the culture media and stirred well using sterile glass rods; thus, the yeasts were distributed uniformly throughout the media and incubated at 37 ℃ for a specified time [5]. Then, fermented tomato pomace was harvested and stored in freezer for further analysis and yeast activity prevention. Biochemical analysis was carried out based on standard procedures.

Solid-state cultivation in tray bioreactor

In this study, a tray bioreactor was designed. This bioreactor included a multi-tray compartment with the ability to adjust distance between the trays, which could operate in aerated and non-aerated ways. Figure 1 shows a simple 50-l reactor consisting of shallow trays with the upper surface of the trays exposed to air. The most characteristic of the tray bioreactor is the fine-tuning ability of all the operational variables in the bioreactor to achieve the maximum protein content of tomato pomace. Then, 100 g of the tomato pomace with three various particle sizes were poured into three 1-l beakers. Due to the optimum moisture content of 70% (w w^{-1}) of the previous step, a sufficient quantity of DW was added to the tomato pomace. Then, 1 N NaOH was added to the wet tomato pomace and stirred for a few minutes to achieve an almost uniform environment. The resulting culture medium was sterilized at 121 °C for 20 min. After autoclaving, the necessary inoculum was added to the culture media under sterile conditions and mixed well to distribute the yeast in the media. The prepared culture medium was poured to a favorite bed height on trays at a favorite distance. Then, the bioreactor vessel was incubated at 37 °C for 5 d. The fermented tomato pomace was harvested and stored in the freezer for further analysis. This step was carried out in aerated (0.5 vvm) and non-aerated conditions with two replications [7].

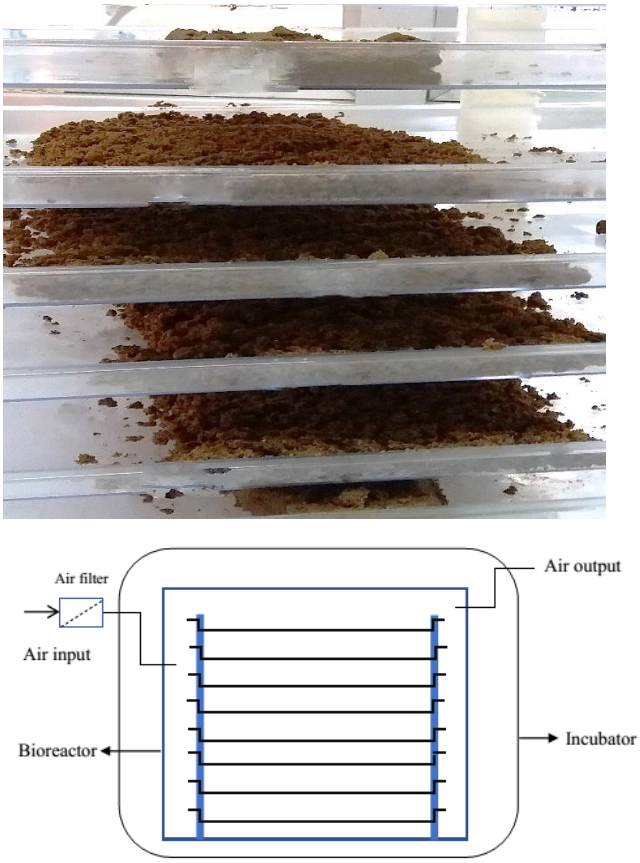

Figure 1. Inside view and schmatic of tray bioreactor for tomato pomace SSF

Analysis

For pH measurement, 5 g of the sample were thoroughly mixed with 50 ml of freshly boiled DW and set to settle. Then, the upper solution pH without smoothing was read using pH meter previously calibrated. The sample moisture content was assessed by the sample weight loss measurement at 105 nm and calculating the weight loss proportion. The sample protein content was assessed by calculating total nitrogen of the sample and multiplying it by 5.7 (approved by plant sources) using Kjeldahl method [21]. Soxhlet method was used to assess the sample fat. Soxhlet system (Novin Pyrex, Tehran, Iran), including extractor, refrigerant and distillation balloon, was dried and weighed. In this method, lipids were extracted continuously using petroleum ether. Fat proportion was assessed via weight loss of the prototype from fat extraction [22]. The proportion of ash weight of the sample was assessed via by completely burning the tomato pomace samples in several steps and assessing the rest of ash weight. Carbohydrate quantity of the tomato pomace sample was assessed by calculating the residual proportion after measuring other components:

Carbohydrate (%) = 100 - moisture (%) - protein (%) - fat (%) - ash (%)

Research methodology

The research was carried out in three stages: 1) selection of further appropriate yeasts by assessing effects of two types of industrial probiotic yeasts and fermentation time in the moisture content of 75% tomato pomace with mesh 30 on the protein content of tomato pomace (Table1,2) determination of optimum conditions for increasing the protein content of tomato pomace by assessing effects of four parameters of the quantity of yeasts, initial moisture content, substrate particle size and cultivation time in three levels (Table 2) based on the Taguchi method in nine experiments in laboratory scale, 3) increase of the protein content of tomato pomace in bench scale tray bioreactor by investigation of three factor substrate depth, distance between the trays and substrate particle size in three levels and one aeration factor in two levels (Table 2) based on Taguchi method. Selection of the levels of the investigated variables in each stage of the research was based on the authors' previous experiences and results of the research linked to the SSF of food industry waste. In deed, results of each stage were effective in choosing type and level of variables for further analyses.

Satistical analysis

After optimizing experiments of Steps 2 and 3, variance analysis of the results (protein content of tomato pomace as target function) was carried out using Qualitek-4 Software (Neutek, Canada). The optimal levels of each factor were assessed by considering the contribution of each factor on the protein content of tomato pomace. Then, the protein content of tomato pomace was verified by experimenting under the suggested optimal conditions.

The noise factor (a lack of protein production) was included in an outer array, which converted results into S/N ratios to select the optimal combination of the variables. The term signal (protein content) indicated the favorite value (the mean response) and noise showed the unfavorite value or standard deviation standard deviation. The highest S/N ratio was the most preferred. In this study, the goal was to further increase of protein content of tomato pomace. Therefore, the optimized characteristic of the response variable was bigger-the-better. The S/N ratios were calculated using the Equation (1) [18, 23]:

$$\frac{S}{N}(W_{CO_2}) = -10\log\left[\frac{1}{n}\sum_{i=1}^{n}\left(\frac{1}{Y_i^2}\right)\right] \quad \text{Eq. 1}$$

Where, Y_i was the response at each run and n was the number of replicates. Orthogonal arrays that made-up only a fraction of full factorial experiments demonstrated self-balancing characteristics. Quantitative characteristics were shown as the mean standard deviation. A p-value of less than 0.05 was considered as statistically significant difference. Experiments were carried out based on these designs at least twice. In analysis of variance, the percentage contribution of each parameter in the total sum of squared deviations included the ratio of the sum squared deviations of each parameter to the total sum of squared deviations and was used to assess importance of the factors on the performance characteristics [23].

Table 1. Experiment conditions and results of the study on the effects of industrial yeast type and fermentation process time on tomato pomace protein content

Factor Number	Yeast	Moisture (ww^{-1})%	Particle size (μm)	Process time (day)	Protein content in dry matter ($w\ w^{-1}$%)
1	*S. boulardii*	75	≤500	3	14.78 ± 0.73
2	*S. cerevisiae*	75	≤500	3	14.41 ± 0.71
3	*S. boulardii*	75	≤500	4	13.50 ± 0.67
4	*S. cerevisiae*	75	≤500	4	15.55 ± 0.78
5	*S. boulardii*	75	≤500	5	14.90 ± 0.75
6	*S. cerevisiae*	75	≤500	5	17.22 ± 0.85

S. boulardii= Saccharomyces. Boulardii, S. cerevisiae= Saccharomyces.

Table 2. Factors and levels addressed to increase the protein content of the tomato pomace solid-state fermented in the flask and bench-scale tray bioreactor

Scale	Factor	Level 1	Level 2	Level 3
Flask	the amount of yeast (g of dry yeast per gram of tomato pomace)	0.01	0.02	0.03
	moisture content ($w\ w^{-1}$)%	70	75	80
	Particle size (μm)	250-500	150-250	≤150
	Process time (day)	4	5	6
bench-scale tray bioreactor	Bed depth (cm)	0.6	0.9	1.2
	Tray distance (cm)	3	4	5
	Particle size (μm)	≤1000	250-500	≤250
	Aeration	no	yes	----

3. Results and Discussion

Setup the fermentation process and selection appropriate industrial probiotic yeasts

Results on the study of the effects of industrial yeast type and fermentation process time on tomato pomace protein content showed that *S. cerevisiae* included the best performance with 5-d fermentation time and therefore used similar yeasts to increase protein content of the tomato pomace (Table 1).

Optimization of the solid-state cultivation in laboratory scale

To increase protein content of the tomato pomace in laboratory scale, four factors of the quantity of yeast, moisture content, particle size of the tomato pomace and process time were investigated simultaneously in three various levels based on the L_9 orthogonal arrays of Taguchi approach. Table 3 shows conditions of the experiments. In all experiments, protein content of the fermented tomato pomace was assessed after the end of each cultivation. Results of the experiments were presented for three levels of variables (Table 3). The highest protein content of the fermented tomato pomace (23.96 g per 100 g of tomato pomace) was achieved in Experiment 7 with the quantity of yeast of 0.03 gram of dry yeast per gram of tomato pomace, substrate moisture level of 70% (w w^{-1}), tomato pomace particle size less than 150 µm and cultivation time of 5 d.

Effects of yeast content on the protein content of tomato pomace

The protein content of *S. cerevisiae* is estimated between 35 and 45% with balanced essential amino acids, vitamins, minerals, carbohydrates and nucleic acids [24]. It has been shown that by decreasing sugar consumption of fruit pomaces, protein and biomass generation significantly increase during the solid-state fermentation with *S. cerevisiae* [24,25]. Figure 2a shows increases in the quantity of yeasts from 0.01 to 0.03 g of dry yeast per gram of tomato pomace have increased protein content. A part of this increase was linked to fermented yeasts and another part was linked to increases in biomass of yeasts due to the consumption of carbohydrates from tomato pomace. The optimal spot in the quantity of yeasts of 0.03 g of dry yeast per gram of tomato pomace was achieved. The steep slope of the increasing protein content after 0.02 g of dry yeast per gram of tomato pomace of the yeasts indicated significant effects of this parameter in fermentation operation. The slight increase in the protein content by increasing the quantity of yeasts from 0.01 to 0.02 was mostly due to the increases in the quantity of the added yeast protein. By further increases in the quantity of added yeasts, available sugars and carbohydrates of the tomato pulps were fermented by the yeasts and with further increases in the biomass, the protein content increased significantly. Increases in the quantity of primary yeasts did not include much effects on increases in the protein content of tomato pomace due to the limitation of available sugars and carbohydrates. Similar results have been reported by other researchers, investigating effects of the quantity of yeasts on increasing the protein content of various wastes [25-27].

Effects of moisture on the protein content of tomato pomace

Moisture content is one of the critical factors in SSF, which is associated to the biosynthesis and secretion of enzymes, attributing to the interference of humidity in physical characteristics of solid particles. In general, the moisture level of solid-bed fermentation processes varies between 30 and 85% and includes significant effects on the growth kinetics of the fermentative microorganisms [25,28]. High substrate moisture levels lead to decreased porosity, less oxygen release, increased risk of bacterial contamination, increased formation of aerial mycelia, decreased gas volume and exchange and changes in lignin decomposition. Similarly, low moisture levels may result in decreased nutrient solubility of the solid substrates, lower swelling degrees and higher water drag. Therefore, initial moisture plays important roles in microbial growth and activity during SSF. Most microbial growth and product formation occur at or near the surface of the solid substrate; therefore, it is critical to provide optimal water levels that control the water activity of the fermentation substrate to achieve the maximum yield [7,27]. In addition, chemical composition of the solid substrates shows its ability to maintain sufficient water resources to support growth [29].

Table 3. Results of the optimization experiments of solid-state fermentation for protein content increase of tomato pomace in laboratory scale based on the orthogonal array of L_9

Factor Number	amount of yeast (g.g^{-1} of tomato pomace)	Moisture (w w^{-1})%	Particle size (µm)	Process time (day)	Protein content without moisture%
1	0.01	70	250-500	4	13.11 ± 0.63
2	0.01	75	150-250	5	17.26 ± 0.84
3	0.01	80	≤150	6	21.75 ± 0.95
4	0.02	70	150-250	6	18.30 ± 0.89
5	0.02	75	≤150	4	19.01 ± 0.91
6	0.02	80	250-500	5	14.91 ± 0.71
7	0.03	70	≤150	5	23.96 ± 1.03
8	0.03	75	250-500	6	16.25 ± 0.77
9	0.03	80	150-250	4	17.64 ± 0.85

Figure 2b demonstrates the average effect of tomato pomace moisture content in increasing protein contents. Based on the findings, the highest protein content of tomato pomace was achieved from 70% moisture content of the substrate. It was observed that by increases in the volume of added water, quantity of the crude protein decreased. Based on the literature, high moisture contents lead to low substrate porosity, which then prevents oxygen penetration. However, low moisture contents may lead to poor nutrient availability and thus delay microbial growth [26,27].

Effects of substrate particle size on the protein content of tomato pomace

Particle size is one of the most important physical parameters in SSF. Particle size distribution affects surface-to-volume ratio of the particles that are initially available to the microorganisms as well as packing density in the surface mass [7]. The interparticle space is occupied by a continuous gas phase [7, 28] and size of the substrate particles controls the pore space occupied by air. This space helps gas exchange as well as heat and mass transfer between the particles. Since the rate of O_2 transport into the pore space affects the microbial growth, the substrate must contain particles of the appropriate size to increase mass transfer [28, 30]. In general, smaller substrate particle sizes provide larger surfaces for the microbial attack and is therefore a favorite factor [31]. However, use of very small substrate particles may lead to substrate aggregation, which may interfere with microbial respiration/aeration, resulting in poor microbial growth. In contrast, larger particles provide better breathing/aeration efficiency due to increased spaces between the particles. However, they provide a limited surface for the microbial attack. This needs compromised particle sizes for specific processes as well as a specific substrates [28,31].

In this study, particle sizes of 250-500, 150-500 and less than 150 µm were used to investigate effects of substrate particle size on the protein content of tomato pomace fermented by SSF. Figure 2c shows the highest protein content of the fermented tomato pomace achieved when using tomato pomace with a particle size of less than 150 µm. Smaller particle sizes provide further surface areas for each volume and allows further contacts of microorganisms with nutrients; however, the oxygen emission is affected. Larger particle sizes provide small surface areas to volume ratio and cause excellent oxygen diffusion; however, microorganism contacts with nutrients decrease. Appropriate particle size must meet mycelial growth and demand for oxygen and nutrients [7]. In a study, Azabou et al. [16] reported maximum lycopene extraction from tomato processing by solid-state fermentation of *Fusarium solani* pisi with particle sizes of 0.8-1.25 mm.

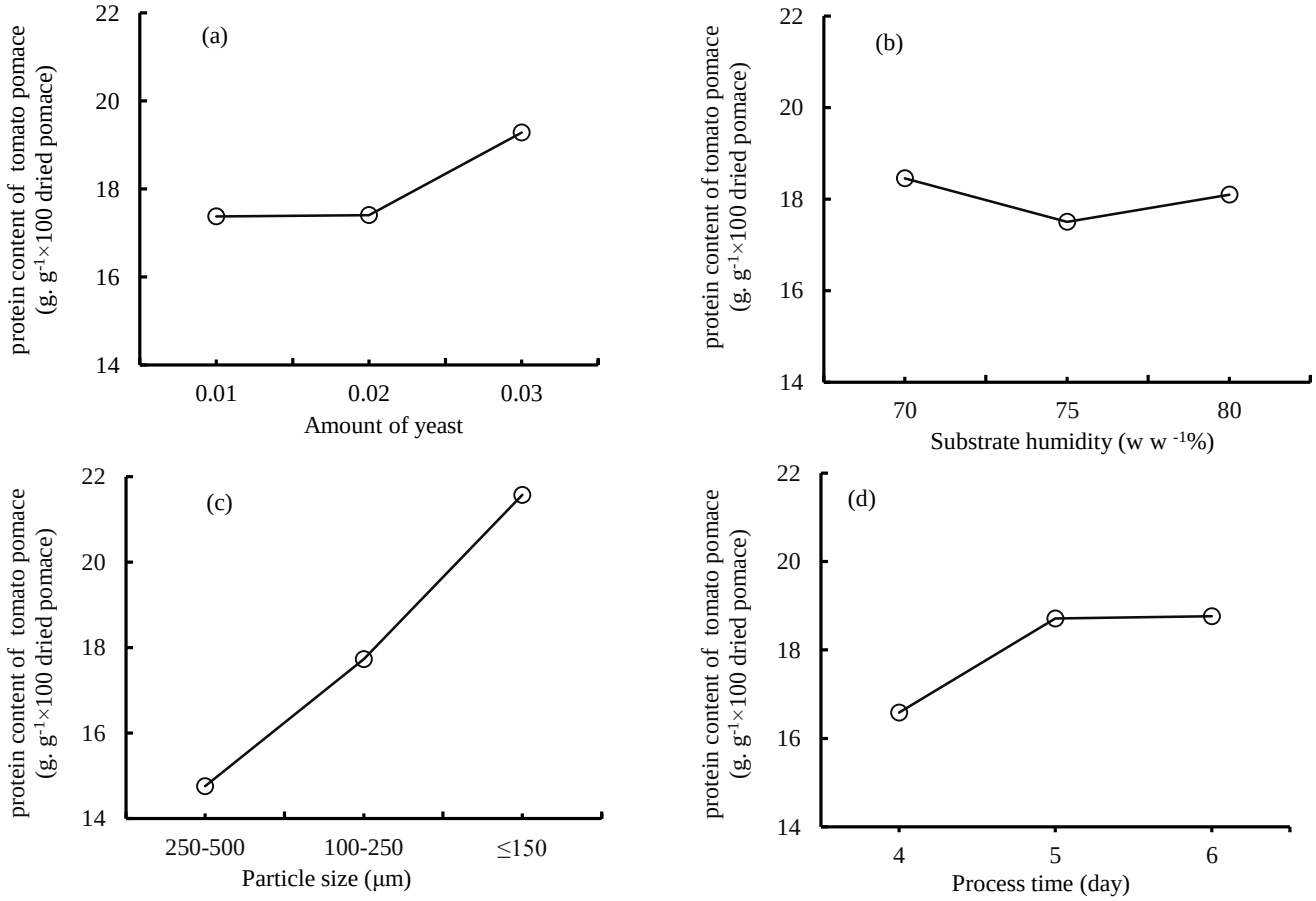

Figure 2. The average effects of yeast content (a), subtrate humidity (b), particle size (c) and process time (d) on the protein content enhancement of tomato pomace in laboratory-scale solid-state fermentation

Effects of fermentation time on the protein content of tomato pomace

Fermentation time needed to achieve the maximum biomass and subsequently the maximum protein content of the pomace depends on all the factors that affect growth rate of the microorganisms in SSF. By changing these factors, including type and size of the substrate particles, quantity of moisture, type and quantity of the microorganisms and other process conditions that affect the growth kinetics, fermentation time needed to reach the highest productivity should be optimized [29,32,33]. Based on the results from the first stage of this study and by changing the particle size and humidity of the substrate and the quantity of inoculum, effects of fermentation time in three quantities of 4, 5 and 6 d on protein content of the fermented tomato pomace with solid state culture were investigated. Figure 2d shows the average effect of time on increases in the protein content. It was observed that by increasing the fermentation time from 4 to 5 d, protein content of the fermented tomato pomace increased; however, no significant changes were observed with further increases in the fermentation time. Based on the findings, the highest protein content of the fermented tomato pomace. Roja et al. [5] achieved the highest protein content after 6 d of fermentation with *S. boulardii*, which is 24 h longer than the time in this study to increase the protein content of tomato pomace via solid-state cultivation.

After experiments based on orthogonal arrays of the Taguchi statistical method, analysis of variance was carried out using Qualitek-4 Software to specify contribution of each factor in protein content increase of the fermented tomato pomace, determine the optimal values of factors and predict protein content of the fermented tomato pomace under the optimal conditions. Table S1 shows the achieved parameters from ANOVA of the results. Moreover, Table S1 demonstrates that all the four factors are involved in increasing the nutritional value of tomato pomace; however, the particle size factor includes the most share. A calculated error of zero shows good accuracy of the experiments. Table 4 shows the optimal conditions and contribution of each factor. Protein content of the fermented tomato pomace under optimal conditions was 24.15% (by weight), which is nearly 70% more than the initial protein quantity.

Table 5 demonstrates results of the experimental design analysis on the optimal sample, comparing with the results from a study by Roja et al. [5]; in which, the quantity of protein increased significantly. In Roja's study, only effects of the type of microorganisms on protein content of the fermented tomato pomace in SSF were investigated and no optimization of fermentation conditions was carried out. This study showed how much optimization of SSF conditions could be effective in increasing the yield of solid-state culture. Results of the optimized protein proportion, compared to the value of primary tomato pomace protein of 14.21%, showing that the protein content increased by more than 74% due to fermentation. Moreover, protein content approved in optimum conditions was higher than the optimal value predicted by the software.

Optimization of the tomato pomace solid-state fermentation in bench scale

The tray bioreactor is the most widely used bioreactor for SSF. Tray bioreactors are the oldest and the simplest systems used under static conditions without mechanical stirring and forced aeration on solid beds. Thickness of the substrate on the tray is the major limiting parameter [12,34]. This type of system only holds a limited quantity of the solid substrate for fermentation because only thin layers should be used to avoid overheating and maintain aerobic conditions. It was detected that bed thickness, surface area and temperature of the chamber included positive effects on metabolic activity and could improve metabolic heat and gas transfer. Usually, trays are located in the incubation room, where the temperature and humidity are controlled for the optimal growth. Trays are arranged one on the top of the other with appropriate distances between them. Since usually no forced aeration is available in the environment, mass and heat transfer occur via diffusion and natural convection [27,34]. The substrate particle size can be effective on density and porosity of the substrate and hence oxygenation and metabolic activity of the microorganisms due to its immobilization on the trays [12,29]. Therefore, it is necessary to reassess its effects on increasing protein content of the tomato pomace based on its physicochemical characteristics and moisture content.

Table 4. Optimal conditions and contribution of each agent to increase protein content of the tomato pomace in laboratory-scale solid-state fermentation

Factor	Level desciption	Level	Contribution%
Amount of yeast (g of dry yeast/ g of dry tomato pomace)	0.03	3	1.262
Moisture content ($w.w^{-1}$)%	70	1	0.435
Particle size (μm)	≤150	3	3.552
Process time (day)	5	3	0.745

Table 5. Comparison of the results from this study achieved under ptimal conditions for the protein content increase of tomato pomace with those from other studies

Factor	Optimal sample	The research of Roja et al. [5]
Protein content (w w^{-1})%	24.72	17.78
Fat (w w^{-1})%	3.29	8.34
Ash (w w^{-1})%	16.45	3.05
Carbohydrate (w w^{-1})%	55.52	30.51

Results of the experiments carried out in the flask showed that the particle size of tomato pomace was the most effective factor in increasing protein content of the fermented tomato pomace. Additionally, fine particle sizes are further costly and time-consuming in operational preparations. Hence, it is preferable to use large particles to decrease costs if higher protein contents are achieved. Moreover, increasing the bed depth in tray bioreactors leads to further compression of the bed, decreasing its porosity and increasing the limit of mass transfer in the bed. Scale-up of SSF is often associated to higher metabolic heat production, higher bed density and lower oxygen mass transfer. Therefore, investigation of the effects of aeration on the tray bioreactor performance is essential.

In this step, effects of various operating conditions of the built tray bioreactor, including aeration of the solid culture media, height of the bed and distance between the trays, on protein content of the fermented tomato pomace in solid-state cultures were investigated under the optimal conditions from the previous step. To increase protein content of the tomato pomace in bench scale, effects of three factors of bed depth, tray distance and substrate particles size in three levels and aeration in two levels (Table 3) on the quantity of protein were investigated. Experiments were carried out based on Taguchi's statistical design method and L_9 orthogonal array using 50-l tray bioreactor in two modes without aeration (six runs) and with aeration (three runs). At the end of each experiment, fermented tomato pomace was harvested and its protein content was assessed. Table 6 shows the 9-test conditions with protein content of the fermented tomato pomace with two replications.

Effects of bed depth

Solid bed height or bed depth is one of the factors affecting the performance of SSF processes, especially in the tray and packed bed bioreactors and upper scales [29,34]. In fact, its effects can be various depending on the humidity and particle size of the substrate, adhesion degree of the substrate particles to each other due to the presence of free sugars (majorly in pulps of sweet fruits) and type of bioreactors. Hence, effects of solid-bed height on the SSF performance should be investigated and optimized for each substrate with each microorganism [6,10,13,27,29]. In this study, effects of bed height in three values of 0.6, 0.9 and 1.2 cm on protein content of the fermented tomato pomace in SSF were investigated. The value of 0.6 cm was equivalent to the height of the bed in the flask stage, which increased up to two times with the increase of the bed surface in the tray bioreactor.

Graph slope of the average effect of bed height changes on protein content of the fermented tomato pomace in SSF showed that in the studied range, bed height did not include significant effects on increasing the protein content of tomato pomace (Figure 3a) possibly because physicochemical characteristics and low stickiness of the tomato pomace particles caused a little compaction due to increases in the bed height. Additionally, increasing surface of the bed in the trays provided possibility of the microorganism access to oxygen despite increases in height of the bed. However, with increases in solid-bed height, useful space of the bioreactor increases and production costs decrease. However, large increases in the bed height cause thickened bed and decrease penetration of oxygen into the bed, resulting in decreased growth of the microorganisms and decreased maximum accessable protein contents.

However, with increases in height of the solid bed, useful working volume of the bioreactor increases and the production cost decreases. In deed, large increases in the bed height cause thickened beds and decrease penetration of oxygen into the beds; thus, decreasing the growth of microorganisms and the maximum achievable protein content of the fermented tomato pomace in SSF. This study completed data linked to increased scale of the effects of bed height at higher values, as well investigating effects of the ratio of height-to-bed area on the protein content of the tomato pomace.

Effects of distance between the trays

Distance between the trays is one of the parameters affecting performance of the tray bioreactors in solid state cultures because it can affect useful workload of the bioreactors and the air circulation inside the bioreactors. In fact, it is necessary to assess effects of this factor with height of the bed at the same time, as carried out in the present study [27,29,34]. Based on the dimensions and limitations of the current tray bioreactor, effects of the distance between the trays in three values of 3, 4 and 5 cm on protein content of the fermented tomato pomace was investigated in the present study. In this study, distance between the trays included no significant effects on the protein content enhancement of the fermented tomato pomace and mildly increased by increases in distance between the trays (Figure 3b). By further increasing the height of the bed and decreasing the actual distance between the solid beds, effects of the distance between the trays are better and further accurately observed, which can be investigated in further studies. By decreasing distance between the trays, number of the trays and hence performance of the bioreactor could significantly increase. Based on the experiments, a distance of 3 cm between the trays (the minimum achievable distance in the bioreactor) was reported as the optimal value.

Table 6. Results of the optimization experiments carried out using solid-state fermentation for the protein content increase of tomato pomace in bench-scale tray bioreactor with two replications based on orthogonal array L_9

Number / Factor	Bed depth (cm)	Tray distance (cm)	Particle size (μm)	Aeration	Protein content (1) (w.w^{-1})%	Protein content (2) (w.w^{-1})%
1	0.6	3	≤1000	no	15.66	15.33
2	0.6	4	250-500	yes	14.51	15.20
3	0.6	5	≤250	no	17.69	21.51
4	0.9	3	250-500	no	14.23	15.39
5	0.9	4	≤250	no	19.29	17.26
6	0.9	5	≤1000	yes	14.23	14.54
7	1.2	3	≤250	yes	17.75	18.90
8	1.2	4	≤1000	no	15.39	17.26
9	1.2	5	250-500	no	15.55	15.50

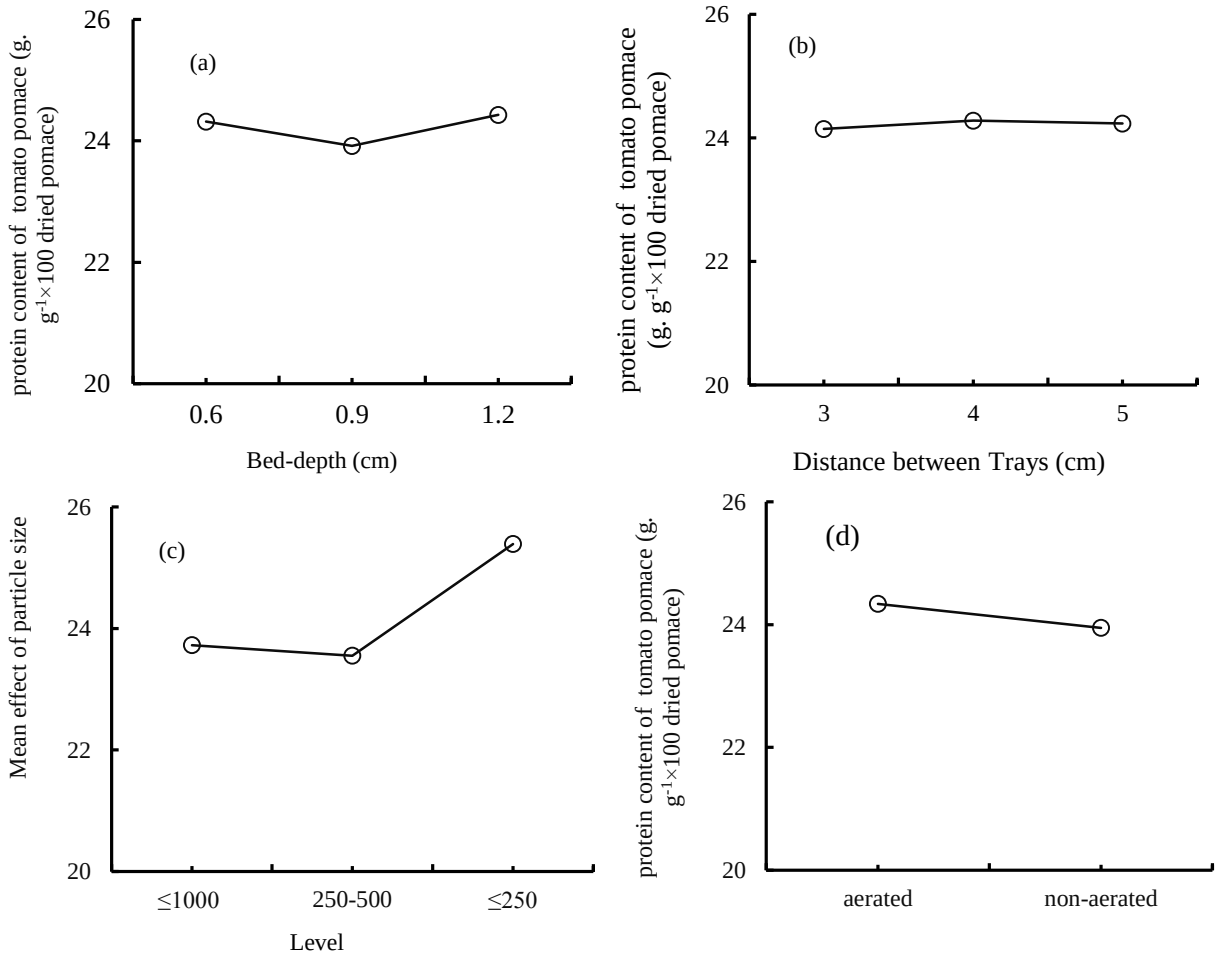

Figure 3. The average effects of bed depth (a), distance between trays (b), particle size (c) and aeration (d) on increased protein content of the tomato pomace using solid-state fermentation in bench-scale tray bioreactor

Effects of the substrate particle size

In the previous section, it was shown that the size of the particles included great effects on increasing protein content of the tomato pomace fermented with SSF due to its good effects on the substrate compaction and subsequent oxygenation of the microorganisms as well as availability of its fermentable compounds. It was expected that by increasing the scales and basically increasing height of the bed, its effects were further expressed and the optimal particle size in the bioreactor was various from the flask. Therefore, effects of the size of larger particles from tomato pomace in three values of less than 250, 500-250 and less than 1000 μm (grind and non-sized particles of the tomato pomace) on its protein content were investigated. Figure 3c demonstrates that the particle size includes great effects on increasing protein content of the fermented tomato pomace. A particle size of less than 250 μm (60 mesh) significantly increases availability of the microorganisms to the substrate nutritional components.

Effects of aeration

Aeration basically includes two functions: 1) providing oxygen for the aerobic metabolism and 2) removing CO_2, heat, water vapor and volatile components produced during the metabolism [35]. In general, gas diffusion increases with pore size and decreases with decreasing particle diameter due to substrate packing [36,37]. In tray bioreactor systems, gas transfer is diffusion limited, causing major problems in heat and mass transfers [38]. Therefore, SSF process assessments were carried out in the tray bioreactor in two modes of aerated and non-aerated. It was seen that aeration in the studied bioreactor included no significant effects on protein content of the tomato pomace (Fig.3d). Lack of aeration effects despite the aerobic nature of bakery yeasts might be due to the existence of sufficient free space containing air inside the tray bioreactor or low aeration speed. In fact, carrying out the SSF process at higher bed heights and decreasing distances between the bioreactor trays and subsequently increasing the oxygen demand, increasing metabolic activity and producing high CO_2 gas, effects and needs of of aeration are further specified, which can be investigated in further studies.

Contribution of each factor in protein content increase of the fermented tomato pomace, the optimal values of the factors and prediction of protein content of the fermented tomato pomace under the optimal conditions were specified using ANOVA and Qualitek-4 Software. Table S2 shows the achieved parameters from variance analysis. Moreover, only two factors of the bed depth of particle size of the tomato pomace involved in increasing nutritional value of the tomato pomace and aeration and distance between the tray did not include significant effects. Furthermore, the particle size factor included the most contribution. A low calculated error showed good accuracy of the experiments (Table S2). Table 7 shows the optimal conditions and contribution of each factor. All experiments were analyzed using Qualitek-4 Software based on S/N analysis. Prediction of the expected results in optimum conditions by the software included 25.792% (w w^{-1}).

Results of the experimental design analysis of the optimum sample in tray bioreactor showed the highest protein content of 25.82% (w w^{-1}) with fat content of 3.23% (w w^{-1}), ash content of 16.19% (w w^{-1}) and carbohydrate content of 54.75%. The quantity of protein content of the fermented tomato pomace under optimal conditions in the fermenter indicated that the quantity of protein increased by more than 80% via fermentation, compared to protein content of the original tomato pomace (14.21% w w^{-1}). Additionally, quantity of the protein approved under optimal conditions was higher than the optimal quantity predicted by the software. Table 8 shows results of protein content from flask and bioreactor decomposition, comparing to other studies. It can be seen that despite scale-up of the SSF process, optimizing conditions of the tomato pulp in the tray bioreactor not only did not decrease quantity of the production compared to the flask but also increased by nearly 5%. It is expected that by investigating effects of various additives, especially carbon and nitrogen sources, as well as carrying out the SSF process with higher bed heights and shorter distances between the trays with aeration while further increasing content of the fermented tomato pulp and decreasing production costs, accurate and more complete data can be achieved to scale-up this process.

4. Conclusion

In this study, nutritional values of the tomato pomace significantly increased at low costs, using inexpensive and accessible industrial microorganisms and SSF process. Based on the analysis of the results, protein content of the tomato pomace achieved under optimum conditions of its SSF by industrial bakery yeasts in bench-scale tray bioreactor is the highest protein content ever reported for the agricultural and food wastes fermented via solid-state cultivation. This study has converted an environmental waste problem to a valuable product using the simplest technology at the lowest cost while significantly increasing the nutritional value of tomato pomace. However, it is necessary to further study scale-up parameters of the SSF developed in this study at higher scales to achieve necessary information for its industrialization. In addition, strategy used in the present study can be used to increase the nutritional values of solid wastes in food and agricultural industries. In deed, it is necessary to optimize the SSF conditions of each substance based on the composition of the desired substance and industrial probiotic microorganisms used in various scales.

Table 7. Optimal conditions and contribution of each agent to increase the protein content in bioreactor

Factor	Level desciption	Level	Contribution%
Bed depth (cm)	1.2	3	0.207
Tray distance (cm)	4	2	0.060
Particle size (mesh)	60	3	1.168
Aeration	no	1	0.137

Table 8. Results of protein contents from the Erlenmeyer flask and bioreactor analyses compared to those from other studies

Substrate	Fermentation conditions	Protein content%	References
Tomato pomace	Bread Yeast, 6 days	17.78	2017 [5]
Cassava waste	*Aspergillus niger*, 8 days	22.61	2018 [24]
Tomato pomace (Erlen)	Bread Yeast, 5 days	24.72	Recent study
Tomato pomace (bioreactor)	Bread Yeast, 5 days	25.82	Recent study

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This study did not receive any specific grants.

7. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that can affect the current study.

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افزایش محتوای پروتئین تفاله گوجه فرنگی در تخمیر حالت جامد با مخمر نانوایی صنعتی

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چکیده

سابقه و هدف: تفاله گوجه فرنگی به عنوان ضایعات اصلی رب گوجه فرنگی از نظر خواص تغذیه‌ای و فناوری قابل استفاده در فرمولاسیون غذا است. هدف از این تحقیق توسعه‌ی روشی ارزان و ساده برای افزایش محتوای پروتئین تفاله گوجه فرنگی است که بتواند به عنوان یک منبع غذایی ارزان و کارآمدتر برای دام و طیور مورد استفاده قرار گیرد.

مواد و روش ها: پژوهش در سه مرحله انجام شد: ۱) انتخاب مخمر مناسب‌تر با بررسی تأثیر دو نوع مخمر صنعتی *ساکارومایسس سرویزیه* و *ساکارومایسس بولاردی* و زمان تخمیر بر روی تفاله گوجه‌فرنگی با رطوبت ۷۵ درصد و اندازه ذرات کمتر از ۵۰۰ میکرومتر بر میزان پروتئین تفاله گوجه فرنگی، ۲) تعیین شرایط بهینه برای افزایش محتوای پروتئین تفاله گوجه فرنگی با بررسی تأثیر چهار پارامتر مقدار مخمر، رطوبت اولیه، اندازه ذرات سوبسترا و زمان کشت در سه سطح، بر اساس روش تاگوچی در ۹ آزمایش در مقیاس آزمایشگاهی، ۳) افزایش محتوای پروتئین تفاله گوجه فرنگی در بیوراکتور سینی در مقیاس آزمایشگاهی با بررسی ۳ عامل عمق بستر، فاصله بین سینی ها و اندازه ذرات سوبسترا در سه سطح و یک غامل هوادهی در دو سطح بر اساس روش تاگوچی.

یافته‌ها و نتیجه‌گیری: *ساکارومایسس سرویزیه* و زمان کشت ۵ روزه برای ادامه تحقیق انتخاب شد. در شرایط بهینه در مقیاس آزمایشگاهی، ۰/۰۳ مخمر (گرم مخمر خشک/گرم تفاله گوجه فرنگی)، رطوبت ۷۰ درصد (وزنی/وزنی)، اندازه ذرات کمتر از ۱۵۰-۲۵۰ میکرومتر (شماره الک ۱۰۰) و فرآیند در زمان ۵ روز، محتوای پروتئین ۲۴/۷۲ درصد با محتوای چربی ۳۲/۹ درصد، خاکستر ۱۶/۴۵ درصد و کربوهیدرات ۵۵/۵۲ درصد (وزنی/وزنی) به دست آمد. در شرایط بهینه بیوراکتور سینی‌دار شامل عمق سوبسترا معادل ۱/۲ سانتی‌متر، فاصله سینی ۴ سانتی‌متر، اندازه ذرات ۲۵۰-۵۰۰ میکرومتر (شماره الک ۶۰) و بدون هوادهی، حداکثر محتوای پروتئین ۲۵/۸۲ درصد وزنی-وزنی به دست آمد که در مقایسه با محتوای پروتئین تفاله گوجه فرنگی اولیه (۱۴/۲۱ w/w%) بیش از ۸۰ درصد بود. این بالاترین میزان پروتئینی است که تاکنون برای تفاله گوجه فرنگی با استفاده از ساده‌ترین فناوری با کمترین هزینه گزارش شده است.

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

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واژگان کلیدی

- تفاله گوجه فرنگی
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- روش تاگوچی
- بیوراکتورهای سینی
- غنی سازی پروتئین

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