# Simulation of Fermented Wheat Germ Extract Production with High Content of 2,6-dimethoxybenzoquinone by Industrial Bakery Yeast

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**ABSTRACT:** *The anti-cancer properties of fermented wheat germ extract (FWGE) have been proven due to the active ingredient benzoquinones, especially 2, 6-dimethoxybenzoquinone (2, 6-DMBQ). In this research, the development of the FWGE production process with a higher content of 2, 6-DMBQ with respect to the bench-scale was considered and simulated by SuperPro Designer (SPD) software. To evaluate the effect of wheat germ granulation, the fermentation process was performed in 250 mL and 2-liter shake flasks and 3.6- and 13-liter bioreactors. Then, the possibility of yeast complete separation in the final product was investigated by combining centrifugation in 3000 g and pressure filtration with linen, polyester, polypropylene, and cellulose membranes. A comparison of the dryer type effect including oven, spray dryer, freeze dryer, and rotary vacuum dryer in terms of drying time and final moisture content of FWGE showed that the spray dryer gives the product with the least humidity 5 (w/w)% in the lowest time 15 min. Examination of the effect of granulation also showed that at higher scales, non-granulated wheat germ produces more 2,6-DMBQ. Yeast complete removal from the final product was achieved using initial centrifugation at 3000 g and then a filter press with a combination of polypropylene membranes 8-10 µm, polyester 5 µm, and a polymer membrane 1-2 µm. Finally, the production process on scales of 10, 100, and 1000 liters was simulated by SPD software. Based on investment cost, the Return Rate of Investment (ROI) equaled 4, 1.9, and 0.4 years on scales of 10, 100, and 1000 liters, respectively. These results showed that the scale-up of the FWGE production process significantly decreases the ROI and can be considered a high-value-added production line at higher scales.*

**KEYWORDS:** *Fermented wheat germ extract; 2, 6-dimethoxybenzoquinone; Industrial bakery yeast; Isolation and purification; Simulation.*

## INTRODUCTION

Wheat germ is a by-product of the wheat milling process, which accounts for 2.5 to 3.8% of the total weight of wheat grain. Wheat germ has been mentioned by many nutritionists as a useful natural food due to its abundance of proteins, fats, vitamins, and other nutrients [1,2]. Fermented Wheat Germ Extract (FWGE), due to its effective materials such as 2-Methoxy benzoquinone (2-MBQ) and 2,6-Dimethoxybenzoquinone (2,6-DMBQ), is known as an anticancer material that increases the immune system performance and autoimmune disorder treatment. It is known as Avemar globally and is also produced with different commercial brands [3-5].

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*1021-9986/2023/4/1305-1313 9/$/5.09*

Extensive studies have been reported on the production and effectiveness of FWGE most of which have been performed on a lab scale to optimize effective parameters for the production increase of effective substances 2-MBQ and 2,6-DMBQ [6-8]. However, no research was done on the economics of the production process, and the Hidvegi report on an industrial scale has been the primary basis of all studies, that production of 0.4 mg 2,6-DMBQ/g of dried FWGE has been reported [9]. It also makes no mention of the production process economically.

It is essential to understand the process of economics before commercialization [10]. Accordingly, all effective parameters of the economy of the process must be considered, and the total capital cost, including fixed and working capital costs, must be estimated. If the estimated costs are more detailed, the error percentage of process commercialization reduces [11]. Effective parameters in process economics have been reported in Peters' book, "Plant Design and Economics for Chemical Engineers" [12]. One of the most critical levels of cost estimation of a process is to simulate the specific production process according to reported parameters [13]. Simulation of industrial units using different software to improve production conditions, processing materials, and various products has been beneficial. It can also be used to optimize these products effectively as much as possible. Of course, obtaining the optimal process conditions eventually leads to higher quality products, lower production costs, and saved time.

According to the production process, the required raw materials and heavy and light equipment types of the process are obtained to estimate the yield of the production process by simulation [14]. One of the most widely used software in biological process simulation is SuperPro Designer software. The data obtained from the software includes the schematic of the whole process, the amount of the entire produced products according to defined reactions, economic estimates (constant capital, circulating, and capital return rate …), and the environmental effects of the process. [15,16].

*Parsazad et al*. have reported the production optimization of 2,6- DMBQ using wheat germ fermentation on a laboratory scale. In this study, an industrial baker yeast strain was used, and after optimization, 2.58 mg/g of 2,6-DMBQ was obtained [17]. Recently, *Hassani et al*. have reported the 2,6-DMBQ production enhancement on a bench scale. They used the Taguchi experimental design to optimize the fermentation conditions, which included three variables initial pH, fermentation temperature, and stirring speed in the 3.6-liter bioreactor. Under optimal conditions, pH of 6, fermentation temperature of 32 °C, and agitation rate of 80 rpm, a maximum of 1.527 mg/g of 2,6-DMBQ was produced [18].

No study has been reported on the simulation of the FWGE production process so far. The purpose of this research was to the economical evaluation of the FWGE production process based on our previous and recent studies. In this research, first, the production process was performed at a higher scale to verify 2,6-DMBQ production at the reported scales. Then the fermentation process was carried out in the lab and bench scales to investigate the scale-up effect on the 2,6-DMBQ content of FWGE. Then, by combining centrifugation and pressure filtration with different membranes, a suitable process was developed for the complete separation of yeast in the final product. Then the effect of the dryer type on FWGE drying was compared in terms of FWGE final humidity and drying time, and an economical and optimal drying process was chosen. Then, based on recent results on the bench scale, for a feasibility study of FWGE industrial production, the production process was simulated on three different scales by SuperPro Designer.

## EXPERIMENTAL SECTION

### *Materials*

The wheat germ was purchased from Ardfred Takestan Company, industrial yeast powder from Razavi Company (*S.cerevisiae* ATCC 24909), 2,6-DMBQ from Sigma Aldrich, chloroform from Merck and maltodextrin from Jahan Shimi Company. Linen and polymer membranes (polyester and polypropylene) and cellulose membranes have been purchased from Safi Aran Company. Fermentation was carried out in 3.6-liter and 13-liter fermenters (INFORS AG Bottmingen CH-4103). Yeast and other solids were separated from FWGE by centrifugation (SIGMA 3K30) and filter press composition. An optical microscope (Moticam 3000) and a Neubauer counting chamber were used for cell counting. To select a suitable dryer, the yeast-free FWGE was dried using a vacuum freeze dryer (ALPHA 2-4 CHRIST), a vacuum evaporator (Buchi), an oven (Behdad), and a spray dryer (Buchi B-290). 2,6-DMBQ analysis was performed

by HPLC (Knauer) according to Peters [12]. Simulation and economic analysis of the production process at scales of 10, 100, and 1000 liters were performed by SuperPro Designer software [12].

***Analysis***

*2,6-DMBQ analysis*

The 2,6-DMBQ content of FWGE was analyzed based on the report of *Rizzello et al*. [6]. 0.5 g of the dried FWGE sample was dissolved in 50 mL of distilled water completely. In 3 steps, 25 mL of chloroform was added to the solution each time, the solution shaken for 30 minutes, and then kept for 30 minutes for separation of the two phases. In 3 stages, each time 25 mL of chloroform was added to the solution, the solution shaken for 30 minutes, and then the solution was kept for 30 minutes for separation of the two phases. At the end of the third stage, the separated phase was concentrated to 5 to 10 mL at 30°C in the oven. Afterward, 20 µl of the concentrated solution was injected into the HPLC BDS Hypersil C18 column (5 µm×250 mm×4.6 mm), and related plots were extracted.

*Cell counts*

First, Neubauer lam and lamel were cleaned. Then, after each separation process, a sample was injected into Neubauer's chamber and lamel placed on it. The cells were counted under a light microscope in 1000x magnification. The number of available yeasts was counted for every 10 squares of Neubauer's chamber.

***Research methods***

*Launching the production process in a laboratory scale*

Fermentation was carried out in two flasks of 250 mL and 2 liters containing 3 (w/v)% of the wheat germ of 60 mesh particle size and 6 (w/v)% industrial baker yeast at 32°C and 150 rpm for 18 hours. At the end of the process, the samples were centrifuged at 3000 g to remove the yeast. Subsequently, the 2,6-DMBQ content of samples was analyzed by HPLC.

*Granulation effect on 2,6-DMBQ production*

The effect of wheat germ granulation on 2,6-DMBQ production was evaluated in a 3.6-liter bioreactor with a working volume of 2-liter in two different granulation conditions, one with a particle size of 60 mesh and another one without granulation. According to the obtained results, the 2,6-DMBQ production process was assessed on a larger scale in a 13-liters bench-scale bioreactor with a working volume of 10 liters. These experiments were carried out at 32°C and 80 rpm for 18 hours.

***Fig. 1: filter Press system, including transfer pump, storage tank, and membrane holder***

*The separation of baker's yeast from FWGE*

At the end of the fermentation process, the yeasts must be removed from the FWGE. Hence, yeasts were counted using a light microscope (Moticam 3000) and a Neubauer lam. According to the current standard in report of Mate Hidvegis, the number of yeasts in the Neubauer lam should be a maximum of 1 per 10 squares. According to *Hassani et al*. [18], a centrifuge cannot completely remove yeast from the culture medium. Hence, by fermented culture medium was first centrifuged at 3000 g and then it was filtered by a filter press. In the filter press system, the various parameters such as pressure drop, filtration rate, yeast separation rate, hydrophilicity, and hydrophobicity membranes are usually investigated. Accordingly, the various membranes include a linen membrane with a pore size of 20 µm, a polyester membrane with a pore size of 10 to 15 µm, a polypropylene membrane with a pore size of 8 to 10 µm, and a cellulose membrane with a pore size of 1, 2, and 8 µm, were used in this study. In the following, highly performant membranes were used together.

*Investigating the method and the time of the final product's drying*

Four different dryers were investigated in this study to evaluate their drying time, sample shape after drying, and sample moisture content. These four dryers include a vacuum freeze-dryer at -70°C, an oven at 70°C, a vacuum evaporator at 60°C, and a spray dryer with the inlet and

***Table 1: Amount of materials used in 10, 100, and 1000-liter fermenters***

| Fermenter Volume (lit) / Raw materials | 10 | 100 | 1000 |
|---|---|---|---|
| Wheat Germ (g) | 0.6 | 6.0 | 60.0 |
| Industrial yeast powder (g) | 0.3 | 3.0 | 30.0 |
| Drinking water (L) | 11.0 | 101.0 | 1013.0 |
| Sunflower oil (L) | 0.1 | 1.0 | 10.0 |
| Maltodextrin (g) | 0.164 | 1.64 | 16.4 |
| NaOH(g) | 0.832 | 8.32 | 83.2 |

outlet air at 120℃ and 90℃, respectively. In more detail, 100 mL sample was used for each dryer.

*Simulation of FWGE production process*

Simulation and economic analysis of the FWGE production process were performed using SPD software. Fig. 2 is a general schematic of the process performed and Table 1 is the type and amount of raw materials used in the FWGE production process simulation. In this simulation, the experimental results obtained in a bioreactor with a working volume of 10 liters were also used. Then, according to the cost of raw materials and equipment required, the total capital cost of the FWGE production line at this scale was calculated. The total capital cost of set-up the production line was calculated according to fixed and working capital costs. The return of investment (ROI) was calculated according to the selling price. To economic evaluation, the production process on a larger scale, simulations, and cost estimations were performed at scales of 100 and 1000 liters. Sunflower oil as an antifoam agent, maltodextrin as an agent that cannot absorb water and prevent stickiness FWGE to the dryer, NaOH, and a disinfectant agent were used in the system.

## RESULT AND DISCUSSION

### *2,6-DMBQ production in bench-scale bioreactors*

Fig. 3 compares the amount of 2,6-DMBQ produced under different conditions in mg/g of the final dried FWGE. Fig. 4-a shows a 2,6-DMBQ standard chromatogram. Fig. 4-b indicates the chromatogram results of the granulation effect area under the curve of 1061289 (b). Fig. 4-c is a chromatogram of 2,6-DMBQ without the granulation effect of wheat germ with the area under the curve of 1168745. These results demonstrated that on larger scales, the granulation decreases of 2,6-DMBQ content of the FWGE In the shake flask,

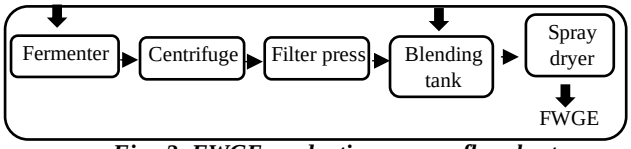


***Fig. 2: FWGE production process flowchart***

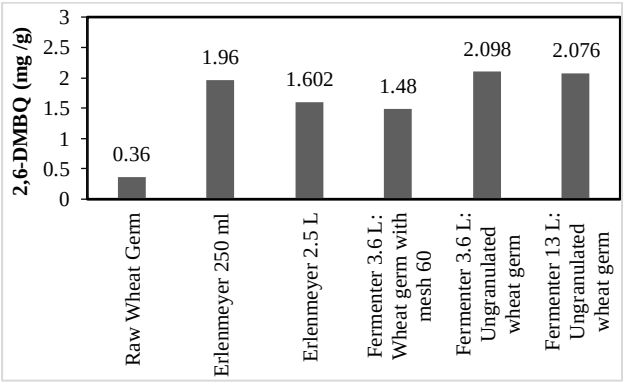


***Fig. 3: Comparison of production amounts of 2 and 6-DMBQ under different conditions***

culture medium's stirring type can create a laminar flow. Consequently, aeration might be disturbed by floated large particles in the culture medium. Moreover, some particles do not participate in the fermentation process, which can reduce 2,6-DMBQ production. Conversely, in turbulent flow, granulation causes the particles to stick together, reducing the mass transfer of $O_2$ and nutrients, thereby reducing the production efficiency of 2,6-DMBQ. The similarity of the results of the 13-liter bioreactor with 3.6-liter fermentation showed that 2,6-DMBQ content of FWGE increases on a larger scale without granulation of wheat germ particles. Accordingly, one of the cost segments was removed by eliminating granulation on the larger scale.

### *Separation and Purification Process Development of FWGW with 2, 6-DMBQ content*

To get the best combination of centrifugation and filtration, first, the outlet of the fermenter was centrifuged at 3000 g. Then, the separated supernatant was then filtered through membranes of different pore sizes to obtain a suitable filtration process for the complete removal of the remaining FWEG yeast. Table 2 indicates the filtration results.

In the analysis of each membrane, filtration velocity, turbidity, and yeast count of the output sample were investigated. The ideal filtration conditions for the complete removal of the remaining FWEG yeast are minimum pressure drop, the highest turbidity of the output sample, and the output product free of yeast [19]. Higher

***Table 2: Results of passing FWGE samples through different membranes***

| Parameters / Membrane type (size: μm) | Initial volume (mL) | Final volume (mL) | Turbidity (620 nm) | Pressure drops (bar) | Number of yeasts per 10 squares |
|---|---|---|---|---|---|
| Linen: 20 | 400 | 400 | >1 | Insignificant | 7-10 |
| Polypropylene (8-10) | 400 | 400 | 0.986 | Insignificant | 5-8 |
| Polyester (5) | 400 | 400 | 0.821 | Insignificant | 4-6 |
| Cellulose (8-10) | 400 | 400 | 0.591 | Insignificant | 8-10 |
| Cellulose (1-2) | 400 | 350 | 0.215 | ≥2 | 0-1 |
| Polypropylene + polyester | 400 | 400 | 0.667 | Insignificant | 4-8 |
| Polypropylene + cellulose (1-2) | 400 | 300 | 0.258 | ≥2 | 0 |
| Polypropylene + polyester + cellulose (1-2) | 400 | 300 | 0.268 | ≥2 | 0 |

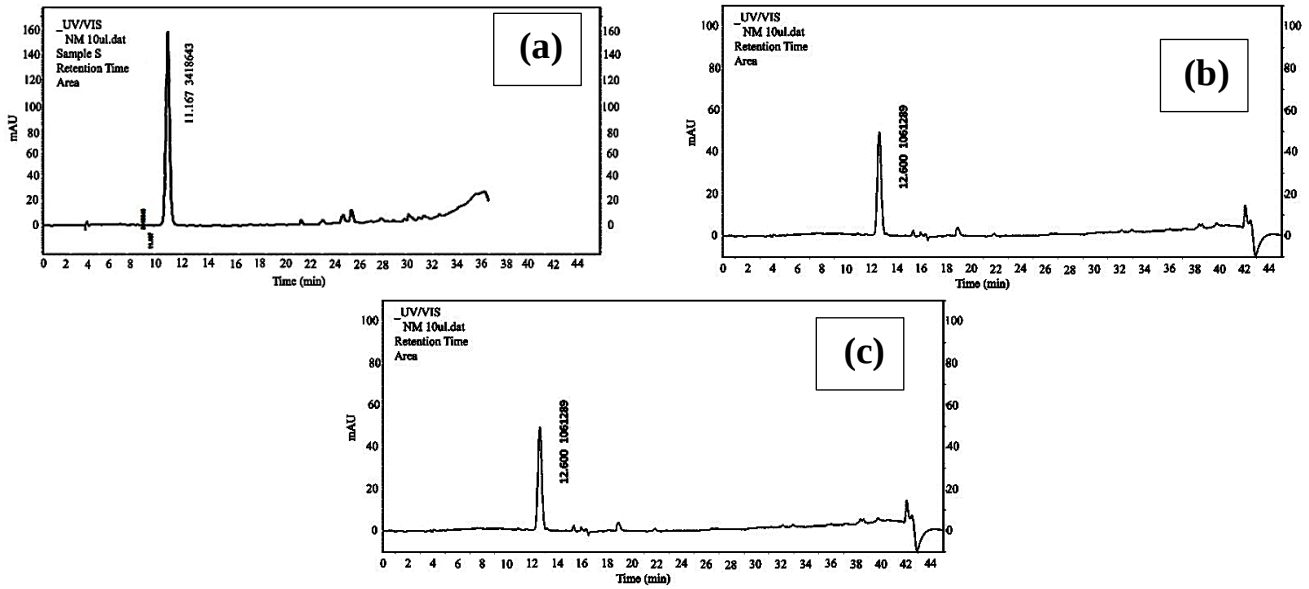


***Fig. 4: Chromatogram of samples: (a) Standard of 2,6-DMBQ. (b) Fermented sample with a mesh size of 60. (c) Fermented sample without granulation***

turbidity means more nutrients due to the higher dry weight of the final FWGE.

Therefore, it is better to use a hydrophobic membrane because the solution is aqueous. According to the results shown in Table 2, the linen membrane initially had an outlet due to the larger pore size than other membranes. Still, over time due to hydrophilicity and water absorption, the pores were blocked and caused a significant pressure drop that no longer had an outlet. The passage of the solution through polypropylene and polyester membranes is constant due to hydrophobicity. It does not cause a significant pressure drop in the process, but due to the size of the pores, the number of yeasts counted in 10 square meters was more than the standard [9]. The FWGE filtered by cellulose membrane (pore size 1-2 μm) was yeast-free, but due to the hydrophilicity of the membrane, a significant pressure drop was created in the system and the solution volume passed from the membrane decreased over time. Filtration results recommend the use of combined membranes including polypropylene 8-10 μm, polyester 5-μm, and polypropylene 2-μm for the fermented wheat germ filtration.

### *Results of dryers*

Four different dryers were used to dry the final output of the separators. According to the drying results of the samples in Table 3, the drying time by Freeze dryer was at least 48 hours, while for the oven at 70 °C was 12 hours, the rotary vacuum at 60 °C was about 3 hours, and the spray dryer was about 15 minutes. The lowest Moisture

Table 3: Dried samples results

| parameters / Type of dryer | Drying time (hours) | Final sample form | Moisture content (w/w) % |
|---|---|---|---|
| Freeze dryer | 48 | bulk | 21.46 |
| Oven (70°C) | 12 | bulk | 6 |
| Rotary Vacuum Evaporator (60 °C) | 3 | bulk | 6.5 |
| Spray dryer | 0.4 (15 min) | powder | 5 |

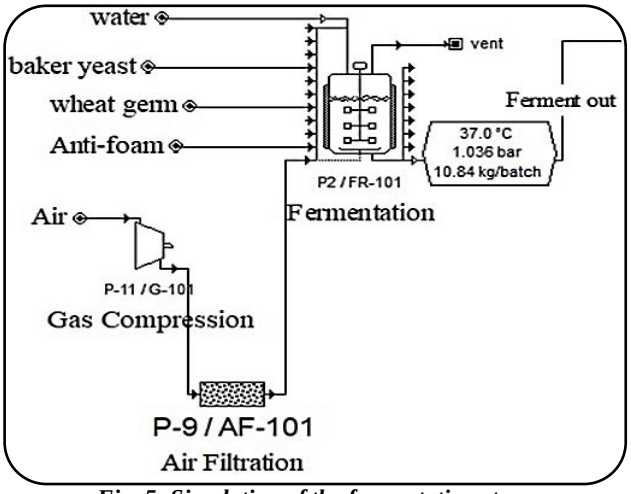


***Fig. 5: Simulation of the fermentation stage***

content in the spray dryer was about 5 (w/w)%, and the type of output material in the spray dryer was in powder form, which indicates that no grinder is needed after using the spray dryer. Therefore, especially at higher scales for drying, an FWGE spray dryer is the most appropriate choice, which is also recommended in the patent of Mate *Hidvegi et al*. [9]. The amount of dry matter produced in the spray dryer per 100 mL of the initial solution was about 2.5 g, based on which the conversion rate was about 28%.

***Simulation results***

Process simulations were performed at a scale of 10 liters in the fermentation, separators, and dryer sections until the final dried sample was reached.

*Fermentation stage*

Typically, all raw materials and equipment related to fermentation must be sterilized before the fermentation process. However, since sterilization at high temperatures reduces the activity of the enzyme beta-glucosidase [20] and this reduction in activity diminishes the production efficiency of 2,6-DMBQ, only bioreactors are sterilized using the SIP system and water is sterilized using microfiltration.

The raw materials are entered into the bioreactor according to Table 1. An air compressor with an air filter is used for aeration. Fig. 5 indicates the simulation of the fermentation stage. This process includes the following steps:

1- CIP
2- Entry of raw materials
3- Agitation (with the entry of raw materials)
4- Aeration (after the entry of raw materials)
5- Anti-foam entry (after the entry of raw materials)
6- Fermentation (with conversion rate of 28% of input materials)
7- Exit

*Separator's stage*

According to the separation results, a combination of centrifuge and filter press was considered for the complete separation of the yeast from FWGE. Fig. 6 shows that the fermented wheat germ entered the disk stack centrifuge after leaving the bioreactor. Solid particles less than 10 microns leaving the centrifuge, including the yeast remaining in the supernatant, are transferred to the filtration system for further separation. The outlet supernatant from the centrifuge is entered the filter press with a combination of suitable membranes from larger to smaller pore sizes for complete separation of the yeast. The maximum thickness of the cake formed on the membrane has been considered based on the standard filter press in the SPD itself (4 cm). Water is used to wash the cake on the filter press. To remove about 80% of yeasts and insoluble solids was performed using a centrifuge and their complete separation in a filter press. Fig. 6 shows the separation processes.

*Drying stage*

Due to faster drying, lower humidity, and final product form, a spray dryer was used to dry FWGE. Prior to spray drying, maltodextrin is added to the FWGE solution to reduce adhesion to the desiccant surface and to absorb moisture from the dried FWGE powder [9]. Maltodextrin is

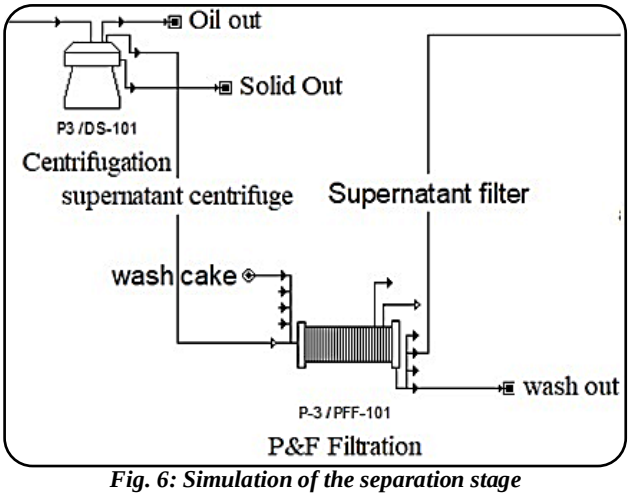


*Fig. 6: Simulation of the separation stage*

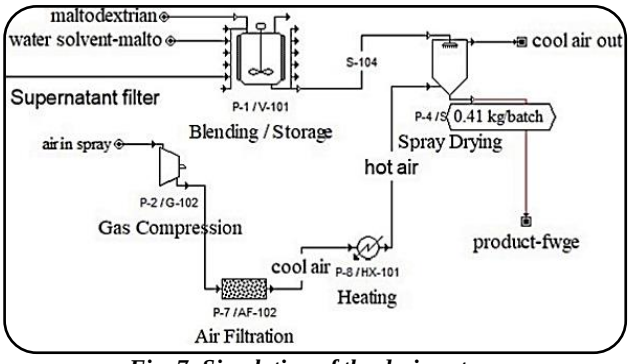


*Fig. 7: Simulation of the drying stage*

first added to high-temperature water and stirred thoroughly. The filter is then added to the maltodextrin solution and stirred thoroughly. All this process is done in the mixing tank. After complete mixing, the solution is transferred into a spray dryer with an inlet and outlet temperatures of 120 °C and 70 °C, respectively. An air compressor equipped with a heater supplies the hot air entering the spray dryer and then is passed through an air filter. The considered final moisture content of the FWGE is 5 (w/w)%. Fig. 7 shows the simulation of the drying stage. The FWGE production amount containing maltodextrin at a scale of 10 liters is 0.41 kg per batch, and the total amount of dry effluent produced in the separation stage is about 1.2 kg.

*Simulation results at higher scales*

All processes performed on a 10-liter scale, considering the required equipment capacity, were simulated to investigate the scale-up to volumes of 100 and 1000 liters. According to Table 4, taking into account the conversion rate of 28% and also the presence of maltodextrin, the amount of dried FWGE per batch per kilogram on the scale

***Table 4: Products per batch***

| product | 10 | 100 | 1000 |
|---|---|---|---|
| Produced effluent (kg) | 1.2 | 12 | 120 |
| dried FWGE (kg) | 0.41 | 4.1 | 41 |

***Table 5: Results of economically effective simulation parameters obtained from the fermenter***

| Parameter | Scale (Lit) | | |
|---|---|---|---|
| | 10 | 100 | 1000 |
| Raw material cost (USD) | 1,264.52 | 11,963.2 | 118,666.7 |
| Required equipment cost (USD) | 28,600 | 105,520 | 145,600 |
| Required fixed capital (USD) | 68,504.5 | 319,325.7 | 348,750.3 |
| Working capital (USD) | 7,611.6 | 35,480.6 | 38,750.03 |
| The total cost of production (USD) | 106,248.1 | 495,053 | 1,046,551.4 |
| Number of batches per year | 150 | 150 | 150 |
| Annual production amount (kg) | 61.5 | 615 | 6,150 |
| Selling price (USD / kg) | 400 | 400 | 400 |
| Annual selling price (USD) | 24,600 | 246,000 | 2,460,000 |
| Production effluent per year (kg) | 104 | 1,040 | 10,400 |
| ROI (year) | 4 | 1.9 | 0.4 |

of 100 and 100 liters is 4.1 and 41 kg, and the total amount of dry production effluent is about 12 and 120 kg. Fig. 8 shows the simulation of the FWGE product line.

*Results of economic analysis of FWGE production process*

Table 5 shows the results of the economic parameters of the FWGE production process. According to this table, the total production cost increases by about 78% with a scale-up from 10 to 100 and by about 50% from 100 to 1000 liters, due to the increase in equipment buying costs and other equipment-related costs. Of course, by process scale-up, the annual production amounts further increase than the total production cost.

Hence, the rate of ROI decreases, which Table 4 well illustrates. So the ROI rate on a scale of 10 liters is estimated at 4 years and on a scale of 100 and 1000 liters 1.4 and 0.4 years. The other point to consider is the amount of effluent produced. Production effluent includes industrial yeast powder and wheat germ isolated in the solid phase separation stages. The calculations of ROI rate have been regardless of the value of the effluent produced (Table 5). This significant effluent can be used as a recycling stream for the FWGE production process, in which case a substantial part of the cost of raw materials can be reduced. Also, due to the use of industrial yeast

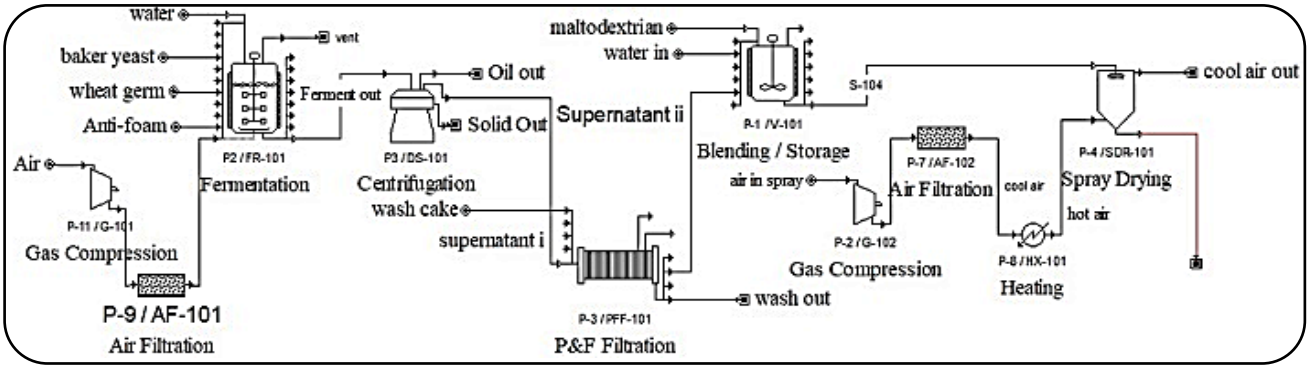


***Fig. 8: General FWGE production process***

powder and nutrients in wheat germ, the produced effluent can be used as a product with high nutritional value in various applications, including feed animals, in which case, considering the value of the effluent produced, the rate ROI will become lower too.

## CONCLUSIONS

Production of FWGE with a high content of 2,6-DMBQ was performed by industrial baker's yeast in flasks and bioreactors. Then, based on the experimental results, the FWGE process production was simulated by SuperPro Designer software. The simulation aimed to design and economically estimate the FWGE production process in 10, 100- and 1000-liter scales. The results of the economic evaluation of the process showed that with the scale-up of the FWGE production process, the rate of ROI decreases and can be considered as a high-value-added production line at higher scales.

*Received: Apr. 22, 2022 ; Accepted: Jul. 25, 2022*

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