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Optimization of 2,6-Dimethoxy Benzoquinone Production through Wheat Germ Fermentation by *Saccharomyces cerevisiae*

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Abstract

Background and objective: Nowadays, anticancer effects of 2,6 dimethoxy benzoquinone are verified. Optimization of 2,6 dimethoxy benzoquinone content of fermented wheat germ extract was carried out by investigating effects of the various effective factors on wheat germ fermentation by *Saccharomyces cerevisiae*.

Material and methods: Effects of controlling concentration of dissolved oxygen in fermentation media were studied on 2,6 dimethoxy benzoquinone content of fermented wheat germ extract. To increase the quantity of 2,6 dimethoxy benzoquinone in fermented wheat germ extract, simultaneous effects of four effective variables including wheat germ particle size, agitation rate, dry materials to water ratio and yeast to wheat germ ratio at three levels were investigated using Taguchi statistical design. Then, effects of fermentation time and increased scale on the content of 2,6 dimethoxy benzoquinone of fermented wheat germ extract were assessed using bench-scale fermenter. Concentration 2,6 dimethoxy benzoquinone was assessed using HPLC. Molecular weight patterns of the fermented wheat germ extract proteins and total protein of fermented wheat germ extract were assessed using gel electrophoresis and Kjeldahl methods, respectively.

Results and conclusion: Control of dissolved oxygen concentration of the fermentation process decreased 2,6 dimethoxy benzoquinone content to 0.135 mg g^{-1}. Investigation effects of particle size of wheat germ, agitation rate, dry materials to water ratio and yeast to wheat germ ratio on 2,6 dimethoxy benzoquinone production showed that 2,6 dimethoxy benzoquinone concentration increased to 2.58 mg g^{-1} (dry material), one of the top concentrations ever reported. Effects of fermentation time in bench-scale bioreactor showed that the highest quantity of production was achieved within 16 h. Study of the protein patterns and total protein of fermented wheat germ extract and comparisons between these values and commercial samples showed that production improvement of 2,6 dimethoxy benzoquinone did not include significant effects on quality and quantity of proteins of fermented wheat germ extract. Results of this study demonstrated that fermentation conditions could significantly affect 2,6 dimethoxy benzoquinone contents of fermented wheat germ extract.

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1. Introduction

Wheat germ (embryo and sprout axis) includes nearly 2.5-3.8% of the overall weight of the wheat grain and is an important side-product of the flour industries [1]. Various studies have indicated that fermented wheat germ by *Saccharomyces* (*S.*) *cerevisiae* yeasts includes anti-inflammatory and antioxidant properties as well as anti-cancer activity in several cancers, including testicular, colon, NSCLC (non-small-cell lung carcinoma), melanoma, leukemia and stomach cancers [2]. Yeast FWG includes hundreds to thousands of various

molecules. Based on the current studies, two quinones in the wheat germ, including 2-methoxy benzoquinone (2-MBQ) and 2,6 dimethoxy benzoquinone (2,6-DMBQ) , play major roles in biological properties of FWG [3]. Of plant foods, wheat germ is the greatest source of inactive glycosylated form of 2-MBQ and 2,6-DMBQ. The inactive forms of these two compounds include antimicrobial properties and are immune stimulants. Transformation of glycosylated to non-glycosylated form needs activity of *β*-glucosidase, which results in significant increases in chemical functional activity, including anticancer effects [4]. These benzoquinones were achieved by substituting β-1,6 glycoside-linked of hydroquinones in wheat germs [5]. In fermentation, the ß-glucosidic bond of hydroquinone glucoside is broken down by *β*-glucosidase from *S. cerevisiae* and hence 2,6-dimethoxy-p-hydroquinone and methoxy-p-hydroquinone are formed. Then, the two hydroquinones are oxidized to 2-MBQ and 2,6-DMBQ by wheat germ peroxidase [6]. However, Lot6 (YLR011wp) is a soluble quinone reductase from *S. cerevisiae* that can react with both benzoquinones and reconvert them into hydroquinone. It has been shown that activity of wheat germ peroxidase is affected by molecular oxygen in the media [7].

Lactobacillus (*L.*) *rossiae* LB1 and *L. plantarum* LB5 were combined to ferment the wheat germ. After incubation for 24 h at 30°C, 2,6-DMBQ increased from 0.035 mg g^{-1} in wheat germ to 0.225 mg g^{-1}. In 2010, Rizzello et al. [4] showed that during the incubation, liberation of 2-MBQ and 2,6-DMBQ was completed within nearly 24 h. Up to 24 h, concentration of the two quinones increases and the highest improvement is achieved within 12-18 h of incubation. After 28 h of incubation, concentration of 2-MBQ and 2,6-DMBQ was reported constant [4]. The *L. plantarum dy-1* was used to ferment wheat germs within 24 h at 30°C, resulting in 0.181 mg g^{-1} 2.6DMBQ in fermented wheat germ extract [8]. In another study, fermentation of wheat germs with a mixed culture of *Pichia pijperi* and *L. zeae* for 48 h increased concentration of 2-MBQ to 0.46 mg g^{-1} ±0.07. This concentration was approximately 1.6-fold higher than that achieved by pure culture of *L.* zeae; however, concentration of 2,6-DMBQ did not significantly increased [9]. In another study, a positive relationship was found between the fermentation time and production of MBQ and 2,6-DMBQ of fermented wheat germ extract by *S. cerevisiae* [10]. In the study of Zheng et al. [11], effects of agitation rate, initial pH, temperature and time of fermentation of wheat germs were assessed for enhanced production of MBQ and 2,6-DMBQ [11]. In a patent registered by Dr. Hidvegi, *S. cerevisiae* was used to produce a food supplement, which ultimately included 0.4 mg of 2,6-DMBQ per g of the final dry product [11]. Therefore, conditions of the fermentation process can affect activities of peroxidase and *β*-glucosidase, and hence total quantities of MBQ and DMBQ in fermented wheat germ extract.

Various statistical methods, especially Taguchi methods and response surface methodology, are widely used to optimize fermentation processes [12]. Taguchi design estimates effects of the factors on response and diversity using orthogonal array. Use of Taguchi method decreases cost and number of tests while saves time, compared to other statistical methods [12,13].

The aim of this study was increasing quantities of 2,6-DMBQ of fermented wheat germ extract by investigating effects of various factors on wheat germ fermentation using industrial *S. cerevisiae*. Therefore, growth effects of an industrial baker yeast (*S. cerevisiae* ATCC24909) on wheat germs and 2,6-DMBQ production of fermented wheat germ extract were assessed. Investigating effects of an industrial yeast type on 2,6-DMBQ production, an appropriate strain with ability of higher production was selected. Effects of controlling concentration of dissolved oxygen on production of 2,6-DMBQ were assessed using fermenter. Based on the results from other studies, effective variables linked to the production of 2,6-DMBQ were highlighted, including wheat germ particle size, agitation rate, dry material to water ratio, and yeast to wheat germ ratio, using Taguchi statistical design. Then, effects of fermentation time and increased scale on 2,6-DMBQ contents of fermented wheat germ extract were investigated using fermenter. Furthermore, protein, fat, ash, moisture and pH of the fermented wheat germ extract were assessed.

2. Materials and methods

Powder wheat germ was purchased from Takestan Ard-Fard, Iran, and stored in refrigerator until use. Industrial yeast powder was purchased from Persian Razavi Dough (A, *S. cerevisiae* ATCC 24909), Razi (B, *S. cerevisiae* ATCC9763), Khuzestan (C, *S. cerevisiae* ATCC7921) and Iran Molasses (D, *S. cerevisiae* ATCC9027). The 2, 6-DMBQ was purchased from Sigma Aldrich, USA. Other materials, including chloroform, methanol, acetic acid, sulfuric acid, boric acid, Methyl red, copper sulfate, sodium sulfate, hydrochloric acid, petroleum ether, agarose, SDS, tris, glycine, Coomassie brilliant blue, APS, temed, mercapto ethanol, acrylamide and bis-acrylamide were purchased from Merck, Germany.

2.1. Analysis

2.1.1 Measurement of 2,6-DMBQ using HPLC chromatography

To measure 2,6-DMBQ, 0.5 g of the lyophilized sample was liquefied in 50 ml of distilled water and the solution was extracted using 25 ml of chloroform with three times of

stirring. Lower heavy phases, containing chloroform, were collected at three steps of extraction and then were mixed together. Then, chloroform was evaporated using vacuum evaporator. Remaining dry matter was dissolved in a combination of 76% methanol in water (v v^{-1}) containing 5% acetic acid (v v^{-1}) as mobile phase and then clarified using 0.22-μm PTFE filters. Then, 20 μl of the clarified material were injected into a HPLC column of BDS Hypersil C18 (5 μm × 250 mm × 4.6 mm) [8], with 1 ml min^{-1} flow rate and UV-V detector at 289 nm. Pure 2,6-DMBQ was liquefied in the moving phase and injected into the column as standard. The standard concentration was 0.1 mg ml^{-1}. Measurement of 2,6-DMBQ concentration was carried out using standard curve. All measurements were carried out at three replications. Results of the production of 2,6-DMBQ were reported as mg g^{-1} of the final dry weight of fermented wheat germ extract.

2.1.2. Assessment of the protein content and SDS-PAGE

Quantity of the protein in fermented wheat germ extract was assessed using method of Kjeldahl. Transformation factor of 5.70 was used [14]. Purity and size of proteins in fermented wheat germ extracts were investigated using SDS-PAGE. To carry out electrophoresis, aqueous solutions of the lyophilized fermented wheat germ extract and wheat germ extract were mixed with a quarter of sample buffer volume (0.5% bromophenol blue, 10% SDS, pH 6.8, 0.25M tris-HCl, 5% β-mercaptoethanol, 50% glycerin) and heated at 100°C for 5 min using water bath. After centrifugation (10 min at 6000 ×g), 10 µl of the supernatant were electrophoresed in 12.5% gel SDS-PAGE using mini cell device (BioRad, USA) at 90 mA for 3 h and colored with Coomassie brilliant blue R-250 for 2 h, followed by decoloring with acetic acid/methanol/water at a ratio of 7.5:10:82.5 [8].

2.1.3. Investigation of baker yeast growth and production of 2,6-DMBQ

First, 10 g of the wheat germ were grinded and passed through a mesh no. 60 (0.25-0.6 mm) and added to an Erlenmeyer flask containing 100 ml of water and 3.33 g of the baker yeast. Flask was incubated in a shaking incubator at 150 rpm for 18 h at 30°C. Then, culture media was centrifuged at 3060 ×g and the supernatant was dried using freeze-drier. The yeast growth was investigated at the beginning and the end of the fermentation process by counting yeast cells using optical microscope. Production of 2,6-DMBQ in the supernatant was assessed at the end of the process using HPLC.

2.1.4. Other analyses

Fat, ash, moisture and pH of the wheat germ flour and FWG were assessed based on an improved method of the American Grain Chemistry Association (AACC, 2003).

2.1.5. Effects of industrial yeast type

To select industrial yeasts with abilities of further production of 2,6-DMBQ from industrial yeasts in Iran, four types of available industrial yeasts (*S. cerevisiae* ATCC 24909, *S. cerevisiae* ATCC 9763, *S. cerevisiae* ATCC 7921, *S. cerevisiae* ATCC 9027) were investigated. Fermentation was carried out in shaking flasks to produce 2,6-DMBQ. Wheat germs with particle sizes 0.25-0.6 mm with a ratio of 1:3 to yeast and a 1:9 ratio of dry to water were incubated in shaking incubator at 150 rpm for 18 h at 30°C [10]. After fermentation, the supernatant was collected from the culture media by centrifugeation at 3060 ×g for 15 min and lyophilization for further analyzes. Moreover, the 2,6-DMBQ content of fermented wheat germ extract was compared with that of crude wheat germs under identical situations.

2.1.6. Effects of controlling oxygen concentration

To control concentration of the dissolved oxygen, the process of fermentation was carried out using bench-scale fermenter with a soluble oxygen concentration of 20% ±10 saturation, which was higher than the limiting concentration of yeast growth. The batch culture was set in a 3-l stirred fermenter with a working volume of 2 l at 100 rpm and 30°C with no pH control with the same media composition of the previous step. The growth rate of yeasts and the quantity of 2,6-DMBQ were assessed at various times and compared with the achieved quantity in shaking flasks. Dissolved oxygen concentration was controlled at 20% ±10 saturation with a change impeller speed and aeration rate. Oxygen concentration was measured using oxygen sensor. Foam formation was inhibited by adding silicon antifoam.

2.1.7. Effects of various conditions on production of 2,6-DMBQ

Since fermentation process conditions can affect β-glucosidase activity and wheat germ peroxidase and hence affect total 2,6-DMBQ content of fermented wheat germ extract, effects of fermentation process conditions on increased production of 2,6-DMBQ were investigated. Therefore, effects of the four factors on 2,6-DMBQ production, including the particle size of wheat germ, ratio of dry matter to water, weight ratio of yeast to wheat germ and agitation rate on increased production of 2,6-DMBQ in fermented wheat germ extract were investigated at 3 levels (Table 1). Experiments were designed based on the Taguchi statistical approach with an orthogonal L_9 array with three replications. Wheat germ fermentation was carried out using industrial bakery yeast. To investigate effects of fermentation time on quantity of 2,6-DMBQ of fermented wheat germ extract, sampling of the fermentation process was carried out at 16, 18, 24 and 28 h.

Table 1. Names and levels of the various factors studied in production increase of 2,6 dimethoxy benzoquinone

Factor Level	Particle size (mesh no.)	Agitation speed (rpm)	Ratio of dry material to water	Weight ratio of yeast to wheat
1	30	100	1 to 7	1 to 2
2	60	150	1 to 9	1 to 3
3	90	200	1 to 11	1 to 4

2.1.8. Investigation of 2,6-DMBQ production in fermenter

To investigate 2,6-DMBQ production in fermenter under optimal conditions in shaking flasks, batch fermentation wheat germ was set in a 3-l fermenter with a working volume of 2 l with no pH control and dissolved oxygen, aeration rate of 0.1 vvm (volume per minute of air per volume of media culture) and agitation rate of 80-100 rpm. To investigate the optimal time of maximum production of 2,6-DMBQ during fermentation process, quantity of 2,6-DMBQ was assessed at 16, 18, 20 and 22 h after initiation of the fermentation.

3. Results and discussion

3.1. Selection of the productive starter culture

Growth assessment in shaking flasks revealed that at the process end of fermentation, the number of yeast cells was only double and production of 2,6-DMBQ included 0.463 mg g^{-1}. Study of the strain effects showed that Strain A included the highest production rate of 2,6-DMBQ (Table 2). Therefore, Strain A was used in this study. Furthermore, quantity of 2,6-DMBQ included 0.115 mg g^{-1} in fermented wheat germ extract with no yeasts. As a result of fermentation, quantity of 2.6-DMBQ in wheat germ extract increased from an initial value of 0.115 mg g^{-1} to a final value of 0.47 mg g^{-1}.

Table 2. Selection results of the industrial yeast production with the highest production rate of 2,6-DMBQ

Culture medium	The production amount of 2,6 DMBQ (mg g^{-1})
Wheat germ without yeast	0.115 ± 0.002
Wheat germ with yeast A	0.47± 0.008
Wheat germ with yeast B	0.425± 0.002
Wheat germ with yeast C	0.435± 0.003
Wheat germ with yeast D	0.42± 0.007

3.2. Effects of controlling concentration of dissolved oxygen

Cell density in the fermenter increased by nearly 2.5 times as a result of controlling dissolved oxygen concentration, compared to that in shaking flasks. The highest growth was seen at early hours of the fermentation of wheat germs in bioreactor and did not change significantly six hours later. Based on the HPLC analysis, concentration of 2,6-DMBQ at 3, 6 and 18 h of the fermentation process included 0.12, 0.155 and 0.135 mg g^{-1}, respectively. Results showed that control of the oxygen concentration by simultaneous altering impeller speed and aeration rate during the fermentation process decreases production of 2,6-DMBQ. Moreover, no significant relationships were seen between the yeast cell density and 2,6-DMBQ production. This was possibly due to the fact that production of 2,6-DMBQ was mildly dependent on oxygen concentration and the higher production in shaking flasks occurred under low aeration. In contrast, peroxidase activity of the wheat germ in the presence of molecular oxygen decreased greatly. Therefore, released hydroquinones in the culture media could not be converted to benzoquinone [7].

3.3. Optimization of 2,6-DMBQ production

To optimize the production conditions, nine experiments were carried out with three replications in shaking flasks (Table 3). Optimal values of the factors for the production of 2,6-DMBQ were predicted using variance analysis of the results and Taguchi test. No significant differences were observed between the predicted quantity of 2,6-DMBQ (2.576 mg g^{-1}) and the achieved actual data from optimal conditions, including mesh no. of 60 (0.25-0.6 mm) of particles, agitation speed of 150 rpm, ratio of dry material (wheat germ and yeast) to water of 1:11, weight ratio of the yeast to wheat germ of 1:2 (Table 4 and Figure 1). The quantity of 2.58 mg g^{-1} of 2,6-DMBQ was one of the highest quantities already reported. Particle size is important because of its great effects on bioavailability of materials for the microorganisms. By increasing the particle size, decreases occurred in production (Figure 1a). This was because by increasing size of the substrate and subsequently decreasing surface to volume ratio of the solid particle nutrients, the availability of microorganisms to nutrients in the wheat germ decreased. Alternatively, small size of the wheat germ particles decreased production, possibly because small size of the wheat germ particles aggregated wheat germs; hence, microorganisms could not access them. Therefore, it was necessary to further production of 2,6-DMBQ the particle size of wheat germ is optimized. In this study, the particle size of mesh no. 60 (0.25-0.6 mm) was introduced as optimal, which included the greatest effect on production of 2,6-DMBQ, following the speed of agitation.

Table 3. The experiments and results of the study on the effects of various conditions on production of 2,6 dimethoxy benzoquinone based on the orthogonal arrays L_9

Factor Runs	Particle size (mesh no.)	Agitation speed (rpm)	Ratio of dry material to water	Weight ratio of yeast to wheat germ	Amount of 2,6 DMBQ per gram of final dry powder (mg g^{-1})
1	30	100	1 to 7	1 to 2	0.918± 0.045
2	30	150	1 to 9	1 to 3	2.15± 0.032
3	30	200	1 to 11	1 to 4	1.83± 0.039
4	60	100	1 to 9	1 to 4	0.337± 0.006
5	60	150	1 to 11	1 to 2	2.58± 0.086
6	60	200	1 to 7	1 to 3	1.99± 0.029
7	100	100	1 to 11	1 to 3	0.49± 0.065
8	100	150	1 to 7	1 to 4	1.91± 0.033
9	100	200	1 to 9	1 to 2	1.25± 0.09

2,6 DMBQ=2,6 dimethoxy benzoquinone

Table 4. The optimum production conditions and analysis of variance of results on orthogonal arrays L_9

Independent variable	Optimum level	Optimum amount	Contribution	Sum of sqrs (S)	Variance (V)	Percent (P%)
Agitation speed	2	150 rpm	0.718	4.163	2.081	85.294
Particle size	2	60 mesh	0.14	0.35	0.175	7.176
Dry to water ratio	3	1:11	0.138	0.282	0.141	5.874
Weight ratio of yeast to wheat germ	1	1:2	0.86	0.085	0.042	1.741

Figure 1b and Table 3 show that the agitation speed included the greatest effect on production of 2,6-DMBQ. By increasing the stirring rate from 100 to 150 rpm, production of 2,6-DMBQ increased and then decreased. This phenomenon might be due to the increased availability of wheat germs to yeast cells by increased agitation. Thereby, release of intracellular peroxidase, hydroquinone glucoside and *β*-glucosidase enzymes within the culture media increased. The hydroquinone glucoside was hydrolyzed by *β*-glucosidase to agglutinins and the hydroquinones produced by peroxidase were oxidized to benzoquinones. Zheng et al. [11] showed that production of benzoquinones increased by the agitation rate enhancement. They reported the optimum agitation speed of 142 rpm [11].

It was observed that the weight ratio of dry matter to water and the weight ratio of wheat germ to yeast included low effects on production of 2,6-DMBQ, respectively (Figure 1c and 1d).

Effects of time on production of 2,6- DMBQ (on the optimum sample) demonstrated that increased time up to 18 h increased the production. By increasing the fermentation time from 18 to 24 and 28 h, production quantity of 2,6-DMBQ slowly decreased (Table 5). Therefore, a time of 18 h was chosen as the optimal time for the overproduction of 2,6-DMBQ in fermentation process of wheat germs. Within 18 h, wheat germ peroxidase included a higher activity than that the Lot6 did and *S. cerevisiae* could produce further Lot6 after 18 h. Lot6 protects *S. cerevisiae* against cytotoxic effects of quinones via two-electron decreasing through mechanism of ping-pong bi [13]. Therefore, reduction activity of peroxidase after 18 h might lead to decreases in benzoquinones. In the study of Zheng et al. [11], this occurred after 40 h of fermentation of wheat germ by *S. cerevisiae* and increased fermentation time more than 40 h decreased production of benzoquinones of fermented wheat germ extract. In that study, 2-MBQ and 2,6-DMBQ were measured together [11].

3.4. Effects of fermentation process on 2,6-DMBQ production in bench-scale bioreactor

Fermentation process was carried out under optimum conditions of low aeration (0.1 vvm), slow stirring (50 rpm) and 1-l working volume in a 3-l fermenter. Quantity of the production in these conditions included 2.62 mg g^{-1}, which was approximately similar to the quantity achieved in shaking flasks, but within a less time (16 h), compared to that achieved in Erlenmeyer flasks. Accordingly, production decreased with increasing time in flasks (Table 6).

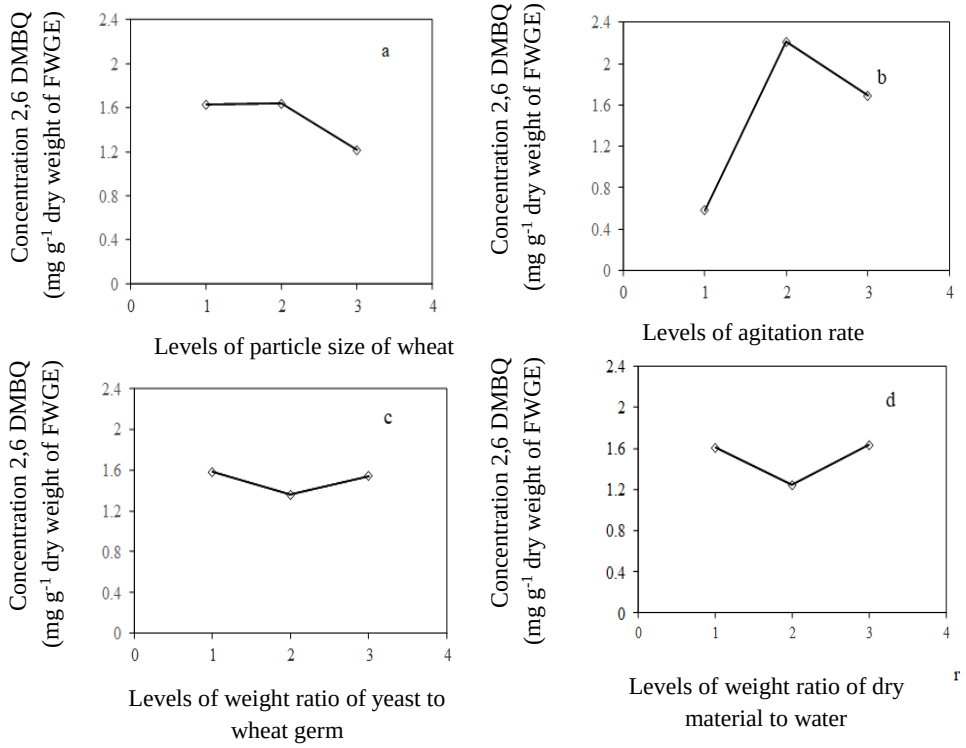

Figure 1. The average effects of various factors at three levels on the overall efficiency of 2,6-DMBQ. a) particle size, b) agitation rate, c) weight ratio of yeast to wheat germ, and d) weight ratio of dry material (yeast and wheat germ) to water.
FWGE =fermented wheat germ extract

Table 5. Effects of time on the production of 2,6 dimethoxy benzoquinone

Time of fermentation process (h)	The amount of 2,6 DMBQ in mg g^{-1}
16	2.42± 0.077
18	2.58± 0.086
24	1.9± 0.045
28	1.27±0.05

Table 6. Production of 2,6 dimethoxy benzoquinone in a fermenter with low aeration and effects of time on the production

Fermentation time (h)	The amount of 2,6 DMBQ in mg g^{-1}
16	2.62± 0.039
18	2.07± 0.064
20	1.86± 0.051
22	1.62± 0.044

3.5. Results of the verification analysis

Protein content of the raw wheat germ included 23.51% of dry matter (DM), fat content of 10.757% of dry matter, ash of 5.96% of dry matter, pH 6.848 and moisture content of 6.66%. The values for lyophilized sample ofthe fermented wheat germ (optimized specimen) included protein content of 38.594% of dry matter, fat content of 3.082% of dry matter, ash of 10.612% of dry matter, pH 4.9 and moisture of 21.66%. In the study of Zhang et al. [8], protein content of the raw wheat germ included 32.9% of dry matter and protein content of the freeze-dried powder of the fermented wheat germ extract included 34.36% of dry matter [9]. The SDS-PAGE electrophoresis was used to achieve information on molecular weight and protein distribution patterns. Protein bands ranged 22–100 kDa. As seen in Figure 2, electrophoresis patterns of the raw wheat germ and the fermented wheat germ extract were approximately similar. This demonstrated that most of the protein components of the wheat germ were present in fermented wheat germ extract. In 2015, Zhang et al. [8] studied electrophoresis patterns of the crude wheat germ and FWG with *L. plantarum*. They observed that the raw wheat germ extract included a protein band in the range of 15-100 kDa while the fermented wheat germ extract included a band of 15-40 kDa. This showed that in the study of Zhang, only low molecular weight proteins were present in fermented wheat germ extracts [8]. Zhang and et al. also reported a fat content of the raw wheat germ of 11.91% [8]. In report of Rizzello, quantity of fat in raw wheat germ included 7.95% [4].

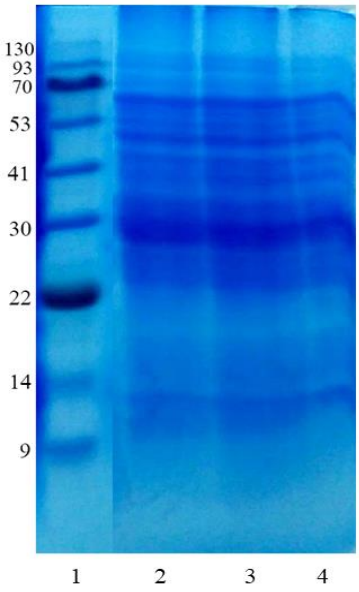

Figure 2. The electrophoresis patterns of raw wheat germ and fermented wheat germ extract. Vertical axe is molecular weight of proteins (kDa). Column 1, molecular weight marker; Column 2, raw wheat germ; and Columns 3 and 4, freeze-dried powder of the fermented wheat germ

In another study in 2010, fat content of the used wheat germ included 7.99% [15]. In the current study, fat content of the used raw wheat germ was similar to the reported content. Based on the experiments with two replications, moisture content of the crude wheat germ was 6.66% and moisture content of the lyophilized powder of the fermented wheat germ extract was 21.66%. Zhang et al. reported a moisture content of 12.58% in wheat germ [8]. Rizzoli reported moisture content of the raw wheat germ as 11.08 and 11.11% [8,15]. Moisture content of the lyophilized powder was compared to that of the raw wheat germ, indicating that the lyophilized method was not appropriate for drying the fermented wheat germ extract. Rizzello et al. reported the quantity of ash in crude wheat germ as 3.77% [15]. Zhang reported ash of wheat germ as 7.68% [8]. However ash content of the lyophilized powder has not been reported, the value (10.612% of dry matter) showed that minerals were more than the minerals in raw wheat germs. The pH of raw wheat germ was 6.48 and the pH of lyophilized powder was 4.9. Rizzello et al. reported pH of the raw wheat germ as 6.34. Moreover, pH of lyophilized powder of the fermented wheat germ was reported as nearly 4.2 [15]. In another study by Rizzello, pH of the raw wheat germ was 6.36 and pH of the lyophilized powder was 4.16 [4].

4. Conclusion

In this study, fermentation of wheat germ with commercial yeast powder of *S. cerevisiae* was carried out to optimize conditions for increasing production of 2,6-DMBQ in final products. It has been shown that constant feeding of oxygen decreased production of 2,6-DMBQ while increasing yeast growth. Therefore, production of this chemical was mildly dependent on dissolved oxygen concentration and rate of growth. It was reported that the commercial yeast powder (*S. cerevisiae* ATCC 24909) included the highest production rate of 2,6-DMBQ within the four commercially available yeasts. To increase quantity of 2,6-DMBQ production, optimization was carried out with four variables using Taguchi method, including wheat germ particle size, agitation speed, dry matters to water ratio and yeast to wheat germ ratio. Optimization results showed that the agitation speed included the greatest effect on the production of 2,6-DMBQ followed by the particle size. Effects of fermentation time on the production were shown to include the highest 2,6-DMBQ production at 18 h, and increases in time decreased production. Quantity of 2,6-DMBQ in this study was the highest quantity already reported and the optimized condition was a cost-effective approach that could be used for scaling up.

5. Acknowledgements

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6. Conflict of interest

The authors declare no conflict of interest.

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بهینه سازی تولید ۲، ۶-دی متوکسی بنزوکینون از طریق تخمیر جوانه گندم توسط *ساکارومایسس سرویزیه*

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چکیده

سابقه و هدف: امروزه، اثرات ضد سرطانی ۲، ۶-دی متوکسی بنزوکینون (2,6-DMBQ) به اثبات رسیده است. بهینه‌سازی محتوی 2,6-DMBQ عصاره جوانه گندم تخمیر شده (FWGE) با بررسی تأثیر عوامل مؤثر بر تخمیر جوانه گندم توسط *ساکارومیسس سرویزیه* انجام شد.

مواد و روش‌ها: : اثرات کنترل غلظت اکسیژن محلول در محیط تخمیری بر محتوی 2,6-DMBQ عصاره جوانه گندم تخمیر شده مورد مطالعه قرار گرفت. به منظور افزایش مقدار 2,6-DMBQ در عصاره جوانه گندم تخمیر شده، اثر همزمان چهار متغیر مؤثر شامل اندازه ذرات جوانه گندم، سرعت همزدن، نسبت مواد خشک به آب و نسبت مخمر به جوانه گندم در سه سطح با استفاده از طرح آماری تاگوچی بررسی شد. سپس، اثرات زمان تخمیر و افزایش مقیاس بر محتوای 2,6-DMBQ در عصاره جوانه گندم تخمیر شده با استفاده از فرمانتور رومیزی ارزیابی شد. غلظت 2,6-DMBQ توسط HPLC تعیین شد. الگوی وزن مولکولی پروتئین‌های عصاره جوانه گندم تخمیر شده و پروتئین کل عصاره جوانه گندم تخمیر شده به ترتیب با روش ژل الکتروفورز و کجلدال تعیین شد.

یافته‌ها و نتیجه‌گیری: کنترل غلظت اکسیژن محلول در فرآیند تخمیر باعث کاهش میزان 2,6-DMBQ به ۰/۱۳۵ $mg\ g^{-1}$ می‌شود. بررسی اثرات ذرات جوانه گندم، سرعت همزدن، نسبت ماده خشک به آب و نسبت وزنی مخمر به جوانه گندم در تولید 2,6-DMBQ نشان داد که غلظت 2,6-DMBQ به ۲/۵۸ $mg\ g^{-1}$ (ماده خشک) افزایش می‌یابد که این یکی از بالاترین غلظت‌هایی می‌باشد که تاکنون گزارش شده است. اثرات زمان تخمیر در بیوراکتور رومیزی نشان داد که بیشترین میزان تولید در ۱۶ ساعت به‌دست می‌آید. بررسی الگوی پروتئین‌ها و پروتئین کل عصاره جوانه گندم تخمیر شده و مقایسه آنها با نمونه تجاری نشان داد که بهبود تولید 2,6-DMBQ تأثیر معنی‌داری بر کیفیت و کمیت پروتئین‌های عصاره جوانه گندم تخمیر شده ندارد. نتایج این مطالعه نشان داد که شرایط تخمیر می‌تواند به میزان قابل توجهی بر محتوای 2,6-DMBQ عصاره جوانه گندم تخمیر شده تأثیر بگذارد.

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

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واژگان کلیدی
- 2,6-DMBQ
- عصاره جوانه گندم تخمیر شده
- روش تاگوچی
- تخمیر جوانه گندم

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