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Full Length Article

Optimization of Wheat Embryo Globulin Fermenting Process and Variation in Properties during Fermentation

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Abstract

In this study, an efficient preparation technology of fermented wheat embryo globulin (FWEG) was developed, and the changes of nutritional characteristics and structure of FWEG were studied during the fermentation process. Protein concentration was selected as the evaluation index, and Box-Behnken experiment design was employed for optimizing the preparation conditions of FWEG. The nutritional characteristics of FWEG during fermentation were dissected through SDS-PAGE and free amino acid assay. The structural changes of FWEG during fermentation were analyzed through circular dichroism, sulfhydryl detection, and electron microscope scanning. The optimum fermentation conditions were determined. The protein concentration of FWEG reached 2.09 ± 0.15 mg mL^{-1} under the optimal conditions. Acidity, contents of protein and protease increased while the content of FWEG decreased in the fermentation. The concentration of small molecule protein and free amino acids went up along with the fermentation. Due to changes in the secondary structure of FWEG and massive released of sulfhydryl groups, the surface of FWEG became uneven during the fermentation. The optimal fermentation process was noticed after Baker's yeast fermentation, lasting for 3.2 h; *Lactobacillus plantarum* was inoculated, and the fermentation continuous for 14.8 h, in which the ratio of baker's yeast to *L. plantarum* (V:V) was 1:2. The nutritional quality of wheat embryo globulin was improved, and its structure was changed during fermentation, which increased the absorption of FWEG and enhanced its suitability as a food ingredient.

Key words: Fermentation; Globulin; Nutritional quality; Wheat embryo

Introduction

Wheat embryo (wheat germ) is a by-product of the flour milling industry (Ogawa and Tabuchi, 2015). The wheat embryo is highly nutritive. Wheat embryo comprises of 2–3% of the total weight of wheat. However, it contains fat, proteins, vitamins and other bioactive compounds (Gomez *et al.*, 2012; Wang *et al.*, 2017). The defatted wheat embryo contains many proteins, and several studies have indicated that wheat embryo proteins have pharmacological properties, like antioxidant, antihypertensive, calcium binding and immunomodulating properties (Yang *et al.*, 2011). Albumin and globulin are composed of more than half of the wheat germ protein, and they also have a well-balanced amino acid composition (Wu *et al.*, 2017).

Wheat embryo protein has been categorized as a with superiorly effective vegetable protein, Wheat embryo protein contains 17 amino acids, especially amino acids, like lysine, methionine, and threonine, which are not present in many cereal grains. Therefore, the wheat embryo is potentially a nutritious food supplement, containing natural Quality proteins and amino acid (Novikova *et al.*, 2018). Wheat embryo globulin has many physiological functions, especially it provides nutrition and enhances immunity (Wu *et al.*, 2017).

Recently, the wheat embryo has been utilized for various purposes in addition to its use as an animal feed. The wheat embryo is considered as a high-quality plant protein resource. In a specific growth environment, microorganisms can transform substrates into bioactive substances through metabolism or biotransformation. The process of microbial fermentation of active substances of the wheat embryo has been studied extensively. Many studies show that fermented wheat embryo possesses potential antitumor activity against the caners in colon, testis, thyroid, ovary, NSCLC, breast, stomach, head and neck, liver, and cervix, as well as glioblastoma, melanoma, and Neuroblastoma (Abdelmonem *et al.*, 2015; Yang *et al.*, 2016; Imir *et al.* 2018). Apart from the anti-proliferative activity against cancer cells, the fermented wheat embryo can enhance the activity of tamoxifen in estrogen-receptor-positive breast cancer cells. At present, many investigations

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are focused on the functional characteristics of small molecules, such as quinones in the fermented wheat embryo, while the nutritional composition and structural characteristics of macromolecular substances, such as proteins, have been rarely studied. After the microbial enzymatic reaction, the water-insoluble proteins in the wheat embryo and the proteins closely linked to cellulose are converted into soluble proteins, which can be easily absorbed in the body (Kuo *et al.*, 2017). In this study, the defatted wheat embryo was studied to explore the optimal fermentation process and the changes in the nutritional composition and structural characteristics of wheat embryo globulin during the fermentation process were measured for developing new technologies for diversified development utilization of wheat embryo resources.

Materials and Methods

Materials and Chemicals

In this experiment, all microbial strains belonged to the edible microorganism, which were approved by the Ministry of Agriculture of the People's Republic of China in 2013. *Lactobacillus rhamnosus* CICC®20257 was purchased from CICC (China Center of Industrial Culture Collection, CICC); *Aroma producing yeast, L. plantarum, L. acidophilus* and *Bifidobacterium* isolated in our laboratory; Baker's yeast, Angel yeast and *Saccharomyces cerevisiae* were purchased from angel yeast Co., Ltd., Direct Vat Inoculation was procured from Shanenkang biotechnology (Suzhou) Co., Ltd. The defatted wheat embryo was purchased from Henan Kunhua biotechnology Co., Ltd. (Henan, China). All other chemicals and solvents were of analytical grade.

Optimization of Preparation Process of FWEG

According to the 1:10 fermentation mixture ratio, defatted wheat embryo and sterile water were selected as fermentation substrate. The ratio of fermentation substrate to bacterium suspension was 20:1. After inoculation, the yeast (10^8 CFU/mL) continued to ferment for 2–6 h, followed by 6% glucose and Lactobacillus (10^8CFU/mL) which fermented for 12–16 h successively. Once fermentation was completed, 0.9% NaCl was added, and the supernatant was centrifuged at 8000 g for 10 min after 2 h of low-temperature oscillation. Fermented wheat embryo globulin (FWEG) was used as the evaluation index, and on the basis of a single factor experiment, the process parameters were optimized by response surface analysis method.

Composition Changes in Fermentation Systems

Samples were taken every 3 h during the fermentation process, and then the contents of protein and protease, starch contents, living bacteria number, pH and TTA were determined. Protein concentration was determined using Bradford reagent method (Cheng *et al.*, 2016). The protease was assayed according to GB/T 23527-2009 protease preparation method (Novozymes Biotechnology Co., Ltd. *et al.*, 2009). Starch contents were assayed using anthrone colorimetric method (Laurentin and Edwards, 2003). The viable count was determined through plate counting method. Total acid (TTA) and pH was determined according to AACC method (American Association of Cereal Chemists International, 2000).

The Nutritional Quality of FWEG during the Fermentation Process

SDS-PAGE and free amino acid analyses were performed in samples fermented for 0, 9 and 18 h. SDS–PAGE was performed by the method of Laemmli (1970), and the free amino acid was assayed using an amino acid analyzer (Biluca *et al.*, 2019).

The Structural Changes of FWEG for Fermentation Process

CD measurements were implemented using Bio-Logic MOS-500 circular dichroism spectroscopy (France) at room temperature. The far-UV (190–250 nm) spectra were recorded through a step size of 1 nm and a 1.5 nm (Li and Hirst, 2017). The free group of sulfhydryl content was determined according to Beveridge's method (Shimada and Cheftel, 1989). The samples were observed using a scanning electron microscope with an accelerating voltage of 5 kV after freeze-drying. Before observation, the sample was sprayed with gold (15 nm) on the surface of the FWEG with an anion ejector.

Data Processing

Response surface was designed in Design Expert Software (Static Made Easy, Minneapolis, M.N., USA, Version 6.0.5, 2001). Statistical analyses (ANOVA) were using SPSS 25.0 (SPSS Inc., Chicago, 600 IL, USA). The difference between the means was considered significant at $P < 0.05$. Origin Pro 8.0 (Origin Lab., Inc., Northampton, M.A., USA) and Microsoft Office Excel 2016 were used for process optimization, nutritional quality changes, structural changes and data consolidation. All data are presented as the mean ±SEM of at least three replications.

Results

Fermentation Process Optimization

In fermentation anaphase, the protein concentration of FWEG was significantly higher ($P < 0.05$) for fermenting with *L. plantarum* and *L. acidophilus* than that with other *Lactobacillus* spp. (Fig. 1). *Lactobacillus* was added at the 3rd hour, which showed significantly higher protein concentration than other groups ($P < 0.05$). The protein

concentration of FWEG by the ratio of lactic acid bacteria to yeast (V:V) exhibited a significant difference ($P < 0.05$). Amongst 5 groups, the fermentation systems at b and d group showed the maximum amount of protein concentration of FWEG. Generally, the protein concentration of FWEG did not differ significantly for different amounts of yeast ($P > 0.05$), which indicated that yeast species was not an important factor.

Central composite design (CCD) was used to constructing a three-factor, a three-level rotatable model consisting of 17 experimental runs with four replicates at the central point (Table 1). Species and addition time of *Lactobacillus* and the ratio of yeast to lactic acid bacteria (V:V) were optimized according to the mathematic model built by Software on the rationale of response surface methodology.

The fermentation condition was optimized using different variable combinations according to the rotatable CCD model in Table 1. The experimental data were subjected to Response Surface Methodology (RSM), and the suitability of the model was analyzed by linear regression and ANOVA (Table 2). The coefficient of determination (R^2) from multiple correlation coefficients represented the relationship between the predicted and the actual values in each quadratic equation. The high R^2 values from the protein concentration model (0.7407) suggested a high degree of correlation between the observed and predicted values. The significance of each coefficient was assessed by F-test and Student's *t*-test. A large F-value associated with a small p-value is desired, as it indicates that the effect on the respective response variables is significant. The F-value from ANOVA showed the relative contribution of the model variance to the residual variance. The model demonstrated that X_1, X_2, X_3, X_1^2 X_2^2 and X_3^2 significant influenced the protein concentration of FWEG ($P < 0.05$) (Table 2). The validation of the goodness of fit was measured by the lack of fit test. The F-value of the lack of fit, and p-value from the fermentation model, were 0.43389 and 0.7407 respectively. These results implied that this model was insignificant as compared to pure error, which also suggested the quadratic model was applicable. The insignificance of lack of fit suggests that the model fits the data well. Thus, the fermentation model can represent the correlation between experimental and response variables and, hence, can be applied for optimizing the fermentation process.

For better visualization of the interactions of different experimental factors variables on the effects of response variables, three-dimensional response surface and contour plots are illustrated in Fig. 2. The plots demonstrated the effect of two experimental variables on a single response, while the third variable was constant. It showed the effects of *Lactobacillus* species, *Lactobacillus* addition time and the ratio of Yeast to *Lactobacillus* (V:V) on the protein concentration of FWEG (Fig. 2).

After analyzing the data with the design-expert software, the optimal process conditions were predicted by

Table 1: The design and results of response surface experiment

The serial number	X_1	X_2	X_3	The protein concentration of FWEG (mg mL^{-1})
1	0	0	0	2.09179
2	0	-1	-1	1.814148
3	1	0	-1	1.78242
4	-1	0	1	1.679681
5	0	1	-1	1.445506
6	-1	1	0	1.493733
7	-1	-1	0	1.744737
8	0	0	0	2.115433
9	1	-1	0	1.355203
10	0	0	0	2.119943
11	0	0	0	2.361363
12	0	0	0	2.361636
13	1	0	1	1.26249
14	0	1	1	1.324724
15	-1	0	-1	1.759721
16	0	-1	1	1.591445
17	1	1	0	1.216272

X_1: *Lactobacillus* species, X_2: Lactobacillus addition time and X_3: The ratio of yeast to lactic acid bacteria (V:V)

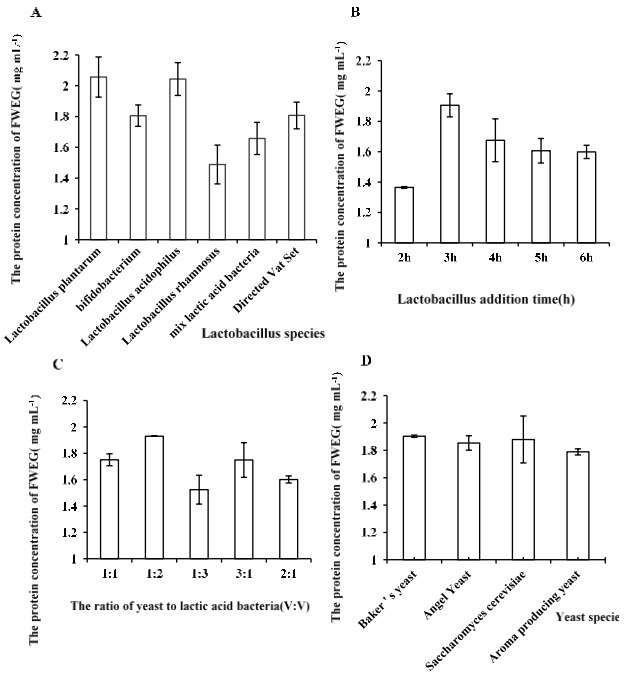

Fig. 1: Single_factor_experiment_(**A**: The effect of *Lactobacillus* species on the protein concentration of FWEG. **B**: The effect of Lactobacillus addition time on protein concentration of FWEG. **C**: The effect of the ratio of yeast to lactic acid bacteria (V:V) on protein concentration of FWEG. **D**: The effect of yeast species on protein concentration of FWEG.)

the quadratic regression equation, and the optimal process parameters were derived from the design-expert software. The optimal fermentation condition was: after 3.2 h *Baker's yeast* fermentation, *L. plantarum* was inoculated, and the continuous fermentation lasted for 14.8 h, in which the ratio of *Saccharomyces cerevisiae* to *L. plantarum* was 1:2 (V:V), and the theoretical protein concentration of FWEG was 2.23 mg mL^{-1}. The validation test was performed under this

Table 2: Variance analysis of response surface experiment

Sources of variation	sum of squares	degree of freedom	mean square	f value	p value	Significant
X_1	0.140844	1	0.140844	9.669046	0.0171	*
X_2	0.131404	1	0.131404	9.020986	0.0198	*
X_3	0.111263	1	0.111263	7.638295	0.0279	*
$X_1 X_2$	0.00314	1	0.00314	0.215568	0.6565	
$X_1 X_3$	0.048376	1	0.048376	3.321025	0.1112	
$X_2 X_3$	0.002597	1	0.002597	0.178284	0.6855	
X_1^2	0.487345	1	0.487345	33.45648	0.0007	**
X_2^2	0.733339	1	0.733339	50.34414	0.0002	**
X_3^2	0.260518	1	0.260518	17.88469	0.0039	**
residual	0.101966	7	0.014567			
lack-of-fit test	0.025035	3	0.008345	0.43389	0.7407	
error	0.076931	4	0.019233			
sum	2.184996	16				

Equation Y_1=2.21-0.13 X_1-0.13 X_2-0.12 X_3+0.028 $X_1 X_2$-0.11 $X_1 X_3$+0.025 $X_2 X_3$-0.34 X_1^2-0.42 X_2^2-0.25 X_3^2。

*P < 0.5 significant effect ; **P < 0.01 remarkable influence. (X_1: *Lactobacillus* species, X_2: *Lactobacillus* addition time and X_3: The ratio of yeast to lactic acid bacteria (V:V))

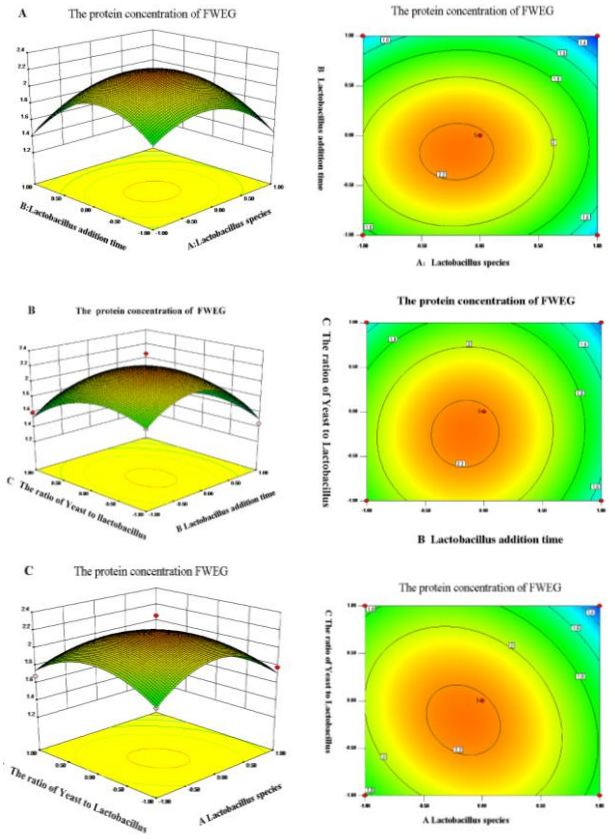

Fig. 2: Surface plots of response surface experiment

experimental condition, which was repeated for 3 times. The protein concentration of FEWG was 2.09 ± 0.15 mg mL^{-1}, and the actual value was close to the theoretical value, indicating that the model was nearly perfect, and the predicted result was relatively accurate.

Composition Changes in Fermentation Systems

Under the optimal fermentation conditions, the protein concentration of FWEG increased gradually in the first six h but decreased afterward, and near the end of the fermentation process, the protein concentration was elevated suddenly (Fig. 3A). At the early stage of fermentation (0~3 h), the starch content in the fermentation broth declined rapidly during the Baker's yeast process. With the progress of fermentation, the decreasing rate of starch content gradually decreases (Fig. 3B). As the microbial metabolism decelerated, the decrease rate of starch content was also gradually slowed down. At the initial stage of fermentation (0–3 h), the growth of the yeasts reached a maximum value of 8.23 Log CFU mL^{-1}, and it slowly decreased after the inoculation of lactic acid bacteria. *L. plantarums* grew rapidly, and then slowly decreased after reaching the highest value of 8.96 Log CFU mL^{-1} at 9 h. (Fig. 3C). The acidification rate of *L. plantarum* was low before fermentation (0~3 h) without inoculation. With the addition and continuous amplification of *L. plantarum*, the acidification rate increased. When the pH value of fermentation broth dropped to about 4.5 and remained stable, the total acidity still grew at a high rate (Fig. 3D). After 12 h, both the pH and total acidity remained stable.

Changes in Nutrient Composition during Fermentation

The molecular weight distribution of FWEG was examined by SDS-PAGE (Fig. 4). SDS-PAGE electropherogram clearly showed that the fermentation significantly influenced the protein distribution of the samples. Differences in protein quantity were determined by densitometric analysis. The results displayed that the protein-banding profiles of lane 1 presented a non-fermented wheat embryo globulin with the main bands of 35 –100 kDa. In case of FWEG, the bands of large-sized proteins disappeared (>63 kDa) and more small-sized proteins or peptides (<48 kDa,) appeared and with the fermentation time prolonged, this phenomenon was more significant than 0 h.

The total free amino acid content of wheat embryo, especially Asp, Ser, Leu and Lys surged 3.62 times after fermentation, *i.e.*, 11.26, 35.89, 12.47 and 20.97 times,

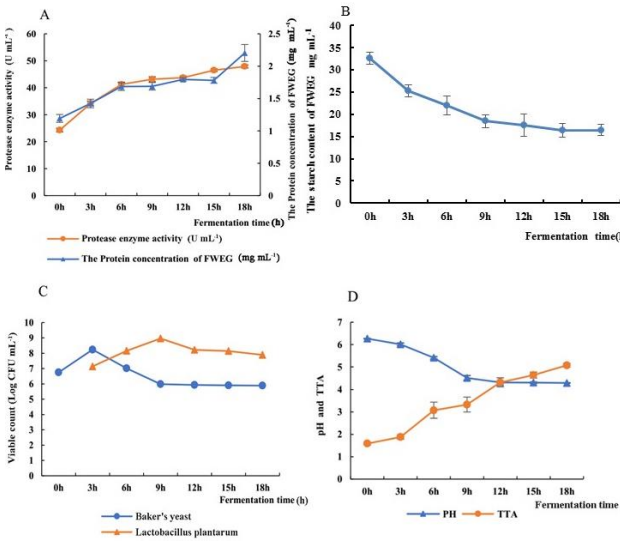

Fig. 3: Effects of the microbial fermentation on the environment. (A: Changes in the protease activity and protein content during the fermentation period. B: Change in the starch content during fermentation period. C: The viable count changes during fermentation period. D: Changes in pH and TTA during fermentation period.)

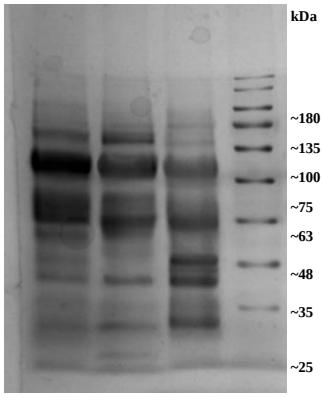

Fig. 4: Changes in the molecular weight during fermentation of FWEG

respectively of the corresponding values before fermentation. The content of free amino acid was significantly enhanced (Table 3).

Structure Change of FWEG during Fermentation

Proteins being chiral molecules show circular dichroism. By determining circular dichroism, the structural characteristics of these compounds can be explained. As the duration of the fermentation was lengthened (Fig. 5), the peaks at 208 nm shifted towards negative spike, whereas the intensity of the peak at 195 nm gradually diminished. These changes suggested the secondary structure of the protein is more regular. The intensity of the positive peak at 195 nm

Table 3: Amino acid composition of wheat embryo protein during fermentation Ug mL^{-1}

	fermentation time (h)		
	0	9	18
Asp	4.96±0.98c	8.59±0.71b	55.90±3.64a
Thr	25.89±5.49a	29.83±0.45a	12.50±0.7b
Ser	13.62±1.33c	262.01±16.11b	488.67±7.85a
Glu	103.25±2.23c	259.73±2.71b	376.44±4.84a
Gly	91.17±0.30b	70.18±0.23c	164.42±1.26a
Ala	34.63±0.37c	73.89±0.05b	204.50±4.29a
Cys	23.65±0.57b	99.20±0.02a	73.18±3.56a
Val	14.37±0.29c	52.47±0.01b	69.41±1.43a
Met	0	13.96±0.07b	17.35±0.02a
Ile	2.33±0.22c	9.25±0.05b	35.40±0.60a
Leu	3.04±0.24c	12.60±0.58b	37.96±0.66a
Tyr	0	6.16±4.38b	43.37±0.28a
Phe	3.85±0.14b	2.97±1.65b	46.55±0.56a
His	73.87±5.17b	172.26±5.61a	25.97±5.17c
Lys	6.23±1.42b	57.16±1.47ab	130.68±1.00a
Arg	218.53±0.18b	142.57±9.7b	968.48±8.92a
Pro	141.62±6.57a	272.34±6.40a	6.48±0.28b
sum	761.02±12.95c	1283.15±26.60b	2757.24±16.50a

Mean ± standard deviation. Values with same letter differ non-significantly ($P > 0.05$)

Table 4: Secondary structural of FWEG samples estimated from circular dichroism spectra

Fermentation	secondary structure			
time/h	α-helical	β- sheet	β-turn	random coil
0 h	0.021±0.0006b	0.377±0.0140a	0.217±0.0074a	0.427±0.0120a
9 h	0.554±0.0170a	0.148±0.0070b	0.163±0.0048b	0.116±0.0039b
18 h	0.554±0.0600a	0.151±0.0045b	0.164±0.0024b	0.118±0.0027b

Mean ± standard deviation. Values sharing same letters differ non-significantly (P>0.05)

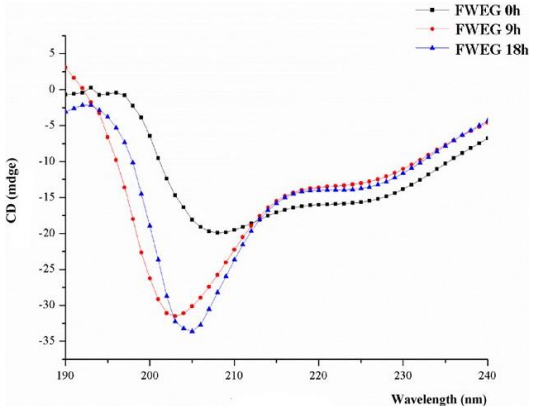

Fig. 5: Circular Dichroism spectrum of FWEG during fermentation

reduction in the CD spectrum of the fermentation sample, and the moderate change in the CD spectrum also suggested that the β-sheet content was reduced. This phenomenon was consistent while analyzing with software CD-Pro. The data of CD were processed by CD-Pro analysis software, and the contents of α-helix, β-folding, β-angle and random coil in FWEG were calculated (Table 4). Compared to WEG after 9 h and 18 h fermentation, the content of α-helix in secondary structure was significantly increased ($P < 0.05$), whereas the content of α-folding, β-turning angle ($P < 0.05$) and random coil ($P < 0.05$) were significantly decreased ($P < 0.05$).

Sulfhydryls (-SH) is an important functional group in wheat embryo protein. The content of the sulfhydryl groups is related to the denaturation of the protein. Changes in the content of sulfhydryl groups would cause corresponding changes in other functional characteristics. The sulfhydryl groups can be divided into those situated at the core of the molecule and those present at the protein surface. The content of the latter was enhanced significantly ($P < 0.01$) and continued to increase as the fermentation proceeded, compared with the samples 0 h (Fig. 6).

The microstructural changes in WEG were observed under a scanning electron microscope during the fermentation process (Fig. 7). The sample fermented for 0 h contained many irregular flaky structures and a few dense and smooth surfaced spherical structures. With the progress of the fermentation, the samples fermented for as long as 9 h did not have smooth surfaces; many holes were present on their surfaces, and they showed irregular flaky structures. At the end of fermentation, the density of pores in the microstructure of the sample significantly increased, and the surface structure was completely destroyed. The structure of FWEG was altered from the original flaky one to a block-like type containing many pores.

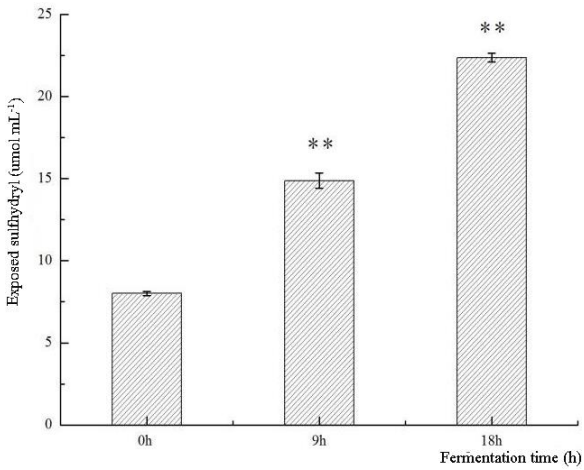

Fig. 6: Exposure to sulfhydryl groups of FWEG during fermentation (∗∗$P < 0.01$, comparing with 0 h.)

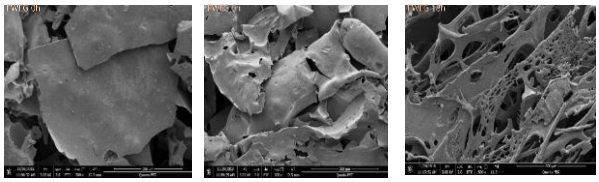

Fig. 7: SEM images of FWEG samples (magnified 500 times)

Discussion

RSM has been successfully employed in optimizing the extraction of polyphenols, polysaccharides, and proteins in plants, and the extract has been correlated to the highest biological activity (Gorguc *et al.*, 2019). The optimum fermentation conditions were determined by a single factor experiment and Box-Behnken experiment design optimization. Under the optimum fermentation conditions, FWEG concentration was 2.09 mg mL^{-1}. This phenomenon also explains the necessity of optimization of the experiment, so that appropriate strains and fermentation conditions were adopted for the reaction (Villarreal-Soto *et al.*, 2018). In an optimized experiment, the increase in protein content may be associated with microbial growth and its secondary metabolites.

In the study, adequate nutrients help microorganisms multiply and produce more protease (Divakar *et al.*, 2018). Yeast fermentation is employed at an early stage because yeast can effectively convert the starch into other nutrients (Yang *et al.*, 2019). After saccharification, starch is transformed into other sugars, organic compounds, *etc*. so as to lay the material basis and provide energy source for the growth of micro microorganisms. Degradation of starch and the reduced interaction between starch and protein might enhance the protein solubility of FWEG (Kasprowicz-Potocka *et al.*, 2016) because, during the fermentation process, the contents of protein and protease increased, starch content decreased, the number of bacteria increased and gradually stabilized. As the fermentation process progressed, TTA of the fermentation environment gradually increased, and pH decreased. Many metabolites were formed due to the growth of *Lactobacillus* spp., while some buffering substances in wheat embryo protein, such as phosphate and protein affected the change of pH value of the fermentation broth. All these phenomena indicated that microbial growth and fermentation changed the composition of the fermentation system.

The protein in the wheat embryo is difficult to be digested or absorbed due to their large molecular weight and compact structure. Fermentation enhanced the small molecular protein, which are more easily absorbed by the body (Lee *et al.*, 2018). Lee et al. reported that *Bacillus subtilis* and *Aspergillus oryzae* caused enhancement in the concentrations of small-size proteins from 5 to 63% and from 5 to 35%, respectively in fermented soybean, while large-size proteins were reduced from 40 to 2% and from 40 to 8%, respectively (Lee *et al.*, 2018). Saccharomycete and lactic acid bacteria produce proteases (peptide enzyme, aminopeptidase, dipeptide enzyme, *etc*.). Proteins were hydrolyzed by those protease that made free amino acids substantial growth in the fermentation system. Wheat embryo was pregnant with endogenous protease which can hydrolyze insoluble protein into soluble protein, polypeptide and amino acid (Mumford *et al.*, 1981). Different proteases cleave at different sites, so the types of peptides and amino acids produced by hydrolysis were also different. In the fermentation process, the macromolecular protein was decomposed into polypeptides with the combined action of endogenous protease and microbial secreted protease, and the polypeptides continued to

hydrolyze into free amino acids along with the action of exopeptidase, thus raising the content of free amino acids. Aspartate a synthetic precursor of Lysine, Threonine and other amino acids as well as purine and pyrimidine based *in vivo*. It could be used as an adjutant in curing heart disease, liver disease and hypertension, and was effective in relieving fatigue (Hansen and Behrman, 2016); Serine had a wide range of medical applications, functioning as fat and fatty acid metabolism boosters or serving as normal immune function maintenance (Abbas, 2017); Leucine and its metabolites had regulatory effects on nutrient metabolism, neuroendocrine regulation, gene expression, signal transduction and immune function *in vivo* (Johnson *et al.*, 2018). As an essential amino acid, Lysine can help drive human development, enhanced immune function, and improved the function of central nervous tissue (Passioura *et al.*, 2019). The increase of these amino acids also indicated that fermentation improved the nutritional characteristics of wheat embryo.

As the fermentation reaction progresses, α-helix content was increased and transformed β-turn into β-sheet. This phenomenon happened because microbial fermentation changed the arrangement of the hydrogen bonds (as α-helix is maintained by hydrogen bonds between peptide chains or peptides) (Zheng *et al.*, 2017). Therefore, secondary structure of wheat embryo turned from disorder to order and in turn altered the original secondary structure of WEG.

Meanwhile, more sulfhydryl groups were exposed as fermentation destroyed the inner structure of protein molecules, unfolded a part of the structure, and exposed the sulfhydryl groups to the surface. Wheat embryo protein hydrolysate showed strong antioxidant capacity and effectively inhibited the formation of sulfhydryl group and disulfide bond (Dordevic *et al.*, 2010). Therefore, the content of the free sulfhydryl group was increased causing protein denaturation.

Scanning electron microscopic images suggested that the surface of wheat embryo protein became uneven as the fermentation progressed, forming some corrosive holes on the surface and damaging the structure. The presence of corroded pores on the surface of the sample indicated that microbial activity degraded the wheat embryo proteins, producing small protein molecules, peptides and amino acids. The accumulation of lactic acid increases the acidity of the media, and the protein surface structure was extremely damaged. The changes in FWEG reactivity by fermentation was not solely associated with changes in the primary structure (degradation) but also related to the changes in the higher structure (Rui *et al.*, 2019). Protein degradation was mediated by both endo- and exopeptidases, which degraded the peptide termini (John *et al.*, 2019). The degradation started at the surface and then migrated inside, exposing free amino acids and mercapto groups, which increased the content of low molecular weight proteins.

Fermentation offers unique nutritional advantages by making the protein of wheat embryo more digestible. The bioactive compounds are reduced, and structures are changed (Worku and Sahu, 2017). In summary, fermentation could dramatically improve the nutritional quality and structural characteristics of wheat embryo globulin, making its absorption easier, and nutritionally enriching the raw materials or food ingredients, which is beneficial for efficient utilization of wheat embryo. Further studies are needed for investigating the possible functional characteristics of FWEG.

Conclusion

Under optimal fermentation condition, *L. plantarum* was inoculated after 3.2 h of *baker's yeast* fermentation, and the fermentation was continued for 14.8 h; the ratio of baker's yeast to *L. plantarum* was 1:2 (V:V). Under this fermentation condition, FWEG protein concentration reached 2.09 mg mL^{-1}, and the fermentation environment changed significantly. The results showed that the nutrient composition improved, and structure changed significantly during fermentation which indicated that FWEG was the development of health food quality natural raw materials.

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