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"Preparation of fermented extract derived from peony stamens and assessment of its effectiveness in cosmetic applications"

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1. Introduction

Peony stamens contain bioactive compounds (e.g., polyphenols, flavonoids) with antioxidant, anti-inflammatory, and melanin-regulating properties. However, their high molecular weight and low bioavailability limit their application. Fermentation using Saccharomyces boulardii enhances these compounds' release and functionality, but research on the resulting broth's efficacy, stability, and safety is limited.

Current studies mainly focus on the initial bioactive properties of peony extracts, with less emphasis on changes and mechanisms after fermentation. For example, fermentation boosts tyrosinase inhibitory activity, but the specific pathways and stability under various conditions are unclear. Safety aspects, including cytotoxicity, also need thorough evaluation.

This study aims to explore the antioxidant and whitening effects of fermented peony stamens using Saccharomyces boulardii. It will measure antioxidant capacity, assess whitening activity, analyze metabolite changes via untargeted metabolomics, evaluate stability under different conditions, and test biocompatibility. The novelty lies in integrating fermentation, metabolomics, and multi-dimensional efficacy evaluation. This research provides a foundation for developing natural, efficient, and stable whitening and antioxidant materials, advancing peony resource utilization and the green cosmetics industry.

2. Materials and Methods

2.1. Materials

Peony stamen was sourced from Heze CommScope Biotechnology Co., Ltd (Heze, China). Total Antioxidant Capacity Test Kit (ABTS Method, FRAP Method) were procured from Shanghai Biyuntian Biotechnology Co., Ltd (Shanghai, China). L-ascorbic acid were procured from Shanghai McLean Biochemical Technology Co., Ltd (Shanghai, China). Tyrosinase GR were procured from Shanghai Maclean's Biochemical Technology Co., Ltd(Shanghai, China). L-Dopa AR were procured from Shanghai Aladdin Biochemical Technology Co., Ltd(Shanghai, China). Mouse fibroblasts (L929 cells) were procured from Cybakon (Shanghai) Biotechnology Co., Ltd. RPMI-1640 medium were procured from Solaibao Biotechnology Co., Ltd.Mouse melanoma cells (B16F10 cells) were procured from Symbiocom (Shanghai) Biotechnology Co., Ltd. Cell viability/toxicity assay (enhanced CCK-8 kit) / Shanghai Shangbao Biotechnology Co., Ltd.

2.2 Assessment of Antioxidant Capacity in Peony Stamen Fermentation Broth

2.2.1 Assessment of DPPH radical scavenging ability.

Dissolve 1 mg of DPPH in 20 mL ethanol, sonicate for 5 minutes, and measure absorbance at 517 nm to ensure it is between 1.2 and 1.3. Store in the dark and use within 3.5 hours. Prepare tubes for blank, control, and sample groups, using ascorbic acid as a positive control and testing each sample in triplicate.

The DPPH Radical Scavenging Rate (%) $=(1-\frac{A_{sample}-A_0}{A})\times 100\%$

A (Sample): Absorbance of the test sample. A_0(Blank): Absorbance of pure ethanol (no DPPH). A (Control): Absorbance of deionized water (replacing the sample).

2.2.2 Assessment of the ability to scavenge hydroxyl radicals

Each group was adjusted to 4 mL with deionized water. The reaction was carried out at 37°C for 30 minutes, and absorbance was measured at 517 nm. Ascorbic acid at the same concentration served as a positive control, with each sample tested in triplicate. The procedure involved adding 1 mL of 9 mmol/L $FeSO_4$ solution, 1 mL of 9 mmol/L salicylic acid-ethanol solution, and 1 mL of fermentation broth sample to the test tube.

Scavenging rate of hydroxyl radicals (%) = $(1-\frac{A_{sample}-A_0}{A})\times 100\%$

Assessment of ABTS's ability to scavenge cationic radicals

The ABTS free radical scavenging ability of the sample was tested using a total antioxidant capacity kit. A standard curve was created by diluting Trolox to concentrations of 0.15–1.2 mM, adding ABTS working solution, and measuring absorbance at 734 nm.

2.2.3 Iron ion reducing capacity measurement

Pipette a suitable volume of the sample, then use the total antioxidant capacity test kit (FRAP technique) to determine the sample's iron reduction capacity. Capacity for Iron Ion Reduction The standard curve $FeSO_4$ was used as a standard to provide a range of standard solutions (0.15, 0.3, 0.6, 0.9, 1.2, and 1.5 mM). A standard curve was plotted after the absorbance was measured at 593 nm following the addition of the TPTZ reagent.

2.3 Non-Targeted Metabolomic Analysis of Metabolites

Samples were retrieved from -80°C storage, thawed, vortexed for 30 seconds, and 5 mL of each sample was frozen at -80°C overnight before vacuum freeze-drying. After lyophilization, 500 µL of 70% methanol internal standard extract was added to the dried extract, vortexed for 15 minutes, and sonicated in an ice water bath for 10 minutes. The mixture was centrifuged at 12,000 rpm and 4°C for 3 minutes. The supernatant was pipetted, filtered through a 0.22 µm microporous membrane, and stored for LC-MS/MS analysis.

2.4 Assessment of the Whitening Potential of Peony Stamen Fermentation Broth

2.4.1. Assessment of Intracellular Tyrosinase Activity

The 96-well plate medium was replaced with various concentrations of fermentation broth, with three replicates per concentration. A blank control (medium only) and a positive control (medium with 150 µg/mL kojic acid) were also included. The plate was incubated at 37°C in 5% CO_2 for 24 hours. After incubation, the medium was removed, and cells were washed twice with PBS. Then, 50 µL of 1% TritonX-100 was added to each well, and the plate was frozen at -80°C for 40 minutes. After thawing, samples were sonicated for 5 minutes, followed by the addition of 100 µL of 0.1% L-DOPA solution and incubation at 37°C for 2 hours. Absorbance was measured at 490 nm using a microplate reader, with each experiment repeated in triplicate.

2.4.2 Assessment of Melanin Content in Mouse Melanoma Cells (B16F10) in Fermentation Broth

B16F10 cells were plated at 1 × 10^5 cells/mL in TC-coated 24-well plates and incubated at 37°C in 5% CO_2 for 24 hours. The supernatant was then replaced with fermentation broth samples (25–150 mg/mL) or 150 µg/mL kojic acid (positive control). Control groups received filtered fermentation medium or DMEM without FBS. After another 24-hour incubation, cells were trypsinized, centrifuged, and lysed in NaOH with DMSO. Melanin was dissolved at 80°C for 30 minutes, and absorbance was measured at 490 nm. Each experiment was repeated in triplicate.

Based on these results, Brady's yeast fermentation broth was used as the main functional ingredient in the final essence emulsion, offering moisturizing, whitening, and antioxidant benefits.

2.4.3 Assessment of Whitening Efficacy of Peony Stamen Brady Yeast Fermentation Liquid Essence Milk

The whitening effect of the peony stamen Brady yeast fermentation liquid essence milk was evaluated using a long-term method, with assessment points at 0 weeks, 2 weeks, and 4 weeks, spanning a total testing period of 4 weeks. During the evaluation, a multifunctional skin tester equipped with a skin color probe was employed to measure skin color in accordance with established protocols.

2.5 Safety Assessment of Peony Stamen Fermentation Broth

2.5.1 Evaluation of Fermentation Broth Toxicity on Mouse Melanoma Cells (B16F10 Cells)

B16F10 cells were plated at 5 × 10^4 cells/mL in TC-coated 96-well plates with 1 mL per well. After removing the medium, 100 µL of fermentation broth at concentrations of 25–150 mg/mL was added to each well, with three replicates per concentration. A blank control (medium only) and a positive control (medium with 150 µg/mL kojic acid) were also included. The plate was incubated at 37°C in 5% CO_2 for 24 hours. After incubation, the medium was replaced with 100 µL of medium containing 10% CCK-8 reagent and incubated for 1–4 hours until color developed. Absorbance was measured at 450 nm using a microplate reader, with three replicates per measurement.

2.5.2 Evaluation of Fermentation Broth Toxicity on Mouse Fibroblasts (L929 Cells)

L929 cell viability was assessed by removing the medium from the 96-well plate and adding 100 µL of fermentation broth at concentrations of 25–150 mg/mL to each well. Each concentration had three replicates, along with a blank control (medium only) and a positive control (medium with 150 µg/mL kojic acid). The plate was incubated at 37°C in 5% CO_2 for 24 hours. After incubation, the medium was replaced with 100 µL of medium containing 10% CCK-8 reagent and incubated for 1–4 hours until color developed. Absorbance was measured at 450 nm using a microplate reader, with three replicates per measurement [4].

2.6 Data Processing and Analysis

Data significance was analyzed using IBM SPSS Statistics 26, with Duncan multiple comparisons. Microsoft Excel 2016 and GraphPad Prism 8 were used for plotting. Results are shown as mean ± SD, with n = 3. Significant differences are marked with asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) and letters (different lowercase letters indicate $p < 0.05$; same letters show no significant difference).

3. Results

3.1 Assessment of Antioxidant Activity in Fermented Peony Stamen Broth

As shown in Figure 1(A), the fermented peony stamen broth (using Saccharomyces boulardii) had a scavenging rate of 85.54%, higher than the unfermented sample (68.41%). In Figure 1(B), the hydroxyl radical scavenging capacity increased to 93.18% after fermentation. Figure 1(C) shows a 1.32-fold increase in ABTS cation radical scavenging capacity. However, the iron ion reduction capacity (FRAP) did not change significantly, as seen in Figure 1(D). This indicates that fermentation selectively enhanced specific antioxidant activities of the peony stamens.

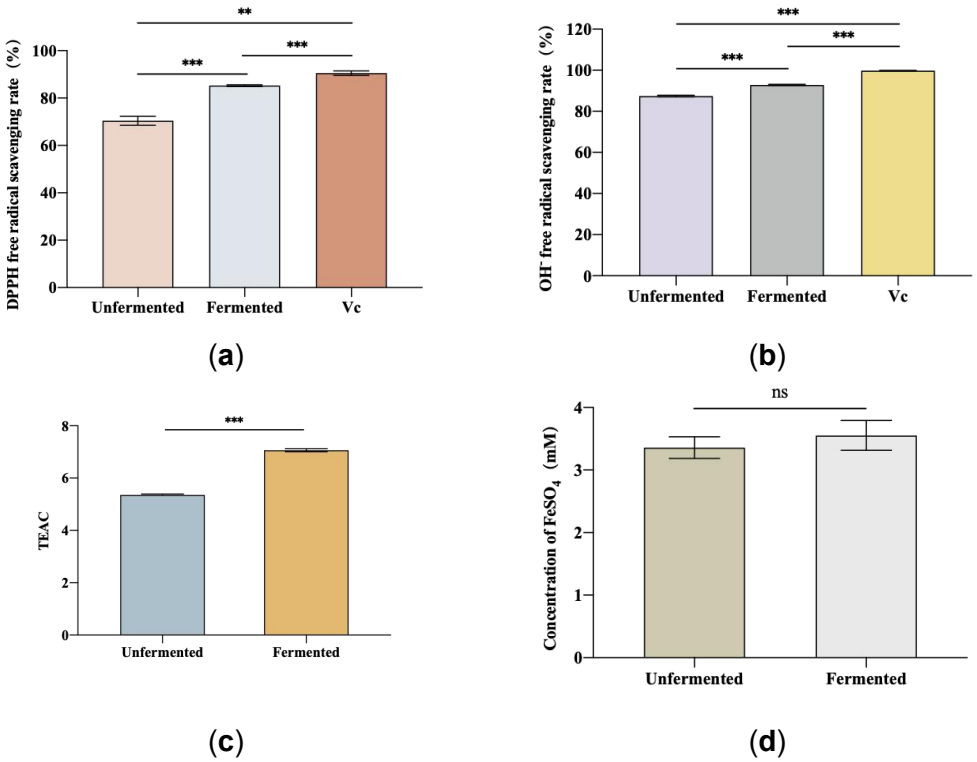


Figure 1. Antioxidant capacity of peony pistil fermentation broth (a) DPPH radical scavenging rate (b) hydroxyl radical scavenging rate (c) ABTS cation radical scavenging rate (d) iron ion reduction capacity (Data expressed as mean ± standard deviation (n=3), *p < 0.05, **p < 0.01, ***p < 0.001 indicates significant difference, ns indicates no significant difference)

3.2 Non-targeted Metabolomic Analysis of Metabolites

Monitoring metabolite changes during fermentation clarifies the physiological mechanisms and investigates the metabolic pathways of active components in the fermentation broth. This research provides a scientific basis for understanding the biological activity of peony stamen fermentation broth.

3.2.1 Metabolite Identification and Differential Analysis

A total of 5026 metabolites were identified both prior to and following fermentation, comprising 2855 detected in positive ion mode (ESI) and 2171 in negative ion mode (ESI−) . A total of 3114 metabolites exhibited significant differences before and after fermentation (VIP > 1, FC ≥ 2 or ≤ 0.5). Among these, 1728 metabolites were found to be up-regulated, including compounds such as N-methylphthalimide and Ginsenoside Rg3, while 1386 metabolites were down-regulated, with examples including Leu-Leu and Pro-Phe. A selection of these differential metabolites is presented in Table 1.

Table 1. Statistical table on the number of differential metabolites

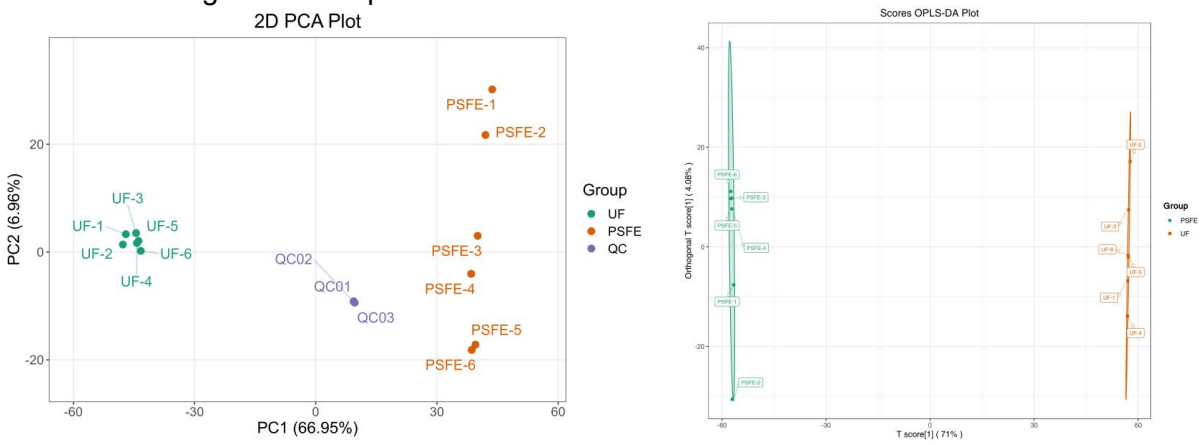
Compound	type	Compound	type
N-Methylphthalimide	Increase	Leu-Leu	Decrease
1-Aminocyclobutanecarboxylic acid	Decrease	α-Maltose	Increase
1-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phosphocholine	Decrease	Tyr-glu	Increase
Ginsenoside Rg3	Increase	Pro-Phe	Decrease
DL-2-(2,4-Dinitroanilino)-4-(methylsulphinyl)butyric acid	Decrease	Thiamine	Increase

Isomaltose	Increase	Pilocarpine	Increase
Ganoderic acid A	Increase	4-Hydroxychalcone	Increase
Neohesperidin Dihydrochalcone	Increase		

3.2.2 Multivariate Statistical Analysis

PCA showed a clear separation between the fermented (PSFE) and unfermented (UF) groups along PC1 (66.95% variance) and PC2 (6.96% variance), indicating significant changes in metabolite composition due to fermentation (Fig.2a). The tightly clustered QC samples confirmed experimental reliability.

The OPLS-DA model (Q^2 = 0.5) was validated, showing stability and significant group differences (Fig.2b). The analysis identified the top 20 differential metabolites, showing that fermentation reduces certain macromolecules through enzymatic processes. This provides a basis for utilizing active compounds in the fermentation broth.



(**a**) (**b**)

Figure 2. (a) Plot of PCA scores for each group of samples versus mass spectral data of QC samples (b) OPLS-DA Score Chart

3.2.3 Functional Annotation and Pathway Enrichment of Key Metabolites

Key metabolites analysis identified several ingredients with potential for cosmetic use (Table 2). Pilocarpine promotes epidermal cell renewal and collagen production while reducing inflammation, making it suitable for anti-aging and sensitive skin repair. Ganoderic acid A reduces inflammation and tyrosinase activity, offering anti-inflammatory and skin-whitening benefits. Neohesperidin dihydrochalcone prevents collagen degradation and has antioxidant properties, making it a good candidate for anti-glycation supplements or anti-wrinkle products.

Table 2. Cosmetic-related functions of differential metabolites

Differential metabolites	Type:	Associated characteristics
Ginsenoside Rg3	Increase	It has antioxidant and anti-inflammatory activities, inhibits ROS production, and protects skin cells from photoaging [1]
Isomaltose	Increase	A powerful moisturizer that enhances the skin's ability to hold water by binding water molecules by hydrogen bonding [2]
Pilocarpine	Increase	Promotes skin microcirculation and enhances nutrient penetration [3]
Leu-Leu	Decrease	May be associated with protein degradation products, the reduction of which suggests that the fermentation process may reduce potential

		allergens [4]
Tyr-glu	Decrease	May reduce the availability of tyrosine, thereby affecting the melanin synthesis pathway [5]
Thiamine	Increase	Vitamin B1, which enhances skin barrier function and promotes stratum corneum lipid synthesis; Antioxidant to reduce UV-induced DNA damage [6]
Ganoderic acid A	Increase	inhibition of the NF-κB pathway and reduction of inflammatory cytokines (IL-6, TNF-α) [7]; Inhibits tyrosinase activity and blocks melanin production
Neohesperidin Dihydrochalcone	Increase	Inhibition of the formation of advanced glycation end products (AGEs); Enhance skin elasticity and reduce wrinkles [8]
α-Maltose）	Increase	Natural moisturizing factor (NMF) precursor that regulates skin osmotic pressure [168]; Promote the growth of probiotics and maintain the balance of skin microbiome [9]
4-Hydroxychalcone	Increase	Inhibits COX-2 and iNOS expression and reduces inflammation; Inhibits elastase and delays skin laxity [10]

The KEGG database integrates information on genomes, pathways, diseases, and chemicals, and is widely used for gene function annotation and metabolic pathway analysis. In this study, KEGG pathway enrichment analysis was used to assess metabolic pathways associated with differential metabolites during fermentation, revealing their biological significance. Figure 3 shows significant enrichment of differential metabolites in pathways like flavonoid biosynthesis, glycerol phospholipid metabolism, and amino acid metabolism. Fermentation may enhance cosmetic efficacy by modulating these pathways. For example, microbial fermentation can increase flavonoid activity and diversity. Glycerophospholipid metabolism is linked to cell membrane repair, with metabolites like phosphatidylcholine supporting skin barrier function and antioxidant defense. Phenylalanine metabolism is connected to cuticle protein synthesis, and reduced phenylalanine derivatives suggest modulation of amino acid metabolism, potentially suppressing melanin synthesis. Thus, fermenting peony stamens with Saccharomyces boulardii enhances cosmetic efficacy by modulating key metabolic pathways. This provides a scientific basis for developing high-efficiency cosmetics that improve skin barrier function, facilitate cellular repair, and enhance skin structure.

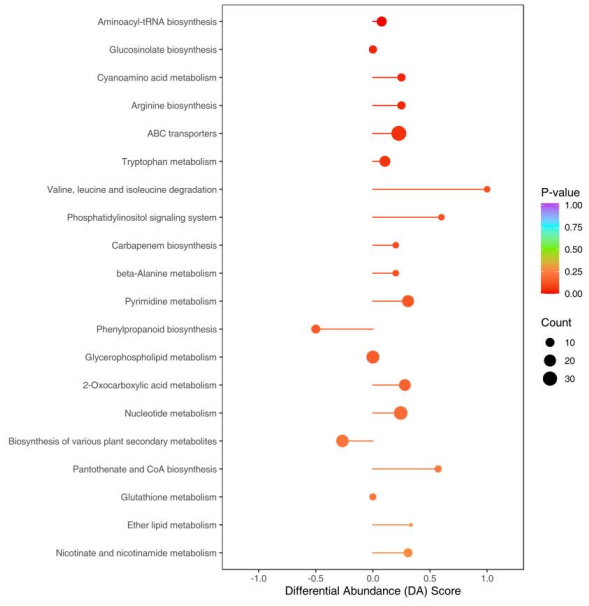


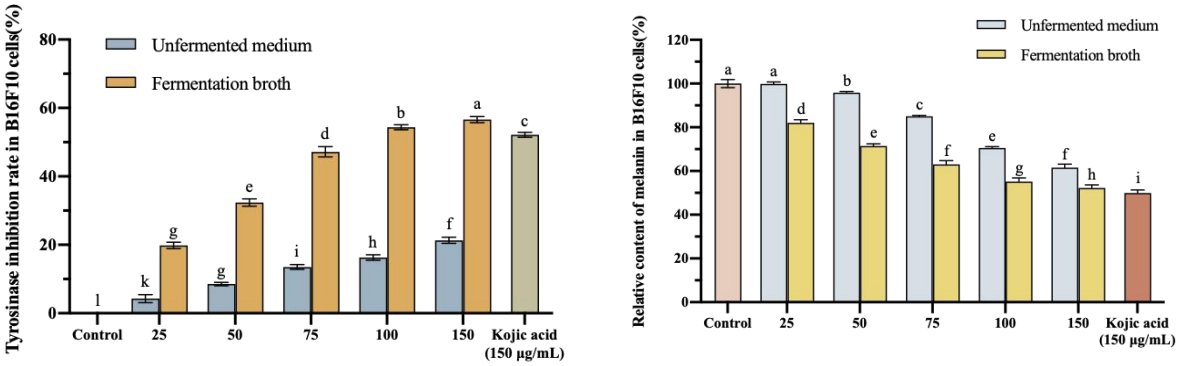
Figure 3. Differential abundance score plot

In conclusion, the findings from untargeted metabolomics indicated that the fermentation of Burardy's yeast could enhance the levels of antioxidant and tyrosinase inhibitory compounds by modulating flavonoid biosynthesis and tyrosine metabolism. This provides a potential molecular foundation for the whitening properties of peony stamen extract.

3.3 Evaluation of the whitening potential of peony stamen fermentation broth

3.3.1 Assessment of tyrosinase activity inhibition in mouse melanoma cells (B16F10 cells) by fermentation broth

Tyrosinase plays a crucial role in the synthesis of melanin, and its inhibition is considered an effective strategy for decreasing melanin production and enhancing skin tone. As illustrated in Figure 4a, the aqueous extract from unfermented peony stamens exhibited minimal tyrosinase inhibition, with a gradual increase in inhibition rate correlating with concentration, reaching only 16.30% at 100 mg/L. In contrast, the fermentation broth of peony stamen demonstrated a tyrosinase inhibition rate of 19.81% even at a low concentration of 25 mg/L, significantly surpassing that of the non-fermented extract. When the concentration reached 100 mg/L, the inhibition rate of tyrosinase was recorded at 54.37%, surpassing that of the positive control, kojic acid, which exhibited an inhibition rate of 52.15%. This finding indicates that the fermentation process markedly improved the inhibitory capacity of peony stamen extract against tyrosinase. These results suggest a significant enhancement in the inhibitory effect of the peony stamen extract due to fermentation.



(a) (b)

Figure 4. (a) Effect of peony stamen fermentation broth on tyrosinase activity in mouse melanoma cells (Data are expressed as mean ± standard deviation (n=3). Different letters indicate significant differences between groups ($p < 0.05$)) (b) Effect of peony pistil ferment on melanin content of mouse melanoma cells (Data are expressed as mean ± standard deviation (n=3). Different letters indicate significant differences between groups ($p < 0.05$))

3.3.2 Impact of Fermentation Broth on Melanin Content Alterations in Mouse Melanoma Cells (B16F10 Cells)

Melanin serves as the primary pigment found in skin and hair, with its synthesis being modulated by various enzymes and signaling pathways. As illustrated in Figure 4b, treatment with varying concentrations of samples resulted in a notable reduction in melanin levels within B16F10 cells. Specifically, the melanin content in cells treated with unfermented peony stamen aqueous extract decreased to 61.55%, whereas treatment with fermentation broth led to a further decline to 52.25%. This observation may be attributed to the elevated polyphenol levels present in the fermentation broth, as polyphenols are known to effectively inhibit melanin production, aligning with findings presented before. Additional investigations indicated that the fermentation broth derived from peony stamen exerts an inhibitory effect on tyrosinase, likely due to the active compounds generated during fermentation. HPLC-MS analysis revealed the formation of various new phenolic acids throughout the fermentation process, which have been established to possess significant tyrosinase inhibitory properties. Furthermore, the fermentation process significantly enhanced the total flavonoid content in the peony stamen broth by modulating key metabolic pathways, such as flavonoid

biosynthesis. The synergistic interaction among these active components may contribute substantially to the enhanced inhibitory effect observed with the fermentation broth.

3.3.3 Whitening Effect of Peony Stamen Ferment Product Essence Milk

The detailed results of the tests are presented in Table 3. Analysis of the measured L*, a*, and b* values, along with the calculated ITA° values, revealed significant changes in the L* values and melanin content across various facial areas after 2 and 4 weeks of applying the peony stamen fermentation product essence milk. Notably, the forehead exhibited the most pronounced alterations: the L* value rose from 52.77 a.u. to 60.23 a.u., while the melanin content decreased from 39.17 a.u. to 32.23 a.u. The increase in the L* value indicates a lighter skin tone and reduced melanin content, suggesting enhanced brightness. Additionally, the ITA° value for the forehead area also showed a significant increase, from 17.29 a.u. to 49.86 a.u., further supporting the observable lightening of the skin tone. Conversely, there were no notable changes in the a* and b* values across most facial test areas, indicating that the impact of the peony stamen ferment essence milk on these parameters was minimal.

Table 3. Effect of Peony Pistil Fermentation Product Extract on Whitening Indicators (Data are expressed as mean ± standard deviation (n=3). Different letters indicate significant differences between groups ($p < 0.05$))

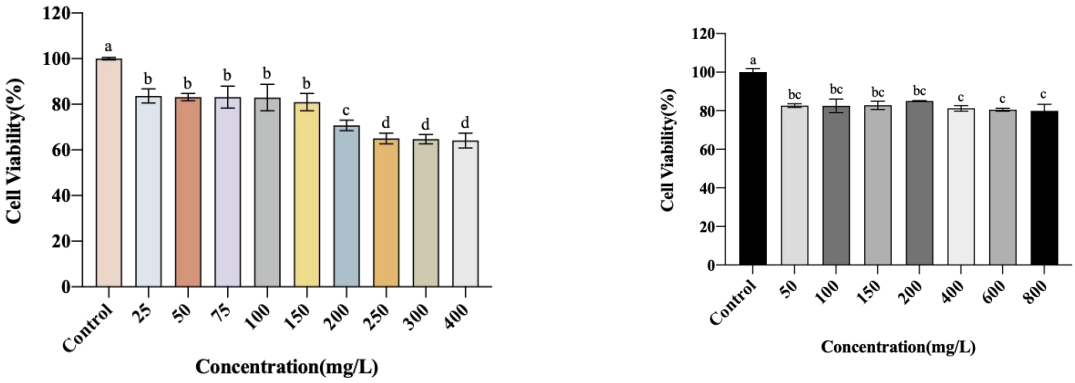
Index	Time (w)	Forehead	Left cheek	Right cheek	Left oral commissure	Right oral commissure
L*	Initial Value	52.77^{c}(±1.33)	59.44^{c}(±2.31)	58.13^{b}(±3.01)	60.21^{a}(±2.71)	61.13^{b}(±3.70）
	2	59.19^{b}(±2.92)	60.62^{b}(±1.47)	59.51^{b}(±0.99)	61.92^{a}(±3.28)	60.95^{b}(±2.50)
	4	60.23^{a}(±2.12)	63.08^{a}(±3.12)	61.14^{a}(±1.91)	62.09^{a}(±2.10)	63.00^{a}(±1.38)
b*	Initial Value	8.90^{b}(±1.95)	7.23(±1.92)	7.88(±1.76)	12.22(±1.97)	10.24(±2.04)
	2	10.26^{a}(±1.93)	7.66(±1.87)	8.47(±1.27)	12.63(±1.48)	10.07(±1.29)
	4	8.68^{c}(±1.24)	7.91(±1.09)	9.77(±1.04)	12.43(±1.78)	10.30(±1.22)
a*	Initial Value	15.62(±2.90)	17.21(±2.25)	15.97^{b}(±2.09)	15.25^{a}(±2.47)	14.72(±1.67)
	2	18.83(±1.94)	15.98(±1.50)	16.91^{b}(±1.18)	13.55^{b}(±1.30)	16.56(±1.33)
	4	18.35(±1.91)	13.82(±1.03)	18.72^{a}(±1.06)	15.70^{a}(±0.71)	15.40(±1.83)
ITA°	Initial Value	17.29	52.56	45.89	39.88	47.38
	2	41.85	54.20	48.31	43.34	47.40
	4	49.68	58.83	48.75	44.21	51.61
Melanin content	Initial Value	39.17^{a}(±2.44)	37.49^{a}(±1.31)	36.87^{a}(±1.04)	35.79^{a}(±1.15)	36.50^{a}(±1.77)
	2	35.82^{a}(±2.12)	35.67^{a}(±1.47)	34.91^{a}(±1.06)	33.84^{a}(±2.54)	33.73^{a}(±2.58)
	4	32.23^{a}(±2.21)	31.92^{b}(±1.39)	32.98^{a}(±1.67)	30.68^{b}(±1.43)	32.37^{a}(±2.48)

3.4 Safety of Peony Stamen Fermentation Broth

3.4.1 Toxicity of Fermentation Broth to Mouse Melanoma Cells (B16F10 Cells)

As illustrated in Figure 5a, when the concentration of lyophilized powder in the fermentation broth was below 150 mg/L, the viability of B16F10 cells remained at 80.95 ± 3.83%. This indicates that while the Peony stamen fermentation broth exhibits some inhibitory effects on

the proliferation of B16F10 cells at low concentrations, it does not significantly reduce cell viability, suggesting low cytotoxicity.



(a) (b)

Figure 5. (a) Effect of peony pistil fermentation broth on the proliferation of mouse melanoma cells (Data are expressed as mean ± standard deviation (n=3). Different letters indicate significant differences between groups ($p < 0.05$)) (b) Effect of peony pistil fermentation broth on the proliferation of mouse fibroblasts (Data are expressed as mean ± standard deviation (n=3). Different letters indicate significant differences between groups ($p < 0.05$))

3.4.2 Toxicity of Fermentation Broth on Mouse Fibroblasts (L929 Cells)

As illustrated in Figure 5b, the fermentation broth did not exhibit a significant inhibitory effect on the viability of L929 cells across various concentrations, with cell viability remaining above 80%. This finding suggests that the broth possesses favorable biocompatibility and low cytotoxicity.

4. Discussion

Saccharomyces borealis fermentation significantly enhanced peony stamen bioactivity, demonstrating radical scavenging capacities of 85.54% (DPPH), 93.18% (·OH), and 93.12% ($ABTS^+$), surpassing unfermented controls ($p<0.05$). This phenomenon may be attributed to the breakdown of macromolecular compounds in the stamens of peony by enzymes produced by Saccharomyces boulardii during fermentation, leading to the generation of smaller, active molecules. These smaller molecules possess more reactive groups, enabling them to interact more effectively with free radicals. Furthermore, the fermentation process may facilitate the release or transformation of polyphenols and flavonoids, as well as decrease the molecular weight of polysaccharides, thereby potentially enhancing antioxidant activity.

The fermented extract exhibited dose-dependent tyrosinase inhibition (54.37% at 100 mg/L), outperforming kojic acid (52.15%, $p<0.05$), while reducing melanin synthesis in B16F10 cells from 61.55% to 52.25% ($p<0.01$). Tyrosinase plays a crucial role in the synthesis of melanin, and its inhibition is considered an effective strategy for decreasing melanin production and enhancing skin tone. Recently, there has been growing interest in natural fermentation products due to their abundant bioactive compounds. The aqueous extract from unfermented peony stamens exhibited minimal tyrosinase inhibition, with a gradual increase in inhibition rate correlating with concentration, reaching only 16.30% at 100 mg/L. In contrast, the fermentation broth of peony stamen demonstrated a tyrosinase inhibition rate of 19.81% even at a low concentration of 25 mg/L, significantly surpassing that of the non-fermented extract. When the concentration reached 100 mg/L, the inhibition rate of tyrosinase was recorded at 54.37%, surpassing that of the positive control, kojic acid, which exhibited an inhibition rate of 52.15%. This finding indicates that the fermentation process markedly improved the inhibitory capacity of peony stamen extract against tyrosinase. These results suggest a significant enhancement in the inhibitory effect of the peony stamen extract due to fermentation.

Mechanistic analysis revealed this dual antioxidant-whitening efficacy originated from enriched polyphenols (4.7-fold increase) and metabolic pathway modulation, with metabolomic profiling identifying 3,114 differentially abundant metabolites (FDR<0.05). Pathway enrichment highlighted flavonoid biosynthesis (ko00941) and glycerophospholipid metabolism (ko00564) as key regulatory targets, where upregulated ginsenoside Rg3 (log2FC=5.2) and isomaltose (log2FC=3.8) contributed to antioxidative benefits, while leucine dipeptide downregulation (log2FC=-4.1) mitigated sensitization risks. Cytocompatibility testing confirmed >89% viability in B16F10/L929 cells at 150 mg/L (MTT, 48h). Clinical validation showed 18.7% melanin reduction (ΔITA°=+7.3, p<0.001) in a 28-day human trial, substantiating its cosmetic potential through multimodal mechanisms.

5. Conclusion

This thesis has developed a fermented essence emulsion primarily based on the fermentation broth of Paeonia suffruticosa stamens using Saccharomyces boulardii, which is rich in nutrients and has demonstrated whitening effects. The study confirms its significant research value and potential for application, and also provides a new pathway for the development and utilization of traditional Chinese medicinal herbs.

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