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An investigation of the antioxidant, whitening and acne-removing effect of blueberry-rose compound ferment in cosmetic field

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Abstract:

On account of the increasing emphasis given to appearance, the modern society is witnessing the booming market of cosmetics. Therefore, in order to cater to consumers' demand, finding and researching for a suitable and effective cosmetic ingredient is essential. This project will evaluate the efficacy of an ingredient called ferment, which refers to the functional microbial fermentation products. Owning to its abundant nutrients, ferment is expected to have very comprehensive efficacy.

In this study, different proportions of blueberries and roses were mixed as raw materials, *Pediococcus acidilactici* and *Metschnikowia pulcherrima* were added for fermentation. The five types of ferment solution to be tested include (1)100% blueberry (2)70% blueberry and 30% rose (3)50% blueberry and 50% rose (4)30% blueberry and 70% rose (5)100% rose.

The first efficacy of ferment to be investigated is the antioxidant activity, DPPH free radical clearance rate test will be carried out. The result of this experiment shows that all five ferment has DPPH clearance rate higher than 90%, indicating remarkable antioxidant activity. The following experiment involves testing tyrosinase inhibition rate. The result shows that all five ferment has tyrosinase inhibition rate in the range from 20% to 40%, indicating certain whitening effect. Finally, by measuring the diameter of bacterial inhibition ring, the acne-removing effect of ferment will be evaluated. The experimental result reveals that the ferment has weak effect on inhibiting *Staphylococcus aureus* but certain effect on inhibiting *Escherichia coli*.

All experiments have proved that ferment is more suitable for compound fermentation, as most compound ferment shows better antioxidant and antibacterial effects than single-material ferment. Among all five types of ferment, ferment 4 (70% rose and 30% blueberry) has the most comprehensive ability, since it ranks at the top twice and the second twice in four experiments.

Keywords: ferment, blueberry, rose, antioxidant activity, whitening effect, acne-removing, fermentation

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1. Introduction

Ferment refers to the functional microbial fermentation products, containing abundant vitamins, amino acids, enzymes, minerals and secondary metabolites and other nutrients. Ferment is produced by one or more kinds of fresh vegetables, fruits, mushrooms or Chinese herbs after the fermentation by a variety of probiotics. By intermediary metabolism or cross metabolism, raw materials produce many complex metabolite and new bioactive components, including more than 100 kinds of new biological enzymes [18]. Therefore, ferment not only preserves the original nutrients of the raw materials, but also contains some new bioactive components through fermentation metabolism.

The various nutrients containing in ferment are beneficial to the human body, such as polyphenols (flavanols, flavonoids, anthocyanins, catechins, phenolic acids, styrene, etc.), organic acids (gallic acid, succinic acid, pyruvic acid, malic acid, etc.) and a variety of sugars [28]. According to the research, ferment has numerous effects. For example, it can inhibit the formation of melanin, remove acne, and delay the skin aging by clearing free radicals in the skin. In the past decade, ferment has been popular in Japan, the United States and some European countries because of its significant effects.

In modern society, more and more people begin to pursue beautiful appearance, such as fair and smooth skin. Consequently, products with good antioxidant, whitening and acne-removing efficacy have been widely favored and concerned by people. In terms of skin oxidation, the excess free radicals, such as DPPH, can induce the denaturation of collagen and elastin in our skin, which leads to wrinkles, brown spots, cutis laxa and other signs of senility [5,20]. When it comes to skin whitening, the color of human skin is mainly determined by the amount of melanin. Since tyrosinase is the primary catalyst during the formation of melanin, it is necessary inhibit tyrosinase activity to have fair skin. As for acne treatment, the proliferation of acne-related pathogens is a vital cause for the growth of acne. Therefore, as a nutrient-rich microbial fermentation product, ferment has the ability to remove free radicals, inhibit tyrosinase activity, and inhibit bacteria [3], which determines its high research value in the field of cosmetics.

In China, rose is considered to have high medicinal value. Rose petals are rich in flavonoids, polyphenols, and anthocyanins. Among them, anthocyanins possesses certain natural antioxidant capacity. Rose flowers have been widely used in food, wine, tea, cosmetics and traditional Chinese herbs. According to the relevant literature, eating rose has the effect of promoting blood circulation, removing blood stasis and nourishing skin. Blueberry fruit is rich in nutrition as well, in addition to sugar, protein, fat, organic acids, it is also rich in anthocyanins, polyphenols, vitamin C, vitamin E, minerals and a variety of trace elements. As a result, blueberry has earned the title “king of berries”, and it is also one of the five healthy fruits recommended by Food and Agriculture Organization.

In China, the majority of studies related to ferment focus on only one type of fruit in the food industry, while there is little research about ferment in the field of cosmetics, especially whitening and antibacterial efficacy.

Therefore, in this study, different proportions of blueberries and roses were mixed as raw materials, *Pediococcus acidilactici* and *Metschnikowia pulcherrima* were added for fermentation to prepare the blueberry-rose compound ferment. The antioxidant, whitening and acne-removing effect of each compound ferment will be measured and evaluated. The aim of this study is to develop a compound ferment with comprehensive efficacy, making up for the gap of ferment in the field of cosmetics.

2. Literature Review

2.1 History of ferment

In 1985, the father of the ferment, American medical doctor Edward Howell first proposed the “ferment nutrition theory”. The discovery of ferment is also considered as a vital milestone in the field of human life science [13].

Ferment research was first launched in Japan at the beginning of the 20th century. At that time, ferment was given high value in Japan and soon entered the application stage, enabling Japan to occupy the main market for ferment. Since Japanese ferment is currently the most advanced and complex one at home and abroad, many countries tried to introduce ferment from Japan to learn about this technology [1]. Besides Japan, ferment has also gradually become prevalent in the United States, Europe and other places these years [2,12,27]. In the 1980s, people began to use modern biotechnology to extract ferment that is beneficial to the skin and add it to cosmetics to improve skin conditions. Therefore, the status of ferment in the field of cosmetics is gradually improving.

2.2 Ferment efficacy

2.2.1 Antioxidant activity

Most human diseases have been shown to be associated with reactive oxygen species and free radicals, including cancer, diabetes and arteriosclerosis. If excessive free radicals are produced, the internal balance system of the body will be broken, thus triggering oxidative stress reaction, resulting in cell damage and even cellular death [9].

In the study of Fan [6], in order to study the antioxidant activity changes in the fermentation process, rose was used as the main raw material, yeast and acetic acid bacteria were added during fermentation. The total phenol concentration and DPPH free radical clearance were determined as the indexes of antioxidant changes. It is registered that the total phenol concentration of rose ferment was 88 mg/mL and DPPH free radical scavenging rate was 85%, under the conditions that the proportion of rose ferment was 8%, yeast was 0.8%, acetic acid bacteria was 1%, and white sugar was 16%, after fermentation at 28 ℃ for 45 days.

Jayabalan *et al*. [11] investigated the fermentation period of Kombucha ferment, whose results showed that Kombucha ferment had a high antioxidant activity, and the antioxidant activity increased with fermentation time. The reason for its high antioxidant activity is due to the microorganisms involved in fermentation, mainly including yeasts and bacteria, which process the substrate structure of enzymes.

Similarly, Jiang [12] also studied the changes of antioxidant ability of blueberry ferment during natural fermentation. The results indicated that blueberry ferment showed high antioxidant properties during fermentation, with an increasing trend over time, which was closely related to the proportion of phenolic substances.

According to Dong *et al*. [4], Pitaya ferment which contains abundant nutritional ingredients had strong scavenging ability of superoxide anion free radical, hydroxyl free radical and DPPH free radical. It is also tested that the activity of SOD in the ferment was 300U/mL, with certain activities of amylase and lipase.

In the research from Li *et al*. [15], the rank of the concentrations of 4 main antioxidant components in ferment from highest to lowest was: ascorbic acid, anthocyanidin, flavone and polyphenol. Compared with pitaya ferment, walnut peel ferment showed stronger antioxidant ability but poor chelating ability to metal ions.

2.2.2 Whitening effect

Referring to the research of Tanaka *et al*. [22], plum ferment decomposition, Clairju, effectively inhibited the phagocytosis of keratinocytes. In human experiment, the melanin index of the skin on which the products containing Clairju was applied dropped obviously, the improvement of the pigmented spots was also effective.

According to the experiment of Ren *et al*. [21], in vitro and in vivo experiments, the ferment had obvious whitening and anti-aging effects. The inhibition rates of 1%, 2% and 5% paste ferment on tyrosinase were 88.41%, 96.35% and 99.87% respectively. The antioxidant experiment and skin vein experiment also proved that the ferment had good antioxidant and anti-aging ability.

2.2.3 Antibacterial activity and acne removing effect

Ferment has been widely proven by research to have antibacterial effects. Dong *et al*. [3] conducted experiments on the bacteriostasis of pasty and the powder microbial ferment. The research showed that the pasty ferment had high inhibition rates on *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, especially on acne-related pathogens. Besides, both of the pasty and powder microbial ferment had the ability of anti-germ.

Ferment contains a large amount of protease and lipase, which, as cosmetic additives, can effectively clean the excessive sebum secreted by sebaceous gland, prevent it from being blocked, and remove the breeding environment of acne-related pathogens. As a result, ferment can effectively prevent acne generation [16].

In addition, Xia [24] measured the antibacterial activity and stability of rose ferment. Using *Escherichia coli* and *Staphylococcus aureus* as indicator bacteria, the antibacterial activity of rose ferment was determined by Oxford cup method. The experimental results showed that rose ferment had certain inhibitory effect on *Escherichia coli* and *Staphylococcus aureus*, in which the inhibition effect on *Staphylococcus aureus* was better.

2.3 Existing studies on ferment production

Mo and Wu [17] investigated the best process of preparing rose ferment by pure fermentation. The results showed that rose ferment with the highest SOD activity could be obtained by fermentation of yeast and lactic acid bacteria.

In a very valuable article of Xia [24], taking SOD activity and total phenol concentration as indexes, the optimal fermentation process was as follows: Add sucrose 17.8g, Rose 14g, lactose 4g, under the optimal conditions (fermentation time 68h, initial pH5.4, inoculum 0.67%, temperature 32 ℃). Thus, the SOD enzyme activity of the rose ferment obtained was 132 U/mL

2.4 Gaps in existing research and technology

Although rapid development and remarkable research results have been achieved, compared with the world's cutting-edge technology, our research on ferment is still relatively backward. The majority of studies on ferment currently focus on fermentation process and application in food industry, which means that there are many secrets about ferment that are worthy of our exploration. Firstly, it is still unclear how the bioactive ingredients in ferment are generated, and the methods to increase their concentration still need to be explored. Besides, since the fermented products appearing on the market mostly use fruits and vegetables as raw materials, the types of fer-

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ment are scarce. Additionally, there are few researches on the antibacterial ability and whitening effect of ferment as well as the application of ferment in cosmetics field. Moreover, only a few researchers have studied the mechanism behind the efficacy of ferment.

3. Methodology

3.2.1 preparation

Blueberry ferment, rose ferment, *Pediococcus acidilactici*, *Metschnikowia pulcherrima*, white granulated sugar, 5* conical flask, centrifugal tube, incubator, super clean bench, inoculating loop, UV Spectrophotometer, pipette, 1mL cuvette, homogenizer, Shaker, anhydrous ethanol, DPPH ethanol solution, Autoclave, Sterilized glass rod, refrigerator, Sterilized saline, Oxford Cup, *Staphylococcus aureus*, *Escherichia coli*, Nutrient Ager(NA) medium, Nutrient Broth(NB) medium, MRS medium, YPD medium,ph6.8 PBS buffer, L-tyrosine solution, 5kU tyrosinase, water bath kettle, volumetric flask, balance

3.2 Prepare five types of ferments

Table 1 Composition of five ferment

Number	composition	blueberry ferment/mL	water/mL	rose ferment/mL	total volume/mL
1	100% blueberry	50	150	0	200
2	100% rose	35	150	15	200
3	70% blueberry + 30% rose	25	150	25	200
4	50% blueberry + 50% rose	15	150	35	200
5	30% blueberry + 70% rose	0	150	50	200

3.3 Further Fermentation

Firstly, under sterile conditions, add white granulated sugar to each type of ferment until the concentration of sugar (by mass) reached 6.4%. After sterilization, inoculate 0.15% of the *Pediococcus acidilactici* and *Metschnikowia pulcherrima* by volume. All five flasks of ferment are under fermentation for 1 day under 37 degree Celsius. Subsequently, filter with gauze and centrifuge the ferment. The supernatant obtained is transferred into bottles and sealed.

Figure 1 Five bottles of ferment solution after further fermentation

3.4 Antioxidant activity test (DPPH clearance rate)

According to Li [14], the experimental methods are shown below.

3.4.1 Take 2mL of the sample solution and transfer it to a 10mL centrifugal tube, then add 2mL of 0.2mmol/L of DPPH ethanol solution, mix it fully, let it stand for half an hour. Measure its absorbance at 517nm at room temperature and record it as A1.

3.4.2 In blank group, under the same conditions, mix 2mL anhydrous ethanol and 2 mL DPPH ethanol solution, measure the absorbance and record as A0.

3.4.3 Under the same conditions, mix 2mL anhydrous ethanol and 2 mL sample solution, measure the absorbance and record as A2.The test is repeated for 6 times.

3.4.4 DPPH free radical clearance rate (%)=[1-(A1-A2)/A0]×100%

Where:
A0 -- absorbance of ethanol and DPPH mixed solution
A1 -- absorbance of sample and DPPH mixed solution
A2 -- absorbance of sample and ethanol mixed solution

3.5 Whitening efficacy (Tyrosinase inhibition rate)

According to Zhang [29], the experimental methods are shown below.
Accurately suck up and add L-tyrosine, PBS buffer (pH6.8) and sample solution with pipette according to the Table 2. Mix fully and place in a water bath at 37℃ for 10min. Tyrosinase was added, quickly placed in a water bath at 37℃ for 10min. The absorbance was measured at 475nm by UV spectrophotometer.

Table 2 Composition and volume of reaction solution for determination of tyrosinase activity

Reagent	AT1/mL	AT2/mL	AT3/mL	AT4/mL
L-tyrosine	1	1	1	1
PBS buffer	3	2	2	1
sample	0	0	1	1
tyrosinase	0	1	0	1
Total	4	4	4	4

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Tyrosinase activity inhibition rate=[1-(AT4-AT3)/(AT2-AT1)]×100%

Where:

AT1-- absorbance of solution without sample and without tyrosinase

AT2-- absorbance of solution without sample and with tyrosinase

AT3-- absorbance of solution with sample and without tyrosinase

AT4-- absorbance of solution with sample and with tyrosinase

3.6 Acne-removing effect (Antibacterial test)

According to Xia [24], the experimental methods are shown below.

Drop 100μL *Staphylococcus aureus* suspension separately on three NA medium. The sterile Oxford cup was gently placed on the medium using tweezers, and 0.2 mL of the ferment solutions were sucked up into the Oxford cup, respectively. With sterile water as the blank control, the culture medium was placed in incubator at 37℃ for 18 h. This experiment is repeated with *Escherichia coli* suspension under the same condition. The diameter of the bacterial inhibition ring was measured.

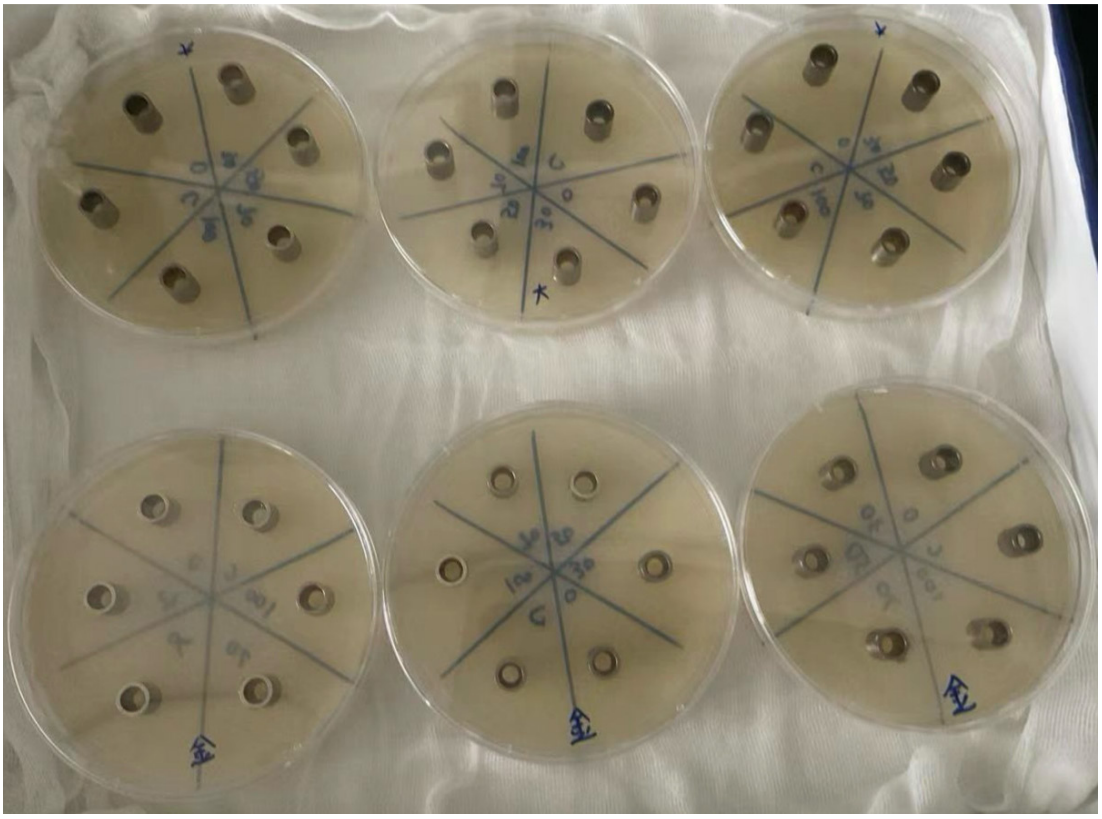

Figure 2 mediums of *Staphylococcus aureus* and *Escherichia coli* with ferment solution in Oxford cup

4. Experimental results

4.1 Antioxidant activity test

Table 3 DPPH clearance rate (antioxidant ability) of ferment

	A1	A0	A2	DPPH clearance rate/%
100% blueberry + 0% rose	0.200	0.550	0.159	92.5
70% blueberry + 30% rose	0.152	0.550	0.118	93.8
50% blueberry + 50% rose	0.127	0.550	0.095	94.2
30% blueberry + 70% rose	0.106	0.550	0.087	96.5
0% blueberry + 100% rose	0.139	0.550	0.101	93.1

The antioxidant ability of ferment is shown by their DPPH clearance rate.

As shown in the Table 3, in general, all five ferment has DPPH clearance rate higher than 90%, indicating remarkable antioxidant activity. For single-material ferment, the antioxidant activity of pure rose ferment is better than that of pure blueberry ferment. The antioxidant activities of the compound ferment obtained after mixed fermentation are better than that of single fruit ferment. Meanwhile, the antioxidant activity increases with the rise of rose proportion, among which the ferment 4 (70% rose and 30% blueberry) has the best antioxidant properties (96.5%).

4.2 whitening effect

Table 4 Tyrosinase inhibition rate of ferment

	A1	A2	A3	A4	Tyrosinase inhibition rate/ %
100% blueberry + 0% rose	-0.017	0.253	0.100	0.275	35.2
70% blueberry + 30% rose	-0.017	0.253	0.116	0.303	30.7
50% blueberry + 50% rose	-0.017	0.253	0.089	0.287	26.7
30% blueberry + 70% rose	-0.017	0.253	0.110	0.297	30.7
0% blueberry + 100% rose	-0.017	0.253	0.045	0.236	29.3

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The whitening effect of ferment is shown by the tyrosinase inhibition rate.

As shown in the Table 4, overall, all five ferment has tyrosinase inhibition rate in the range from 20% to 40%, indicating certain whitening effect. Among them, there is a maximum tyrosinase inhibition rate at 35.2% for the ferment 1 (100% blueberry), which is followed by ferment 2 (70% blueberry + 30% rose) and ferment 4 (30% blueberry + 70% rose), reaching 30.7% for both. On the contrary, the result of ferment 3 (50% blueberry + 50% rose) shows relatively poorer whitening effect (26.7%).

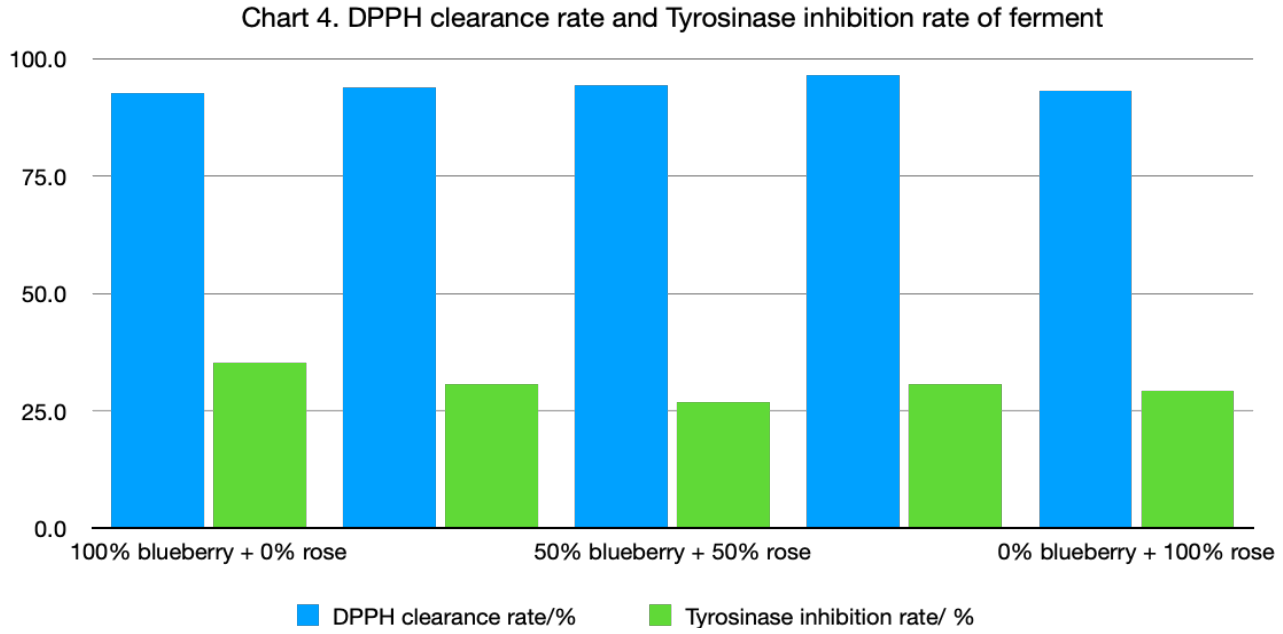

Chart 1 DPPH clearance rate and Tyrosinase inhibition rate of ferment

A brief comparison between the antioxidant activity and whitening effect is shown by the Chart 1.

4.3 antibacterial activity

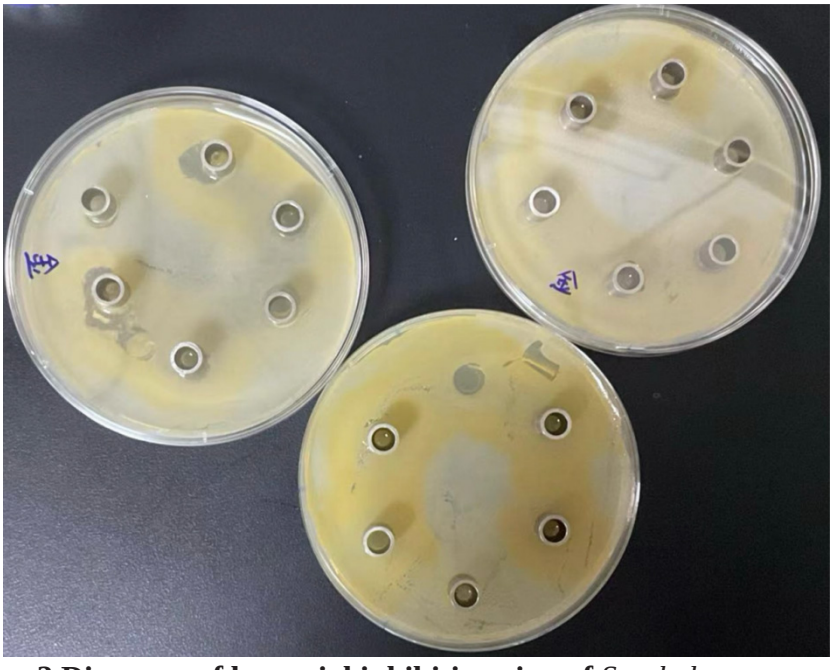

Figure 3 Diameter of bacterial inhibition ring of *Staphylococcus aureus*

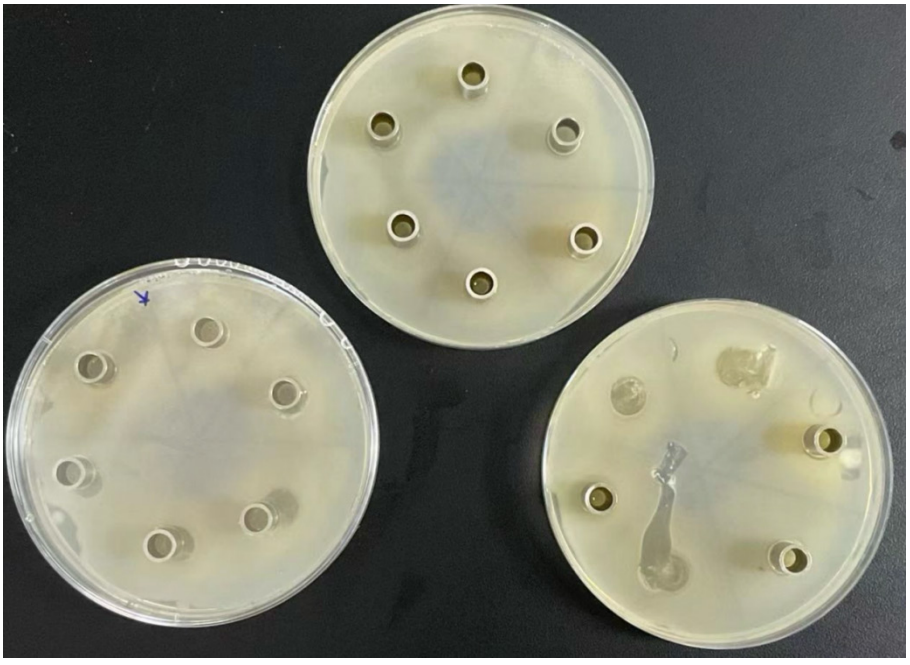

Figure 4 Diameter of bacterial inhibition ring of *Escherichia coli*

Table 5 Diameter of bacterial inhibition ring of ferment

	Staphylococcus aureus/mm	Escherichia coli/mm
100% blueberry + 0% rose	8.25±0.25	9±0
70% blueberry + 30% rose	9±1.0	9±0.5
50% blueberry + 50% rose	8.5±0.5	9±1.0
30% blueberry + 70% rose	8.5±0.5	10.5±1.0
0% blueberry + 100% rose	8±0	10±1.0
control	8±0	8±0

The antibacterial activity of ferment is shown by their diameter of bacterial inhibition ring.

As shown in the Table 5, in general, most of the ferment has the effect of inhibiting bacterial growth and reproduction. When it comes to the inhibition of *Staphylococcus aureus*, the single-material ferment, including ferment 1 (100% blueberry) and ferment 5 (100% rose), shows little antibacterial activity. By contrast, all three compound ferment has certain antibacterial activity, among which the diameter of bacterial inhibition ring of ferment 2 (70% blueberry and 30% rose) is largest at 9mm.

The inhibitory effect of ferment on *Escherichia coli* was slightly stronger than that of *Staphylococcus aureus*. The minimal effect is shown by ferment 1 (100% blueberry) at 9mm, while the inhibition effect of ferment 5 (100% rose) ranks the second at 10mm. For the compound ferment, the diameter of bacterial inhibition ring increases with the rise of rose proportion, among which the ferment 4 (70% rose and 30% blueberry) has the best inhibitory effect on *Escherichia coli* (10.5mm).

5. Discussion

5.1 Antioxidant activity test

According to the Table 3, the experimental results are quite consistent with the expected results. Many studies have proved that ferment has high antioxidant activity. According to Jayabalan *et al*. [11], the reason for its high antioxidant activity is due to the microorganisms involved in fermentation, mainly including yeasts and bacteria, which process the structure of substrates during fermentation. Ferment is also rich in phenols, flavonoids, vitamin C and enzymes, such as superoxide dismutase, protease and lipase. Phenols can easily give a hydrogen ion and be stabilized by resonance hybridization, which is the main reason for the high radical scavenging ability [7].

By intermediary metabolism and cross metabolism, mixed

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rose and blueberry can produce more complex metabolite and more bioactive components, which may contribute to relatively higher antioxidant activity compared with single-material ferment.

The reason why antioxidant activity increases with the rise of rose concentration is that rose itself contains many functional and antioxidant ingredients, such as alkaloids, vitamins, polysaccharides and so on. For instance, polysaccharide extracted from rose has been proved to have high antioxidant effect, which is regarded as an excellent natural antioxidant [24]. Wang [23] used rose bud as raw material and ethanol as solvent to extract flavonoids from rose. The antioxidant capacity of extracted flavonoids was evaluated by measuring total antioxidant (FRAP), indicating significant free radical scavenging effect of rose. After fermentation, many of the original antioxidant ingredients in roses are retained, contributing to the high antioxidant activity of the ferment.

The results about antioxidant activity are quite in line with that in the previous research. For instance, the DPPH clearance rate of the rose ferment reached 70.99% after fermentation for 72 hours in the study of Xia [24]. Similarly, the figure of the rose ferment after dilution for 100 times was 78.9% after fermentation for 10 days, showed in the study of Li [14]. Referring to the blueberry ferment, its DPPH free radical scavenging rate increased to 96.90% after it was fermented for 30 days and was diluted 10 times [8]. In the research of Jiang *et al*. [13], the DPPH clearance rate of blueberry ferment rose to the peak at 94.41% on the 48th day.

5.2 Whitening effect

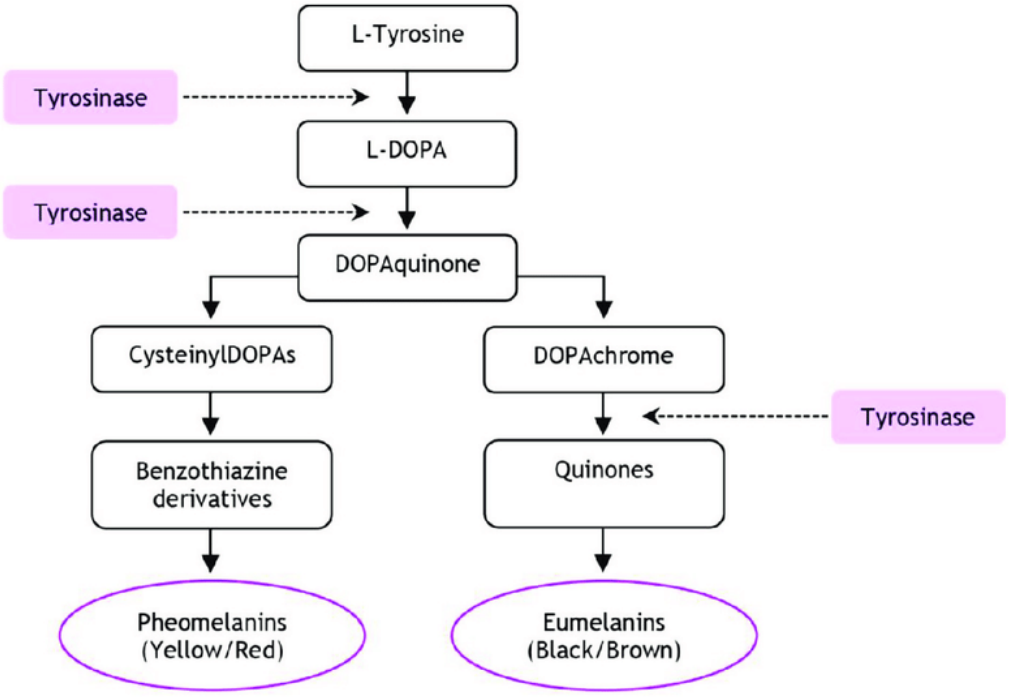

Figure 5 Pathway of melanin synthesis

Human skin color is closely related to the amount and distribution of melanin produced by melanocytes in the skin. Tyrosinase is an oxidoreductase containing copper, which acts as a rate-limiting enzyme for the production of melanin. According to Figure 5 from Raoufinejad *et al*. [19], during the pathway of melanin synthesis, the first two steps are catalyzed by tyrosinase, in which L-tyrosin converts to L-DOPA and then L-DOPA changes to a quinone which spontaneously polymerizes to form melanin pigments. Therefore, by inhibiting the activity of tyrosinase, the ferment can effectively reduce the production of melanin, thus having a strong whitening effect.

Another reasonable explanation for the whitening effect of ferment is that the ferment blocks the keratinocytes from ingesting melanocytes, thereby preventing the keratinocytes from turning black [22]. Melanin produced by melanocytes will be transferred to the surrounding epidermal cells (keratinocytes) through various pathways, and the emitted melanin is ingested by the keratinocytes by phagocytosis However, the experimental results have shown that ferment can block these pathways.

According to table 4, the pure blueberry ferment has max-

imum tyrosinase inhibition rate, mainly owning to the abundant vitamin C containing in blueberry [25]. Vitamin C can not only inhibit the activity of tyrosinase, but also reduce dark melanin to colourless.

5.3 Antibacterial activity

One prominent cause for the antibacterial activity of ferment is that there is a large amount of lysozyme in ferment. Lysozyme can break down β-1,4- glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in the cell wall, so that the insoluble mucopolysaccharide of the cell wall is decomposed into soluble glycopeptides, resulting in cell wall rupture, escape of the contents and bacteriolysis [16].

The antibacterial effect of ferment is also closely associated with its bacteriostatic composition and mechanism. Ferment contains a large number of bioactive ingredients, such as antioxidants, organic acids, vitamins, amino acids, mineral elements, which enable ferment to have certain antibacterial effect.

However, some *Staphylococcus aureus* cell walls are coated with capsular antigens, which are covalently combined by polypeptide chains and peptidoglycans. Therefore, it is speculated that bioactive substances with antibacterial properties can not easily cross the capsular membrane and function to the interior of *Staphylococcus aureus*. Therefore, the inhibiting effect of the ferment on it is very weak [26]. In addition, *Staphylococcus aureus* itself is likely to develop drug resistance, which means that it may also become resistant to the ferment.

The experimental results shown in the Table 5 are similar with some the previous studies. The study of Dong *et al*. [3] showed that the powdered ferment only had a certain inhibitory effect on *Escherichia coli* and was almost ineffective against *Staphylococcus aureus*. Similarly, according to Yan [26] almost all types of ferment had weak inhibitory activity against *Staphylococcus aureus*, while all of the samples had noteworthy inhibitory activity against *Escherichia coli*. However, the experimental data does not completely conform to some research results, involving the research from Xia [24], which showed that rose ferment had a significant inhibitory effect on both *Escherichia coli* and *Staphylococcus aureus*, and the inhibitory effect on the latter was slightly stronger than that of the former. The diameter of inhibition ring of rose ferment against *Escherichia coli* and *Staphylococcus aureus* was 19.75 mm and 20.13 mm respectively.

6. Conclusion

In conclusion, all five ferment has remarkable antioxidant activity (more than 90% clearance rate of DPPH free radical), moderate whitening effect (ranging from 20% to 40% of tyrosinase inhibition rate), weak effect on inhibiting *Staphylococcus aureus* and certain effect on inhibiting *Escherichia coli*. It is noteworthy from Chart 1 that the ferment has stronger antioxidant activity compared with its whitening effect. In addition, Table 5 shows that the inhibitory effect on *Escherichia coli* of ferment is generally better than that on *Staphylococcus aureus*. However, the antibacterial effect of ferment is found to be unstable and may be affected by many other factors.

All three experiments have proved that ferment is more suitable for compound fermentation, as most compound ferment showed better antioxidant and antibacterial effects than single-material ferment.

When choosing one type of ferment according to a specific function, ferment 4 (70% rose and 30% blueberry) has the best antioxidant properties (96.5%) and the best inhibitory effect on *Escherichia coli* (10.5mm). Ferment 1 (100% blueberry) has maximum tyrosinase inhibition rate (35.2%). Ferment 2 (70% blueberry and 30% rose) has the best inhibition ability on *Staphylococcus aureus* (9mm).

If all performance of the ferment is considered, it seems that ferment 4 (70% rose and 30% blueberry) has the most comprehensive ability, since it ranked at the top twice and the second twice in four experiments.

On account of the abundant and diverse biological active ingredients, ferment has excellent ability in the field of cosmetics and becomes a very promising microbial product. Hopefully, there will be more relevant research as well as more application of ferment on ferment in cosmetics and even other fields in the future.

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