EFFICACY OF RICE BRAN FERMENTATION IN COSMETICS AND SKIN CARE PRODUCTS

EFICÁCIA DA FERMENTAÇÃO DE FARELO DE ARROZ EM COSMÉTICOS E PRODUTOS PARA CUIDADOS COM A PELE

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ABSTRACT: This study aims to develop rice bran-based skin care products with moisturizing, whitening and anti-wrinkle effects similar to Pitera (a natural by-product of sake lees fermentation) but without alcohol irritation for sensitive skin. To achieve this objective, bran from organic indica rice was fermented by lactic acid bacteria in a safe and pollution-free environment. In terms of anti-oxidation, the DPPH · free radical scavenging ability of 100.0 mg/mL bran fermentation solution was 71.4% of that of vitamin C of the same concentration; and its Fe²⁺ chelating ability was 79.0% of that of EDTA of the same concentration. Moreover, the superoxide anion scavenging ability of 10.0 mg/mL bran fermentation solution was equivalent to 42.9% of that of BHT of similar concentration. With respect to inhibition of melanin synthesis, the bran fermentation solution's ability to inhibit the synthesis of dopachrome, the intermediate of melanin, was positively correlated to its concentration, i.e., the higher the concentration of the bran fermentation solution was, the better the inhibition ability was. The IC_{50} of bran fermentation solution was 9.23 mg/mL while, for comparison, that of arbutin was 0.52 mg/mL. Furthermore, according to the cell survival assay, no obvious cytotoxic effect was found with the increase of the concentration of the bran fermentation solution. As for whitening evaluation, the whitening improvement rate was 9.29% in 20% dilution, 5.36% in 15% dilution, 3.69% in 10% dilution, 2.43% in 5% dilution, 0.35% in 1% dilution in a 30-day test. In the moisturizing evaluation, the moisturizing improvement rate was 44.31% in 20% dilution, 20.48% in 15% dilution, 7.68% in 10% dilution, 6.02% in 5% dilution and 2.02% in 1% dilution. Based on the experimental results, the alcohol-free rice bran fermentation solution not only did not cause irritation but also had antiaging, melanin synthesis inhibition, whitening and moisturizing effects. Therefore, it is advisable to add rice bran fermentation solution to cleaning mousse, shower gel, serum and essence to turn bran from compost of agricultural waste (cradle to grave) into a natural raw material (cradle to cradle) of the cosmetic industry, creating new value of rice bran.

KEYWORDS: Rice Bran. Lactic acid bacteria. Alcohol-free fermentation. Anti-oxidation. Anti-aging. Whitening. Cosmetics.

INTRODUCTION

Rice bran is the waste in the rice polishing process, which turns brown rice to polished white rice. It mainly consists of yellowish outer layers of pericarp, seed-coat and part of germ. According to literature (FU, 2001; TSAI, 2014; HE, 2014; KAYAHARA *et al.*, 2000; VILLAREAL *et al.*, 1991), the calorie of brown rice and white rice is similar. However, at least 50% of nutrients, including fat, fiber, ash, calcium, phosphorus, iron, vitamins A, vitamins B1, B2, B6, vitamin E, amino acids and nicotinic acid are lost from the polishing process. The rice bran contains the lost nutrients that can effectively prevent dry skin, delay skin aging and avoid pigment deposition. It is wasteful to discard the valuable rice bran as waste or compost.

Based on literature (YANG, 2011; CHANG, 2014; PENG, 2007; CHEN, 2014; WU, 2007; LIU, 2012; TU, 2010; CHAO et al., 2006) and users' experience of lees-based beauty products on the market (SHEN, 1990; CHANG et.al., 2012; HUANG, 1991; CHANG, 1998), we used rice bran from organic rice as the experimental material and applied yeast fermentation technique to produce alcohol-free rice bran fermentation solution, which is different from the white rice-based and yeastfermented lees. We evaluated the whitening, moisturizing, anti-oxidation, melanin synthesis inhibition, and mutation effects of the rice bran fermentation solution before developing cleansing and skin care products, such as shower gel, cleansing mousse and essence as the overture of the rice bran legend (Figure 1).

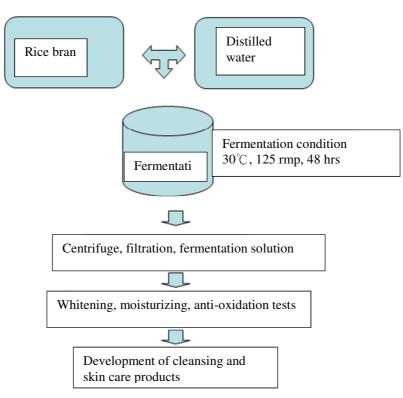


Figure 1. Development of cleansing and skin care products from rice bran fermentation

MATERIAL AND METHODS

Preparation of rice bran fermentation solution

First, 0.2 g lactic acid bacteria were added to 10 mL of fermentation solution (formula as Table 1) for 24-hour culture. Then, 3 mL culture medium was added to the fermentation solution of Table 1 to make 1 liter solution for 48-hour fermentation at 30°C and 125rpm. (The rice bran fermentation solution must be sterilized by an autoclave and then cooled down to room temperature before inoculation.).

 Table 1. Bran fermentation formula

Table 1. Dran termentation formata	
Approximate	Per Liter
Rice Bran	50.0 g
Proteose Peptone No.3	10.0 g
Yeast Extract	5.0 g
Dextrose	20.0 g
Polysorbate 80	1.0 g
Ammonium Citrate	2.0 g
Sodium Acetate	5.0 g
Magnesium Sulfate	0.1 g
Dipotassium Phosphate	2.0 g

DPPH · free radical scavenging ability

The method was performed as described by Yamaguchi et al. (1998). A mixture of 100 μ L rice bran fermentation solution, 400 μ L of 100 mM Tris-HCl buffer (pH=7.4) and 500 μ L of 250 μ M DPPH \cdot free radical ethanol solution was well mixed in a microcentrifuge tube before the tube was placed in a 25°Cthermostatic reactor for 20 minutes followed by OD₅₁₇ measurement of the solution by a UV- Vis

spectrophotometer. This test was repeated for three times. The DPPH \cdot free radical scavenging rate is calculated as follows:

DPPH \cdot free radical scavenging rate (%) = [1-(OD₅₁₇ of sample/OD₅₁₇ of blank solution] ×100%

Fe²⁺ chelating ability

The method was performed as described by Dinis *et al.* (1994). First, 0.1 mL rice bran

fermentation solution, 3.7 mL of 95% ethanol and 0.1 mL of 2 mM FeCl₂·4H₂O solution were added to a sample bottle and was left for 30 seconds at room temperature. Next, 0.2 mL of 5 mM ferrozine solution was added to the sample bottle and was left for reaction for 10 minutes at room temperature. OD₅₆₂ of the sample was measured by the UV- Vis spectrophotometer. This test was repeated for three times. The Fe²⁺ chelating ability is calculated as follows:

The Fe^{2+} chelating ability (%) = [1-(OD₅₆₂ of sample/OD₅₆₂ of blank solution] ×100%

Superoxide anions scavenging ability (O_2)

The method was performed as described by Fried et al. (1996). First, 0.25 mL rice bran fermentation solution and 1 mL mixture solution (pH 7.4, 0.1 M phosphate buffer added with 100 µl of 10 μ M PMS, 50 μ l of 78 μ M NADH and 100 μ of 50µM NBT to 1 mL) were added to a sample bottle, and then the sample bottle was placed in 25°C thermostatic water bath for 10 minutes. Then, 0.25 mL of 1.2 units/mL xanthine oxidase was added to the bottle, and the solution was left for reaction for two minutes in the 25°C thermostatic water bath. OD₅₃₂ of the sample was measured by the UV- Vis spectrophotometer. Next, xanthine oxidase was replaced with 0.1 M Tris-HCl buffer (pH=7.4), and the absorbance of the sample at 532 nm measured was A₁.

Next, sample was replaced by DMSO, and the absorbance of the sample at 532 nm measured was A_b . Finally, the sample and xanthine oxidase were replaced by DMSO and phosphate buffer solution, respectively, and the absorbance of the sample at 532 nm measured was A_b . This test was repeated for three times. The O_2^- scavenging rate is calculated as follows:

 O_2^{-1} scavenging rate = [$(A_b - A_0) - (A_t - A_1)/(A_b - A_0)$]×100%

Melanin synthesis inhibition ability

The method was performed as described by Lee *et al.* (1997). A mixture of 1 mL rice bran fermentation solution, 0.9 mL of phosphate buffer solution (pH = 6.8) and 1 mL of 0.03% tyrosine aqueous solution was well mixed in a sample bottle, and then the bottle was placed in 37°C thermostatic water bath for 10 minutes. Next, 0.1 mL of 350 units/mL tyrosinase was added to the solution, and the well-mixed solution was reacted in the thermostatic water bath for 25 minutes. The absorbance of the solution at 475 nm measured by the UV-Vis spectrophotometer was A_t. Then, the tyrosinase was replaced by phosphate buffer solution, and the absorbance of the solution at 475 nm measured was A_1 . Next, the rice bran fermentation solution was replaced by deionized water, and the absorbance of the solution at 475 nm measured was A_b . Finally, the rice bran fermentation solution and tyrosinase were replaced by distilled water and phosphate buffer solution, respectively, and the absorbance of the solution at 475 nm measured was A_0 . This test was repeated for three times. The O_2 removal rate is calculated as follows:

Dopachrome inhibition rate (%) = $[(A_b - A_0) - (A_t + A_0)]$

$$-A_1)/(A_b - A_0)] \times 100\%$$

 IC_{50} (50% inhibition concentration) is the concentration of rice bran fermentation solution that inhibits 50% of dopachrome synthesis, and was obtained by a linear regression curved constructed based on the dopachrome inhibition rate under different concentration of the rice bran fermentation solution.

Cell survival assay

The method was performed as described by Kazuho Abe *et al.* (1999). Cell survival assay is usually performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl- tetrazolium bromide (MTT). Therefore, this assay is called MTT test or Tetrazolium assay. MTT is a yellow water-soluble substance, which can be metabolized by dehydrogenase in mitochondria in cells. The activity of dehydrogenase is positively correlated with cellular respiration. Thus, the cell activity can be measured following this principle.

When the tetrazolium ring of MTT is cut off by dehydrogenase, the MTT is reduced to purple crystal formazan (3-[4,5-dimethylthiazol-2-yl]-2,5diphenyl-formazan), accumulating in the cells. Formazan is soluble in DMSO and its concentration can be measured by a spectrophotometer at 570 nm. The higher the cell survival rate is or the more the cell number is, the more accumulated purple crystal is.

Therefore, MTT test is often used to assess the cell survival rate. Five milligrams of 3-(4,5dimethylthiazol-2-yl)-2,5-diph-enyl-tetrazolium bromide (MTT) was dissolved in 1 mL sterile PBS and filtered by a 0.45 μ m membrane before it was stored at 4°C. Mouse fibroblast 3T3 cells were cultured in a 96-well plate (1 × 105 cells/0.1 mL/well). After 24 hours, the old culture medium was aspirated and culture media of different concentration were added to the plate. After 24 hours of incubation, the old culture media were aspirated. The cells were rinsed with PBS twice and 100 μ L MTT (0.5 mg/mL) was added to the cells. Efficacy of rice bran...

Then, the cells were incubated in a 37°C incubator with 5% CO₂ for one hour. Finally, the old culture medium was aspirated before 100 μ L DMSO was added to the culture, and then the culture left for 10 minutes. The absorbance of the culture was measured by a spectrophotometer at 570nm.

Cell proliferation (%) = (O.D sample/O.D control) \times 100%

Whitening and moisturizing assay

(1) Instrument name: three-in-one skin analyzer (SSC3);

(2) Brand: Courage-Khazaka Electronic Gmbh (CK), Germany

(3) Test methods

1. The protocol number of the Human Research Ethics Committee for this research: 201705ES002

2. Sampling: 2 mL of 20%, 15%, 10%, 5%, 1% rice bran fermentation solution each.

- Test spots: forehead and cheek.
- 4. Test age/skin: 18 to 20 years old females.

5. Test environment: thermostatic indoor temperature at 22°C.

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6. Test area: the test areas were divided into experimental area and control area. Right site was experimental area (for cleansing mousse with rice bran fermentation solution), and the left side was control area (for cleansing mousse without rice bran fermentation solution).

7. Application time: the whole face was cleaned with clean water. After 30 minutes, the first test was conduction. Cleansing mousses without and with rice bran fermentation solution were applied to the left side and right side of the face, respectively, and rinsed off with water. The second test was conducted after another 30 minutes.



3.

Figure 2. Three-in-one skin analyzer

RESULTS AND DISCUSSION

Anti-oxidation ability of rice bran fermentation solution

DPPH • free radical scavenging ability

DPPH \cdot free radicals are stable free radicals containing odd number of electrons. When they are combined with other free radicals or reduced by antioxidants, DPPH \cdot free radicals are scavenged (DPPH \cdot +AH \rightarrow DPPH-H + A \cdot), and the color is turned from purple to light yellow, which in turn reduces the absorbance.

The lower the absorbance is, the stronger the sample's DPPH \cdot scavenging ability is and the

stronger the anti-oxidation ability is. DPPH \cdot ethanol solution has very strong absorbance at 517 nm visible light.

Figure 3 shows the DPPH \cdot scavenging ability of rice bran fermentation solution on DPPH \cdot free radicals. The DPPH \cdot free radical scavenging ability of rice bran fermentation solution increases with the increase of the concentration of rice bran fermentation solution. The DPPH \cdot free radical scavenging ability of 10.0 mg/mL rice bran fermentation solution was 23.9%, and that of 50.0 mg/mL and 100.0 mg/mL rice bran fermentation solution was 37.4% and 64.6%, respectively.

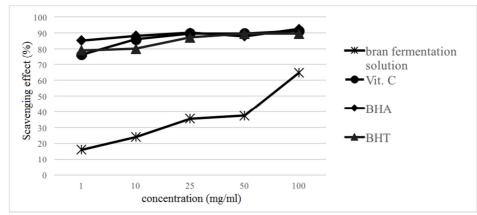


Figure 3. The free radical scavenging ability of rice bran fermentation solution

Fe²⁺ chelating ability

In addition to the formation of hydroxyl radicals ('OH) by Fenton reaction, the ferrous ions (Fe^{2+}) in the body also reacts with lipid peroxides (LOOH), resulting in oxidative lipid free radicals (LO'). Therefore, substrates with iron chelating ability can also act as antioxidant synergists. Therefore, if the rice bran fermentation solution has iron chelating activity, it can act as an antioxidant.

 Fe^{2+} can form a complex with ferrozine. The maximum absorbance of such complex is at 562 nm. The lower the absorbance is, the stronger the

sample's Fe^{2+} chelating ability is. Figure 4 shows the rice bran fermentation solution's chelating ability against Fe^{2+} . Such ability increases with the increase of the concentration of the rice bran fermentation solution. When the concentration of the rice bran fermentation solution was 100.0 mg/mL, the Fe^{2+} chelating ability was equivalent to 79.0% of that of EDTA. However, further studies are required to identify which component in the rice bran fermentation solution is related to Fe^{2+} chelating, like the chromatography.

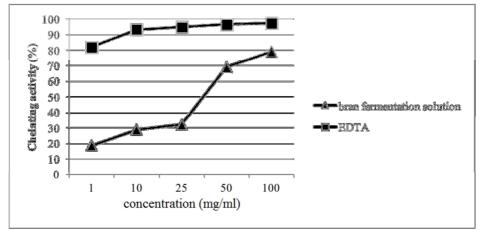


Figure 4. Fe²⁺ chelating ability of rice bran fermentation solution

Superoxide anion scavenging ability

In the process of in vivo metabolism, an oxygen molecule forms a superoxide anion with an electron through reduction reaction. The electron transfer chain reaction taking place on the inner mitochondrial membrane in the cytoplasm also results in superoxide anion formation. These processes give rise to O_2 free radicals, which cause damage to human cells.

Therefore, O_2^- scavenging substrates can act as antioxidants to reduce oxidative damage. The oxidation of xanthine by xanthine oxidase generates O_2 , which reduces nitroblue tetrazolium in the reaction reagent to formazan. The maximum absorbance of formazan is at 532 nm. The lower the OD_{532} is, the better the sample's scavenging ability is. Figure 5 shows the O_2 scavenging ability of rice bran fermentation solution, which increases with the increase of the concentration of rice bran fermentation solution. When the concentration of rice bran fermentation solution was 10.0 mg/mL, the O_2 scavenging ability of rice bran fermentation solution was 10.0 mg/mL, the O_2 scavenging ability of rice bran fermentation solution was 10.0 mg/mL the same concentration.

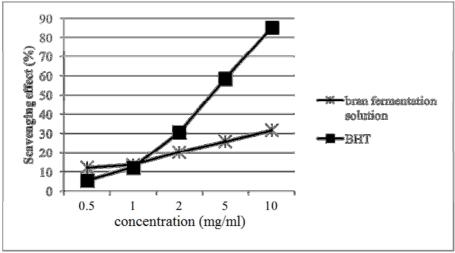


Figure 5. O_2^- scavenging ability of rice bran fermentation solution

The ability of rice bran fermentation solution to inhibit melanin synthesis

In the process of melanin synthesis, tyrosine is turned into dopaquinone through the catalysis of tyrosinase. Dopaquinone then forms melanin through a series of reactions. Dopachrome is a more stable intermediate in the process of melanin synthesis, and its maximum absorbance is at 475 nm. The lower the OD_{475} is, the better the sample's ability to inhibit melanin synthesis.

Figure 6 shows the ability of rice bran fermentation solution to inhibit melanin synthesis. Such ability increased with the increase of the concentration of rice bran fermentation solution, indicating a positive correlation. The IC_{50} of the rice bran fermentation solution was 9.23 mg/mL (IC_{50} of Arbutin was 0.52 mg/mL).

 Cu^{2+} is a cofactor of tyrosinase, which catalyzes the oxidation reaction of melanin synthesis. If the sample has metal ion chelating ability, it can inhibit melanin synthesis. Based on the results in the anti-oxidation test, the rice bran fermentation solution was able to chelate metal irons. Therefore, it was inferred that the ability of rice bran fermentation solution to inhibit dopachrome synthesis is related to its Cu^{2+} chelating ability.

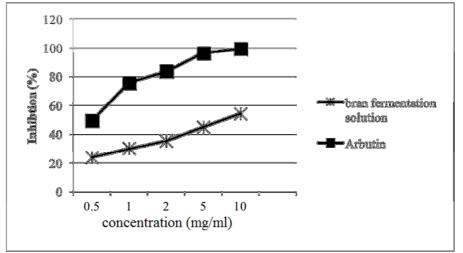


Figure 6. The ability of rice bran fermentation solution to inhibit melanin synthesis

Cell survival assay of rice bran fermentation solution

MTT is a yellow water-soluble substrate, which can be reduced to blue-violet crystals by dehydrogenase in mitochondria during cellular respiration. The blue-violet crystals accumulated in the cells can be dissolved by DMSO and its absorbance can be measured at 540 nm. The higher the cell survival rate is, the more the blue-violet crystals are produced and the higher the OD_{540} value is.

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In this study, the rice bran fermentation solution is tested with the cell survival assay. The objective was to observe whether the rice bran fermentation solution is toxic to the cells. Figure 7 is the survival rate of 3T3 cells in the rice bran fermentation solution of different concentration. The results indicated that there was no obvious cytotoxic effect on the cells as the concentration of the rice bran fermentation solution was increased.

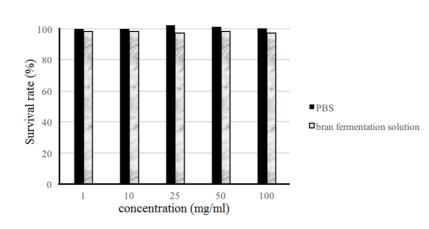


Figure 7. Cytotoxicity of the rice bran fermentation solution

Assessment of moisturizing effect of rice bran fermentation solution

The rice bran fermentation solution was diluted with distilled water, and then filtered and centrifuged. A series of 20%, 15%, 10%, 5% and 1% dilution were applied to 20 subjects for the assessment of the moisturizing effect of the rice

bran fermentation solution. The average values of the 20 subjects are summarized in Table 2 and Figure 8, and the moisturizing improvement rate is illustrated in Table 3 and Figure 9.

Table 2. Moisturizing data of experimental group and control group of the rice bran fermentation solution (average values of 20 subjects)

Concentration of		Day 1		Day 8		Day 15		ay 23		Day 30
rice bran solution	Control	Experimental								
20%	68.6	67.3	70.3	69.5	63.5	74.8	59.0	81.6	58.0	83.7
15%	54.2	58.7	51.1	61.4	62.4	62.3	58.4	63.9	57.6	69.4
10%	57.7	59.3	57.2	61.7	55.2	61.9	58.4	62.8	58.6	63.1
5%	53.5	56.4	58.8	58.3	53.5	58.7	55.2	58.7	58.1	61.6
1%	55.6	56.5	57.4	58.8	57.8	58.9	59.8	60.1	59.5	60.7

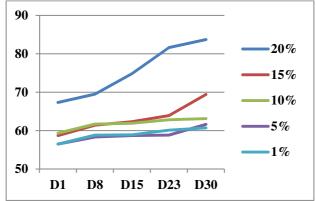




Table 3. Moisturizing improvement rate of rice bran fermentation solution after	30 days
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Variable			Value		
Diluted solution	20%	15%	10%	5%	1%
Moisturizing improvement rate	44.31%	20.48%	7.68%	6.02%	2.02%

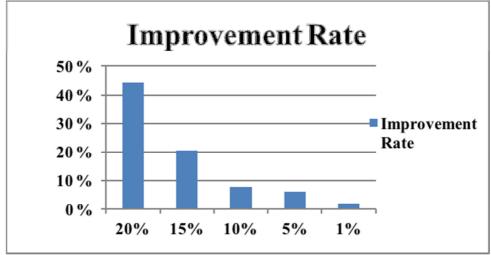


Figure 9. Moisturizing improvement rate of rice bran fermentation solution after 30 days

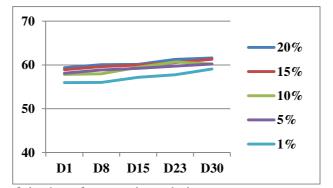
After 30 days of test on 20 females aged 18 to 20, the results showed that the higher the concentration of the diluted rice bran fermentation solution is, the better the moisturizing effect is. Based on the experimental data, the 20% rice bran fermentation solution had the best moisturizing effect.

7. Whitening assessment of rice brand fermentation solution

The rice bran fermentation solution was diluted with distilled water, and then filtered and centrifuged. A series of 20%, 15%, 10%, 5% and 1% dilution were applied to 20 subjects for the assessment of the whitening effect of the rice bran fermentation solution. The average values of the 20 subjects are summarized in Table 4 and Figure 10, and the moisturizing improvement rate is illustrated in Table 5 and Figure 11.

Table 4. Whitening data of experimental group and control group of the rice bran fermentation solution (average values of 20 subjects)

Concentration	Day 1		Day 8		Day 15		Day 23		Day 30	
of rice bran solution	Control	Experimental								
20%	58.6	59.4	59.0	60.1	58.3	60.1	58.1	61.2	56.4	61.6
15%	58.6	58.9	59.0	59.6	59.8	60.0	59.8	60.5	58.2	61.3
10%	56.9	57.8	57.6	58.0	58.9	59.5	57.0	60.7	58.1	60.2
5%	56.9	58.1	58.0	58.9	58.0	59.2	57.8	59.7	58.8	60.2
1%	55.7	56.0	55.0	56.0	56.4	57.2	55.1	57.8	58.9	59.1



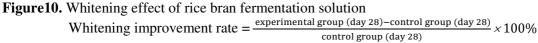


Table 5.	Whitening	improvement	rate of rice	bran ferme	ntation s	olution	after 30 d	lays

Variable	Value				
Diluted solution	20%	15%	10%	5%	1%
Whitening improvement rate	9.29%	5.36%	3.69%	2.43%	0.35%

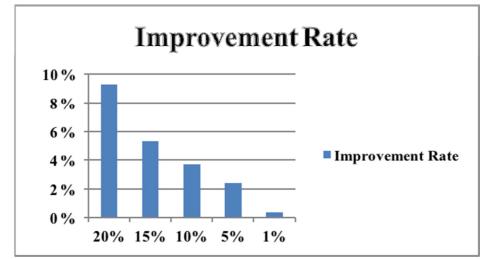


Figure 11. Whitening improvement rate of rice bran fermentation solution after 30 days

After 30 days of test on 20 females aged 18 to 20, the results showed that the higher the concentration of the diluted rice bran fermentation solution is, the better the whitening effect is. Based on the experimental data, the 20% rice bran fermentation solution had the best moisturizing effect.

According to the results of other studies, the whitening improvement rate of 7.8% sake lees was 3.24%; and that of 8.43% beer lees and 3.16% rice wine lees was 2.26% and 1.33%, respectively. Based on our experimental results above, when the concentration of the rice bran fermentation solution was between 5% and 10%, the whitening

improvement rate was between 2.43% and 3.69%, which was comparable to other lees. Moreover, the rice bran fermentation solution is alcohol-free, and thus it does not irritate skin. In addition to whitening, the effects of moisturizing, antioxidation, melanin synthesis inhibition were proved by experiments and usage. As a result, the rice bran fermentation solution can be added to cosmetics formula as a natural raw material of skin care products. The use of rice bran to obtain the fermentation solution is also an environmentally correct alternative, since it reduces its disposal in nature. Em termos de antioxidação, a capacidade de eliminação radical de DPPH.free de 100,0 mg / mL de solução de fermentação de farelo foi de 71,4% da vitamina C da mesma concentração; E sua capacidade de quelação Fe2 + foi de 79,0% da EDTA da mesma concentração. Além disso, a capacidade de eliminação de aniões superóxido de 10,0 mg / mL de solução de fermentação de farelo era equivalente a 42,9% da BHT de concentração similar. Com relação à inibição da síntese de melanina, a capacidade da solução de fermentação do farelo de inibir a síntese do dopachrome, o intermediário da melanina, correlacionou-se positivamente com sua concentração, ou seja, quanto maior a concentração da solução de fermentação do farelo, melhor a capacidade de inibição estava. A solução de IC50 de fermentação de farelo foi de 9,23 mg / mL enquanto que, para comparação, a arbutina era de 0,52 mg / mL. Além disso, de acordo com o ensaio de sobrevivência celular, nenhum efeito citotóxico óbvio foi encontrado com o aumento da concentração da solução de fermentação de farelo. Quanto à avaliação do branqueamento, a taxa de branqueamento foi de 9,29% na diluição de 20%, 5,36% na diluição de 15%, 3,69% na diluição de 10%, 2,43% na diluição de 5%, 0,35% na diluição de 1% em um teste de 30 dias . Na avaliação hidratante, a taxa de melhora hidratante foi de 44,31% em 20% de diluição, 20,48% em diluição de 15%, 7,68% em diluição de 10%, 6,02% em diluição de 5% e 2,02% em diluição a 1%.

Com base nos resultados experimentais, a solução de fermentação de farelo de arroz sem álcool não só não causou irritação, mas também teve anti-envelhecimento, inibição da síntese de melanina, branqueamento e efeitos hidratantes. Portanto, é aconselhável adicionar solução de fermentação de farelo de arroz para mousse de limpeza, gel de banho, soro e essência para transformar o farelo do composto de resíduos agrícolas (berço a túmulo) em uma matéria-prima natural (berço para berço) da indústria de cosméticos, criando Novo valor do farelo de arroz.

PALAVRAS-CHAVE: Brilho de arroz. Bactérias de ácido lático. Fermentação sem álcool. Antioxidação. Anti-envelhecimento. Branqueamento. Cosméticos.

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