

Antioxidant Activity and Whitening Efficacy of Makgeolli Fractions

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막걸리 분획물의 항산화활성 및 미백효능

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Abstract This study aimed at evaluating the potential of makgeolli, which is widely consumed as beverage, as a functional cosmetic ingredient, based on analyses on its antioxidant activity and tyrosinase activity inhibitory effect. The sample was extracted by concentrating the suspension obtained after adding MeOH (3 L) to the residue of a commercial makgeolli and then adding EtOAc (3 L) to the concentrate, which was subjected to fractionation. The upper layer of the fractions was used as the final sample. In MTT assay assessments, no cytotoxicity was observed at a concentration range of 10 to 1,000 $\mu\text{g/mL}$; the antioxidant activity of the extract showed a concentration-dependent tendency and it had a high activity with an EC_{50} of 7.008 mg/mL. Also, in a tyrosinase activity inhibitory effect assessment, the extract showed an IC_{50} value of 39.22 mg/mL. These results confirmed that this sample has potential as a functional cosmetic ingredient.

Key Words : Antioxidant, Cosmetic ingredient, Makgeolli, MTT assay, Whitening Efficacy

요 약 본 연구에서는 식품으로 널리 애용되고 있는 막걸리의 항산화효능 및 티로시나아제 활성 억제효과를 연구하여 기능성 화장품 소재로서의 가능성을 평가하고자 하였다. 시료는 시판 막걸리 잔사에 MeOH 3 L를 가한 후 얻어진 현탁액을 농축한 후 EtOAc 3 L를 가하고 분획하여 상층을 최종시료로 사용하였다. MTT assay 평가에서는 10~1,000 $\mu\text{g/mL}$ 범위의 농도에서 세포독성이 전혀 나타나지 않았고, 항산화활성은 농도 의존적 경향을 보였으며, EC_{50} 은 7.008 mg/mL로 높은 활성을 보였다. 또 티로시나아제 저해 활성 결과 추출물의 IC_{50} 값은 39.22 mg/mL로 나타났다. 이상의 결과로 본 시료는 기능성화장품 원료로서의 가능성이 있음을 확인하였다.

주제어 : 항산화물질, 화장품성분, 막걸리, MTT평가, 미백효능

1. INTRODUCTION

Makgeolli is an alcoholic beverage made of rice and leaven and a pure brewed wine produced in two processes: the saccharification process of rice starch by

the amylase of mold among leaven microorganisms and the fermentation process involving the conversion of fermentable sugar into ethanol by yeast with alcohol fermentation function. In other words, it is made through parallel complex fermentation by the

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harmonized enzyme reactions of various microorganisms[1].

Makgeolli is made with leaven. When it ripens, it is filtered through a sieve so that lees are removed and then roughly brewed in which process water is added. The milky opaque color of makgeolli is attributed to this making process. However, it has a richer flavor and higher nutritional value than other alcoholic beverages[2]. Meanwhile, cheongju, which is made in the same method with that of makgeolli, has a relatively clear and transparent color than makgeolli, as it is thoroughly filtered through sieves, so that lees are completely removed. Makgeolli's popularity has been rapidly rising due to economic downturn and media's spotlight on makgeolli's anticancer effects in recent years, which has aroused enthusiasm towards makgeolli among Koreans. However, the sales growth has become slow due to the influx of various foreign alcoholic beverages and a high popularity of imported beer[3].

Various studies on the functional efficacies of western wine have been actively conducted, and in the case of sake, which is a representative alcoholic beverage of Japan and similar to Korean cheongju made of rice, the Japanese government designated sake its national alcoholic beverage for its globalization and has been aggressively supporting overseas marketing. This led to the record high exports of sake despite the aftermath of the recent earthquake in the eastern part of Japan[4]. Although there have been media reports that Korea's makgeolli exports are more than double the imports of sake, the world demand of sake is still twice greater than that of makgeolli[5]. However, amidst the recent makgeolli boom, in 2011, makgeolli exports exceeded 5 million dollars, close to the Japanese sake exports in 2005[2]. Korea started to promote makgeolli's globalization only a few years ago, and it has made a huge progress in a short time. Makgeolli, which has recently gained popularity in Japan through the Korean Wave, is also emerging as a

new ingredient for cosmetics. Products using cheongju have already been released by Korean cosmetics companies since several years ago. There is also a growing interest in the active ingredients and cosmetic effects of makgeolli in the Asian market[6]. Cosmetics made with makgeolli are being released targeting Asian markets by Korean mid - to low- priced cosmetics brands. However, in overseas markets, there is still no representative Korean traditional alcoholic beverage that has a high profile as do wine, beer, and sake. Nevertheless, as consumers' interest in fermented foods has increased in recent years, research on makgeolli has steadily increased. As such, makgeolli has been recognized as a well-being food in terms of nutritional and functional aspects and has attracted attention from domestic and foreign consumers[7]. Makgeolli contains 10-25 times more anticancer and antineoplastic substances such as Farnesol than wine or beer. Several studies have reported the anticarcinogenic, antiinflammatory, antioxidant and immunomodulatory properties of these substances in Makgeolli[8]. In addition, makgeolli also contains essential amino acids such as phenylalanine, threonine and leucine as well as glutathione with an antioxidant effect. Since it was found that free radicals are involved in arteriosclerosis, brain and cardiovascular system disorders, aging and carcinogenesis, the effects and efficacies of antioxidants have recently attracted attention[9]. One of these antioxidants, glutathione is a polymer composed of the three amino acids of glutamic acid, cysteine, and glycine. Its reduced form is referred to as the abbreviated form GSH. GSH reacts with free radicals instead of other molecules and is oxidized removing free radicals.

Domestic previous studies on Korean traditional alcoholic beverages include the followings: a study on yeast and fermentation methods[10], changes in the ingredients of makgeolli[11], production and quality characteristics of Korean traditional alcoholic beverages using black rice[12], a study on the taste substances of

organic acids, etc[13]. In addition, studies on the physiological activities and functions of Korean traditional alcoholic beverages include: a study on the quality and antioxidant characteristics of *yakju* made of different grape varieties[14], a study on the isolation and identification of the antioxidants in makgeolli[15], and others.

Studies on Korean traditional alcoholic beverages have evolved from studies on fermentation microorganisms and advanced into those analyzing the effects of physiologically active substances. However, only limited studies have been conducted on makgeolli; there is a lack of scientific study findings on the differences in physiological activities and mechanism of action due to differences in manufacturing methods. As such, there is not enough scientifically-proven data on makgeolli. In particular, few research on its application as a cosmetics ingredient has been carried out. Therefore, this study aimed at assessing the antioxidant activity and whitening efficacy of the fractions of makgeolli, which is one of Korean traditional alcoholic beverages, to determine its potential as a new cosmetics ingredient.

2. MATERIALS AND METHODS

2.1 Reagents and apparatus

The reagents used are as follows. Ascorbic acid(Duksan Pure Chemical, Korea), DPPH(1,1-Diphenyl-2-picrylhydrazyl, Wako, Japan). And, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT), were purchased from Sigma-Aldrich(St. Louis, MO, USA). Homogenizer (PT-3100D, Kinematica, Swiss), Concentrator(R-205, BUCHI labortechnik AG, Swiss), UV/VIS spectrophotometer(Optizen POP, Mecasys, Korea), CO₂ incubator(MCO-17AC, Sanyo electric, Osaka, Japan) was used.

2.2 Sample Extraction

The makgeolli of S brand used in the experiment was purchased from a supermarket on the day of shipment. The sample was extracted in the following process: First, the commercial makgeolli (3 L) was filtered through filter paper to separate the residue. To the obtained residue, MeOH (3 L) was added, followed by a 3 h homogenization. The obtained suspension was filtered again and concentrated, to which EtOAc (3 L) was added. The mixture was subjected to solvent fractionation 3 times and then the upper layer of the fractions was separated and concentrated to obtain the final sample.

2.3 DPPH assay

Evaluating the anti-oxidative effect using the DPPH method of Brand-williams. Two types of extracts were prepared at 125, 250, 500, 1000 mg/mL respectively, and the DPPH samples were prepared at concentrations of 1×10^{-4} M, 2.5×10^{-4} M, 5×10^{-4} M, 6.5×10^{-4} M. Four kinds of samples of 1 mL were added to each of 4 DPPH solutions of 1 mL with different concentration, respectively; the absorbance was measured at 540 nm for 5 min at intervals of 10 sec through UV/VIS spectrophotometer(Optizen POP, Mecasys, Korea); and then the reaction was caused for 25 min in the dark room to measure the absorbance value 30 min later. Measurement of all samples was conducted per 3 set using the same method.

2.4 Inhibition of Tyrosinase Activity

The sample was dissolved in ethanol and diluted to a concentration range to inhibit tyrosinase activity. To a test-tube, 220 μ L and 20 μ L of sodium phosphate buffer, and 20 μ L of mushroom tyrosinase solution were put one at a time. After this, 40 μ L of 1.5 mM tyrosine solution was added and this solution was let react at 37°C for 10 to 15 min. Absorbance was measured at 490 nm using an UV/VIS spectrophotometer(Optizen POP, Mecasys, Korea).

Instead of the sample solution, 0.1 M sodium phosphate buffer was used as a sample blank.

2.5 Safety Test

The MTT assay, which is a cytotoxicity test, was used to evaluate safety as a cosmetic ingredient. Quantification was performed by modifying the Mosmann method. B16 F10 mouse melanoma cells were cultured at a density of 1×10^4 cells/ml for 24 h; replaced with fresh medium supplemented with the sample of the extract diluted at a concentration of 10–1,000 $\mu\text{L}/\text{mL}$; and cultured again for another 24 h. After adding 20 μL of MTT to each well, the formazan formed after a 2 h incubation at 37°C in a 5% CO_2 incubator was dissolved in 200 μL DMSO and absorbance was measured at 595 nm with an ELISA reader (PowerWave XS2, BioTek, USA). Cell viability was calculated by the following formula.

$$\text{Cell viability (\%)} = [(\text{Exp.} - \text{Blank}) / \text{Control}] \times 100$$

Exp: Absorbance of the extract containing cells

Blank: Absorbance of the extract containing no cells

Control: Absorbance of distilled water containing cells

3. RESULTS AND DISCUSSION

3.1 Antioxidant activity

Some of the theories that explain skin aging point to ultraviolet and free radicals as major causes of aging[16]. In addition, since melanin, which is a skin pigment, is produced by a continuous oxidation process of tyrosine by the activity of tyrosinase, a substance having a high antioxidative effect is likely to inhibit the oxidation process in melanin production[17]. The most widely used method based on this mechanism to determine the potential as an ingredient for functional cosmetics of a substance is the method of measuring antioxidant capacity using DPPH reagent. The extract showed an oxidizing power of 7.038~60.37% at 1.25~

10 mg/mL concentration. The higher the concentration of the extract was, the greater the activity level became; thus, it can be said that its antioxidant activity had a concentration-dependent tendency. It showed an EC_{50} of 7.008 mg/mL, which was lower than that of the control group Vitamin C (EC_{50} =3.89 ppm), but higher than the values reported in the previous studies[15] using the conventional processing methods.

Unlike ordinary alcoholic beverages, makgeolli contains various nutrients such as protein, saccharide, and vitamin B group, and raw yeast; also, it is reported that it has special effects such as anticancer and antiinflammatory effects as well as a high nutritional value[18]. In addition, makgeolli has a high content of phenolic compounds, which are abundant in plant foods. Phenolic compounds have hydroxyl (-OH) groups and are reported to have antioxidant and anticancer effects[19]. That is, the hydroxyl (-OH) groups located in the ring structure of phenolic substances donate electrons to free radicals and reduce them. Although it cannot be definitely claimed that the content of phenolic compounds is proportional to the antioxidant capacity, it is considered that the significant antioxidant activity of this extract is exhibited partially due to this mechanism.

3.2 Inhibition of Tyrosinase Activity

This study intended to assess the whitening efficacy of the extract by identifying its inhibitory effect on tyrosinase activity. Tyrosinase is a copper-containing enzyme that plays an important role in the formation of melanin in the skin. It is a tyrosine hydroxylase that produces DOPA by oxidizing tyrosine within the melanosome, and acts as a DOPA oxidase that oxidizes DOPA to DOPA quinone, thereby acting as an important enzyme in synthesizing melanin polymers[16]. In the experiment, the extract's inhibition of tyrosinase enzyme activity was measured by the IC_{50} value obtained after letting the substrate tyrosine react with the enzyme tyrosinase. As shown in <Table 1>,

as a result of measuring the inhibition rate of tyrosinase activity from a concentration of 200 mg/mL, the IC₅₀ value of the extract was 39.22 mg/mL and the IC₅₀ value of vitamin C used as a positive control group was 239.01 µg/mL.

<Table 1> Effect of Makgeolli Fractions on inhibitory Tyrosinase

Sample	Makgeolli	Vitamin C
IC ₅₀	39.22 mg/mL	239.01 µg/mL

IC₅₀ : Inhibitory activity is expressed as the mean of 50% inhibitory concentration of triplicate determines, obtained by interpolation of concentration-inhibition curve.

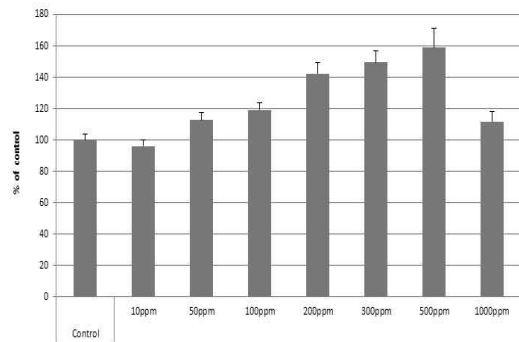
Generally, typical substances known for inhibiting tyrosinase activity include Vitamin C, arbutin, kojic acid, and mulberry extract[7]. Arbutin was extracted from wild cowberry growing in mountains, but it can now be synthesized in an artificial way. Kojic acid, which is a γ-pyrone compound, is mainly produced in the fermentation broth of mold such as the *Aspergillus* genus and *Penicillium* genus, which are fungi, and are highly contained in soybean paste and alcoholic beverages[17]. As described above, makgeolli is a total fermentation product including yeast and lactic acid bacteria involved in the fermentation process unlike other general alcoholic beverages. Makgeolli has distinctive characteristics because as it contains the decomposition products of leaven, which is a representative fermentation substance[18].

According to the study of Baek(2015)[19] that compared the whitening efficiencies of natural leaven and makgeolli, the group of the experiment subjects who applied makgeolli showed the greatest changes in their melanin levels after 4 weeks of the application. In addition, the study of Seo et al.(2013)[20] reported an excellent inhibitory effect on tyrosinase activity that makgeolli byproducts have. These results are thought to be attributed to the efficacies and properties of the microorganisms produced during fermentation. With

regard to this, further studies on more specific ingredients and mechanisms are deemed to be necessary.

3.3 ActivitySafety Test

The results of the extract's cytotoxicity evaluation are represented in [Fig. 1] in comparison with the control group vitamin C. No cytotoxicity was exhibited at a concentrations range from 10 to 1,000 µg/mL. Since the extract has a high level of safety, it can be used at high concentrations in cosmetic formulations; therefore, it is considered useful in various aspects.



[Fig. 1] Effect of different concentrations on the cell viability of B16 F10 cells. The cytotoxicity of cells was determined by measuring optical density(OD) at 595 nm in MTT assay. Result are means ± SD of triplicate data.

4. CONCLUSION

Makgeolli (rice wine, also known as “takju” is a Korean traditional alcoholic beverage and many Korean researchers have actively conducted studies for the nutritional enhancement of it. This study, however, which reflects the recent trend of discovering functional cosmetic ingredients from diverse substances such as foods and pharmaceuticals, evaluated the potential of the fractions of makgeolli, which is mainly consumed as beverage, as a functional

cosmetic ingredient. First, in a safety assessment using the MTT assay method, makgeolli extract obtained by fractionation showed no cytotoxicity at a concentration range of 10 to 1,000 $\mu\text{g/mL}$ which confirmed that makgeolli fractions are a safe ingredient. Second, in an antioxidant activity assessment, it exhibited a superior antioxidant activity than the conventional makgeolli extracts, with an EC_{50} of 7.008 mg/mL . Third, it had an IC_{50} value of 39.22 mg/mL in a tyrosinase activity inhibitory effect assessment, displaying an excellent whitening efficacy. These results suggest that the makgeolli extract has potential as a functional cosmetic ingredient, which seems to be attributed to various properties of fermenting substances. In this regard, there is a need for follow-up studies on whitening mechanisms through specific ingredient analyses.

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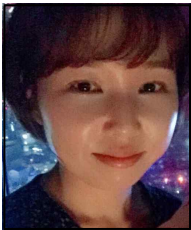
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