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Development of Wine Cake as a Skin-Whitening Agent and Humectant

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ABSTRACT

In this study, 15 wine cakes from various sources were collected for the investigation of whitening and moisturizing effects. The fatty acid and free amino acid contents of the wine cakes were analyzed. The results showed that flattened yellow wine cake had the highest free amino acid contents (630 mg/100 g) among all the wine cakes tested. In addition, the fatty acid content of the wine cakes was higher than that of Japanese sake wine. Since the fatty acids and amino acids of wine cake are similar to the natural moisturizing factors and the intercellular lipids in the human epidermis, the wine cakes could be applied as cosmetic humectants.

The ethanolic extracts of wine cakes were subjected to tests for the inhibition zones of tyrosinase, the inhibition rates on tyrosinase activity, and inhibition of DOPA quinone synthesis via B16 melanoma cell. The inhibition zones of tyrosinase caused by grain sorghum wine cakes and yellow wine cakes were larger than that of arbutin. Reduction of the inhibition rate on tyrosinase activity was in the following order: dry grain sorghum wine cake, flattened yellow wine cake, wet grain sorghum wine cake, dry yellow wine cake and wet yellow wine cake. Moreover, in the tests for inhibition of DOPA quinone synthesis, higher inhibitions were shown for dry grain sorghum wine cake by 1.03-fold, wet grain sorghum wine cake by 0.74-fold, dry yellow wine cake by 0.62-fold, wet yellow wine cake by 0.94-fold, and sake wine cake by 0.78-fold, when compared with arbutin.

Our results of the three *in vitro* inhibition tests for tyrosinase activity indicated that grain sorghum wine cakes and yellow wine cakes can be developed for use as skin-whitening agents and humectants.

Key words: wine cake, natural moisturizing factor, skin-whitening, tyrosinase, humectants

INTRODUCTION

The function of melanin in mammalian species is to protect the skin from UV-induced injury; however, over-expression of melanin would cause hyperpigmentation and skin disorders such as chloasma, freckles and age spots[1,2]. In Northeast Asia, the concept that a white complexion is powerful enough to hide a number of faults was ingrained, especially for women. The mechanism of melanogenesis was extensively studied[2]. Hyperpigmentation was dependent on the activity of melanogenic enzymes and the function of tyrosinase. UV radiation would cause the overexpression of melanin and influence melanogenesis through the autocrine and paracrine regulation process involving keratinocytes[3]. The activating factors include α-MSH (melanocyte-stimulating hormone), ET-1 (endothelin-1), SCF (stem cell factor)/cj-kit and chemical mediator associated with inflammation. In addition, superoxide anion radicals ($\cdot O_2^-$) would promote the hydroxylation of tyrosine and the oxidation of DOPA quinone[4]. In the process of melanosome transfer from melanocyte to keratinocyte, protease-activated receptor 2 (PAR2) would play a role in melanocyte-keratinocyte interaction and would cause hyperpigmentation[5]. Down-regulation of melanin synthesis or melanin transfer is required for recovery of pigmentary disorders.

Tyrosinase is an enzyme catalyzing the rate-limiting reaction in melanin biosynthesis[6]. The enzyme catalyzes two different reactions. The first is hydroxylation of monophenol to *o*-diphenol (monophenolase activity), and the second is oxidation of *o*-diphenol to *o*-quinone (diphenolase activity). The latter is then oxidized and polymerized to produce melanin[7]. Inhibition of tyrosinase, the key enzyme of melanogenesis, is key to developing skin whiting agents[8-10].

Wine cake is claimed to have skin lightening effects and can be applied topically with cotton or cloth on the skin. It has been reported that sake kasu (the lees left over from sake production) functions as a tyrosinase inhibitor, and its activity was similar to that of kojic acid but stronger than arbutin[11]. Topical application of linoleic acid is considered

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to be effective in the treatment of melasma patients because of the inhibition of melanogenesis in *in vitro* tests[12,13]. The fatty acids contained in sake kasu are caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, oleic acid and linoleic acid[11]. Therefore, sake lees, with its water retention effects because of its abundant amino acids and fatty acids, can be used as a moisturizer [14].

In this study, 15 wine cakes from various sources including wet grain sorghum wine cake, dry grain sorghum wine cake, wet yellow wine cake, dry yellow wine cake, flattened yellow wine cake and sake wine cake were collected for the investigation of whitening and moisturizing effects. The objective of this study is to identify the fatty acids and amino acids in wine cakes and evaluate the activity of these wine cakes in tyrosinase inhibition.

MATERIALS AND METHODS

I. *Collection of Wine Cake*

Fifteen types of wine cakes were collected from various liquid distilleries of Taiwan Tobacco and Liquor Corporation. Wet yellow wine cake (A and B), Dry yellow wine cake (C), and flattened yellow wine cake (D) were obtained from Taichung Distillery. Sake wine cake was collected from Puli Distillery (E). Dry grain sorghum wine cake was obtained from Kinmen Distillery (F). Wet grain sorghum wine cakes were obtained from Kinmen (G), Longtian (H) and Chiayi (I); in addition, Chayi Distillery donated 6 more wet grain sorghum wine cakes produced by different process and lots (J1-6). J1-4 were distilled before second fermentation, J5 and J6 were not.

II. *Chemicals*

Arbutin, agar, glycerol, L-tyrosine, 3,4-dihydroxy-L-phenylalanine (L-DOPA), 2-mercaptoethanol, 3-methylbenzothiazolinone hydrazone (MBTH), bromophenol blue and dimethyl sulfoxide (DMSO) were purchased from Sigma chemical (St. Louis, MO, USA). Mushroom tyrosinase was purchased from Fluka Chemie GmbH (Bushs, Switzerland). Dibasic sodium phosphate, monobasic potassium phosphate, monobasic sodium phosphate and sodium chloride were purchased from J. T. Baker, Inc. (Philipsburg, NJ, USA). Potassium chloride, sodium hydroxide and Tween 20 were purchased from Merck (Darmstadt, Germany). Glycine and tris were purchased from USB corporation (Cleveland, OH, USA).

III. *Determination of Fatty Acid and Free Amino Acid of Wine Cakes*

The determination of fatty acid content in wine cakes was carried out according to the AOCS official method Ce-1b-89[15]; and free amino acid content was determined using CNS 12632 N6221[16].

IV. *Wine Cakes Extraction*

Wine cakes weighing 10 g were soaked and extracted twice with 95%, 70% and 50% (w/v) ethanol 50 mL, respectively. Then, the mixtures were extracted twice by sonication, and the filtrates were collected. The filtrate was evaporated to dryness and redissolved with distilled water before use.

V. *Tyrosinase Activity Assay−In vitro Tyrosine-Tyrosinase*

(I) *Assay for Inhibition Zone of Melanin*[11]

The sample solutions were prepared by dissolving the wine cake extracts with distilled water. The series concentrations of sample solutions were 0.652, 1.25, 2.5 and 5.0 g/mL. The agar plate contained agar (2.0%), phenoxyethanol (0.4%), distilled water (97.5%) and L-tyrosine (0.08%)(2400 U/mL) spread uniformly over the surface of the agar. The paper disc was positioned on the agar plate and then loaded with 40 μL sample solution. The plate was incubated at 37°C for 3 h, and the inhibition zone of melanin formation was measured. Kojic acid and arbutin were used as positive controls.

(II) *Enzyme Assay*[17]

A 305 μL sample was combined with 1 mL PBS buffer (NaCl, KH_2PO_4, Na_2HPO_4 and KCl, pH 6.8), 0.03% tyrosine 2 mL and 1000 U /mL tyrosinase 150 μL. The mixture was shaken and incubated at 37°C for 25 min, and absorbance was measured at 475 nm using a spectrophotometer (UV-160A). The preparation of sample solutions was the same as the section of (I) Assay for Inhibition Zone of Melanin. The sample solution and tyrosine were replaced by the same volume of distilled water in the control and blank, respectively. Kojic acid and arbutin were used as positive controls. The inhibitory effects on the enzyme activity by the test samples were represented as % of inhibition.

VI. *Cell Cultures and Cellular Tyrosinase Activity Assay*

B16F10 murine melanoma cells were purchased from FIRDI (Food Industry Research and Development Institute, Hsinchu, Taiwan) and cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum (FBS), 4 mM L-glutamine, 1.5 g/L sodium bicarbonate and 4.5 g/L glucose in a humidified atmosphere containing 5% CO_2 in air at 37°C. Cells were planted in a 12-well plate at 5×10^4 cells per well and incubated for 24 h. After that, the medium was replaced by DMEM containing various concentrations of wine cake extracts. After 24 h, the medium was removed, and the sample was washed with PBS. Then 0.5% Triton X-100 in 50 mM sodium phosphate buffer (pH 6.9) 300 μL was added, and the solution was freeze-thawed by incubating at -80°C for 30 min, followed by exposure to room temperature for 25 min and 37°C for 5 min. After that, a substrate solution (6.3 mM MBTH and 1.1 mM L-dopa in 48 mM sodium phosphate buffer pH 7.1) was added to the

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former solution, and the resulting solution was incubated at 37°C for 1 h. The absorbance was then read at 508 nm using a microplate reader.

VII. *Statistical Analysis*

The values are expressed as mean ± standard deviation (SD). Differences between the amounts obtained with the various treatments were compared by ANOVA followed by Scheffe's test.

RESULTS AND DISCUSSION

I. *Determination of Fatty Acid and Free Amino Acid of Wine Cakes*

The fatty acid contents of the wine cakes are shown in Table 1 and the free amino acid contents are in Table 2. Palmitic acid (C16 : 0), olieic acid (C18 : 1) and linoleic acid (C18 : 2) were most abundant. The content of palmitic acid was low in flattened yellow wine cake (D), but that of cis-10 pentadecanoic acid, which was not found in the other wine

Table 1. The fatty acids composition of yellow and sorghum wine cakes

Fatty acid composition (g/100 g lipid)[a] Fatty acid	Wine cake						
	B	C	D	F	G	H	I
Caproic Acid (C6 : 0)	0.33	0.38	0.11	0.09	ND	ND	0.18
Caprylic Acid (C8 : 0)	1.48	2.39	0.83	1.69	2.49	0.36	0.47
Pelargonic Acid (C9 : 0)	0.15	0.06	0.13	0.36	0.46	0.42	0.50
Capric Acid (C10 : 0)	4.84	4.53	2.17	1.42	1.44	0.28	0.64
Lauric Acid (C12 : 0)	1.45	1.04	0.72	0.36	1.26	0.40	0.93
Tridecanoic Acid (C13 : 0)	0.33	0.09	0.06	ND	ND	0.17	ND
Myristic Acid (C14 : 0)	3.59	2.27	1.87	0.72	1.17	0.70	1.05
Pentadecanoic Acid (C15 : 0)	0.16	0.22	0.21	0.19	0.23	0.18	0.23
cis-10-Pentadecanoic Acid (C15 : 1)	ND	ND	33.41	ND	ND	ND	ND
Palmitic Acid (C16 : 0)	31.67	40.06	1.2	22.25	20.66	22.81	20.88
Palmitoleic Acid (C16 : 1)	0.52	0.54	ND	0.94	0.82	1.93	1.04
Margaric Acid (C17 : 0)	0.19	0.13	0.20	0.22	0.26	0.26	0.28
cis-10-Heptadecenoic Acid (C17 : 1)	ND	0.07	0.12	0.19	0.26	0.27	0.26
Stearic Acid (C18 : 0)	3.17	2.82	4.95	2.47	2.92	4.77	2.86
Olieic Acid (C18 : 1)	13.2	11.96	19.24	22.14	24.64	38.12	24.08
Linoleic Acid (C18 : 2)	29.95	29.58	31.16	40.84	38.16	24.60	40.32
Linolenoic Acid (C18 : 3)	1.38	1.47	1.14	3.69	3.57	2.06	3.64
Arachidic Acid (C20 : 0)	0.38	0.16	0.46	0.46	0.59	0.42	0.94
Gadoleic Acid (C20 : 1)	0.19	0.11	0.17	0.14	ND	0.26	ND
Eicosadienoic Acid (C20 : 2)	0.45	0.17	0.08	0.12	ND	ND	ND
cis-8,11,14-Eicosatrienoic Acid (C20 : 3)	ND	0.05	ND	ND	ND	ND	ND
Arachidonic Acid (C20 : 0)	2.06	0.35	0.25	ND	ND	ND	ND
Behenic Acid (C22 : 0)	3.84	0.60	0.81	0.97	0.76	0.47	1.10
Eruic Acid (C22 : 1)	0.44	0.09	0.11	0.17	0.29	0.26	0.32
Docosapentaenoic Acid (C22 : 5)	ND	0.33	ND	ND	ND	ND	ND
Tricosanoic Acid (C23 : 0)	0.23	0.04	0.05	0.12	ND	ND	0.27
Lignoceric Acid (C24 : 0)	ND	0.51	0.53	0.46	ND	1.14	ND
Nervonic Acid (C24 : 1)	ND	ND	ND	ND	ND	ND	ND
Total	100	100.02	98.98	100.01	99.98	100	99.99

[a]: The relative content of fatty acid in 100 g lipid.
ND: not detectable.

Table 2. The free amino acids composition of yellow and sorghum wine cakes

Free amino acid (mg/100 g)	Wine cake						
	B	C	D	F	G	H	I
o-Phosphorserine	2.07	10.52	ND	10.03	2.43	1.87	2.23
Taurine	ND	14.58	21.65	3.05	1.05	0.94	1.24
L-Aspartic Acid	7.77	29.94	122.19	3.70	2.80	1.43	2.44
L-Threonine	2.39	3.47	19.15	1.51	1.29	0.38	0.84
L-Serine	3.95	5.46	27.55	1.91	1.78	0.55	1.18
L-Glutamic acid	5.75	6.13	74.66	8.60	7.66	3.17	5.46
L-2-Aminoadipic Acid	ND	ND	0.20	ND	ND	ND	0.28
Glycine	4.31	2.98	27.28	3.48	2.94	1.24	2.64
L-Alanine	10.88	13.70	65.56	15.09	9.82	5.04	10.06
L-Citrulline	ND	ND	ND	ND	ND	ND	ND
DL-2-Aminobutyric Acid	ND	0.33	ND	ND	ND	ND	ND
L-Valine	7.02	10.88	41.34	5.97	4.76	3.00	4.81
L-(-)-Cystine	ND	ND	ND	ND	ND	ND	ND
L-Methionine	2.27	3.38	4.30	1.98	1.76	1.78	2.31
L-Cystathionine	1.68	10.04	8.19	1.54	1.50	1.63	1.46
L-Isoleucine	3.82	6.31	28.75	3.62	2.87	1.39	2.50
L-Leucine	7.24	12.46	46.45	7.77	4.95	3.41	6.50
L-Tyrosine	5.55	6.56	15.97	3.59	2.45	1.98	3.70
L-Phenylalanine	5.40	6.17	21.55	3.42	1.91	1.94	3.65
β-Alanine	ND	5.40	16.29	1.48	0.34	0.41	0.66
DL-3-Aminoisobutyric Acid	0.25	ND	1.78	ND	ND	ND	ND
γ-Aminobutyric Acid	2.08	4.38	6.13	2.43	1.36	0.70	1.87
Tryptophan	2.02	1.33	ND	ND	ND	ND	ND
Ethanolamine	0.60	0.25	0.62	0.18	0.24	0.18	0.27
DL-plus allo-δ-Hydroxylysine	0.90	4.87	0.29	0.36	0.45	0.74	0.46
L-Ornithine	0.73	0.00	0.63	1.40	1.10	1.18	2.23
L-Lysine	4.49	2.65	18.65	3.09	2.21	1.61	2.82
L-Histidine	1.22	0.88	2.77	0.45	0.34	0.34	0.76
L-3-Methylhistidine	ND	0.19	ND	ND	ND	ND	ND
L-Carnosine	0.99	5.66	1.17	1.00	0.98	1.20	1.59
L-Arginine	3.93	7.12	14.51	4.42	3.31	2.70	4.70
L(-)-Proline	4.48	4.92	42.16	16.18	7.43	2.65	6.41
Total	91.79	180.6	629.79	106.3	67.73	41.46	73.07

cakes, was high in D. It had been reported that the Japanese sake wine cake was abundant with various fatty acids and amino acids[11]. The contents of Japanese sake wine cake were 0.3, 1.2, 1.0, 3.2, 46.0, 0.2, 3.5, 14.8, 28.8, 0.8, 0.1 and 0.1 for caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, olieic acid, linoleic acid, linolenic acid, gadoleic acid and behenic acid, respectively. In this study, the contents of the above fatty acids, except palmitic acid, in wet grain sorghum wine cake, wet yellow wine cake, dry yellow wine cake, and flattened yellow wine cake were higher than those in the Japanese sake wine cake. The total amount of free amino acids in the flattened yellow wine cake (D) was the highest, since it was fermented, and the amino acids content would increase after fermentation. The free amino acids and free fatty acid in the extracts of wine cakes are similar to the natural moisturizing factors and the wine cakes could be considered as cosmetic humectants.

II. *Extraction of Wine Cakes*

In our preliminary test, the wine cakes were extracted with 50, 70, and 95% alcohol, respectively. The ethanolic extracts of wine cakes were concentrated under reduced pressure to obtain the wine cake extracts. The 50, 70 and 95% alcohol extracts of the wet grain sorghum wine cake

Table 3. The inhibition zone of wine cake extracts on tyrosinase activity

Conc. c (g/mL) / Inhibition zone (cm) / wine cake	5	2.5	1.25	0.625	r^2*
C	1.67 ± 0.12	1.40 ± 0.00	1.37 ± 0.06	1.13 ± 0.06	0.90
D	1.97 ± 0.06	1.67 ± 0.12	1.37 ± 0.12	1.17 ± 0.12	0.95
F	2.83 ± 0.06	2.67 ± 0.06	2.47 ± 0.12	2.07 ± 0. 12	0.78
G	2.87 ± 0.06	2.63 ± 0.06	2.17 ± 0.06	1.77 ± 0.15	0.85
H	2.73 ± 0.15	2.73 ± 0.15	2.13 ± 0.06	1.67 ± 0.06	0.90
I	2.37 ± 0.15	2.03 ± 0.15	1.90 ± 0.00	1.60 ± 0.00	0.93

n = 3
Data present as mean ± SD (cm).
The inhibition zone of arbutin (27.23 mg/mL) and kojic acid (14.21 mg/mL) were 1.3 ± 0.06, and 2.1 ± 0.06 cm, respectively.
* The r^2 means the correlation *coefficient* between the inhibition zone of wine cake and its concentration.

were not significantly different on tyrosinase inhibition. The results of the inhibition of mushroom tyrosinase activity indicated that the inhibition zones of 0.2 g/mL wine cakes were 2.6 - 3.1 mm, and that of arbutin (27.23 mg/mL) was 1.25 ± 0.10 mm. Therefore, the 50% alcohol was selected to extract all wine cakes.

The extracted rates of wine yields by 50% ethanol were 4.8 (A), 3.1 (B), 15.3 (C), 22.0 (D), 13.2 (E), 13.1 (F), 6.9 (G), 6.4 (H), and 6.1 % (I). This result indicated that the extraction rate of the flattened yellow wine cake was the highest. As expected, the extraction rate of the dry wine cakes (C and F) was higher, but the sake wine cake (E) was also high.

III. *Inhibitory Effects of Wine Cakes on Tyrosinase Activity and Melanin Synthesis*

Mushroom tyrosinase and murine tyrosinase from cultured B16F10 cells were used in the present investigations. The inhibition of tyrosinase activity of wine cake extracts (0.625 - 5 g/mL) is shown in Table 3. In comparison to arbutin (27.23 mg/mL, inhibition zone: 1.3 ± 0.06 cm) and kojic acid (14.21 mg/mL, inhibition zone: 2.1 ± 0.06 cm), the inhibition of some wine cakes on tyrosinase was superior to that of kojic acid, especially sorghum wine cakes. In addition, all the tyrosinase activity inhibition of wine cakes was significantly higher than arbutin. Our results indicated that the inhibition zones of wine cake extracts were larger than that of arbutin; furthermore, sorghum wine cakes (F, G and H) were better than yellow wine cakes (C and D).

The IC_{50} (half inhibition concentration) of wine cakes ranged from 9.56 - 44.02 mg/mL (Table 4). The inhibition of tyrosinase activity of various sorghum wine cakes is shown in Figure 1. The results indicated that the inhibitions were 97.4% ± 0.1 to 100.0% ± 0.2 at 100 mg/mL and 82.3% ± 0.88 to 99.7% ± 0.2 at 50 mg/mL. Most of the inhibition rates were dramatically decreased at 25 mg/mL, while the inhibition rates of J5 and J6 were 94.8% ± 0.8 and 95.6% ± 0.6 at 25 mg/mL, respectively. The wine cake was the byproduct after distillation to afford sorghum wine, but J5 and J6 were

Table 4. The IC_{50} of wine cake extracts on mushroom tyrosinase activity

Wine cakes	IC_{50} (mg/mL)	r^2
A	44.02	0.99
B	56.96	0.99
C	20.70	0.99
D	13.13	0.93
F	9.56	0.81
G	16.81	0.81
H	20.15	0.7
I	24.40	0.86
arbutin	2.42	0.99
kojic acid	0.18	0.82

The concentrations of sample solutions within in the range of 6.25 - 70.0 mg/mL, kojic acid were 0.25 - 8 mM (0.04 - 1.14 mg) , and arbutin were 0.625 - 40 mM (0.17 - 10.9 mg).

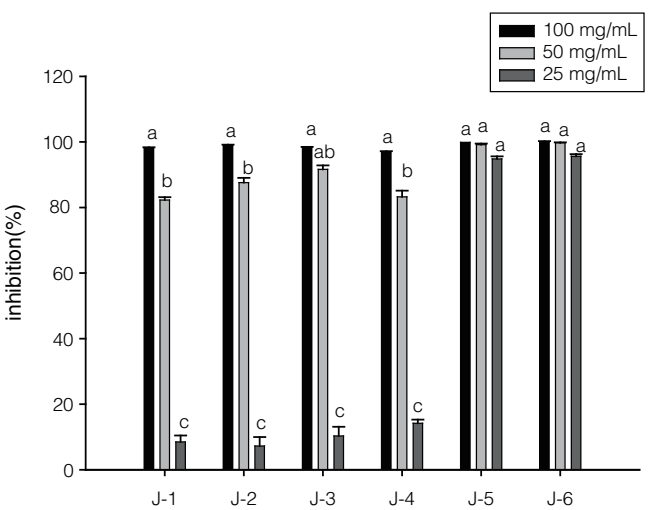

Figure 1. The tyrosinase inhibition rate (%) of different process and lots of wine cakes extracts (25 - 100 mg/mL).
Each value represents Mean ± SD (n = 4).
Means with the same letter are not significantly different.

the wine cakes without distillation. It had been reported that free fatty acids regulated melanogenesis in melanoma cells through modulating tyrosinase degradation [18], additionally, unsaturated fatty acids which was unstable while heating decreased melanin synthesis and tyrosinase activity. We speculated that the lower anti-tyrosinase activity of distilled sorghum wine cake might due to the soluble whitening constituents of that was damaged or loss during distillation. Thus, the inhibition zone method or enzyme assay created by this study could be used for controlling the variety of manufacturing lots.

IV. *The Effect of the Wine Cakes Extracts on Cellular Tyrosinase Activity*

The results of wine cake extracts on cellular tyrosinase activity of mouse B16 melanoma are shown in Table 5. The results indicated that the wine cake extracts showed tyrosinase inhibition activity. Furthermore, a low concentration of wine cake extracts showed more potent cellular tyrosinase activity inhibition than a high concentration. The number of mouse B16 melanoma cells were reduced and their morphology was shrunk after the wet yellow wine cake extract (A) (10 and 2 mg/mL) treatment. In addition, MTT test of wet yellow wine cake extract (A) and dry yellow wine cake extract (C) on human foreskin fibroblasts also showed cytotoxicity while the concentration over 200 μg/mL (data not shown). We suggested that the extracts might influence the physiology or function of melanoma cells. Free fatty acids have been shown to have remarkable regulatory effects on melanogenesis in cultured B16F10 murine melanoma cells by modulating proteolytic degradation of tyrosinase[18]. Fatty acids were the major components of cell membranes with regulation of tyrosinase proteasomal degradation by modulating the ubiquitination of tyrosinase[19]. The number and location of unsaturated bonds of fatty acid would correlate with the inhibition of melanogenesis[14,20]. Unsaturated fatty acids involving oleic acid (C18 : 1), linoleic acid (C18 : 2) and α-linolenic acid (C18 : 3) decreased melanin synthesis and tyrosinase activity, while saturated fatty acids, such as palmitic acid (C16 : 0) and stearic acid (C18 : 0), retarded tyrosinase ubiquination[12,21,22]. Furthermore, topical application of α-linolenic acid and linoleic acid would activate melanocytes to inhibit melanin generation and accelerate the turnover rate of the stratum corneum and then cause hypopigmentation[21]. In this study, the content of unsaturated fatty acids was higher than that of saturated fatty acids, which may have contributed to the tyrosinase inhibition activity.

Table 5. The inhibition of wine cakes on tyrosinase activity of murine B16F10 melanoma cells

	concentration	mean ± SD (optical density)	Inhibition (%)*
arbutin	10 mM	0.23 ± 0.01	100.0
kojic acid	10 mM	0.08 ± 0.02	149.73
	1 mM	0.30 ± 0.01	77.99
	0.1 mM	0.38 ± 0.06	50.16
A	10 mg/mL	0.28 ± 0.05	84.14
	2 mg/mL	0.26 ± 0.04	89.86
	0.2 mg/mL	0.25 ± 0.01	93.74
C	10 mg/mL	0.36 ± 0.02	59.01
	2 mg/mL	0.35 ± 0.03	61.49
	0.2 mg/mL	0.35 ± 0.06	62.03
E	10 mg/mL	0.31 ± 0.01	74.87
	2 mg/mL	0.30 ± 0.01	78.21
	0.2 mg/mL	0.30 ± 0.05	77.89
F	10 mg/mL	0.28 ± 0.04	83.06
	2 mg/mL	0.24 ± 0.01	96.55
	0.2 mg/mL	0.22 ± 0.03	103.13
G	10 mg/mL	0.48 ± 0.07	19.53
	2 mg/mL	0.41 ± 0.01	41.42
	0.2 mg/mL	0.31 ± 0.02	74.22

*The inhibition of arbutin on tyrosinase activity was 100% at 2.72 mg/mL.

Inhibition = 【(control-sample) / (control-arbutin)】× 100

CONCLUSIONS

In our study, extracts from wine cakes, especially sorghum wine cakes, exhibited tyrosinase activity inhibition in murine B16 melanoma cells. The various fatty acids and amino acids found in wine cakes would be a potential ingredient for cosmetics as a humectant.

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