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Research Article

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The Multi-Berry Extracts Improved Aging Skin and Increased Antioxidant Capacity

Yung-Kai Lin[1], Yung-Hsiang Lin[2], Shu-Ting Chan[2], Han-Fang Wu[2] and Chi-Fu Chiang[2*]

[1]Institute of Food Safety and Risk Management, National Taiwan Ocean University, Keelung, Taiwan. Department of Food Science, National Taiwan Ocean University, Keelung, Taiwan. Graduate Institute of Biomedical Engineering, National Chung Hsing University, Taichung, Taiwan

[2]Research & Design Center, TCI CO., Ltd., Taipei, Taiwan

[*]Corresponding author
Chi-Fu Chiang, Research & Design Center, TCI CO., Ltd., Taipei, Taiwan

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Abstract

Skin aging was a complex and continuous process that affected skin functions and appearance. The natural berry extract had antioxidant activity, can capture the superoxide free radicals and hydroxyl free radicals generated by ultraviolet light, so it can prevent skin aging. Several kinds of berries, including grapes, pomegranate, black currant, black chokeberry, to produce multi-berry favor drink. We used multi-berry favor drink to treated B16/F10 cells with blue light to examine melanin and antioxidant ability. Moreover, 40 adult subjects were recruited, and drink daily for 8 weeks, then examine skin condition. The multi-berry favor drink significantly decreased the melanin formation compared with the blue light group in vitro. After taking 8 weeks, the multi-berry favor drink significantly increased SOD activity, and slight increased total antioxidant capacity in human plasma. Multi-berry favor drink significantly improved the collagen, spots, melanin, wrinkles, texture and pores in the facial skin. Taking multi-berry favor drink for 8 weeks can improve the collagen, spots, melanin, wrinkles, texture and pores of the skin. In addition, multi-berry favor drink can increase the capacity of antioxidant and decrease the melanin production.

Keywords: Antioxidant, Berry, Skin Aging, Melanin

Introduction

The skin was the largest organ of the human body, mainly to protect the body. Skin aging was the result of two synergistic mechanisms: intrinsic or temporary aging, which occurred not only on the skin, but also on all tissues; external aging or photoaging was due to the skin being exposed to harmful substances, especially caused by ultraviolet (UV) radiation [1]. Human skin exposure to UV radiation was the main cause of skin diseases such as erythema, inflammation, age-related degenerative changes and cancer [2]. Excessive exposure of the skin to UVA and to a lesser degree of UVB can cause oxidative stress, thereby increasing the production of reactive oxygen species (ROS), leading to lipid peroxidation of cell membranes, damage to tissues by DNA and proteins, inflammation, and keratinocyte apoptosis [3]. ROS also triggered the expression of matrix metalloproteinases (MMP), which degraded extracellular matrix, such as collagen, to maintain the integrity of cells and skin. In recent years, the health care product market advocates nature, and it had become a trend to use natural fruits and vegetables as the source of health care products. Studies had shown that berries can enhance the skin's antioxidant capacity, collagen, and reduce skin wrinkles and pigmentation [4].

Red grapes contain a lot of anthocyanins and quercetin, which can effectively scavenge free radicals and protect cells from damage. The main polyphenolic compound contained in grape seed extract was catechin, which can fight inflammation, suppress immune response and degradation of skin collagen induced by ultraviolet radiation [5]. Grape extract also can increase skin elasticity, moisture and smoothness, reduce skin pigmentation and photoaging [6]. Pomegranate was widely used in traditional medicine as a traditional product for antibacterial, anti-inflammatory, and homeostasis maintenance [7]. The study had showed that the ellagic acid of pomegranate extract can increase the skin's anti-UV effect and improve the skin's melanin precipitation [8]. Black currant can form a skin protective film, which can effectively relieve skin discomfort and improve the smoothness, stability and elasticity of the skin [9]. The black chokeberry was rich in organic acids, flavonoids and phenolic acids, which can eliminate a variety of oxygen free radicals and reduce cellular oxidative damage [10]. However, there were still not many clinical studies on the skin health effects of natural multi-berry, so it was still worth exploring.

In this study, we combined several kinds of berries, including grapes, pomegranate, black currant, black chokeberry, to produce multi-berry favor drink. Here, we used multi-berry favor drink to examine whether can improve skin condition in vitro and in vivo. The key findings of this study provided important insight into the anti-skin aging potentials of multi-berry to be

used as novel sources of anti-aging ingredients for skincare.

Materials and Methods

Cell Culture

Mouse melanoma cell line B16/F10 was obtained from American Type Culture Collection (ATCC), and cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 2 mM of l-glutamine (Gibco), 100 U/mL of penicillin and 100 µg/mL of streptomycin (Nacalai Tesque, Kyoto, Japan). The normal adult human primary epidermal keratinocyte cell line was purchased from the ATCC, and cultured in dermal cell basal media supplemented with 0.4% bovine pituitary extract (BPE), 0.1% recombinant human (rh) TGF-α (0.5 ng/mL), 3% l-glutamine (6 mM), 0.1% hydrocortisone hemisuccinate (100 ng/mL), 0.1% rh insulin (5 mg/mL), 0.1% epinephrine (1.0 mM), 0.1% apotransferrin (5 mg/mL; ATCC PCS-200–400), and 0.1% penicillin (100 U/ml)-streptomycin (100 µg/ml; Thermo Fisher Scientific), in a humidified incubator of 5% CO2 at 37°C.

Detection of Melanin Production

One was used the $1.5x10^5$ cells/well B16F10, and the multi-berry favor drink to treat in a dose depend manner with blue light. Another was used the $1.5x10^5$ cells/well B16F10 co-cultured with $1.5x10^5$ cells/well keratinocyte, and the multi-berry favor drink to treat in a dose depend manner with blue light. After 24 hours of reaction, using 1N NaOH to dissolve the melanin in the B16F10, and then measure the melanin content at a wavelength of 405 nm.

Clinical Trial Design

The clinical study had been approved by Antai-Tian-Sheng memorial Hospital Institutional Review Board (TSMH IRB No.20-082-B), and the study had been registered on ClinicalTrials.gov Identifier: NCT04878523. 40 adult subjects (> 20 years old) were recruited in this trial between August 2020 and July 2021, and informed consent was obtained from all subjects before the study at Chia Nan University of Pharmacy & Science. The subjects divided into a placebo group (n=20) and an multi-berry favor drink group (n=20). Each subject was informed to intake a bottle of multi-berry favor drink labeled 50ml, or a placebo drink daily for 8 weeks and was not allowed to take any other supplement during the intervention period, and was confirmed by telephone interview. The exclusion criteria included: i) skin disease, liver cirrhosis or chronic renal failure; ii) allergy to cosmetics, drugs, or foods; iii) pregnant and breastfeeding; iv) taking chronic drugs; v) people who had any cosmetic procedures (intense pulse light, medical peelings, or laser therapy) before 4 weeks of the study.

Supplement Formulation

The multi-berry favor drink contains: 14% red grapes, 8.5% fructose, 3% pomegranate, 2% black currant, 1% black chokeberry, citric acid, sucralose, water. Placebo drink of the main ingredient: citric acid, sucralose, water. Each subject was required to undergo skin condition checks at 0, 4 and 8 weeks, and blood was drawn at 0 and 8 weeks to analyze antioxidant ability.

Antioxidant Ability Test

The measurement of superoxide dismutase (SOD) and total antioxidant capacity (TAC) in blood were examined by SOD colorimetric activity kit and total antioxidant capacity assay. All experimental procedures were following the recommended protocols

Skin UV Spot Measurement

VISIA® Complexion Analysis (Canfield Scientific) was employed to measure the skin UV spots of full face. The yellow dots in the software indicate the spots. The degree of improvement in skin UV spots is inversely correlated with the numbers of yellow dots.

Skin Melanin Measurement

Soft Plus (Callegari 1930, Italy) was employed to measure the skin melanin on the cheek. The higher the value, the higher the melanin content of the skin.

Skin Collagen Content

DermaLab® Series SkinLab Combo (Cortex) was employed to skin collagen content of upper cheek. The instrument uses ultrasound to analyze the collagen density of upper cheek.

Skin Wrinkles Measurement

VISIA® Complexion Analysis (Canfield Scientific) was employed to measure the skin wrinkles of full face. Wrinkles are characterized by the long and narrow shape areas; the green lines in the software reflect the presence and depth of wrinkles. The degree of improvement in skin wrinkles is positively correlated with the measured value.

Skin Texture Measurement

VISIA® Complexion Analysis (Canfield Scientific) was employed to measure the skin texture of full face. The degree of improvement in skin texture is positively correlated with the measured value.

Skin Pore Measurement

VISIA® Complexion Analysis was employed to measure the skin pores of full face. The purple dots in the software indicate the pores. The degree of improvement in skin pores is inversely correlated with the numbers of purple dots.

Statistical Analysis

The comparison of measurement results for skin parameters among groups and between groups was analyzed by one-way repeated measurement ANOVA and one-way ANOVA, respectively, followed by Tukey's post hoc test through GraphPad Prism, as $P < .05$ was considered statistical significance.

Results

To explore the effect of multi-berry favor drink on melanogenesis, multi-berry favor drink was used to treat B16F10 cells and examined by the melanin after blue light exposure. Figure 1A showed the content of melanin after treatment with different concentrations of multi-berry favor drink (with/without blue light irradiation). The results discovered the blue light exposure in-

creased melanin formation in B16F10 cells. In addition, 0.125%, 0.25% and 0.05% of multi-berry favor drink significantly decreased the melanin formation 29.7%, 28.1% and 28.1% in comparison with the blue light group. Then, we used B16F10 cells co-cultured keratinocytes, and stimulated with blue light. The results showed that 0.25% and 0.05% of multi-berry favor drink significantly decreased the melanin formation 20.6% and 19.7% in comparison with the blue light group (Figure 1B).

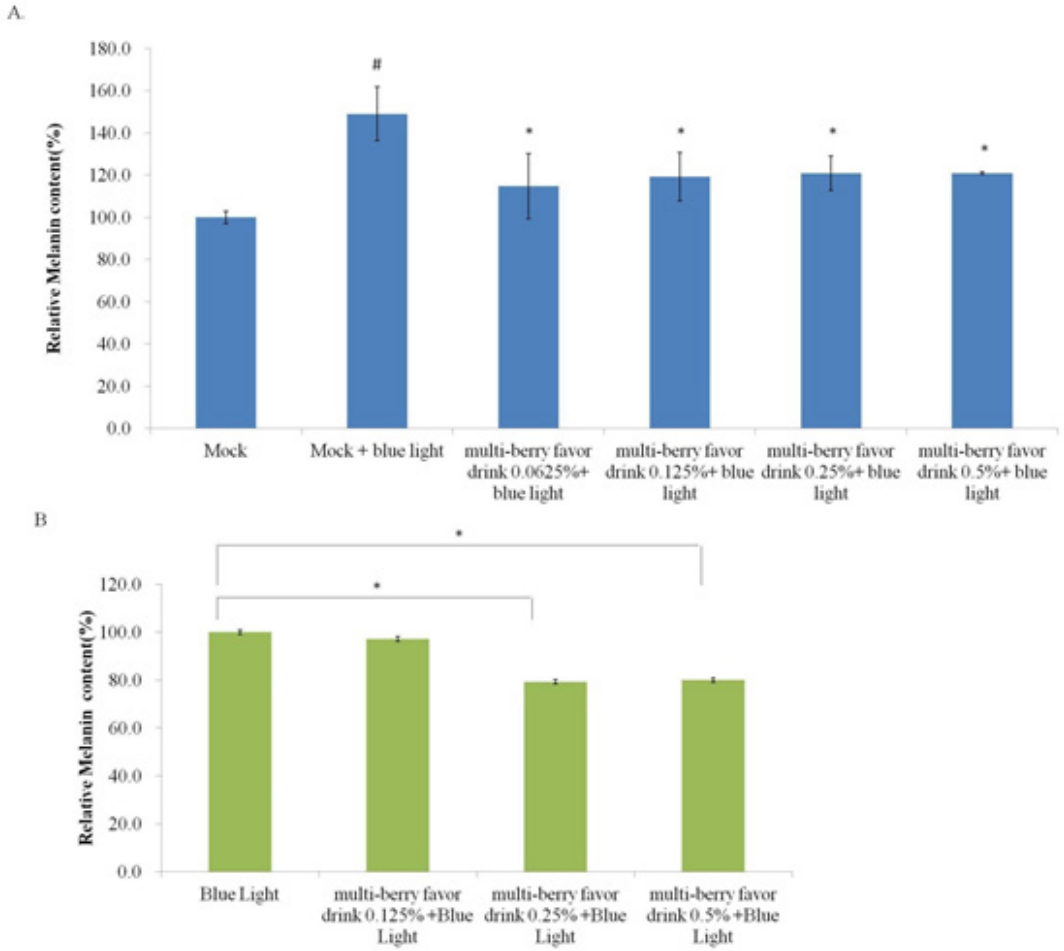

Figure 1: The multi-berry favor drink decreased the melanin formation after blue light exposure. (A) 0.0625%, 0.125%, 0.25%, 0.5% multi-berry favor drink to treat B16F10 cells with blue light then used 1N NaOH to dissolve the melanin in the B16F10 cells. (B) B16F10 cells co-cultured with keratinocytes, and used multi-berry favor drink to treat with blue light, then then used 1N NaOH to dissolve the melanin in the B16F10 cells. (n = 3; mean ± S.D.). *, $p < 0.05$ compared with blue light. #, $p < 0.05$ compared with mock.

ROS playedd an important role involved in the skin function(Okayama 2005). Therefore, we estimated the antioxidant capacity of multi-berry favor drink in human blood plasma. Figure 2A illustrated 8 weeks of multi-berry favor drink increased SOD activity in human blood plasma by 9.7% compared with the group before using; furthermore, we found that SOD activity increased 17.1% after 8 weeks intake multi-berry favor drink compared with 8 weeks of placebo group. In addition, treatment of 8 weeks of the multi-berry favor drink increased the total antioxidant capacity (TAC) by 4.6% in comparison with the group before using (Figure 2B).

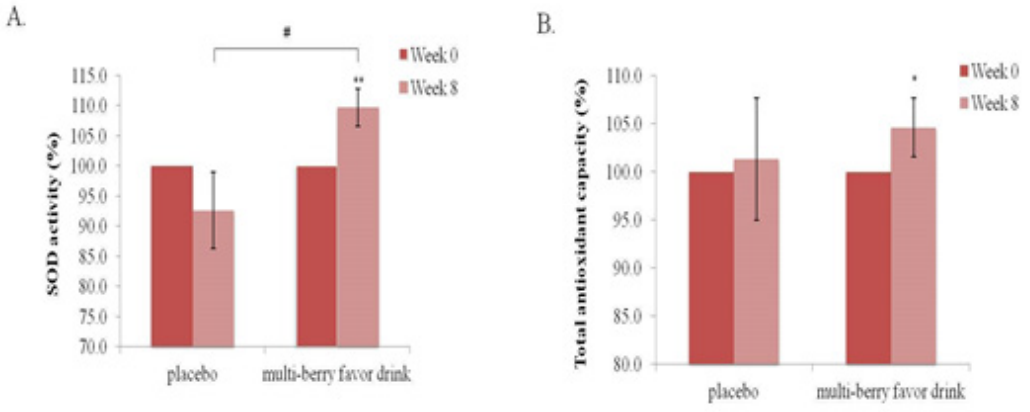

Figure 2: The antioxidant properties on multi-berry favor drink. The subjects were recruited and divided into placebo group and multi-berry favor drink. After drinking for 8 weeks, the effect of (A) SOD activity and (B) total antioxidant capacity after multi-berry favor drink in human blood plasma (n = 20; mean ± S.D.) (*, compared with before using. #, compared with placebo) (*, $p < 0.05$, **, $p < 0.01$) (# , $p < 0.05$)

The Figure 3 showed that collagen density was significantly increased by 7.5%, skin UV spots decreased by 4.4% and the content of melanin decreased by 7.5% compared with placebo group after 8 weeks intake of the multi-berry favor drink. Moreover, the wrinkles, texture and skin pore were decreased by 15.8%, 12.9% and 12.3% after treatment of 8 weeks of multi-berry favor drink in comparison with group before using (week 0), respectively. The images of collagen, spots, wrinkles, texture and pore were showed in Figure 4. In addition, Table 1 indicated the summary of above improvement skin parameter between placebo and multi-berry favor drink after 8 weeks treatment. According to the above results, multi-berry favor drink increased collagen and decrease spots, melanin, wrinkles, texture and pores.

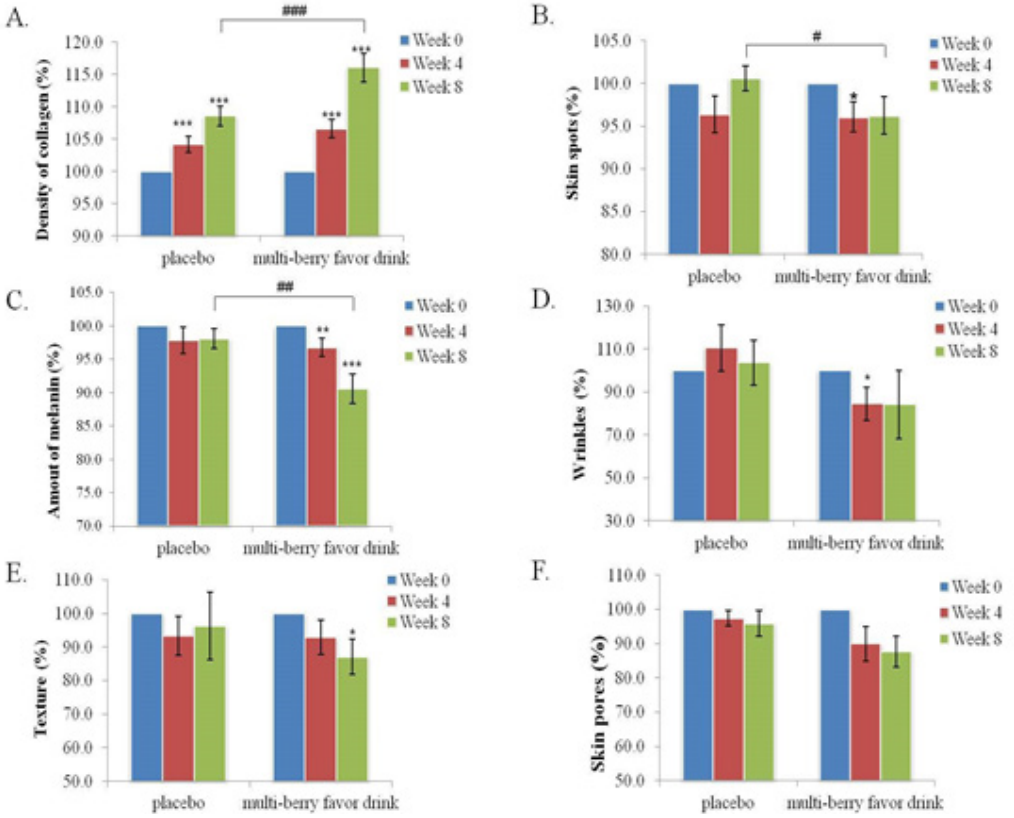

Figure 3: The improvement of skin properties after 8 weeks multi-berry favor drink treatment. The (A) density of collagen, (B) uv spots, (C) melanin, (D) wrinkles, (E) texture, (F) pores. (n = 20; mean value ± S.E.M.) (*, compared with before using(week 0). #, compared with placebo) (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$) (# , $p < 0.05$, ###, $p < 0.001$)

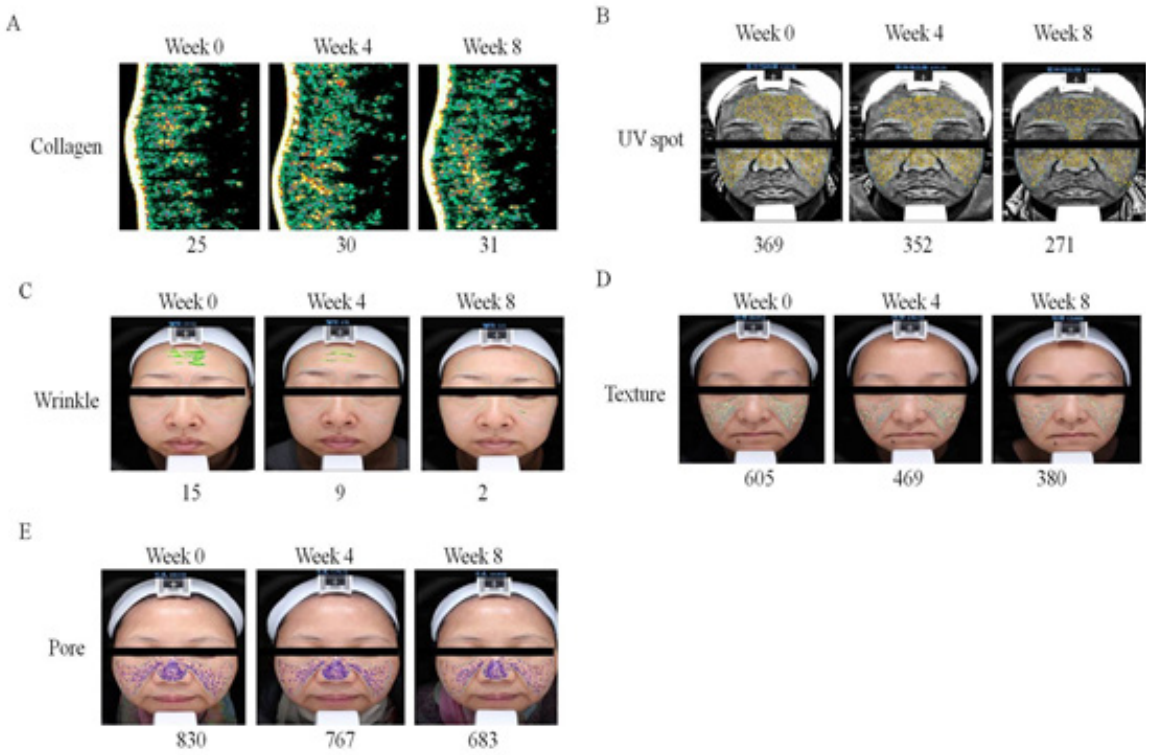

Figure 4: The photo image of skin properties after 8 weeks multi-berry favor drink treatment. The (A) collagen, (B) uv spots, (C) wrinkles, (D) texture, (E) pores.

Table 1: Summary of the improvement in SOD, TAC, spot, melanin, collagen density, wrinkles, texture and pore after 8 weeks treatment of placebo drinks and multi-berry favor drink. (n = 20)

Items	Improvement of the skin parameter (%)	
	placebo	multi-berry favor drink
SOD	-7.40%	9.7%**, #
TAC	1.30%	4.6%*
UV spot	0.60%	-3.8%#
Melanin	-1.90%	-9.4%***, ##
Collagen	8.6%***	16.1%***, ###
Wrinkles	3.70%	-15.60%
Texture	3.70%	-12.9%*
Pore	-4.10%	-12.30%
*, compared with before using. #, compared with placebo *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ # , $p < 0.05$, ##, $p < 0.01$, ###, $p < 0.001$		

Discussion

The blue light increased the production of ROS that induced melanogenesis activation, resulting in skin pigmentation [11, 12]. Previous studies revealed the tyrosinase, a key enzyme in regulating melanogenesis, was increased by the enhancement of ROS in HaCaT cells [13, 14]. Wang et al., demonstrated that the aqueous of acerola fruit have the properties of antioxidant and tyrosinase inhibition [15]. In addition, both black currant and *Aronia melanocarpa* (chokeberry) were reported to decrease the action on tyrosinase [16]. Furthermore, the oleanolic acid from the *Fragaria ananassa* (strawberry) resulting in decreased the cellular tyrosinase activity and further downregulated the levels of melanin [17]. Hence, we speculate that the multi-berry favor drink decreased the melanin through the inhibition of the tyrosinase activity. Accordingly, the multi-berry favor drink alleviated the influence of oxidative stress. Berries, a great source of antioxidants, include *Rosaceae* (strawberry and raspberry) and Ericaceae (blueberry and cranberry) [18]. Evidences revealed the berries containing the anthocyanins, flavonols, and tannins, which had the properties of anti-inflammatory, protection DNA and regulation the cellular metabolism [18, 19]. Wolfe et al., demonstrated the blackberry, raspberry, blueberry and pome-

granate had a relatively high cellular antioxidant activity [20]. The above finding was consistent with the observations in the multi-berry favor drink on our study. In this study, we discovered the capacity of antioxidant was owing to the component of berries in the multi-berry favor drinks.

The main reason of the skin aging including intrinsic- and extrinsic aging was caused by the ROS. Skin aging resulting in the deep wrinkling, loss of elasticity, dryness, laxity, rough textured appearance [21]. The berries had an abundance of ellagic acid exhibited significantly antioxidant which further decreased wrinkles and other signs of skin aging related to sun exposure [22, 23]. Moreover, berries contained resveratrol that had been known to increase the concentration of collagen via stimulating proliferation of fibroblasts [24]. Thus, we speculated multi-berry favor drink improved the skin parameters due to the berries contain the abundance of ellagic acid and resveratrol.

Conclusion

This study demonstrated the effect of 8 weeks of ingestion multi-berry favor drink improve the collagen, spots, melanin, wrinkles, texture and pores of the skin. In addition, multi-berry favor drink increased the capacity of antioxidant and decreases the melanin production. Taken together, we suggested that multi-berry favor drink improve the skin parameters to prevent the skin aging.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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