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"Study of Chinese skin pigmentation and solutions"

Lujia Zhang[1], Xueli Jia[1], Benyue Li[1], Jinming Ye[1], Jie Yang*[1], Fengwei Qi[1]

[1] Shandong Huawutang Biotechnology Co., Ltd., Jinan, China 1;

1. Introduction

The societal preference for fair skin ('white beauty' ideal) has significantly intensified in recent years. This includes concerns regarding localized skin dullness and post-cessation rebound hyperpigmentation following discontinuation of whitening products. Melanogenesis, the biosynthetic pathway of melanin production, serves as the primary regulatory mechanism for skin pigmentation. Notably, ultraviolet (UV) radiation intensity has been established as a critical exogenous factor modulating cutaneous chromaticity.

UV radiation has been demonstrated to promote melanin synthesis through several mechanisms. First, it has been shown to upregulate the tyrosinase mRNA transcript levels in melanocytes[1]. Second, findings show that it activates melanocortin receptor 1 and regulates the cAMP pathway[2]. Third, it can also activate p38 MAPK and induce MAPK phosphorylation[3]. Finally, it has been observed to promote paracrine factor secretion[4]. The occurrence of inflammation results in increased melanocyte activity. The produced melanin is transferred via dendrites to neighboring keratin–forming cells. Simultaneously, melanin infiltrates the dermis via the compromised basal layer, where it is subsequently engulfed by macrophages[5]. Repetitive mechanical actions have been demonstrated to result in friction development between the protruding bone area and the foreign body, which consequently leads to localized pigmentation of the skin[6, 7] accompanied by variations in the severity of pigmentation across different regions[8]. Discontinuation of whitening Products may result in rebound hyperpigmentation, manifesting as cutaneous darkening[9].

Under such multiple demands, we measured the physiological parameters related to pigmented areas and skin color, analyzed different skin pigmentation characteristics under different skin areas, and selected five landmark plant extracts based on the knowledge of oriental herbs. These ingredients include α-bisabolol from German chamomile, Rosa rugosa flower water distilled using the ancient Mediterranean coast method, niacinamide (universal restorative factor), Eriobotrya japonica leaf extracts, and Mentha spicata recorded in the Oriental pharmacopeia. A novel skincare ingredient, designated as HYB, was formulated by integrating contemporary dermatological research findings. This innovative formulation is designed to provide soothing, reparative, and brightening effects on the skin.

α-bisabolol, a naturally occurring terpenoid, has been shown to reduce MITF and tyrosinase gene expression by impeding the cAMP signaling pathway[10], owing to the melanogenesis signaling process obstruction, consequently intercepting melanogenic signals at their origin.

R. rugosa flower water has been shown to exert an inhibitory effect on tyrosinase activity, reducing melanin synthesis and content. Conversely, the flavonoids in R. rugosa flower water (e.g., quercetin, kaempferol) can directly scavenge free radicals, enhancing the efficacy of

whitening agents[11]. Song et al. discovered that R. rugosa flower water significantly increased skin luminosity in a double-blind experiment on humans[12].

Niacinamide has been demonstrated to effectively inhibit melanin's transit to keratinocytes. In cases where melanin reaches the skin surface, niacinamide has been shown to accelerate the epidermal cell renewal rate, promote the shedding of melanin-containing cells, and reduce melanin epidermal deposition[13]. Concurrently, nicotinamide reducing reactive oxygen species (ROS) generation[14]. Additionally, Kawada et al. ascertained that nicotinamide-containing cosmetics were efficacious in diminishing UV–induced skin aging and pigmentation[15]. Kang et al. posited that niacinamide retards deleterious ROS accumulation and other aging hallmarks[16].

Furthermore, the M. spicata and E. japonica leaf extracts incorporated into the product induce autophagy in melanocytes. This process involves the breakdown of melanin produced by cells or accumulated owing to transit obstruction by autophagic lysosomes. Consequently, this reduces the melanin content in melanocytes, mitigating the "rebound pigmentation" associated with niacinamide cessation[17]. Contrastingly, a total of three triterpenoids and four flavonoids have been identified in E. japonica leaf extract, exhibiting high oxygen radical uptake and Trolox-equivalent antioxidant capacity. Furthermore, M. spicata extracts have been shown to possess substantial antioxidant capacity[18].

The focus of this study was on the differences in physiological parameters of different pigmented areas and the development and validation of a new whitening system, "HYB," which provides new ideas for the scientific design and optimization of the efficacy of complex whitening formulations.

2. Materials and Methods

2.1 Study of the Distribution of Physiological Parameters at the Pigmentation Site

2.1.1 Materials

Skin Surface Moisture Loss Tester (Courage & Khazaka (German), TewameterTM300), Skin Melanin Tester (Courage & Khazaka (German), MexameterMX18), spectrophotom eter (KONICA MINOLTA (Japanese), CM-26d).

2.1.2 Participants

(1) 120 healthy Chinese females aged 18–-29 years old; (2) Individual type angle (ITA°) of skin color at the test site ranged from 20–41°; (3) Height ranged from 158cm–168cm, and body mass index was 18.5–23.9. The study was conducted following the tenets of the Declaration of Helsinki. The Ethics Committee Board approved the study protocol.（Ethical Review Approval Number：SECCR/2023-116-01）

2.1.3 Test Methods

The human pigmentation sites[8]see Figure 1, Skin physiological parameters related to skin color were selected for the determination of b* Value、TEWL Value and MI Value.

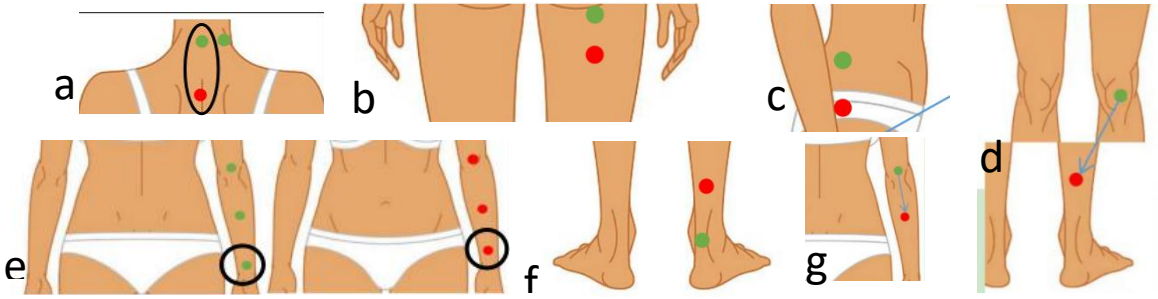


Figure 1 Typical areas of human pigmentation sites
（a. Back of the neck area；b. Under the buttocks；c.Lateral crotch；d. Middle of the knee；e.Front wrist；f. Back heel ；f. Back of the elbow）

2.2 In vitro Verification

2.2.1 Materials

Melanin model culture solution (Guangdong Boshi Biological), PBS (Solepol), Tretinoin (Yuan Ye Biological), Melanin standard (Sigma), Sodium hydroxide (Sinopharm), Anhydrous ethanol (Sinopharm), Ether (Sinopharm), EpiGrowth culture medium (Guangdong Boshi Biological), vitamin E (VE, Sigma), ROS kit (Biyun Tian), DCFH-DA probe (Biyun Tian).

2.2.2 Main Equipment

CO_2 incubator (Thermo, 150I), ultra-clean bench (Su Jing Antai, SW-CJ-1F), UVB irradiator (Philips), colorimeter (CortexTechnology, DSMII), SLR camera (Canon， Fluorescence microscope (Olympus, BX43).

2.2.3 3D Melanin Modeling Testing

In this experiment, a 3D melanin UVB irradiation model (MelaKutis)was used to assess the whitening efficacy of HYB by monitoring alterations in apparent color and lightness (L*), b*, and melanin content. Commencing on the day the model was received (day 0), the negative control (NC), positive control (PC), and sample groups were subjected to daily UVB irradiation (50 mJ/cm^2). The positive control (Kojic acid) and sample groups were administered twice daily on days 3 and 5, respectively, and the delivery method was surface administration. The sample group was administered in 2 and 4 μL volumes. Subsequently, the model was subjected to a seven-day incubation period, after which samples were collected for testing. A comparative analysis was conducted to assess the whitening efficacy variability (L* and melanin content) of 5% niacinamide and HYB.

2.2.4 In vitro Antioxidant Testing

In this study, a 3D epidermal skin model (EpiKutis) of UVB irradiation was used to assess the antioxidant efficacy of the investigated samples by monitoring alterations in ROS levels. The BC, NC, and sample groups added 0.9 mL of model culture solution per well, while the PC group added 0.9 mL of model culture solution containing VE per well. The sample group uniformly applied the original sample on the model surface. The irradiation procedure entailed placing all groups, except the BC group, under a UVB irradiator, with a dose of 600 mJ/cm^2.

2.3 An Evaluation of Human Efficacy

2.3.1 Testing Instruments

Part of the instrumentation is the same as part 2.1.1，Skin 3D imaging analysis system Antera 3D (Miravex, Ireland).

2.3.2 A Real–World Review of Sun Marks

2.3.2.1 Participants

Notably, environmental control or test modeling methodologies were not used in this study. Self–generate color differences within authentic environments (e.g., mountaineering, coastal settings, instructional driving practice, commuting, and outdoor sporting activities, among others). We included 33 healthy Chinese females aged 18–35 years who exhibited an ITA° difference of more than 10° between the exposed and non-exposed areas on both sides of the arm. In this study, Antera 3D was evaluated in a sample of 12 participants.

2.3.2.2 Test Methods

The test product was applied to the exposed area, while the non-exposed area received no product. The contralateral arm served as the control group, receiving no treatment. The test product was administered in the exposed area of the experimental group of participants, with the product applied twice daily. No products were used in the non-exposed areas of the experimental group and in the exposed areas of the control group. Skin darkness, MI value, ITA° value, and pigmentation were measured on day 0, 7 days, 14 days, and 28 days, respectively.

2.3.3 Skin Tone 7 days after stopping whitening products.

2.3.3.1 Participants

Thirteen healthy individuals between the ages of 18-60 were recruited for this study and the test area was pre-treated.

2.3.3.2 Test Methods

The treatment cycle consists of 28 days of product application (morning and evening) followed by 7 days of rest. On D-1, researchers performed 0.75 MED irradiation at 5 upper arm points, with baseline measurements at D0, D1 and D3. Product application began on D4, while the control group received no treatment. Follow-up measurements (MI, b*, ITA°) were taken at D5 (D1'), D11, D32 and D39.

3. Results

3.1 Study of the Distribution of Physiological Parameters at the Pigmentation Site

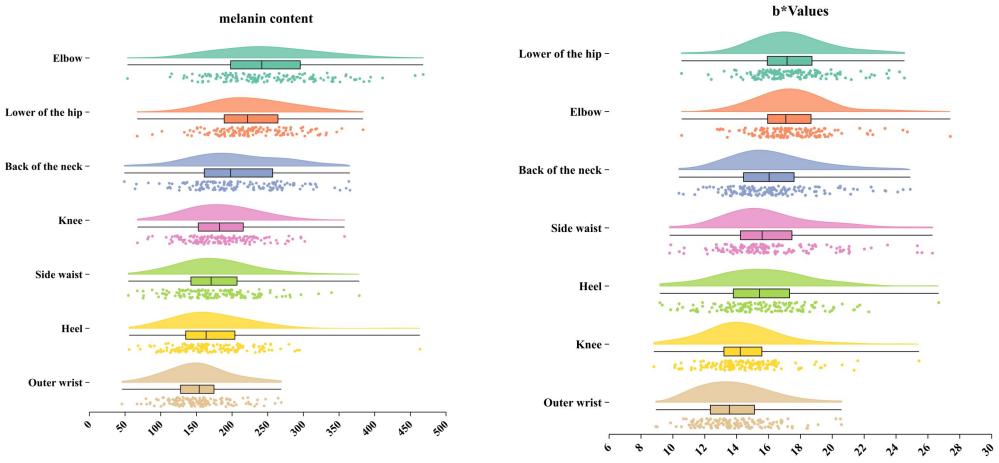
3.1.1 Physiological Parameters of Melanin Distribution Results in Pigmentation Sites

The analysis revealed that the skin melanin content exhibited a pattern of increased levels on the back of the elbows and under the buttocks, while the back of the neck exhibited a slightly lower value compared with under the buttocks. This observation is hypothesized to be attributed to the premise that the melanin deposition caused by friction surpasses the melanin deposition caused by sun exposure.

3.1.2 Results of the Distribution of the Physiological Parameter b* Values at the Site of Pigmentation

The b* value difference is more pronounced in seven specific regions. The b* value of the lower hip and the elbow back exhibited the most significant variation, suggesting greater skin friction potential in these areas. However, existing literature does not provide substantial evidence to support the hypothesis that skin friction impacts skin yellowness.

The preceding studies indicated that the mechanism of localized skin pigmentation (e.g., the nape of the neck, subgluteal folds, knees, and elbows) is characterized by significant regional variability in the formation of localized skin pigmentation. This phenomenon results from the interaction of multiple factors, including mechanical friction, chronic inflammation, and photoaging.



(a) (b)

Figure 2. Distribution parameters in pigmentation sites((a)MI content;(b)b* Values)

3.2 Multi-target Synergistic Mechanism Analysis

Infiltrating HYB into the base material for subsequent analytical testing ensured the consistency of subsequent studies, and standardized samples were prepared in this study.

3.2.1 3D melanin modeling

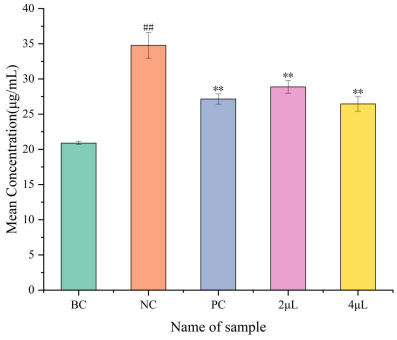
In this study, the whitening efficacy of the test sample was systematically evaluated through a multidimensional assessment system (Figure 3). As shown in Figure 3a, the melanin quantification demonstrating 16.97% and 23.93% ($p < 0.01$), respectively, compared to the (NC). This indicates that the samples were able to inhibit melanin production at the prescribed dosage, thus demonstrating whitening potential.

Quantitative analysis using a colorimeter showed 13.28% and 18.76% enhancements in L* values (lightness axis) compared with the NC group (Figure 3b, $p < 0.01$). Notably, the

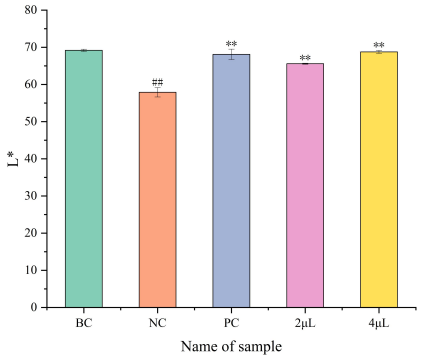
yellow-blue chromaticity axis (b*) analysis revealed substantial yellow pigmentation suppression in sample groups, with inhibition rates reaching 19.01% and 27.50% (Figure 3c).

Subsequently, a comparative assessment of the whitening efficacy was conducted between the HYB composite formulation and the single-component niacinamide (5%) through systematic experimentation. The evaluation protocol incorporated both melanin content quantification and L* value measurements as key efficacy indicators (Figure 3d, e). Experimental results revealed that the HYB formulation is statistically significantly superior in skin luminance enhancement, achieving a 1.33-fold greater improvement in L* value elevation rate compared with the niacinamide control group. Furthermore, the HYB composite showed notably enhanced melanogenesis inhibitory capacity, with its suppression rate reaching 1.82 times that of the niacinamide formulation. These findings collectively indicate the superior performance of the HYB complex in both chromatic brightness improvement and pigment synthesis regulation.

The findings indicated that the HYB complex combined whitening efficacy was significantly superior to that of the single-ingredient group ($p < 0.01$). suggesting that HYB employs a multi-pathway mechanism to block melanogenic signaling and inhibit melanogenesis. A pharmacologic advantage over single therapeutic interventions, providing a comprehensive solution for pigmentation management through simultaneous prevention, interception, and elimination mechanisms.



（a）



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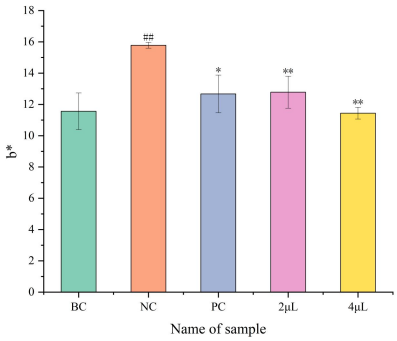
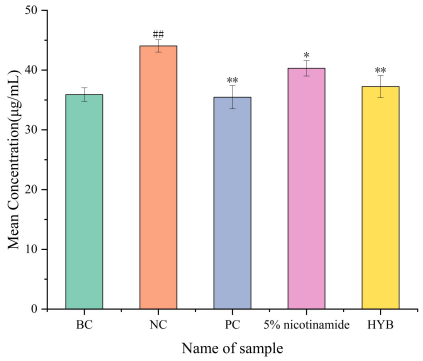
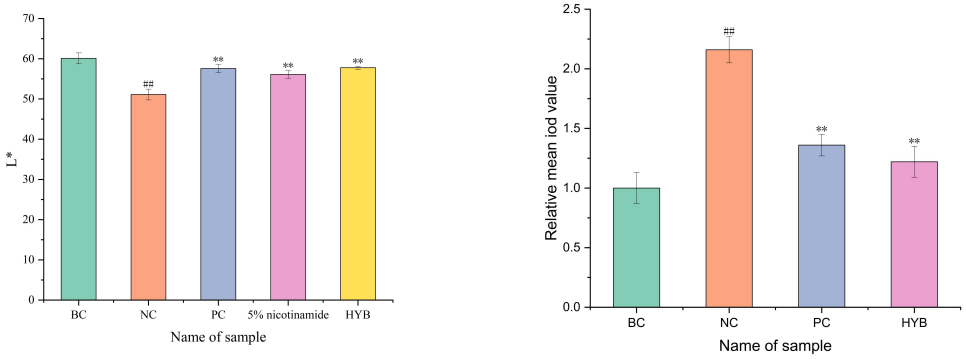


(c)



(d)



(e) (f)

Fig. 3 (a) Melanin Content, (b) L* value, (c) b* value, (d) Comparison between Nicotinamide and HYB- Melanin Content, (e) Comparison between Nicotinamide and HYB-L*, (f) ROS-integrated optical density (IoD) (Note: For statistical analysis using t-test method, significance is indicated using # when compared with BC group, P-value<0.05 is indicated as # and P-value<0.01 is denoted as ##; compared with the NC group, significance is denoted as *, P-value<0.05 is denoted as *, P-value<0.01 is denoted as **, and P-value<0.001 is denoted as ***)

3.2.2 In vitro antioxidant testing

As demonstrated in Figure 3f. Quantitative analysis using a 3D melanogenesis model revealed a statistically significant reduction of 43.52% (p<0.01) in ROS levels in the treatment group compared with NC. This pronounced antioxidant effect is attributed to the formulation's multi-target synergistic antioxidant system: Niacinamide activates endogenous antioxidant defense pathways, and E. japonica leaf extract, enriched with triterpenic acids and flavonoids, possesses synergistic oxygen radical absorbance capacity. M. spicata extract enhances singlet oxygen scavenging efficiency through polyphenolic constituents. Such comprehensive oxidative stress mitigation shows critical therapeutic potential for preventing UV-induced melanogenesis through simultaneous endogenous antioxidant system potentiation, direct free radical neutralization, and secondary oxidation product inhibition.

3.3 Clinically Proven Balance of Fast-acting and Long-lasting Effects

3.3.1 A Real–World Review of Sun Marks

As demonstrated in Figure 4a-c. Following a 4-week test product intervention, the MI values in the exposed areas of the arms of the experimental group underwent a significant decrease of 11.30% compared with the pre-intervention period ($p < 0.001$). A 5.90% significant increase in ITA° values compared with the pre-intervention period ($p < 0.001$). A 4.78% significant decrease in mean pigmentation concentration compared with the pre-intervention period ($p < 0.001$).

Additionally, the MI values, the ITA° values, and the mean pigmentation concentration in the exposed areas of the experimental group exhibited a substantial reduction when compared with both the non-exposed area of the experimental group and the exposed areas of the control group ($p < 0.001$).

The study revealed that the investigated product effectively ameliorated skin pigmentation induced by natural light exposure, suggesting a potential mechanism of action involving melanin synthesis inhibition and keratin metabolism promotion. These findings provide a

scientific foundation for skincare product development designed to target natural light-induced pigmentation.

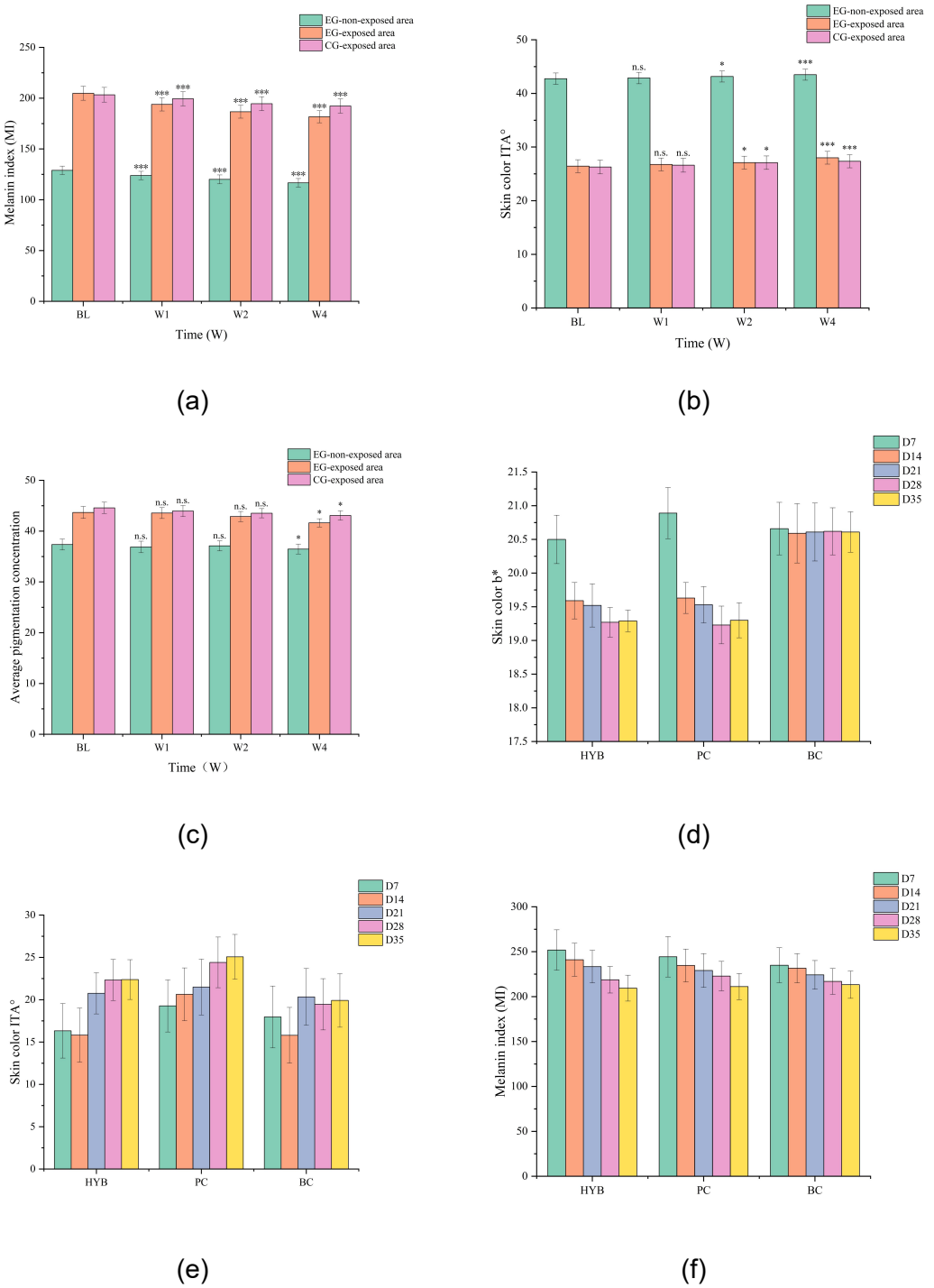


Fig. 4 (a) Melanin Index of the skin MI, (b) Skin color ITA°, (c) pigmentation of the skin,(d) Skin color b*, (e) Skin color ITA°, (f) Melanin Index of the skin MI.

3.3.2 Skin Tone 7 days After Stopping Whitening Products

As demonstrated in Figure 4d-f.After 14 consecutive days of applying the test product on the upper arm (modeling area), the reduction rates of the MI value, b* value, and ITA° value compared with those at 7 days post-modeling were 4.33%, 4.44%, and 3.12%,

respectively(Figure 6.). Following 28 days of continuous use, the MI and b* values showed 13.17% and 6.00% decreases, respectively, compared with those at 7 days post-modeling, while the ITA° value increased by 36.74%.

After the product discontinuation for 7 days, the MI value reduced by 16.89% compared with that at 7 days post-modeling and 4.28% lower than the pre-modeling baseline. The b* value decreased by 5.90% compared with that at 7 days post-modeling. Meanwhile, the skin color ITA° value increased by 37.05% compared with that at 7 days post-modeling, with a slight increase of 0.22% compared with the value before discontinuation.

The present study is predicated on human efficacy test modeling to verify the whitening mechanism and the sustainability of the HYB effect. The study's findings indicated that the skin color did not undergo a rapid darkening process after the cessation of product usage. The findings indicated that HYB not only effectively enhances skin pigmentation through a four-fold synergistic mechanism that inhibits tyrosinase activity, blocks melanin transport, and accelerates keratin metabolism but also promotes melanin autophagy. Moreover, the regulatory effect maintains the homeostasis of the skin's melanin metabolism after discontinuation, providing a crucial theoretical foundation for the production of a stable and durable whitening product.

4. Discussion

The present study examined the relationship between the spectrum of body skin pigmentation of young Chinese women and their physiological parameters, including TEWL, MI value, and b* value indicators. The findings indicated that sun exposure and friction significantly contribute to the dullness of skin color. The study concluded that strategies aimed at reducing melanin production and mitigating inflammation are crucial for addressing the issue of skin color.

The HYB system development represents a significant advancement in the cosmetic dermatology field, overcoming the limitations of traditional whitening ingredients. The system's multifaceted approach, involving "multi-pathway synergistic - cross-dimensional stabilization," is a novel and promising strategy. α-bisabolol has been demonstrated to possess both anti-inflammatory and tyrosinase-inhibiting properties. Niacinamide has been shown to inhibit melanin transport and act as an antioxidant. Rose rugosa flower water has been identified as an antioxidant. E. japonica leaf and M. spicata extracts have been shown to have antioxidant and melanin autophagy-inhibiting properties and constitute an action network that covers the entire process of melanin production, transport, and degradation. This provides a new paradigm for developing highly effective and safe skin whitening products. The absence of rebound pigmentation following 7-day cessation of product use represents a novel biomarker for assessing sustained whitening effects

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