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Development and Optimization of Dimethicone-based Cream Containing Muscat Hamburg Grape Extract: *In-vitro* Evaluation

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Abstract

Background: One of the main subsidizing factors to the rising healthcare costs are related to the design and development of visionary pharmaceutical products. As a consequence, healthcare authorities have identified herbal preventive and therapeutic options as possible alternative strategies. In the developing world, there is an urgent need for the pharmaceutical industry field to invest more time and resources in the research and development of complementary and alternative medicines.

Purpose: The main aim of this study was to contribute to the development of an innovative phyto-based formulation for topical application and determine its thermal stability chronically.

Material and Methods: A cream of water-in-oil type emulsion was prepared and implemented or not (placebo *aka* base) with *Muscat hamburg* black grape extract (active formulation). *M. hamburg*-based cream was first mixed with the emulsifier (i.e. Abile EM90® *aka* Dimethicone) and compared with a placebo. Successful formulations were selected based on their ability to remain significantly ($p<0.05$) stable during a pre-determined period when stored at different temperatures (i.e. 8°C, 25°C, 40°C ± 75% relative humidity). Different physicochemical parameters like eventual changes in color, centrifugation, phase separation, liquefaction, conductivity, viscosity, and pH were assessed immediately after preparation (time 0) and at various time points (i.e. 12 hours 24 hours, 36 hours, 48 hours, 72 hours, 7th day, 14th day, 21st day and 28th day). For viscosity studies, we extended the analysis to 90 days.

Results: In our experimental conditions and from our comparative analyses, we noticed (i) unchanged organoleptic properties in terms of appearance, color and odor; (ii) unchanged properties after centrifugation and phase separation, and in terms of electrical conductivity, liquefaction, or viscosity. Importantly, we showed that both placebo and active formulation had insignificant mean pH (5.12 ± 0.43 *versus* 5.04 ± 0.39, $p>0.05$) when all respective samples were assessed. In spite of a progressive time-dependent and temperature-independent decline of the mean pH in both placebo and active formulation, the mean pH of both emulsions fit the acceptable range of dermal pH (i.e. 4.5-6.5) for 21 days.

Conclusions: The *in-vitro* evaluation of our newly developed dimethicone based cream containing *Muscat hamburg* extract showed satisfactory and promising results for its possible use as a topical semi-solid dosage form for various skin ailments.

Keywords: *Muscat hamburg*; Stability studies; Drug delivery system; Topical application; Complementary and alternative medicine; Phytomedicine; Translational medicine

Abbreviations: ANOVA: Analysis of Variance; DPPH: 1, 1-Diphenyl-2-Picryl Hydrazyl; LSD: Least Significant Difference; *M. Hamburg*: *Muscat Hamburg*; RH: Relative Humidity; ROS: Reactive Oxygen Species; UVR: Ultraviolet Radiation; W/O: Water-in-Oil

Introduction

Emulsion is a dispersed system stabilized by an emulsifying agent, which is constituted of small globules of liquid known as dispersed phase, distributed in another immiscible solvent called dispersion medium [1]. The emulsifying agent possesses hydrophilic and hydrophobic groups [2], adsorbed at the interphase of water and oil, and reduces the interfacial tension. This helps in the distribution of dispersed phase globules [3]. On standing, two immiscible phases can separate but the thin film formed by the emulsifying agent on each globule acts as a barrier, avoiding globules of same liquid to join [4]. Emulsion of water-in-oil type (W/O type) is generally used in cosmetics for dry skin treatment. Abile EM90® (Dimethicone) is a compatible emulsifier and produces W/O emulsion (i.e. Evonik). The oily phase used is comprised of paraffin oil. It is transparent, tasteless and colorless liquid, odorless at cold temperatures but gives faint petroleum like odor when heated [5]. Different methods for formulating an emulsion are available and include dry gum, wet gum, phase inversion, or membrane emulsification strategies [6]. "*Muscat hamburg*" is a black table grape cultivar that belongs to *Vitis vinifera* (L.) (Family: *Vitaceae*), a grapevine crop species which comprises about 60 inter-fertile wild *Vitis* varieties

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distributed in Asia, North America and Europe, under subtropical, Mediterranean and continental-temperature climatic conditions [7].

Nowadays, grape production has gained much attention worldwide due to its great economic impact and health benefits. Indeed, Food and Agriculture organization stated that, in 2004, the global grape production was 65.4 million tone and about 71% of which was used in wine industries [8]. In Pakistan, grapes are cultivated in Baluchistan province, in Quetta and Qalat regions, where the production varies from 5 to 15 tons/hectare in average [9]. Fruits and seeds of *Muscat hamburg*, have been used for thousands of years because of their nutritional and medicinal benefits, due to their rich content in sugars, polyphenols (i.e. flavonoids, anthocyanins and proanthocyanins, tannins), organic acids, mineral salts and vitamins [8,10]. *M. hamburg* grape's peel, seed and juice, especially from the red and black species, contain high amount of resveratrol, a stilben derivative considered as one of the most potent antioxidant [11,12].

Indeed, a recent study has shown that resveratrol was 95% efficient at preventing lipid peroxidation, compared to 37% for ascorbic acid (*aka* vitamin C) and 65% for a-tocopherol (*aka* vitamin E). Other studies have reported the potential benefits of this polyphenol against cardiovascular diseases (i.e. prevention of LDL-cholesterol oxidation, lowering of total cholesterol levels), infection diseases (e.g. anti-bacterial activity), cancers (e.g. prevention of tumorigenesis and carcinogenesis), and a number of skin conditions [13-19].

Therefore, the purpose of this study was to formulate a stable W/O emulsion containing the *M. hamburg* extract for eventual further use as topical application. We also describe the subsequent *in-vitro* physical-chemical characterizations of the original cream.

Materials and Methods

Plant

Muscat hamburg black grapes were obtained from a local market of Bahawalpur, Pakistan, and authenticated by the CIDS (Cholistan Institute of Desert Plants Studies), The Islamia University of Bahawalpur, Pakistan. A voucher specimen (Voucher no. MH-FT-11-18-27) has been kept in the herbarium at the CIDS, The Islamia University of Bahawalpur, Pakistan, for future reference.

Chemicals, solvents and apparatus

Distilled water (Islamia University of Bahawalpur, Pakistan), methanol (Merck, Germany), hydrochloric acid (Merck, Germany), paraffin oil (Merck, Germany), Abil EM 90® (Cetyl dimethicone copolyol with HLB 5, Franken Chemical, Germany), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Chemical Co., USA) were employed in this study.

Apparatus

A thermostatic water bath boiler (HS-21-4, China), electrical balance (Precisa BJ-210, Switzerland), digital humidity meter (TES Electronic Corp, Taiwan), digital pH meter (WTW pH-197i, Germany), digital conductivity meter (WTW COND-197i, Germany), centrifuge machine (Hettich EBA 20, Germany), mechanical mixer (IKA, Germany), refrigerator (Orient, Pakistan), cold incubator (Sanyo MR-153, Japan), hot incubator (Sanyo MR-162, Japan), refrigerator (Dawlance, Pakistan), homogenizer (Euro-Star, KA D 230, Germany), rotary evaporator (Eyela, Co. Ltd. Japan), digital rheometer (Brookfield model DV-III, USA), and UV-Vis spectrophotometer (UV-1800 Shimadzu, USA) were used in this study.

Plant extract

One kg of fresh *M. hamburg* black grapes were de-stemmed and washed to remove dust particles. The skin and seeds were separated, air dried, ground to powder and stored in a well-closed container. 50 mL of fresh solvent (i.e. methanol, water and 1M HCl with percentage ratio of 70:29.5:0.5, respectively) was added to 10g of dried powder in a 100 mL beaker, and the mixture was sonicated for 15-20 min. Then, the solution was filtered through layers of muslin cloth to remove the coarse particles, and the filtrate was again filtered through whatman #01 filter paper in order to get particles free extracts. This process was performed three additional times. The subsequent hydro-alcoholic solutions were eventually transferred into a new and same beaker, and the pooled solution was concentrated up to 1/3 of its initial volume using a rotary vacuum evaporator at 40°C under reduced pressure. The final dark-colored solutions were filtered through whatman # 01 filter paper and stored at 8°C in a freezer.

DPPH assay

DPPH (1,1-diphenyl-2-picryl hydrazyl) method was used to measure the anti-oxidant activity of *M. hamburg* extract. The hydro-alcoholic solutions (range of 0-60 µM) containing *M. hamburg* extract (i.e. test) were added to the DPPH solution. Ascorbic acid (vitamin C), a natural potent antioxidant, was used as external control (i.e. standard). Spectrophotometric absorbances of the test and standard were obtained at λ=517 nm using the appropriate solvent as a blank.

Compared to the standard, absorbance of the test was always lower and decreased in function to higher added concentration. Scavenging activity was expressed as the percentage (%) inhibition calculated using the following formula: Inhibition (%)=$[(A_0-A_1)/A_0] \times 100$ where A_0=absorbance of the control and A_1= absorbance of the extract. Scavenging activity was eventually compared to ascorbic acid, in terms of inhibitory concentration 50 (IC_{50}) (*aka* concentration requested to obtain 50% of free radicals scavenged).

Preparation of emulsions

W/O placebo and active formulation-based emulsions were prepared by adding the aqueous phase (100% pure water for placebo or 98% pure water/2% *M. hamburg* extract for active formulation) into the oily phase (80% pure water/16% paraffin oil/4% Abil EM 90®). Thereby, the oily and water phases were first heated up to 75°C ± 1°C using a water bath. Then, the aqueous phase was gradually added to the oily phase, during continuous mechanical stirring at 2000 rpm, in order to obtain the same temperature for both phases. Once the aqueous phase was fully added into the oily phase, which took about 15 min, the stirring speed was reduced twice (1000 rpm for 5 min and 500 rpm for the next 5 min) in order to allow the emulsion to cool at room temperature. Therefore, the only difference between the "active formulation" and the "placebo or base formulation" (used as a control) was that the aqueous phase contained or not the plant extract. The type of emulsion was determined by dilution test (*aka* miscibility test) which is based on the solubility of external phase of the emulsion, and consists to dilute the emulsion with oil and water separately [20,21]. Briefly, in two different tubes, few drops of water or oil were added separately into few drops of emulsion. If the water distributed uniformly, then it was O/W-type emulsion; if the water separated out as layer, then it was W/O-type emulsion. Opposite effects were eventually observed in case of oil.

Physical-chemical characterizations of the emulsions

The physical-chemical characterizations of the emulsions (i.e.

placebo/base or active formulation) were carried out for a period of 28 days (unless otherwise specified) following established stability methods previously described [22]. First, 5 g of each emulsion was centrifuged for 10 minutes at 5000 rpm and at room temperature (25°C). Then, the emulsion characterizations consisted to assess eventual time-dependent changes after emulsion preparation (i.e. immediately (time 0); 12 hours; 24 hours; 48 hours; 72 hours; 7th day; 14th day, 21st day; 28th day) in major physical-chemical parameters (i.e. colour aspect; liquefaction; phase separation; viscosity; electrical conductivity; pH), when samples were stored/placed at different temperatures (i.e. 8 ± 0.5°C (in refrigerator); 25 ± 0.5°C (in incubator); 40 ± 0.5°C (in incubator); 40°C ± 0.5 with 75% relative humidity (RH) (in incubator)). Since the flow property of the emulsions was highly dependent on its viscosity, the viscosity assays were extended to 90 days, with checking time points at the 60th day and the 90th day. Each sample experiment was performed in triplicate.

Statistical analysis

The statistical tool SPSS version 17 was used to analyze the data ANOVA test was applied to determine eventual variation between different time intervals. Paired sample t-test was used to describe potential differences between the two creams. *Post-hoc* analysis through LSD (*aka* Fisher´s Least Significant Difference) computed "pairwise comparisons" (i.e. the smallest significant difference between two means variation), and enabled to declare significant any difference larger than the LSD. Statistically, a significant difference was considered at a p value of less than 5% ($p<0.05$).

Results and Discussion

Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, cancers, Alzheimer´s disease, or various skin ailments [16,23]. Natural occurring phyto-antioxidants (e.g. polyphenols, flavonoids and phenolic compounds present in spices and herbs) are known to prevent harmful effects of oxidative stress by acting as free radical scavengers or promoting enzymatic-based oxidation [13-19,22].

From our body of experiments which consisted to develop an original W/O emulsion containing *M. hamburg* grape extract (2%) for possible topical preventive and therapeutic applications (e.g. anti-aging, prevention of skin conditions), emulsion samples were stored at different temperatures and analyzed *in-vitro* for their overall physical-chemical stability. Indeed, the stability of emulsions is the critical parameter to take into consideration at the early stage of their production, and checking during the development process is recommended through the use of fast and reliable analytical methods. For instance, it is well-admitted that the temperature and humidity are important environmental factors that increase the risks of emulsion instability [24]. Interestingly, we have previously shown that the Abil EM 90®/Dimethicone and paraffin oil are non-toxic, colorless liquids, and induce no change in the emulsions [25].

Our present results first report free radical scavenging activity of hydro-alcoholic solutions of *M. hamburg* extract, using DPPH assay. We observed that *M. hamburg* extract exert an antioxidant activity, and the IC_{50} of *M. hamburg* extract is obtained at M, which was about 3 folds higher than IC_{50} of ascorbic acid, a powerful anti-oxidant used as standard control (Figure 1). This indicates that *M. hamburg* extract elicited a thrice lower free radical scavenging activity than ascorbic acid. Interestingly, the data showed that the free radical scavenging capacity of *M. hamburg* extract can be total if the concentration of 55 µM is used, although this is still lower (i.e. about 1.7 folds) than the full antioxidant activity exerted by ascorbic acid (i.e. 35 µM),

We then decided to develop active emulsion-based formulation (i.e. with *M. hamburg* extract), which would be stable to various conditions (i.e. color, liquefaction, phase separation, phase centrifugation, viscosity, electrical conductivity, and pH) under pre-determined temperatures (i.e. 8°C, 25°C and 40°C ± 75% RH) and time period (i.e. 28 days, except for viscosity assay which was extended to 90 days). The subsequent stability data were not only intra-analyzed but also compared to placebo (i.e. without *M. hamburg* extract) emulsion prepared in parallel at the same experimental conditions and used as external control (Tables 1-4).

Thereby, we noticed that unchanged color ($p>0.05$) in and between all placebo and active formulation samples. Indeed, the W/O emulsion color was light grey (LG) for both placebo and active formulation samples (Table 1). This indicated that the color aspect of any of the emulsions was thermally stable during the whole study period.

Further, no significant liquefaction changes ($p>0.05$) were noticed in and between both placebo and active formulation samples that were stored at 8°C or 25°C Table 1. Nevertheless, in both placebo and active formulation samples that were stored at 40°C+75% RH, small liquefaction changes were observed on 21st and 28th day Table 1. It is admitted that the liquefaction, which depends on cream viscosity, could be affected by the temperature and time [26]. Our data indicates that: (i) the liquid aspect of the prepared emulsions was overall stable; (ii) the best conditions of liquefaction stability can be obtained at a temperature inferior to 40°C for a period ≥ 21 days, or at 40°C + 75% RH for a period inferior to 21 days; (iii) the grape extract did not influence this parameter stability.

Moreover, placebo and the active formulation samples stored at 8°C, 25°C and 40°C did not show significant phase separation changes ($p>0.05$) during the study period (Table 1). However, both placebo and active formulation samples stored at 40°C+75% RH showed mild-phase separation on the 21st and 28th day. This indicated that: (i) the prepared emulsions are ideally stable at temperature ≤ 40°C for a period longer

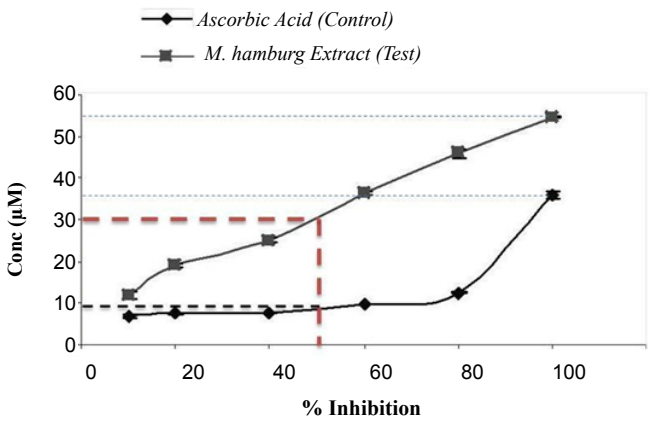

Figure 1: DPPH scavenging activity of *Muscat hamburg* extract. The activity (% inhibition) of a range of concentration (0-60 µM) of *Muscat hamburg* extract (test) was compared to that one of ascorbic acid (standard), one of the most potent naturally-occurring oxidant. The inhibitory concentration of *M. hamburg* extract to scavenge 50% of free radicals (IC_{50}) is mentioned by the red dashed line. IC_{50} of ascorbic acid is represented by the black dashed line. IC_{100} is mentioned in blue dashed line for both compounds.

	Color				Liquefaction				Phase Separation			
Time	A	B	C	D	A	B	C	D	A	B	C	D
0 hr	LG	LG	LG	LG	-ye	-ye	-ye	-ye	-ye	-ye	-ye	-ye
12 hrs	LG	LG	LG	LG	-Ye	-Ye.	-ye	-ye	-ye	-ye	-ye	-ye
24 hrs	LG	LG	LG	LG	-ye	-ye	-ye	-ye	-ye	-ye	-ye	-ye
36 hrs	LG	LG	LG	LG	-ye	-ye	-ye	-ye	-ye	-ye	-ye	-ye
48 hrs	LG	LG	LG	LG	-ye	-ye	-ye	-ye	-ye	-ye	-ye	-ye
72 hrs	LG	LG	LG	LG	-ye	-ye	-ye	-ye	-ye	-ye	-ye	-ye
7 days	LG	LG	LG	LG	-ye	-ye	-ye	-ye	-ye	-ye	-ye	-ye
14 days	LG	LG	LG	LG	-ye	-ye	-ye	-ye	-ye	-ye	-ye	-ye
21 days	LG	LC;	LG	LG	-ye	-ye	+ve	+ye	-ye	-ye	-ye	+ve
28 days	LG	LG	LG	LG	-ye	-ye	+ye	+ve	-ye	-ye	-ye	+ve

Table 1: Characterization of organoleptic properties from active formulation-based water-in-oil (W/O) emulsion Color, liquefaction and phase separation were assessed at the indicated time points for a total study period of 28 days, and at the following pre-determined temperatures: A: 8±0.5°C; B: 25±0.5°C; C: 40±0.5°C; D: 40°C±0.5°C +75%RH. LG: Light Grey; -ve: No change; +ve: Slight change. Identical data were obtained from the placebo-based W/O emulsion.

T (°C) / Time	8±0.5	25±0.5	40±0.5	40±0.5 +75%RH
0 hr	441.09±1.20	441.09±1.20	441.09±1.20	441.09±1.2
12 hrs	481.16±1.14	481.04±1.05	459.93±1.11	480.27±1.05
24 hrs	480.96±1.02	481.51±1.12	444.06±1.13	482.03±1.12
36 hrs	481.09±1.09	482.26±1.05	447.21±1.11	480.57±1.09
48 hrs	478.63±1.12	482.00±1.09	439.86±1.15	479.50±1.13
7 days	474.21±1.32	471.99±1.13	440.06±1.04	481.26±1.01
14 days	474.56±1.08	452.93±1.01	438.56±1.02	478.52±1.08
21 days	472.26±1.07	443.08±1.05	439.70±1.03	481.95±1.09
28 days	474.43±1.22	455.69±1.13	434.81±1.05	461.95±1.12
60 days	473.06±1.32	450.92±1.09	430.16±1.02	471.43±1.07
90 days	473.81±1.14	455.05±1.03	420.16±1.11	443.05±1.09

Table 2: Viscosity stability of the active formulation. The values were expressed in cP. The corresponding water-in-oil (W/O) type emulsion samples were processed at the indicated temperatures and time points for a total study period of 90 days. Similar data (p>0.05) were obtained from the placebo-based W/O emulsion.

T (°C) / Time	8±0.5	25±0.5	40±0.5	40±0.5 +75% RH	Mean±SD	P-value
0 hr	5.44	5.44	5.44	5.44	5.44±0.00	**Ref.***
12 hrs	5.41	5.43	5.42	5.43	5.42±0.01	0.434
24 hrs	5.39	5.42	5.40	5.41	5.41±0.01	0.124
48 hrs	5.37	5.39	5.37	5.38	5.38±0.01	0.090
72 hrs	5.34	5.37	5.33	5.35	5.35±0.02	0.130
7 days	5.29	5.33	5.31	5.32	5.31±0.02	0.059
14 days	4.95	4.85	4.99	4.97	4.94±0.06	0.000
21 days	4.42	4.47	4.51	4.44	4.46±0.04	0.021
28 days	4.30	4.35	4.43	4.34	4.36±0.05	0.000
Mean±SD	5.10±0.45	5.12±0.44	5.13±0.40	5.12±0.44	5.12±0.43	P>0.05
P-value	Ref.*	0.299	0.038	0.209	**PLACEBO FORMULATION**	
	0.299	Ref.*	0.266	0.822		
	0.038	0.266	Ref.*	0.372		
	0.209	0.822	0.372	Ref.*		

Table 3: pH stability of the placebo formulation. The corresponding water-in-oil (W/O) type emulsion samples were processed at the indicated temperatures and time points for a total period of 28 days. *Ref. means reference used for Fisher´s Least Significant Difference (LSD) statistical tests.

than three weeks or at 40°C+75% RH for a time period lesser than three weeks; (ii) the grape extract did not influence this parameter stability.

In agreement with our liquefaction data, viscosity values obtained for both the placebo and the active formulation samples showed insignificant inter-differences (p>0.05), and acceptable stability over the study period of 90 days (Table 2). Nevertheless, a slight increase in viscosity was observed at the 28th, 60th and 90th day in any of the samples subjected to the lowest tested temperature (i.e. 8°C). Conversely, as the time passed, there was creaming in all samples placed at increased temperatures (i.e. 25°C from the 7th day, and at 40°C ± 75% RH from 12 hours), and this fact insignificantly reduced (p>0.05) the emulsion viscosity when compared to fresh prepared formulations (i.e. time 0) or to emulsion samples placed at 8°C). Finally, grape extract did not influence this effect which in line with previous studies in the field [24,25] reporting classical low viscosity and slight phase separation of emulsions at increased temperatures due to displacement of small surfactant molecules from the interface.

T (°C) / Time	8±0.5	25±0.5	40±0.5	40±0.5 +75% RH	Mean±SD	P-value (ANOVA)
0 hr	5.37	5.37	5.37	5.37	5.37±0.00	**Ref.***
12 hrs	5.34	5.33	5.35	5.34	5.34±0.01	0.065
24 hrs	5.32	5.29	5.34	5.32	5.32±0.02	0.002
48 hrs	5.30	5.27	5.32	5.29	5.30±0.02	0.000
72 hrs	5.27	5.21	5.28	5.25	5.25±0.03	0.000
7 days	5.09	4.98	5.14	5.12	5.08±0.07	0.000
14 days	4.89	4.87	4.92	4.90	4.90±0.02	0.000
21 days	4.51	4.45	4.54	4.53	4.51±0.04	0.000
28 days	4.32	4.28	4.37	4.34	4.33±0.04	0.000
Mean±SD	5.05±0.39	5.01±0.40	5.07±0.38	5.05±0.38	5.04±0.39	P<0.05
P-value	Ref.*	0.010	0.026	0.596	**ACTIVE FORMULATION**	
	0.010	Ref.*	0.000	0.000		
	0.026	0.000	Ref.*	0.080		
	0.596	0.000	0.080	Ref.*		

Table 4: pH stability of the active formulation-based water-in-oil (W/O) emulsion. The samples were processed at the indicated temperatures and time points for a total study period of 28 days. *Ref. means reference used for Fisher´s Least Significant Difference (LSD) statistical tests.

Besides, we showed that no one of placebo and active formulation samples display electrical conductivity, and so means that the temperature and time did not have any influence on that parameter data not shown. However, in oil-in-water (O/W) emulsion samples, we previously reported electrical conductivity [27], suggesting a possible alternative physical method to discriminate W/O and O/W emulsion samples.

Eventually, the mean pH of all placebo samples during the whole study period was not significantly different to that one of all active formulation samples (5.12 ± 0.43 *versus* 5.04 ± 0.39, p>0.05) Tables 3 and 4, respectively. These data are encouraging for further topical application tests since they fit within the dermal pH range of 4.5-6.5 [28]. With respect to time and temperature, two-ways ANOVA statistical tests showed that the pH changes were insignificant (p>0.05) in the placebo samples (p=0.160 and p=0.208 for time and temperature changes, respectively) whereas they were significant (p<0.05) in the active formulation samples (p=0.000 and p=0.000 for time and temperature changes, respectively). At a further analysis step using LSD statistical test, we could notice that the mean pH of placebo samples were acceptable at any of the tested temperatures for a period that does not exceed 21 days (Table 3).

Nevertheless, the progressive minimal changes observed in placebo samples during the period study might occur faster at cold temperature. Indeed, it was noticed (Table 3): (i) a progressive decrease in mean pH of freshly prepared placebo samples when compared to mean pH of samples kept to a minimum of 12 hours (e.g. 5.44 ± 0.00 at time 0 to 4.36 ± 0.05 at day 28), *albeit* significant pH changes occurred from day 14 (p<0.05) independently of the temperature set point; (ii) an unacceptable temperature-independent mean pH at day 28 (i.e. 4.36 ± 0.05) when considering physiological dermal pH range; (iii) a significant decrease (p=0.038) in mean pH occurred when samples kept at 8°C (5.13 ± 0.40) were compared to samples kept at 40°C (5.10 ± 0.45). However, this mean pH at 8°C remained acceptable until day 14. With respect to the active formulation samples (Table 4), and similarly to placebo samples, the mean pH of active formulation samples was acceptable at any of the tested temperatures for a period that does not exceed 21 days. Nevertheless, progressive minimal changes can occurred from 24 hours, especially when exposed at room temperature (25°C). Indeed, it was importantly noticed that (Table 4): (i) at a given temperature, mean pH is progressively lowered when samples at time 0 (i.e. freshly prepared samples) were compared to samples kept to a minimum of 12 hours, (i.e. 5.37 ± 0.00 at time 0 to 4.33 ± 0.04 at day 28), *albeit* significant mean pH changes occurred from 24 hours (p<0.05). This significant effect was then precocious compared to that of the one seen in placebo samples (i.e. from day 14); (ii) the mean pH obtained at day 28 (i.e. 4.33 ± 0.04) was out of the acceptable dermal pH range; (iii) significant changes in mean pH (p<0.05) occurred when samples kept at 8°C (5.05 ± 0.39) were compared to samples kept at 25°C (5.01 ± 0.40, p=0.010) or 40°C (5.07 ± 0.38, p=0.026).

However, unlikely seen with the placebo samples, the mean pH of samples kept at the coldest temperature (8°C), or even at the warmest ones (i.e. 40°C ± 75% RH), significantly increased (p<0.05) when compared to the mean pH of samples kept at 25°C. Nevertheless, the mean pH of all of these samples remained acceptable for possible further topical application, considering the dermal pH range. Eventually, the decrease of mean pH, observed both in the placebo and active formulation samples could be attributed, at least partially, to the production of metabolites or decomposition of paraffin oil into aldehydes or organic acids [5].

Taken together, the overall physical-chemical characterizations of our newly developed active cream strongly suggest that its optimal use as stable topical application agent shall be done within 21 days.

Conclusion

M. hamburg black grape extracts contains polyphenolic compounds, including flavonoids and stilbenoids (e.g. resveratrol), are known to possess potent antioxidant activity, as confirmed by the DPPH assay. In our experimental conditions, the W/O cream developed as a carrier for this active ingredient, was found to be overall stable for 21 days, and could be considered for the development of original skin care products. Indeed, such a cream could provide a satisfactory anti-aging effect when applied to the skin frequently. Nevertheless, for efficient and safe translational medicine, our *in-vitro* encouraging results merit further experiments (e.g. cytotoxicity assays, pre-clinical studies).

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