

Research Article

Self Preserving Skin Care Cosmetic Products

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ABSTRACT

Microbiological control of cosmetic products is always of interest to the industry, since microbial spoilage can lead to product degradation and be a potential human health hazard. Preservatives are added to control microbial spoilage, increase shelf life of product and prevent infection in humans. However, their safety and in some cases their environmental impact have been questioned often.

There is a growing interest to develop 'Preservative – free' or 'Self – Preserving' cosmetic formulations. In self preserving products, the traditional preservatives are replaced by 'Multifunctional' cosmetic ingredients which apart from delivering their main functional property also exhibits antimicrobial property.

This paper explores the use of combination of multifunctional and other cosmetic ingredients in leave – on skin care products to deliver microbiologically safe product. Products studied are emulsion based Cream, Lotion and Vanishing cream.

MIC of 69 cosmetic ingredients were screened for antimicrobial property and compared with known preservatives. 17 skin care ingredients were shortlisted and in various combinations used to prepare 69 cream, 51 lotion and 19 vanishing cream formulations. They were evaluated for their efficacy to preserve the product against microbial challenge by Preservative Challenge Test (PCT), as 6 multiple weekly challenge. Formulation with preservative was used as control. Of the formulations screened, 11 cream, 24 lotion and 4 vanishing cream formulations passed our criteria, which was similar to control formulation with preservative. It is possible to develop different options of self preserving skin care products which are as efficacious as products with preservative.

Key Words: Cosmetics, Preservative, Self – preserving, Multi-functional actives, chelators, antioxidant, synergy.

INTRODUCTION

Cosmetic products are not expected to be aseptic, but they need to be safe for consumer use. Microbiological control of cosmetic products is always of interest to the industry since microbial spoilage can lead to product degradation and worse, human health hazard. Preservatives are

chemical antimicrobials used in cosmetics primarily to prevent microbial spoilage of the product, thereby improving the product shelf life and also to protect consumers from any adverse microbial infection. [9,37]

Classes of preservatives which are generally used in personal care products are Parabens, Formaldehyde releasers and Isothiazolinones. In recent times all three classes of preservatives have come under a cloud of suspicion of causing more harm than good to human health. Although declared safe for use by the Cosmetic Ingredient Review, parabens, have been implicated as endocrine disruptors [24,22] and may increase the risk of women developing estrogen positive breast cancer [11]. Formaldehyde releasers like Imidazolidinyl urea and Diazolidinyl urea are thought to cause skin reactions in sensitive individuals [8]. Although the Isothiazolinones (Methyl chlorisothiazolinone & Methylisothiazolinone) are considered non – carcinogenic, they have been associated with frequent causes of contact allergy. They are known contact sensitizers. [1]

Use of certain preservatives which are potent antimicrobial agents as well, may have driven the fixation and spread of certain resistance cassette collectors (class 1 integrons) [16] More indiscriminate use of such preservatives in cosmetic products may drive the selection of further new genetic elements that will aid in the persistence and spread of antimicrobial resistance and thereby limits our treatment options for microbial infections [6]. So it is getting clear that although chemical preservatives prevent microbial growth, their safety is questioned by a growing segment of consumers.

There is a growing interest to develop ‘Preservative-free’ or ‘Self – preserving’ cosmetic formulations. Common definition of ‘Preservative – free’ means that the product does not contain substances that are classified as preservatives according to the cosmetic legislation. The term ‘Self – preserving’ is more appropriate than Preservative free. In Self – preserving formulations traditional preservatives are replaced by other cosmetic ingredients which apart from delivering its primary property also

exhibits antimicrobial property. They are also termed as ‘Multifunctional’ ingredients. [13,14]

In an effort to develop Self-preserving leave – on skin care cosmetic products, this paper explores the use of multifunctional ingredients and identifies efficacious / synergistic combinations which meet regulatory norms of microbiologically safe product. The leave – on skin care products developed for this study are emulsion based cream, lotion and vanishing cream.

MATERIALS AND METHODS:

Materials: The cosmetic ingredients including preservatives used in this study were procured from various leading dealers and suppliers which includes Merck Specialities, India; Schulke & Mayr GmbH Germany; Sigma Aldrich, USA; Inolex CC, USA; Symrise Pvt Ltd, Mumbai; Dow Chemicals, Mumbai; Lonza India; Galaxy Surfactants Ltd, Mumbai; Vivimed Labs Ltd, Hyderabad; Hayashibara Co. Ltd, Japan; Kumar Organic Products Ltd., Bangalore; Croda Chemicals, India; and BASF India.

Microbial Culture: The microbial strains used for the screening studies were the same standard cultures and strains to be evaluated for preservative efficacy studies as recommended by Personal Care Products Council (PCPC) USA. The following microbial strains were used throughout the study.

1. *Staphylococcus aureus* ATCC 6538
2. *Staphylococcus epidermidis* MTCC 435
3. *Escherichia coli* ATCC 8379
4. *Pseudomonas aeruginosa* ATCC9027
5. *Candida albicans* ATCC 10231
6. *Aspergillus niger* ATCC 16404

Preparation of Inoculum:

The bacterial cultures were inoculated in slants of Tryptone Soy Agar for 18 – 24 hours at 36°C ± 1°C for 48 hours. The fungal strains were inoculated on Sabouraud Dextrose Agar and incubated at 23 ± 1°C for seven days respectively.

All the cultures were harvested in sterile saline and diluted to obtain counts of 1×10^8 CFU/ml.

Selection of Multifunctional actives with anti microbial properties:

Several cosmetically approved ingredients from among solvents, solubilizers, emollients, penetration enhancers, Functional actives, cooling agents, chelators, Anti oxidants, fragrance ingredients, preservative boosters, non – preservative antimicrobials were screened for their Minimal Inhibitory Concentrations (MIC) against the micro – organisms listed. In all about 69 ingredients were evaluated. Preservatives approved for use in cosmetics were also evaluated as controls.

Assessing the Minimal Inhibition Concentration:

MIC was determined by macro broth double dilution method for both antibacterial and anti fungal activity, as per the NCCLS guidelines [25]. These assays were done in quadruplicates and inferences were drawn.

Formulations

Leave on skin care products like cream, vanishing cream and lotions were developed with different combinations of the above mentioned multifunctional actives with the following ingredients as listed in the Table 1.

Preservative Efficacy Test (PET) / Preservative Challenge Test (PCT)

The formulations were evaluated for their efficacy to preserve the product by PCT (Preservative Challenge Test). Base formulations with preservatives were used as controls. Unfortunately there is no standard universally accepted method of challenge testing and interpretation of results. Various pharmacopoeias prescribe different methods, but CTFA (Cosmetic, Toiletries and Fragrance Association) – now PCPC (Personal Care Products Council) guidelines are used for cosmetic products. [10]

The PCT as per CTFA guidelines prescribe a single challenge with pathogenic Bacterial, Yeast & Mold cultures. After challenging with the

organisms, the samples of the product are withdrawn at regular intervals and evaluated for microbial load up to 28 days from day of inoculation. The product is considered to pass the test when the bacterial load reduces by 3 logs within 7 days from day of challenge, and no further growth is observed by day 28; and fungal count reduces by 2 logs within 7 days from day of challenge and no further growth occurs by day 28. However, cosmetic products are exposed to recurrent microbial insults, which are not addressed by the single challenge test.

Alternatively, in the Schulke – Koko test [31], the microbial mixture of bacteria, yeast & mold are inoculated 6 times (once a week) into the test product with the goal of keeping the test material germ free for this period. A sample is considered well preserved, if during the 6 challenges in the 6 weeks, the product shows no microbial growth on the test samples. But this method does not establish any resurgence of the organism over a period of time.

In the present study, the preservative efficacy of the product to microbial challenge is evaluated vs. the control product (base + preservative) by a combination of CTFA and Schulke KoKo method. Here the product is exposed to 6 multiple weekly challenges with a combination of bacterial, yeast and mold culture; and after each challenge the product is evaluated at fixed time points for microbial load for upto 28 days. This method ensures a rigorous process to determine the preservative efficacy of the product. A formulation is considered well preserved when it controls microbial growth with each challenge within 7 days and shows no further growth for upto 28 days.

Basically the required quantity of test product was inoculated with mixed microbial cultures (Bacterial mixture made of *S.aureus*, *E.coli* & *Ps. Aeruginosa*; and fungal mixture made of *C.albicans* & *A.niger*) in such a way that the final concentration of the organisms in the product was 1×10^6 CFU / ml for bacteria &

1×10^5 CFU / ml for fungal strains. The samples were mixed well after inoculation. Immediately after inoculation 1 g of the sample was removed and diluted in sterile Lethen broth to an appropriate concentration for colony counting and transferred to petriplates in duplicates. To determine bacterial count TSA was poured on plates, mixed well and allowed to solidify. To determine yeast & mold count molten SDA was used and solidified. The plates were incubated at $36^\circ\text{C} \pm 1^\circ\text{C}$ for 48 hrs for bacterial enumeration and at $25^\circ\text{C} \pm 1^\circ\text{C}$ for 5 – 7 days for fungal enumeration. The samples were evaluated at the following fixed time intervals after inoculation: Day 1 (Immediately after inoculation), Day 2, Day 3, Day 5, Day 7, Day 14, Day 21 & Day 28. On day 7, required aliquot was kept aside to continue microbial enumeration for the remaining time points and to the rest of the product the 2nd microbial challenge was done and the process of microbial enumeration done as explained above repeated. This was done 6 times so that the product underwent 6 weekly multiple challenges. The experiments were performed in triplicate. The product is judged adequately preserved, when bacteria are reduced by more than 99.9% (3 log) after 7 days of challenge, in each of the 6 challenges; yeasts & mold should be reduced by more than 99% (2 log) after 7 days of challenge, in each of the 6 challenges and no further growth for upto 28 days.

STATISTICS

The non-parametric Kruskal -Wallis test was conducted to evaluate the differences among the test products (including control) on the median change in the log count of bacteria and fungi in the substrate at each challenge. For each challenge, the Kruskal-Wallis test was performed across the products at the final time point where non-zero counts have been recorded. Due to the sample size, the chi-square statistic for the Kruskal-Wallis test is only approximate. The observations (log counts) from each test product

are independent of each other. The homogeneity of the samples, with respect to composition and starting log counts, before the treatment of samples are ensured.

If the Kruskal-Wallis test showed statistical significance, it was followed-up with the pair - wise comparisons and these were conducted using the Mann-Whitney U test, which yields identical results with the Kruskal-Wallis test for two independent samples. For each pair - wise comparison, controlling for Type I Error was ensured by adjusting the *a priori* alpha level divided by the number of comparisons (Bonferroni adjustment). The numbers of comparisons vary for each category (Cream, Vanishing Cream, etc). The control (base + preservative) was compared against the different test products in each category.

The statistical analysis was done using the statistical package PASW Statistics 18.

RESULTS

1. Minimal Inhibitory Concentration

Out of 69 ingredients evaluated, 17 ingredients showed MIC values similar to approved preservatives normally used in skin care products. Ingredients which have shown good antimicrobial efficacy are the emollients – Decylene glycol, Glyceryl caprylate & Capraloyl Glycine; Multi-functional ingredients – Undecylenol Phenyl alanine, Zinc latate, Zinc gluconate, para Anisic acid, Azelic acid, Potash alum, n-Butyl resorcinol & Hydroquinone; Chelators – EDTA – Di Sodium (EDTA – DS) & EDTA – Tetra Sodium (EDTA – TS); Antioxidants – Citric acid, Tartaric acid & TBHQ. As shown in Table 2 the MIC values of these 17 ingredients are in similar range as that of approved preservatives.

2. Preservative Efficacy test and multiple challenge Tests

The various combinations, as shown in Table 1 were developed into creams, vanishing creams

and lotions and evaluated for preservative efficacy by multiple challenge studies.

Out of the 69 cream formulations evaluated, 11 formulations passed all the 6 challenges and showed no microbial growth on continued incubation. Of the 51 lotion formulas, 24 passed the multiple challenge criteria effectively with no further growth. Out of the 19 vanishing cream formulations studied, 4 showed good preservative efficacy. As shown in Table 3, all these test formulation options were evaluated Vs base formula with preservative (DMDM Hydantoin (Glydant) or Methyl paraben + Propyl paraben + Phenoxyethanol) and were found to be as efficacious as the preservative based product for the multiple challenge test.

It is observed in our study that when base formulas of cream, lotion & vanishing cream were studied individually with either only antioxidant or chelator or multifunctional ingredient, TBHQ was effective for 1 – 2 cycles of challenge in all the 3 bases and EDTA was effective only in vanishing cream base for 2 cycles of challenge. Among the multifunctional ingredients, para Anisic acid was effective for 2 challenge cycles in cream base, Decylene glycol, Capraloyl Glycine & Undecylenoyl Phenylalanine were effective for 2 -3 challenge cycles; and in the vanishing cream base, the skin lightening active hydroquinone & n-butyl resorcinol were relatively better performing than others as they were effective for 4 challenge cycles.

Similarly, when binary combinations of antioxidants + chelators or combination of 2 antioxidants were studied in all the 3 bases, no further enhancement of preservative efficacy was observed.

But, when multifunctional actives in combination with antioxidant & chelator, or in combination with 2 antioxidants was studied, some synergistic combinations were seen which was effectively controlling microbial load for upto 6 multiple

challenges, thereby making these products pass our criteria.

In creams, para – Anisic acid in combination with any one antioxidant (CA/TA/TBHQ) and any one chelator (EDTA – DS / TS) was effective for 6 challenges. Potash alum in combination with Tartaric acid and EDTA – DS also showed good microbial control for all 6 challenges. Both para –Anisic acid and Azelic acid in combination with 2 antioxidants viz. Citric acid or tartaric acid with TBHQ also shows synergistic behavior, showing good microbial control upto 6 challenges.

In lotions similarly, the multifunctional actives, Glyceryl caprylate, Decylene glycol and Capraloyl glycine in combination with an antioxidant (CA/TA/TBHQ) and a chelator (EDTA – DS / TS) was effective for 6 challenges and they also exhibited synergistic activity in combination with 2 antioxidants viz. Citric acid or tartaric acid with TBHQ.

In vanishing cream formulas, the multifunctional actives Hydroquinone & n-Butyl resorcinol showed synergistic preservative efficacy in combination with antioxidant TBHQ and chelators (EDTA – DS / TS).

The preservative efficacy of these synergistic combinations in creams, lotions & vanishing creams were similar to that exhibited by base product with preservatives as seen in Fig. 1 – 3 which depict microbial load during each challenge of a few representative formulations.

Statistical evaluation among cream & lotion samples (Fig 4 – 7) for bacterial & fungal counts indicates that there is a significant difference in the median log counts. However, pair-wise comparison of control against other test products, after Bonferroni adjustment (adjusted $\alpha = 0.0045$ for cream & 0.0021 for lotion), showed that there is no statistical difference between the median values of each pair. Hence it can be stated that test products are on par with the control. For vanishing cream samples, (Fig 8 – 9) the results of the analysis indicates that there is a

significant difference in the median log counts at all challenges excluding challenge 4 (for bacterial counts) and excluding challenge 2 (for fungal counts). However, pair-wise comparison of control against other test products, after Bonferroni adjustment (adjusted $\alpha = 0.0125$), showed that there is no statistical difference between the median values of each pair. Hence it can be stated that test products are on par with the control.

DISCUSSION

While it is extremely essential to ensure protection of personal care products from microbial spoilage, selecting the right preservative system can be a tough challenge to the formulator. The cosmetic scientist is restricted by legislation to a limited number of chemistries, and further by the level at which these chemistries are allowed to be used in the product [35]. Once a routinely used and rarely questioned ingredient in cosmetic formulations, preservatives are now being viewed with skepticism by consumers.

To look beyond current technologies, formulators are seeking opportunities to apply new preservation principles to develop 'Preservative – free' or 'Self – preserving' formulas. The use of 'Hurdle Technology' is taking center stage in this endeavor. This technology combines a number of preservative factors in order to prevent microbial growth. The different hurdles may result in synergistic rather than additive effects [21,32, 34].

There are many cosmetically approved ingredients which have anti microbial properties. By a careful selection of these ingredients, it is possible to reduce or eliminate the use of regular preservatives and develop self preserving formulas. [34]. These ingredients are termed as 'Multi-functional actives', as they are molecules with more than one beneficial effect on the skin. Some such multifunctional ingredients include glycols, fatty acids and their monoesters;

phenethyl alcohol; Ethyl Hexyl Glycerine; phospholipids, etc.[21,32,34].

Chelators like EDTA are known to have potentiating effects on biocides. By themselves, they have weak antimicrobial effects, but they synergize with antimicrobials and enhance their activity synergistically. This potentiating effect is exhibited across a wide range of antimicrobials (including antibiotics, antifungals and antimalarial drugs) [28,5,18,2]. Chelators exert a lytic action on the outer lipopolysaccharide layer of the bacterial cell wall especially on the Gram negative bacteria. They remove multivalent cations from the cell membrane of the microbes by forming complexes, weakening the outer surface by reducing the membrane stability; thereby making them more vulnerable to biocides [21,31,7,]. Furthermore, strong chelators may inhibit microbial metabolism by binding trace minerals required for cellular reproduction, growth and survival [30].

Antioxidants primarily function to delay autoxidation of unsaturated lipids in products. They do show significant antimicrobial activity. Primary antioxidants are phenolic compounds. Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), and Tert-Butyl Hydroquinone (TBHQ) are commonly used antioxidants in cosmetic products. BHA and TBHQ are effective antimicrobials. Organic acids like citric acid, tartaric acid are also effective antioxidants. Antioxidants have also been reported to enhance the potency of antimicrobials [33,15,26,29,4].

Antimicrobial activity of antioxidants like citric acid and tartaric acid may be attributed to inhibitory mechanism like depression of internal pH of microbial cell by ionization of undissociated acid molecules and disruption of substrate transport by altering cell membrane permeability or reduction of proton motive force [20,12]. Tartaric acid is believed to act by lowering the pH of the cell. It also prevents the production of malic acid a key intermediate in

the process of gluconeogenesis, a primary fuel for the cell [23,3]. The main target of organic acids is cell wall and membrane proteins [33].

Antioxidants are presumed to potentiate efficacy of antimicrobials by protecting them from autoxidation, thereby reducing the rate of spontaneous inactivation and prolonging their biological activity [19]. On the other hand, antioxidant like TBHQ is known for its radical scavenging activity as well as antimicrobial property. Ooi *et al.*, (2013) have demonstrated that TBHQ exhibits antibacterial activity upon degradation and formation of Tert Butylbenzoquinone (TBBQ); which possesses good antibacterial activity. The mechanism of action of TBBQ appears to be by compromising the integrity of bacterial cell membrane without causing cell lysis [27].

In this study we have explored the use of selected multifunctional ingredients which are approved cosmetic ingredients, but not classified as preservatives according to Annex VI of Commission Directive 76/768/EEC, in combination with chelators and antioxidants to develop self-preserving skin care cosmetic formulations. Several types of cosmetic ingredients known to deliver different functional benefits like emollients (Glyceryl caprylate, Decylene glycol, Capraloyl glycine), skin lightening ingredients (Hydroquinone, n-Butyl resorcinol), Anti-inflammatory agent (p-Anisic acid), astringent (Potash alum), anti acne agent (Azelic acid) were selected based on antimicrobial efficacy. These multifunctional ingredients along with Chelators and antioxidants in the formulations shows synergistic antimicrobial efficacy in controlling multiple microbial challenges. Similarly, multifunctional ingredient in combination with 2 antioxidants (one of which has to be TBHQ) also showed good preservative efficacy.

The fact that these formulations have passed multiple microbial challenges effectively, gives great confidence in the robustness of the

microbial stability of the products and guaranteeing declared shelf life to the consumer.

CONCLUSION

The intelligent combination of multifunctional ingredients using synergism and boosting effects can be a solution to protect products from microbial contamination, are friendly to the skin and accepted by consumers. This approach to product preservation helps to eliminate usage of preservatives which may cause skin irritation or contact sensitivity

In conclusion, our study shows that it is possible to develop 'self-preserving' cosmetic skin care products without compromising on product aesthetics and product sensorial. These products can withstand multiple microbial challenges. They exhibit similar efficacy as that of products made with preservatives. However, one must be clear that these products can be self preserving only when GMP conditions are maintained while manufacturing, as like any product made with preservatives, these self preserving formulations will also not be able to control overwhelming contamination by microbes.

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Self Preserving Skin Care Cosmetic Products

Tables

Table 1: Formulations of cream, lotion and vanishing cream studied for PET.

	INGREDIENTS	QUANTITY (%)					
		CREAM		LOTION		VANISHING CREAM	
		Control (Base + preservative)	TEST	Control (Base + preservative)	TEST	Control (Base + preservative)	TEST
1	CCTG (Caprylic Capric Triglyceride)	10	10	5	5	-	-
2	Arlacel 165 (Glyceryl Stearate (and) PEG-100 Stearate)	5	5	3	3	-	-
3	Cetyl alcohol	2	2	3	3	-	-
4	Sepigel (Polyacrylamide & C13-14 Isoparaffin & Laureth-7)	0.75	0.75	1	1	-	-
5	DM water	QS 100	QS 100	QS 100	QS 100	QS 100	QS 100
6	Glydant – Preservative	0.6	-	0.6	-	-	-
7	Zinc Gluconate (ZG)	-	2.0 - 5.0	-	-	-	-
8	Zinc Sulfate (ZS)	-	2.0 - 5.0	-	-	-	-
9	Zinc lactate (ZL)	-	2.0 - 5.0	-	-	-	-
10	Potash Alum (PA)	-	2.0 - 5.0	-	-	-	-
11	Azelic acid (AA)	-	2.0 - 5.0	-	-	-	-
12	p-Anisic acid (pAA)	-	2.0 - 5.0	-	-	-	-
13	Citric acid (CA)	-	0.5 - 1.5	-	0.5 - 1.5	-	-
14	Tert – Butylhydroquinone (TBHQ)	-	0.01 - 0.1	-	0.01 - 0.1	-	0.01 - 0.1
15	Ethylenediaminetetraacetic acid – TS (EDTA – TS)	-	0.01 - 0.1	-	0.05 - 0.1	-	0.01 - 0.1
16	Ethylenediaminetetraacetic acid (EDTA – DS)	-	0.01 - 0.1	-	0.01 - 0.1	-	0.01 - 0.1
17	Tartaric acid (TA)	-	0.5 - 1.5	-	0.5 - 1.5	-	-
18	Glyceryl Caprylate (GC)	-	-	-	3.0 - 8.0	-	-
19	Decylene glycol (DG)	-	-	-	3.0 - 8.0	-	-
20	Capraloyl Glycine (CG)	-	-	-	0.5 - 5.0	-	-
21	Undecylenoyl phenyl alanine (UPA)	-	-	-	0.5 - 5.0	-	-
22	Stearic acid	-	-	-	-	18	18
23	Cetyl alcohol	-	-	-	-	0.5	0.5
24	Potassium Hydroxide	-	-	-	-	0.57	0.57
25	Titanium dioxide	-	-	-	-	0.6	0.6
26	Propylene glycol	-	-	-	-	2.5	2.5
27	Isopropyl myristate	-	-	-	-	1	1
28	Octyl Methoxy cinnamate	-	-	-	-	1.5	1.5
29	BMDM	-	-	-	-	0.75	0.75
30	Dimethicone	-	-	-	-	0.5	0.5
31	Methyl Paraben (MP) - Preservative	-	-	-	-	0.25	-
32	Propyl paraben (PP) - Preservative	-	-	-	-	0.15	-
33	Phenoxyethanol (PE) - Preservative	-	-	-	-	0.5	-
34	Hydroquinone (HQ)	-	-	-	-	-	0.5 - 4.0
35	n-Butyl resorcinol (nBR)	-	-	-	-	-	0.5 - 4.0

Table 2: MIC of skin care ingredients which showed promising antimicrobial activity.

S.No	SAMPLE	MIC ($\mu\text{g} / \text{ml}$)					
		<i>S.aureus</i>	<i>S.epider</i>	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>C.albicans</i>	<i>A.niger</i>
PRESERVATIVES							
1	Glydant	390.62	781.25	1562.5	625	1875	625
2	Methyl Paraben	2500	2187.5	1250	1406.25	703.12	625

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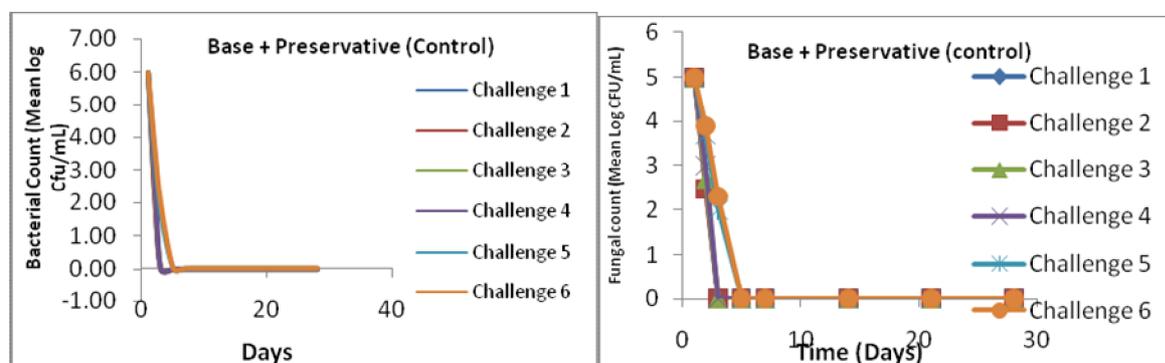
3	Propyl Paraben	546.87	625	390.62	1406.25	273.43	156.25
4	Triclosan	15.86	5.49	390.62	4375	21.97	29.29
5	Phenoxyethanol	5625	8750	8750	10000	10000	5625
EMOLLIENTS							
6	Decylene glycol	195.31	273.43	507.81	175.78	136.71	87.89
7	Capraloyl Glycine	2500	2812.5	5250	2812.5	6250	8750
8	Glyceryl caprylate	195.31	390.62	2500	271.43	781.25	390.62
MULTIFUNCTIONAL INGREDIENTS / FUNCTIONAL ACTIVES							
9	Undecylene Phenyl alanine	273.43	312.5	2500	1093.75	1562.5	1250
10	Zinc lactate	703.12	312.5	1562.5	2500	195.31	195.31
11	Zinc gluconate	781.25	390.62	3750	1562.5	390.62	781.25
12	Para Anisic acid	1562.5	1093.75	3125	2500	195.31	390.62
13	Azelic acid	1562.5	2187.5	2500	2187.5	6250	5000
14	Zinc sulfate heptahydrate	625	546.87	1250	2812.5	156.25	156.25
15	Potassium Aluminium sulfate (potash alum)	1093.75	1093.75	1093.75	5312.5	5000	10000
16	n-butyl Resorcinol	253.9	253.9	390.62	703.12	78.12	195.31
17	Hydroquinone	546.81	175.78	8750	2578.12	5000	8750
CHELATORS							
18	EDTA – DS	68.35	136.71	6250	2187.5	24.41	136.71
19	EDTA – TS	156.25	195.31	6250	1328.12	97.65	546.87
ANTIOXIDANTS							
20	Citric acid	546.87	1250	3125	3125	8750	>10000
21	Tartaric acid	2187.5	1093.75	2500	2187.5	> 10000	> 10000
22	TBHQ	24.41	34.17	781.25	390.62	625	781.25

Table 3: Effective synergistic combinations in different leave –on skin care products

S.No	Combinations	Effective combinations in products			# of PCT cycles passed	Final outcome Pass / Fail
		CREAM	LOTION	VANISHING CREAM		
1	Base – Preservative	Base	Base	Base	0	Fail
2	Base + Preservative	Base + Glydant	Base + Glydant	Base +MP + PP + PE	6	Pass
3	Base + Antioxidant + Multifunctional Active + Chelator	Base +CA / TA / TBHQ + pAA / PA + EDTA – DS / TS	Base +CA / TA / TBHQ + GC / DG / CG + EDTA – DS / TS	Base +TBHQ+HQ / nBR +EDTA – DS / TS	6	Pass
4	Base + Antioxidant + Antioxidant + Multifunctional Active	Base +CA / TA + BHQ+pAA / AA	Base +CA/ TA + TBHQ+ GC / DG / CG	-	6	Pass

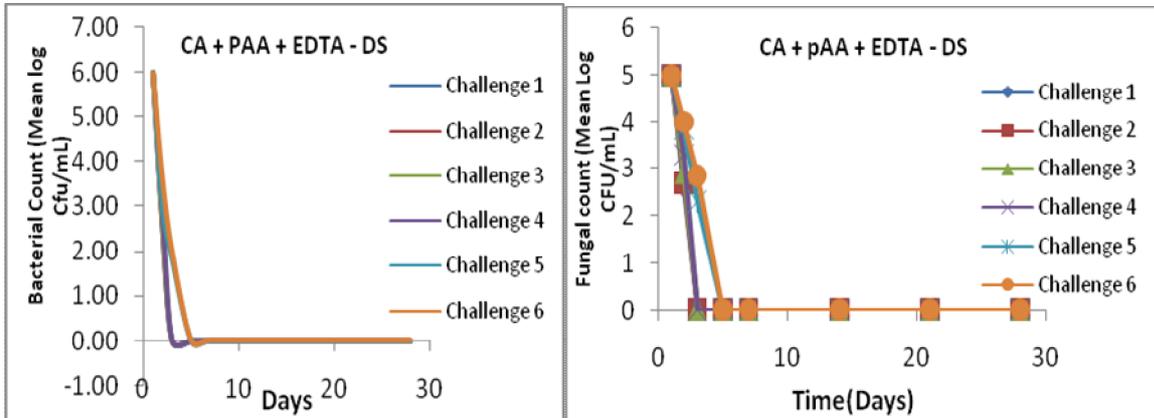
Figures

a) Cream : Base + Preservative (Control)



Self Preserving Skin Care Cosmetic Products

b) Cream : Base + CA + pAA + EDTA – DS



(c) Cream : Base + TA + TBHQ + AA

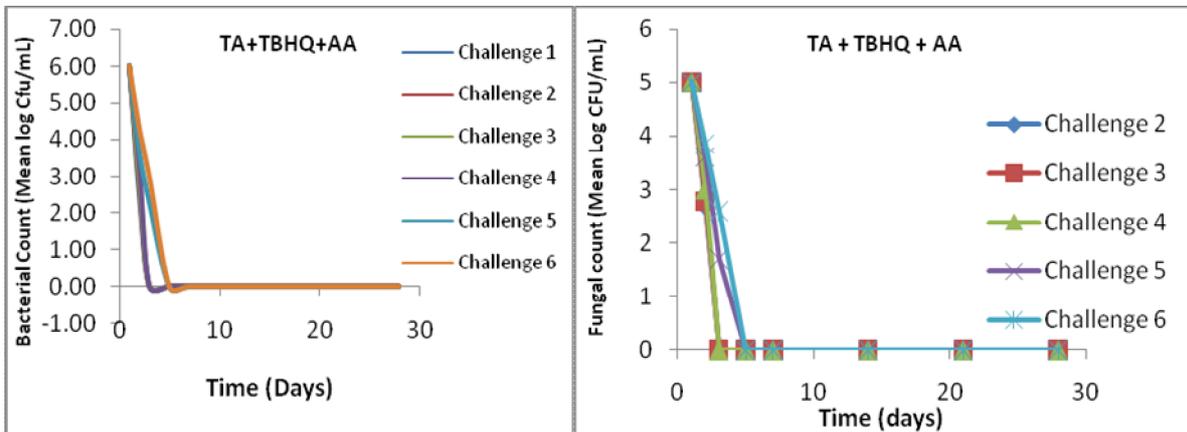
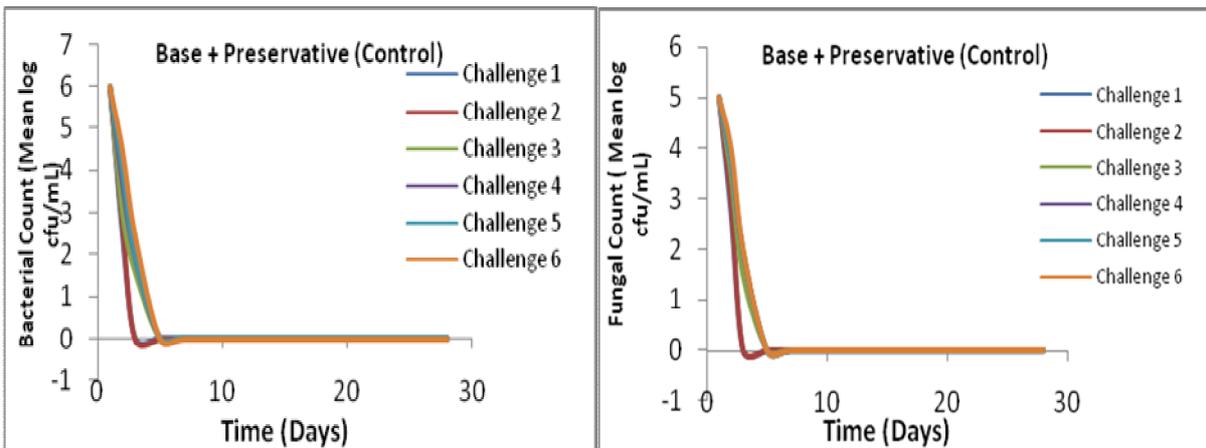


Fig 1 : Microbial Load during each Preservative Challenge of a few representative cream

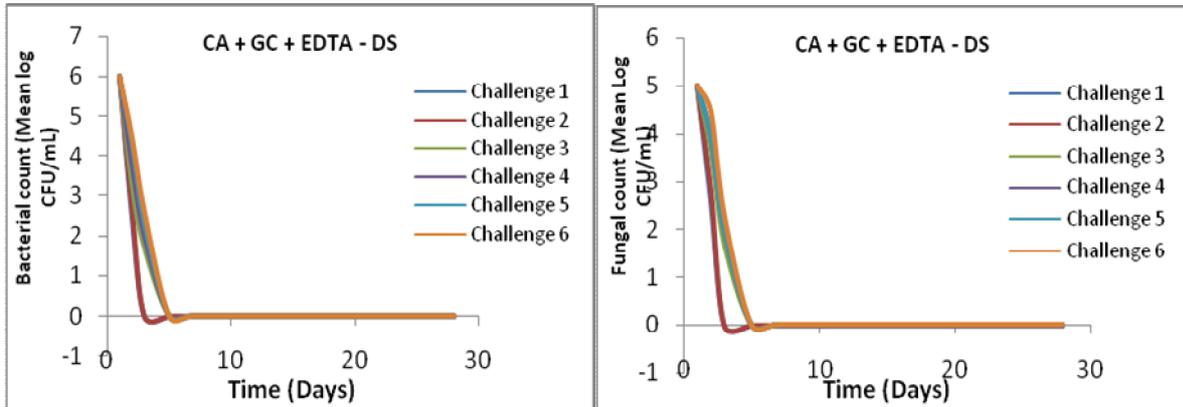
FORMULATIONS.

(a) Lotion : Base + Preservative (Control)

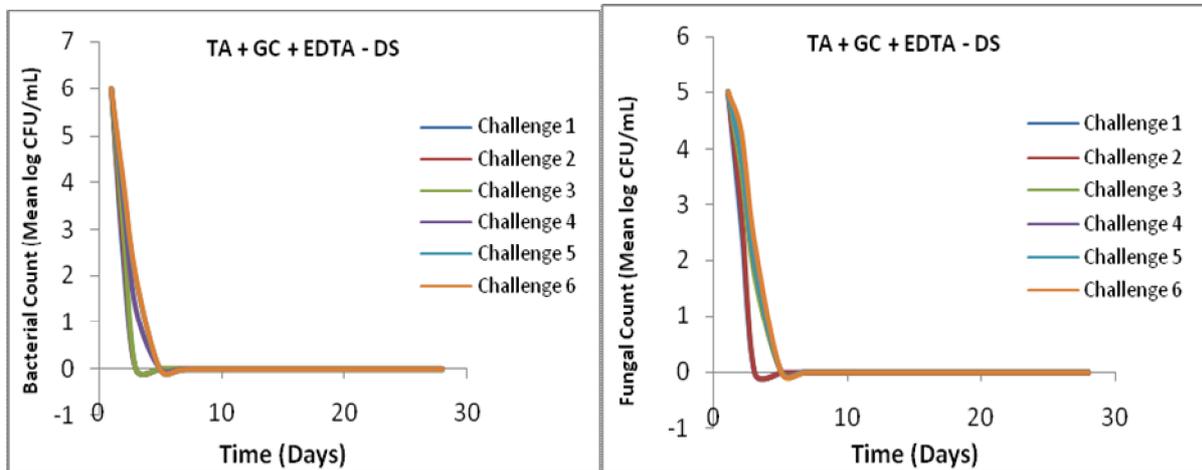


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(b) Lotion – Base + CA + GC + EDTA – DS



(c) Lotion – Base + TA + GC + EDTA – DS



(d) Lotion – Base + TA + TBHQ + CG

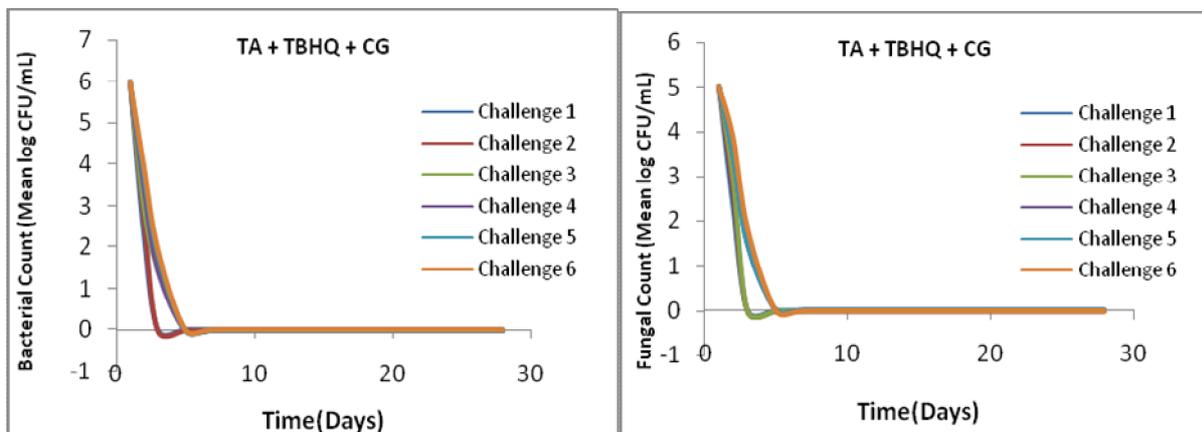
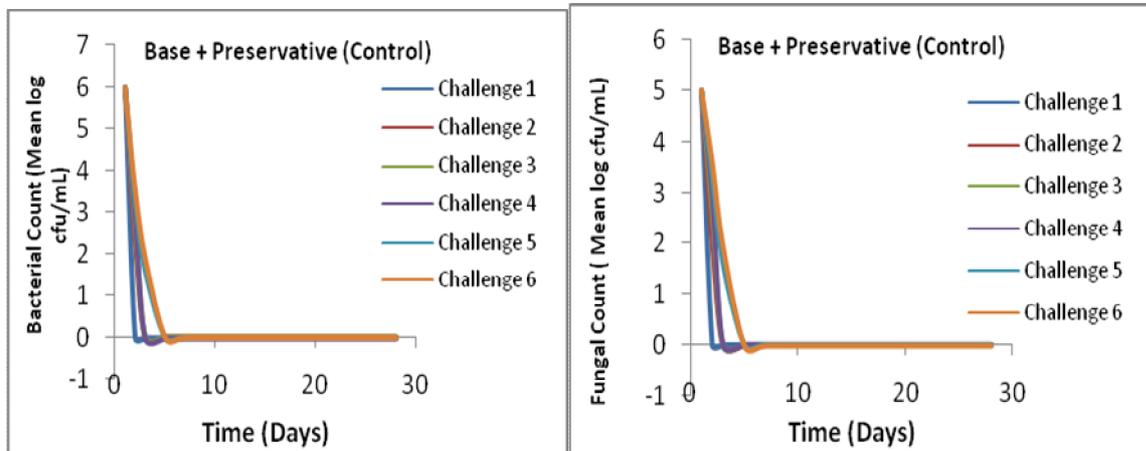


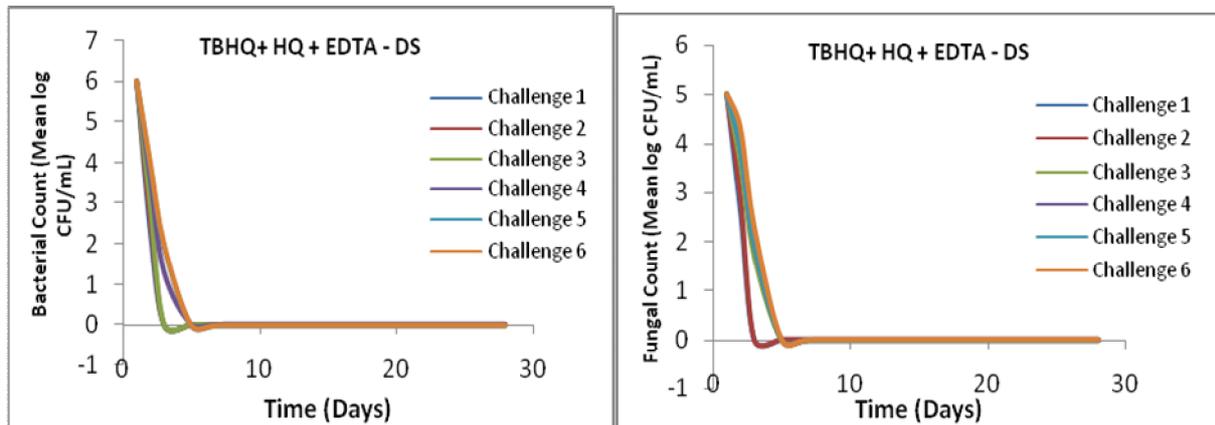
Fig 2 : Microbial Load during each Preservative Challenge of a few representative lotion formulations.

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a) Vanishing cream – Base + Preservative (Control)



b) Vanishing cream – Base + TBHQ + HQ + EDTA – TS



c) Vanishing cream – Base + TBHQ + nBR + EDTA – TS

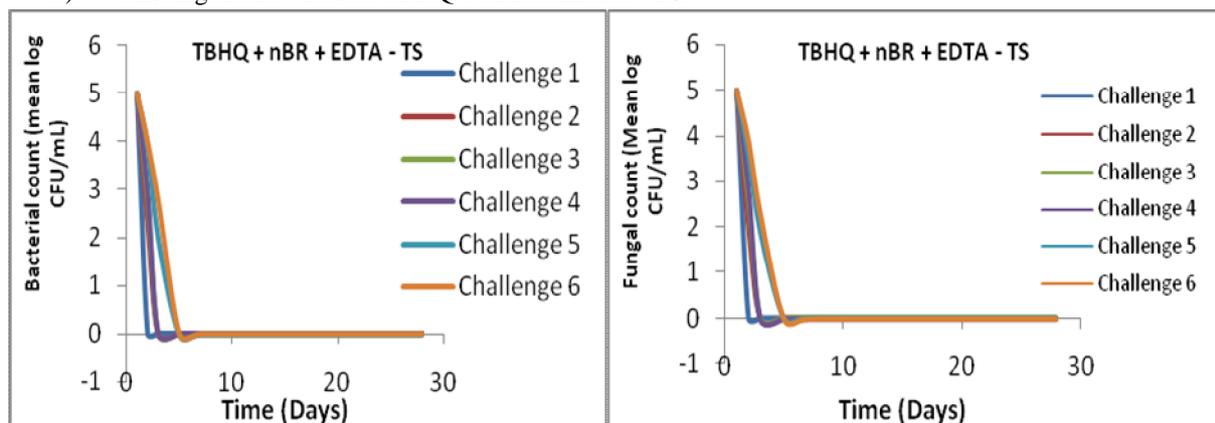


Fig 3: Microbial Load during each Preservative Challenge of a few representative Vanishing Cream formulations.

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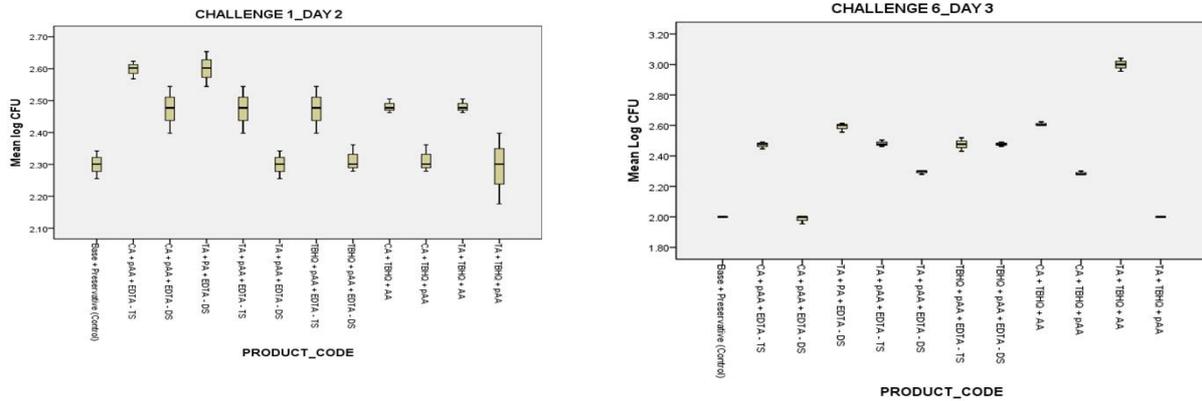


Fig 4: Comparative bacterial load of self – preserving cream formulations between initial challenge (Challenge 1 – day 2) & final challenge (Challenge 6 – day 3).

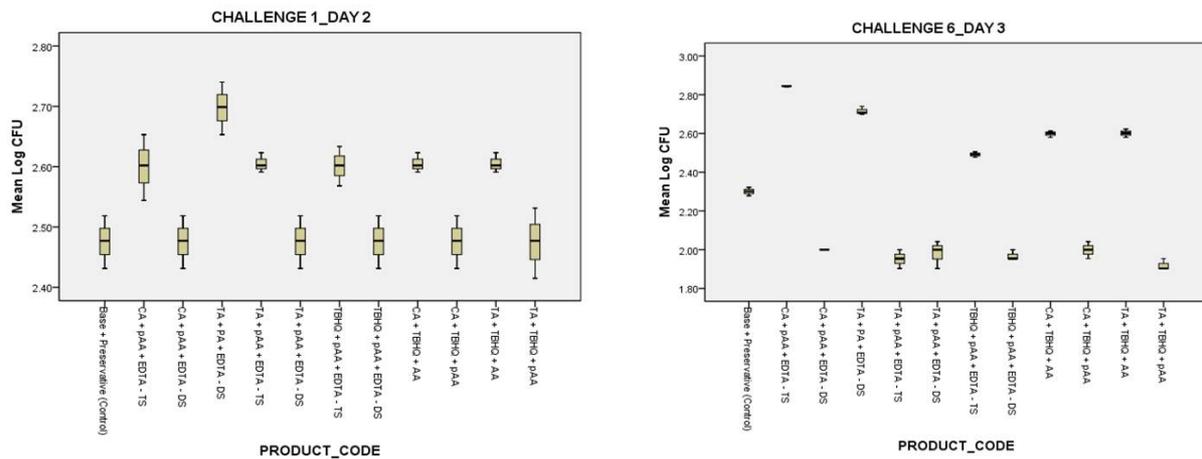


Fig 5 : Comparative fungal load of Self – preserving cream formulations between initial challenge (Challenge 1 – day 2) & final challenge (Challenge 6 – day 3).

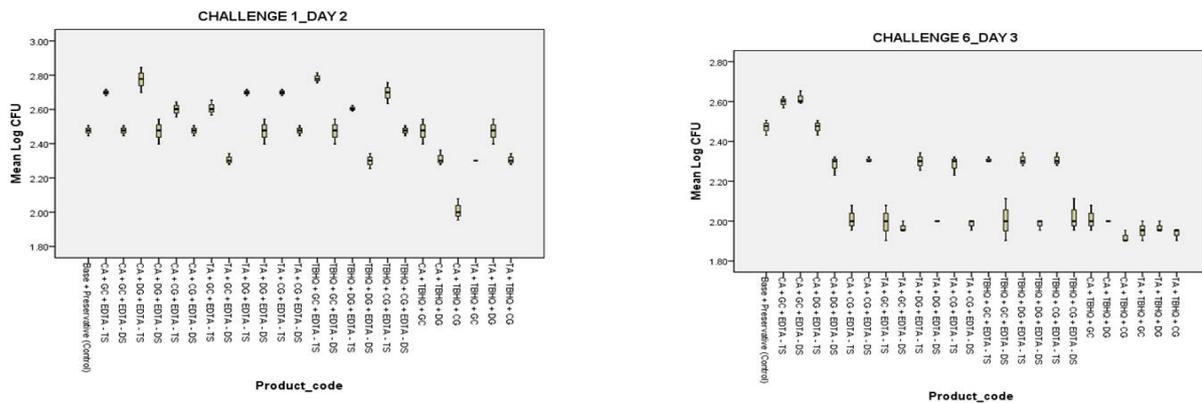


Fig 6: Comparative bacterial load of Self – preserving lotion formulations between initial challenge (Challenge 1 – day 2) & final challenge (Challenge 6 – day 3).

Self Preserving Skin Care Cosmetic Products

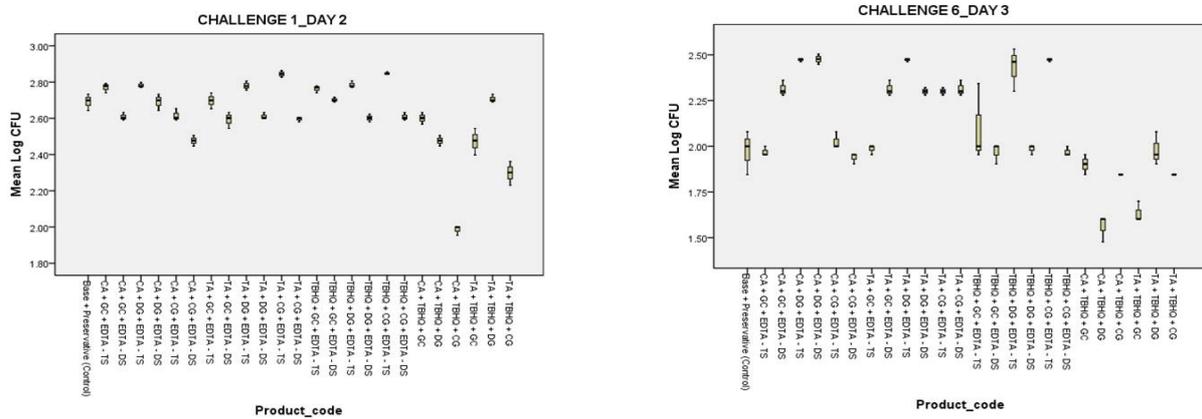


Fig 7: Comparative fungal load of Self – preserving lotion formulations between initial challenge (Challenge 1 – day 2) & final challenge (Challenge 6 – day 3).

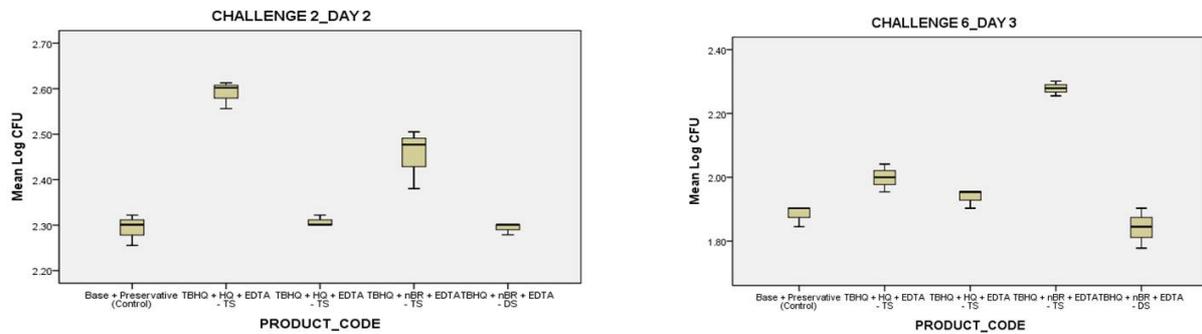


Fig 8: Comparative bacterial load of Self – preserving vanishing cream formulations between initial challenge (Challenge 2 – day 2) & final challenge (Challenge 6 – day 3).

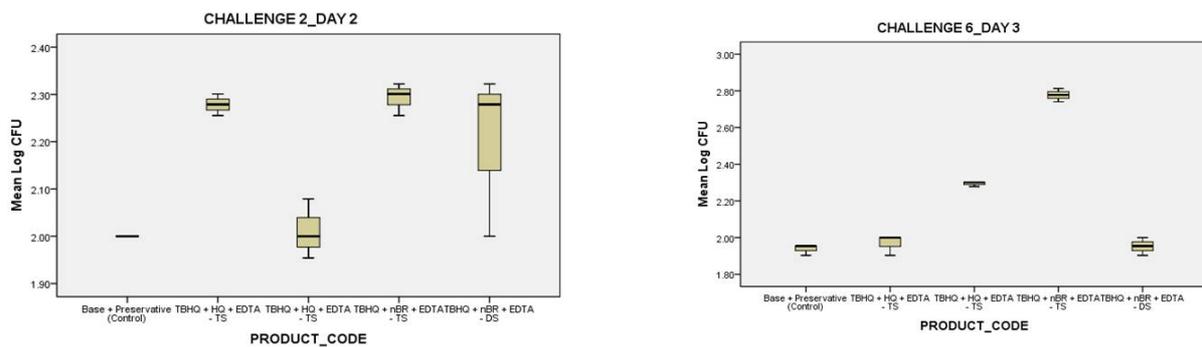


Fig 9 : Comparative fungal load of Self – preserving vanishing cream formulations between initial challenge (Challenge 2 – day 2) & final challenge (Challenge 6 – day 3).