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ORIGINAL ARTICLE / ÖZGÜN MAKALE

Formulation of Mild Shampoos and Investigation of Possible Prebiotic Effects

Hassas İçerikli Şampuan Formülasyonları ve Olası Prebiyotik Etkilerinin Araştırılması

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Abstract

Background: Recently, there has been a significant increase in the application of prebiotics in cosmetic products. Thus, this investigation aims to create two mild shampoo compositions, containing inulin: a distinguished prebiotic, and a reference shampoo.

Materials and Methods: After formulation development, physicochemical (physical appearance, pH, percentage of solid contents, viscosity, density and stability studies) and challenge test were carried out. The efficacy of formulations against strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria, as well as mixed cultures of these two bacteria, was assessed with MIC (Minimum Inhibition Concentration), MBC (Minimum Bactericidal Concentration).

Results: The results showed that the hair and body shampoo formulas displayed good stability and maintained their physicochemical properties under different conditions over time. Furthermore, they were microbiologically safe according to the challenge test and instrumental analysis. Microbial assays indicated that Shampoo-A promoted the growth of *Staphylococcus epidermidis* while inhibiting the growth of *Staphylococcus aureus* in the presence of prebiotic active, whereas Shampoo-B inhibited the growth of both bacteria.

Conclusions: Although further research is required to declare the microbiome-related claims, the development of these products holds promise for positive effects on skin health and microbiome.

Keywords: Shampoo, inulin, formulation, skin microbiome

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Öz

Amaç: Son zamanlarda, kozmetik ürünlerde prebiyotiklerin uygulanmasında önemli bir artış olmuştur. Bu araştırma, seçkin bir prebiyotik olan inülin de dahil hassas içerikli iki şampuan ve referans bir şampuan formülasyonu geliştirmeyi amaçlamaktadır.

Gereç ve Yöntemler: Formülasyon geliştirildikten sonra fizikokimyasal (fiziksel görünüm, pH, katı içerik yüzdesi, viskozite, yoğunluk ve farklı ortamlarda stabilite çalışmaları) ve koruyucu etkinlik testi incelenmiştir. Formülasyonların *Staphylococcus aureus* ve *Staphylococcus epidermidis* bakteri türlerine ve bu iki bakterinin karışık kültürlerine karşı etkinliği MIC (Minimum İnhibisyon Konsantrasyonu), MBC (Minimum Bakterisidal Konsantrasyon) testleri ile değerlendirilmiştir.

Bulgular: Sonuçlar, saç ve vücut şampuanı formüllerinin iyi bir stabilite sergilediğini ve zaman içinde farklı koşullar altında fizikokimyasal özelliklerini koruduğunu göstermiştir. Ayrıca, zorlama testi (koruyucu etkinlik testi) ve koruyucu miktarının değişimini gösteren enstrümantal analize göre mikrobiyolojik olarak güvenlidirler. Mikrobiyal analizler, Şampuan-A'nın prebiyotik aktif varlığında ortamda *Staphylococcus aureus*'un artışını engellerken *Staphylococcus epidermidis*'in artışını desteklediğini, Şampuan-B'nin ise ortamda her iki bakterinin de artışını engellediğini göstermiştir.

Sonuç: Mikrobiyomla ilgili iddiaları beyan etmek için daha fazla araştırma yapılması gerekse de, bu ürünlerin geliştirilmesi cilt sağlığı ve cilt bariyeri üzerindeki olumlu etkilerinden dolayı umut vaat etmektedir.

Anahtar Kelimeler: Şampuan, inulin, formülasyon, cilt mikrobiyomu

INTRODUCTION

In response to the current demand for mild and skin-friendly beauty products, researchers have extensively studied the correlation between skincare products and the microbiota of the skin in recent years. The human skin's microbiota is characterized by a complex and delicate network of interaction between microorganisms and the skin's surface cells. *S. epidermidis, S. capitis, S. caprae, S. hominis, S. lugdunensis, and S. haemolyticus* are prevalent species of coagulase-negative *Staphylococcus* (CoNS) found in the skin microbiome of healthy individuals, commonly considered harmless or even beneficial (1). These gram-positive, facultative anaerobes are distinguishable from coagulase-positive S. aureus. Recent studies have shown that changes in the balance of skin microbiome are often associated with skin problems, including atopic dermatitis, acne vulgaris, and rosacea (2-4). Therefore, maintaining or modulating the skin microbiome has been considered a wise approach for protecting beneficial bacteria in host organisms and promoting skin health. The increasing public awareness of the role of skin microbiome modulation is driving the study and commercial development of cosmetics utilizing prebiotic and probiotic agents for topical application (5). Many plant-derived oligosaccharides, including fructooligosaccharides (FOS), galactooligosaccharides (GOS), mannooligosaccharides (MOS), xylooligosaccharides, oligofructose, and inulin, have the potential to be classified as prebiotics (6). Inulin, due to its efficacy and safety, has become a widely used ingredient. Furthermore, it is used as a stabilizer for emulsions and detergents, and, when combined with fatty acids, provides non-irritating surfactants. Additionally, it forms a thin film layer on the skin, which is recognized as a skin conditioner and protective agent (7).

The aim of the current study is to develop mild hair and body shampoos using prebiotic ingredients, to analyse the physico-chemical properties and stability of the formulations

and to evaluate their antimicrobial activity against specific micro-organisms.

MATERIALS AND METHODS

Formulation of Shampoos

Shampoos were formulated by adding the weighted ingredients as shown in the composition Table I.

Table 1. Composition of formulated shampoos

Shampoo-A	Shampoo-B	Shampoo-C
Aqua	Aqua	Aqua
Disodium-2 Sulfolaurate	Disodium-2 Sulfolaurate	Disodium-2 Sulfolaurate
Inulin (and) Fructose	**Inulin (and) Alpha-Glucan Oligosaccharide**	**-**
Cocamidopropyl Betaine	Cocamidopropyl Betaine	Cocamidopropyl Betaine
Lauryl Glucoside	Lauryl Glucoside	Lauryl Glucoside
Decyl Glucoside	Decyl Glucoside	Decyl Glucoside
Glycerin	Glycerin	Glycerin
Sweet Almond Extract	Sweet Almond Extract	Sweet Almond Extract
Quaternium 22	Quaternium 22	Quaternium 22
Sorbitan Sesquicaprylate	Sorbitan Sesquicaprylate	Sorbitan Sesquicaprylate
Sodium Benzoate	Sodium Benzoate	Sodium Benzoate
Citric acid	Citric acid	Citric acid

Physicochemical Evaluations

Physical appearance/visual inspection:

Developed formulations were evaluated in terms of their clarity, color, and odour.

Determination of pH: The pH measurement was performed on undiluted shampoo by using a digital pH meter at room temperature.

Determination of percentage solids contents: The sample to be analysed was weighed into the aluminium container with a minimum weight of 0.5 g and a maximum weight of 2.0 g. The measurement was done with moisture analyzer (Ohaus MB 45).

Measurement of viscosity: The viscosity of shampoo was measured at 20°C at 53 spindles with 12 rpm by using viscometer (Brookfield DV2T Viscometer)

Stability studies: Developed shampoos were kept under sun, at 40° C, room temperature (25 °C) and refrigerator (4 °C). Their physical appearance, pH and viscosity were checked at one-month intervals for two months. The preservative amounts (sodium benzoate) of the samples stabilised in different media were checked by HPLC (Agilent 1260 Infinity II).

Biological Evaluations

Challenge test

The stages of the preservative efficacy test were conducted according to ISO 11930:2019 - Cosmetics - Microbiology - Evaluation of the antimicrobial protection of a cosmetic product. The test utilized standard strains including *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404. For the experiment, the test product was prepared in five separate sterile containers at a concentration of 20 g/ml (8).

Solutions were prepared with a density of $1x10^7$ - $1x10^8$ cfu/ml for bacteria and $1x10^6$ - $1x10^7$ cfu/ml for yeast and mold strains. To obtain values between 1×10^5 cfu/ml and 1×10^6 cfu/ml for bacteria, and between 1×10^4 cfu/ml and 1×10^5 cfu/ml for C. *albicans* and A. *brasiliensis,* 0.2 ml of inoculum was added to each container. The mixture was thoroughly stirred to achieve homogeneity. The containers containing the inoculated formulation were stored at (22.5 ± 2.5) °C.

On the 7th, 14th, and 28th days, 1 g/ml was taken from each test container and added to a 9 mL neutralizing solution, followed by vortexing. Tryptic soy agar (TSA) was used for bacterial cultures, Sabouraud dextrose agar (SDA) for C. *albicans,* and Potato dextrose agar for A. *brasiliensis*. Incubation

was carried out at 32.5 ± 2.5 °C for 48-72 hours for bacteria and C. *albicans* and at 22.5 ± 2.5°C for 3-5 days for A. *brasiliensis.*

Calculations were made according to the evaluation criteria in Annex B, table B.1 in the ISO 11930:2019 standard. To determine the decrease in the number of microorganisms in the experimental petri dishes on days 7, 14, 28; $R_x = \log N_0 - \lg N_x$ formula was used (8).

In vitro microbial assay

The Clinical and Laboratory Standards Institute (CLSI) procedure was modified, and the method was applied. Shampoos were used at 100 % concentrations. *Staphylococcus aureus* (ATCC 6538- 25923) and *Staphylococcus epidermidis* (ATCC 12228) bacterial strains were used for in vitro microbial activity assays. The bacterial suspension prepared at 0.5 McFarland turbidity was diluted 1:10 and 5 µL was inoculated into the wells to obtain an inoculum density with a final concentration of $5x10^5$ cfu/ml. Microdilution plates were incubated at 35±2ºC for 16-20 hours. After incubation, measurements were made at 630 nm with a Biotek brand 800 TS model microplate reader. (Figure 1) Then, the MBC (Minimum Bactericidal Concentration) test was performed, and the samples taken from the wells were inoculated into TSA medium. Petri dishes were incubated at 35°C for 24 hours (9).

RESULTS

All shampoos had pale yellow colour and characteristic odour. The viscosity range was measured between 2420-3860 cP and the acid balance (pH) was measured at 4.95. The solid composition percentage for each shampoo was identified at 13.27-14.7 as presented in Table II.

Table 2. Characterization of formulated shampoos

Evaluation Parameters	Formulated Shampoos		
	Shampoo-A	**Shampoo-B**	**Shampoo-C**
Color	Light Yellow	Light Yellow	Light Yellow
Odor	None	None	None
Transparency	Transparent	Transparent	Transparent
pH	4.97	4.97	4.97
Viscosity (Cp)	3860	2420	3350
Density	1.027	1.027	1.023
Solid Content (%)	13.51	14.70	13.27

The challenge test involved inoculating the product with Escherichia coli, Straphylococcos aureus, Pseudomonas aeruinosa, Candida albicans and Aspergillus brasiliensis. Table 3 summarizes the microbial challenge test results of shampoo A as an example.

A growth inhibition/promotion assay was conducted to investigate whether the formulas inhibit/promote bacteria growth. The effects of two common skin bacteria on promoting or inhibiting growth were investigated. S. aureus was chosen to represent the deleterious bacteria on the skin whereas S. epidermidis was chosen to represent the beneficial bacteria on the skin.

As shown in Figure 1, samples were taken from the first wells. For Shampoo-B, no growth was observed at a concentration of 100 % in the Petri dishes, while for Shampoo-A, growth results were observed where S. epidermidis suppressed S. aureus at a concentration of 100%.

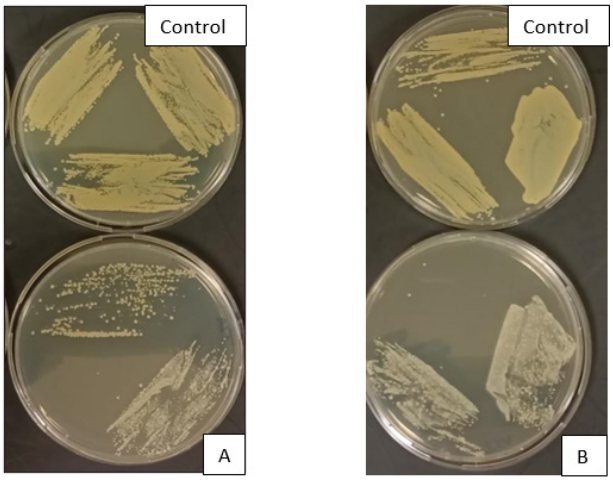

Figure 1. Shampoo A and Shampoo B MBC results comparison to their control group

Table 3. Challenge test results of formulated shampoo A

Quantity of the Initial Numbers of Microorganisms		
Microorganisms	N	N_0
Escherichia coli ATCC 8739	$2.87x10^7$	$2.87x10^5$
Staphylococcus aureus ATCC 6538	$3.10x10^7$	$3.10x10^5$
Pseudomonas aeruginosa ATCC 9027	$2.65x10^7$	$2.65x10^5$
Candida albicans ATCC 10231	$3.17x10^6$	$3.17x10^4$
Aspergillus brasiliensis ATCC 16404	$2.10x10^6$	$2.10x10^4$

*N: number of microorganisms in mL

N0: N/100, number of microorganisms in mL of product at time T0

Shampoo-A Analysis Results										
Microorganisms	7 days (T7)			14 days (T14)			28 days (T28)			Log reduction value
	N	N_0	R	N	N_0	R	N	N_0	R	
E. coli	<10	$2.87x10^5$	5.45	<10	$2.87x10^5$	5.45	<10	$2.87x10^5$	5.45	Accepted
S.aureus	<10	$3.10x10^5$	5.49	<10	$3.10x10^5$	5.49	<10	$3.10x10^5$	5.49	Accepted
P. aeruginosa	<10	$2.65x10^5$	5.42	<10	$2.65x10^5$	5.42	<10	$2.65x10^5$	5.42	Accepted
C. albicans	<10	$3.17x10^4$	4.50	<10	$3.17x10^4$	4.50	<10	$3.17x10^4$	4.50	Accepted
A. brasiliensis	<10	$2.10x10^4$	4.32	<10	$2.10x10^4$	4.32	<10	$2.10x10^4$	4.32	Accepted

*N : Reproducing microorganism at the end of contact*cfu/ml (*cfu: colony-forming unit)

N0 : Final concentration of the microorganism in the sample after inoculation cfu/ml

R : Rx= LgN0 – LgNx

DISCUSSION

Within the shampoo formulas, a very mild surfactant system was preferred; cocamidopropyl betaine, disodium-2-sulfolaurate, lauryl and decyl glucoside. Additionally, glycerin and sweet almond extract were included to promote conditioning effects of the skin and hair. Quaternium 22 was also included as a hair conditioning and antistatic agent, while sorbitan sesquicaprylate was used as a thickener and foam booster. The observation period (3-month stability) demonstrated that organoleptic characteristics of the formulated shampoos and stability were chemically and physically acceptable.

Based on the data from the *in vitro* microbial challenge test results (Table 3), it can be concluded that all shampoos successfully inhibited most of the microbial growth, while preserving the physicochemical properties of the product. Notably, the challenge tests revealed that prebiotic formulated shampoos (A and B) were more effective in comparison to the control shampoo (C). Overall, Shampoo-A inhibited the growth of S.aureus and maintained the growth of skin friendly *S.epidermidis* according to MBC test. Growth inhibition assay has shown promising results for the initial. Further *in vitro*, *in vivo* or 3D skin model studies should be performed as a next step to reinforce the

idea that these formulations have an impact on the human microbiome and to attribute them as microbiome-friendly hair care products.

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Conflict of Interest

The authors declared no conflict of interest regarding this article.

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Ethical Declaration

Ethics Committee approval was not required for this study.

Author Contributions

Idea: BTE, BS Design: BTE, SO, Supervision: BS, Equipment: Eczacıbaşı Consumer Products, Data

collection and processing: BTE, SO, Analysis and commentation: BTE, SO, Literature review: BTE, SO,

Writing: BTE, SO, Critical review: BTE

CONCLUSION

Overall, the findings demonstrated that the hair and body shampoo formula exhibited favorable stability and sustained its physicochemical properties over time despite diverse conditions. Additionally, it was microbiologically safe in line with the challenge test. Microbiological tests displayed that Shampoo-A stimulated the proliferation of *Staphylococcus epidermidis* whilst suppressing the development of Staphylococcus aureus in the presence of prebiotic compounds, whereas Shampoo-B restrained the progression of both bacteria. Though additional inquiry is necessary to assert the validation of microbiome-related assertions, these product advancements are estimated to positively impact skin wellbeing and microbial balance.

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