NEW COSMETICS BASED ON COLLAGEN AND CAFFEINE WITH ANTIMICROBIAL ACTIVITY

MARIA MINODORA MARIN, GEORGETA BUMBENECI, RODICA ROXANA CONSTANTINESCU, CIPRIAN CHELARU, MADALINA GEORGIANA ALBU-KAYA

The National Research & Development Institute for Textiles and Leather - Leather and Footwear Research Institute Division, 93 Ion Minulescu Str., 031215, Bucharest, Romania, icpi@icpi.ro

Cosmetic area has increased worldwide and will continue to expand as long as there are users. At the moment, the interest leads towards cosmetics based on natural ingredients. A common aesthetic problem for majority of the people is represented by dark circles which appear in the infraorbital region. The purpose of this work was to prepare and analyze new cosmetics based on collagen and caffeine with antimicrobial activity that can be used for the treatment of dark circles. The obtained cosmetics are stable at the natural pH of the skin, indicating that new products can be securely used. Images from the optical microscopy evaluation revealed that cosmetics have a soft and foam like appearance. All the obtained cosmetics present excellent stability at 4 and 40°C. The antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* was also performed showing that the cream with collagen hydrolysate and smallest amount of caffeine is the most efficient Some more analysis need to be performed before notification.

Keywords: collagen hydrolysate, caffeine, cosmetic.

INTRODUCTION

Over the last years, the majority of the people no longer view cosmetics as strange products because everyone uses them, regardless of appearance. Cosmetics are defined by the Food, Drug, and Cosmetics Act (FDCA) as items that are brushed, sprayed on, or applied to the human body in order to clean, enhance, and change one's appearance (Shafie *et al*., 2022). Cosmetics are used to take care of the body's parts, the active compounds they contain helping to improve the healthiness, treatment of acne, and anti-aging of human skin (Joshi *et al*., 2018). Cosmeceuticals are the name given to these active substances (Panico *et al*., 2019; Shafie *et al*., 2022). One of these components, collagen hydrolysate, is known to be used in cosmetic formulations to protect the structure and function of the skin as well as to improve its appearance. In addition to the beauty business, collagen is a naturally occurring biological substance that is used in ophthalmology, dentistry, pharmacy, and biotechnology (Yorgancioglu *et al*., 2013).

Dark circles, which appear in the infraorbital region, are characterized as bilateral, ovoid, homogeneously pigmented macules with a multifactorial etiology. There are a variety of treatments available, such as laser therapy, chemical peels, whitening creams, topical retinoid acid, autologous fat transplantation, injectable fillers, and surgery interventions (Eyraud *et al*., 2021). Therefore, it is not surprising that dark rings are a substantial source of aesthetic concern for certain individuals (Friedmann *et al*., 2015).

The naturally occurring methylxanthine caffeine is found in the cherry beans of *Rubiaceae* plants. Caffeine can be consumed by people through foods, drinks, dietary supplements, drugs and cosmetics (Bury *et al*., 2021). Various animal and human data addressing the pharmacological and toxicological effects of caffeine are available. Caffeine is a well-known compound with a wealth of data (Alexander-White *et al*., 2021). Also, the efficacy and tolerance of a cream based on caffeine and used for the improving of the visible, under-eye dark circles, was evaluated for different clinical studies (Ahmadraji *et al*., 2015). Since the dawn of time, cosmetics have played a significant role in human life. Although they are not required to be sterile microbiologically, they must

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be of a proper quality for consumer health (Halla *et al*., 2018). It has been established that these cosmetics are susceptible to contamination by microorganisms from the production environment or from raw materials, particularly water, and that the contamination can happen either during or after production due to unsanitary storage conditions (Halla *et al*., 2018). The fragrance, color, viscosity, and performance of cosmetic products may alter unintentionally as a result of microbial contamination (Halla *et al*., 2018). Due to the elements in their composition, cosmetics are a good growth media for microorganisms (Lundov *et al*., 2009).

The aim of this research work was to obtain and analyze new cosmetics based on collagen and caffeine with antimicrobial activity that can be used for the treatment of dark circles. The obtained cosmetics are stable at the natural pH of the skin, indicating that new products can be securely used.

MATERIALS AND PREPARATION METHOD

Materials

Type I collagen hydrolysate was obtained by acidic hydrolysis as we previously described (Ficai *et al*., 2013). Ingredients: Stearine, Lanoline, White wax, Paraffin oil and methylxanthine caffeine were purchased from a local pharmacy. Nutrient agar and nutrient broth and *Staphylococcus aureus* (*S. aureus*, ATCC 6538), *Escherichia coli* (*E. coli*, ATCC 10536) and Cetyl alcohol were purchased from Novachim (Bucharest, Romania).

Preparation of the Cosmetics

The composition of cosmetics is given in Table 1. For all the samples, the components of phase A and phase B were heated using a water bath and heating-resistant Berzelius beakers, under intermittently homogenization. When both parts reached a temperature of about 70-75°C, they were removed from the water bath; part B was mixed with part A under constant stirring for 10 minutes. The recipient was put in a cold-water bath with continuous stirring for 3 minutes. In the cooled composition, the ingredients of phase C were introduced and mixed gradually.

Table 1. Composition of the obtained samples

Phase	Ingredients	C1	C2	C3	C4
A	Stearine, %	7.8	7.8	7.8	7.8
A	Lanoline, %	2.6	2.6	2.6	2.6
A	White wax, %	1.6	1.6	1.6	1.6
B	Paraffin oil, %	27	27	27	27
B	Cetyl alcohol, %	1	1	1	1
B	Ultrapure water, %	48.3	48.2	48	47,6
C	Collagen hydrolysate, %	1.6	1.6	1.6	1.6
C	Caffeine, %	0.1	0.2	0.4	0.8
C	Triethanolamine, %	1.6	1.6	1.6	1.6

The obtained cosmetics were transferred to a sterile vessel and analyzed.

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METHODS

pH Determination

The pH of the obtained cosmetics was determined using an inoLab pH meter.

Stability Determination at 4 and 40°C

The stability determination of the obtained cosmetics was evaluated using a thermostat that ensures a constant temperature of 4 and 40°C, respectively. 5g of the sample was placed in a weighting Petri, covered with a lid and kept in a thermostat for 8 hours at a temperature of 4°C. The Petri dish was removed from the thermostat; the sample was examined, covered with the lid and reinserted into the thermostat at a temperature of 40°C for 8 hours. After that the sample was examined again. The sample is considered stable if the phases do not separate. All the experiments were performed in triplicate for all the samples.

Optical Microscopy

The microscopic determinations of the designed cosmetics were carried out using a LEICA optical microscope model S8AP0, with an increase power factor of 20-160x.

Antimicrobial Activity

Microbiologic tests were performed using ISO 16187:2013 standard - Footwear and footwear components.

When the test is considered to be effective, the antibacterial activity ratio is obtained according to the following formula:

$$R = \frac{C_t - T_t}{C_t} X100\% \quad (1)$$

R – is the ratio of the antibacterial activity;

C_t – is the average of the number of bacteria obtained from three control samples after an incubation period of 18 to 24 h;

T_t – is the average number of bacteria obtained on three samples with antimicrobial effect after an incubation period of 18 to 24 h.

RESULTS AND DISCUSSION

All new cosmetics presented a homogeneous, stable, and free of phase separation state. The pH of the obtained cosmetics ranged from 5.5 to 6, which is in line with the skin's pH (Dănilă *et al*., 2020).

The stability determination of the obtained cosmetics was evaluated. Figure 1 shows the samples before and after the evaluation of stability at 4 and 40°C during 8 hours.

In Figure 1 can be seen that after the determination of the stability at 4 and 40°C, all the creams were stable at the tested temperatures during the 8 hours period of treatment.

Figure 2 shows the images that were acquired after optical microscope investigation of the cosmetics morphology.

Figure 2 showed that all creams exhibit a “foam-like” appearance. The different caffeine amounts are what caused the variations in the microscopic images.

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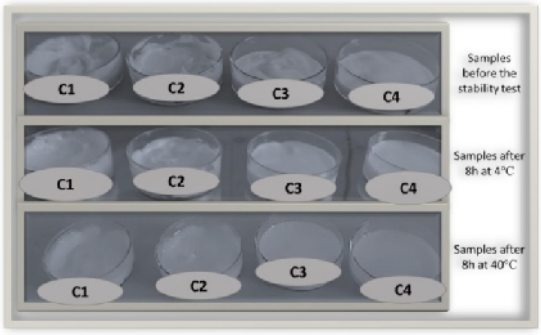

Figure 1. Stability determination at 4 and 40°C of the obtained cosmetics

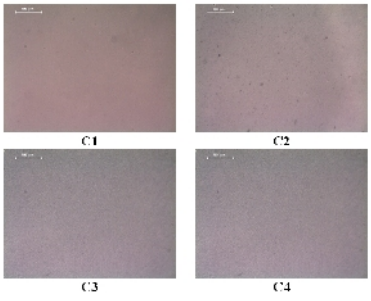

Figure 2. Optical microscopy images of the obtained cosmetics

The most significant feature of cosmetics is to obtain them without developing microbial growth (Dadashi *et al*., 2016). For all obtained cosmetics, the total number of viable aerobic microorganisms was determined. The results can be seen in Table 2.

Table 2. Total number of viable aerobic microorganisms

Microbiological characteristics	Admissibility conditions	Results			
		C1	C2	C3	C4
Total number of aerobic microorganisms (TAMC), CFU/g	Up to 100 CFU/g for topical products Up to 1000 CFU/g for pharmaceutical products	0 CFU	1 CFU	2 CFU	3 CFU
Total number of fungi and filamentous fungi (TYMC) CFU/g	Up to 100 CFU/g for topical products Up to 1000 CFU/g for pharmaceutical products	0 CFU	0 CFU	0 CFU	1 CFU
Staphylococcus aureus	absent	absent	absent	absent	absent
Escherichia coli	absent	absent	absent	absent	absent
Pseudomonas aeruginosa	absent	absent	absent	absent	absent

From the Table 2 can be observed that all tested samples show admissible limits for microbial contamination.

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The antibacterial activity ratio for all new cosmetics is presented in Table 3.

Table 3. Antibacterial activity ratio of the obtained cosmetics (*Staphylococcus aureus ATCC 6538* and *Escherichia coli ATCC 10536*)

Sample	Result (*Staphylococcus aureus ATCC 6538* and *Escherichia coli ATCC 10536*)	R%	Log10 red.
C 1	T0=1,5x105CFU/mL T24= 0 CFU/mL	100%	5,00
C 2	T0=1,5x105 CFU/mL T24=0 CFU/mL	100%	5,00
C 3	T0=1,5x105 CFU/mL T24= 0 CFU/mL	100%	5,00
C 4	T0=1,5x105 CFU/mL T24= 0 CFU/mL	100%	5,00

For all the samples the antibacterial activity ratio was 100 %. Further, in accordance with SR EN ISO 20645/2005—Control of the antibacterial activity, the resulting cosmetics were evaluated by the inhibitory activity. The evaluation of the samples is based on the absence or presence of bacterial proliferation in the contact region between the inoculum and the sample and on the emergence of an inhibition zone surrounding the samples (Figure 3).

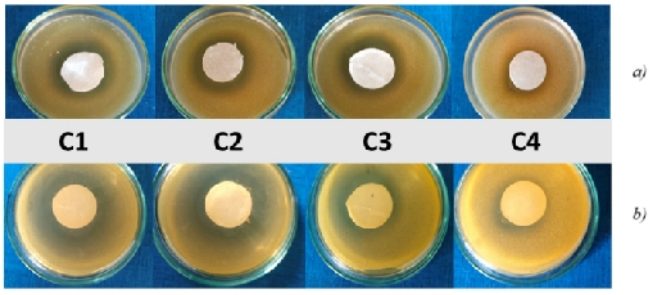

Figure 3. Inhibitory activity against a) *Escherichia coli* and b) *Staphylococcus aureus*

The inhibition areas formed by all the formulations showed diameters varying between 6, and 12.5 mm when tested against *Staphylococcus aureus* and 5.5 and 10 mm against *Escherichia coli* after 24 h of incubation. The samples with the higher concentration of caffeine presented the smallest antimicrobial activity. The best antimicrobial potential against both bacterial strains was obtained for the sample C1 which contain the lowest concentration of caffeine.

CONCLUSIONS

New cosmetics based on collagen and caffeine with antimicrobial activity that can be used for the treatment of dark circles were obtained and evaluated. The obtained cosmetics had a pH that ranged from 5.5 to 6, indicating that the new products can be securely used for skin care. Images from the optical microscopy evaluation revealed that cosmetics have a foam like appearance. All the obtained cosmetics present excellent

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stability at 4 and 40°C. The antimicrobial activity against *Staphylococcus aureus* an *Escherichia coli* was also performed. Further, rheological analyses and clinical tests are necessary for the new obtained cosmetics.

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