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Article

Immediate and long-term effects of polysaccharides-based formulations on human skin

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A new trend in cosmetic formulations is the use of biotechnological raw materials as the polysaccharides from *Klebsiella pneumoniae*, which are supposed to enhance cell renewal, improve skin hydration and micro-relief. Botanical extracts of *Myrtus communis* leaves contain different sugars, which may provide the same benefits. Thus, the objective of this study was to evaluate through objective and subjective analysis the immediate and long-term effects of cosmetic formulations containing polysaccharides biotechnologically-originated and / or the ones contained in *Myrtus communis* extracts. Three polysaccharide-based and placebo formulations were applied on the forearm skin of 40 volunteers. Skin hydration, transepidermal water loss (TEWL), viscoelasticity and skin micro-relief measurements were made before and 2 hours after a single application and after 15 and 30 day-periods of daily applications. Answers to a questionnaire about perceptions of formulation cosmetic features constituted the subjective analysis. All polysaccharide-based formulations enhanced skin hydration. Formulations with isolated or combined active substances improved skin barrier function as compared to placebo, in the short and long term studies. Formulations containing *Myrtus communis* extracts had the highest acceptance. Results suggest that daily use of formulations containing these substances is important for protection of the skin barrier function.

Uniterms: Polysaccharides/cosmetic use. *Klebsiella pneumoniae*/cosmetic use. *Myrtus communis*/cosmetic use. Cosmetic formulations/evaluation. Human skin/hydration. Cosmetology/use of biotechnology. Biotecnological raw materials/use in cosmetology.

Uma nova tendência em formulações cosméticas é a utilização de matérias-primas biotecnológicas como os polissacarídeos de *Klebsiella pneumoniae*, que pode aumentar a renovação celular e melhor a hidratação e micro-relevo da pele. Por outro lado, o extrato vegetal de *Myrtus communis* contém diferentes polissacarídeos, que também podem proporcionar benefícios à pele. Assim, o objetivo do estudo foi a avaliação dos efeitos imediatos e em longo prazo, de formulações cosméticas contendo polissacarídeos obtidos por processo biotecnológico e/ou de extrato de *M. communis* por meio de análises objetivas e subjetivas. Três formulações contendo os polissacarídeos e um placebo foram aplicadas na pele dos antebraços de 40 voluntários. As medidas foram realizadas antes e após 2 horas da aplicação das formulações e após 15 e 30 dias de aplicações diárias em termos de hidratação da pele, perda transepidérmica de água (TEWL), viscoelasticidade e micro-relevo da pele. Para a análise subjetiva, os voluntários responderam um questionário a fim de obter-se informações sobre a percepção relativa à qualidade de cosméticos. Todas as formulações provocaram aumento da hidratação cutânea. As formulações que continham os polissacarídeos melhoraram a função barreira da pele, em curto e em longo prazo. A formulação contendo extrato de *M. communis* apresentou maior aceitação. Os resultados sugerem que o uso diário dos polissacarídeos avaliados é importante na proteção da função barreira da pele.

Unitermos: Polissacarídeos/uso cosmético. *Klebsiella pneumoniae*/uso cosmético. *Myrtus communis*/uso cosmético. Formulações cosméticas/avaliação. Pele humana/hidratação. Cosmetologia/uso da biotecnologia. Matérias-primas biotecnológicas/uso em cosmetologia.

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INTRODUCTION

The use of cosmeceuticals as skin cleansers, moisturizers and sunscreens is well established. Recently a new class of products has been gaining popularity in skin care regimens as adjuvants against inflammatory disorders and skin aging. New substances and technologies are being introduced mainly with anti-aging objectives. However there have been few studies with high methodological quality to support the claimed effects (Hashizume, 2004; Draelos, 2008).

One of the new tendencies in cosmeceuticals formulations is the use of biotechnological raw materials like polysaccharides. Those produced by *Klebsiella pneumoniae* and their derivatives are outstanding due to suggestive effects in skin water content and micro-relief improvement, as well as in the stimulation of cell renewal and glycosaminoglycan biosynthesis (Robert *et al.*, 2003; Isnard *et al*., 2004).

Myrtus communis extracts have traditionally been used as antiseptic agents. Its leaves are rich in flavonoids, especially myricetin glycosides, and hydrolyzed extracts contain different sugars such as galacturonic acid, rhamnose, galactose, glucose, xylose and fructose (Appendio *et al*., 2006; Montoro *et al*., 2006). *In vitro* studies have shown that *Myrtus communis* hydrolyzed extracts reduce collagen glycation as a biological activity and may also interfere in intercellular communication. These properties may induce caveolins gene expression in senescent cells as a response to the accumulation of glycation products (Pageon *et al*., 2007), which in turn may enhance epidermal cell proliferation (Park *et al*., 2000) and skin barrier function (Sando *et al*., 2003). Preservation of water content of the epidermis is also essential for the skin barrier function.

In vitro studies indicate that the effect of caveolins in collagen glycation and enhanced fibroblast apoptosis is probably one of the mechanisms involved in human skin aging (Alikhani *et al.*, 2005). The presence of advanced glycation end (AGEs) products is a well-known predictor of complications in chronic diabetes (Meerwaldt *et al*., 2005). Best studied and most important factors that are involved in chronological skin aging, photoaging, pre-malignant and malignant lesions development are: telomeres shortening and rupture (Yaar *et al.*, 2002), generation of reactive oxygen species (ROS) and chronic, uncontrolled sun exposure [ultra-violet (UV) and infrared radiations (IR)], leading to DNA mutation and degradation of the extracelullar dermal matrix (Fisher *et al*., 2002; Schieke, Schroeder, Krutmann, 2003; Rabe *et al*., 2006).

An evidence-based treatment for chronological skin aging and photoaging is the long-term use of topical retinoids, mainly 0.05% tretinoin (Kang *et al*., 2005; Rabe *et al.*, 2006; Sing *et al.,*2006). However, many other strategies have been proposed against photoaging like sun protection as a primary prevention, topical and oral antioxidants, growth factors and cosmetic procedures (Rabe *et al*., 2006). A combined therapy should be the ideal strategy as photoaging is considered a superposition of UV and IR effects over chronological aging. Additionally, it is well known that skin hydration and cutaneous barrier improvement are essential to epidermal homeostasis and differentiation and to the control of side effects in anti-aging treatments (Draelos, Ertel, Berge, 2006; Short *et al.*, 2007).

If polysaccharides may benefit human skin by acting to ameliorate the above mentioned conditions is a question still to be answered since few clinical trials have been conducted (Robert *et al*., 2005). It is important to determine the *in vivo* efficacy of formulations by biophysical and skin image techniques as an objective evaluation possibly completed by volunteers' information as a subjective analysis (Robert *et al.,* 2005; Dal'belo, Gaspar, Maia Campos, 2006). Based on positive immediate effects and volunteer acceptance it is possible to speculate on the efficacy of prolonged use.

The aim of this study was to evaluate immediate and long-term effects of cosmetic formulations containing biotechnologically –originated polysaccharides and/or *Myrtus communis* hydrolyzed extracts on skin hydration and barrier function.

MATERIAL AND METHODS

Formulations

The basic gel formulations were made of hydrophilic acrylate polymer with carboxylic acid groups and methylphenyl polysiloxane (a silicone micro emulsion, Net FS, Nikko Chemycals), and also contained propyleneglycol, glycerin (humectants), phenoxyethanol and parabens (preservatives), dimethicone and dimethicone crosspolymer, cyclopentasiloxane (silicones), BHT and EDTA (antioxidants). The formulation was also used as control or placebo. The experimental formulations were supplemented with 10% of polysaccharides of biotechnological origin (formulation A) or 3% *Myrtus communis* hydrolyzed extract (formulation B) or a combination of both (formulation C). The radiation-sterilized polysaccharides were produced by a non-pathogenic strain of *Klebsiella pneumoniae* and have an apparent average molecular weight of about 40 kDa (Péterszegi *et al.*, 2003).

Subjects

The study, approved by the Institutional Ethics Committee from Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of Sao Paulo, involved 40 healthy female subjects, who signed informed consents. They were students from the University, 18 to 25 years old. The exclusion criteria were: presence of any dermatitis and/or other skin or allergic diseases and smokers. Volunteers were instructed not to apply any topical products such as moisturizers and sunscreens to the test sites for 2 weeks before and during the study.

Objective analysis

Formulations were applied on the forearm skin of volunteers by using non-invasive methods The analysis of skin conditions were performed before (baseline values) and two hours after a single application and after 15 and 30 day-periods of daily applications. The evaluated parameters were: skin hydration or stratum corneum water content, transepidermal water loss (TEWL), viscoelasticity and skin micro-relief by profilometry (Dobrev, 2000; Dal'belo, Gaspar, Maia Campos, 2006).

Skin hydration was evaluated by a capacitance method (Corneometer CM 825). The device determines water content of superficial epidermal layers, down to a depth of 0.1 mm, in values expressed in arbitrary units (Dal'belo, Gaspar, Maia Campos, 2006). The TEWL is correlated to skin barrier function; it is determined by an evaporimeter (Tewameter TM 210) and the results are registered in $g/m^2.h$ during 2 minutes after probe equilibration on the skin for 30s (Dal'belo, Gaspar, Maia Campos, 2006).

Skin viscoelastic properties were evaluated by suction followed by measurements of the consequent skin deformation (Cutometer SEM 575). The instrument consists of a microprocessor-regulated pneumatic system which applies suction through a 2mm circular opening in the handheld probe. Each measurement is a result of 5 consecutive cycles of 2s-suction followed by 2s-relaxation periods. The suction load was 450 mbars. The viscolelastic / elastic distension ratio (Uv/Ue) was analyzed (Dobrev, 2000).

Skin micro-relief parameters were evaluated by the Visioscan VC 98, which is a special, high resolution UV-A light video camera developed to analyze skin surface directly and by the Surface Evaluation of the Living Skin (SELS) method. The images show skin structure and the dryness degree. The grey level image distribution is used to evaluate the following parameters: skin roughness (Rt), skin smoothness (SE_{sm} – proportional to wrinkles width and type) and wrinkles number and width (Se_w) (Dobrev, 2007).

All devices were from Courage & Khazaka, Electronic GmbH, Khöln, Germany. The measurements were performed after the volunteers were acclimatized for 30 minutes under standard conditions (21-22 ºC and 45-55% humidity).

Subjective analysis (sensorial properties)

The study subjects were asked to answer a questionnaire on their sense and perception concerning the following cosmetic qualities: clammy feeling, spreadability and skin feeling just after the application. Five minutes latter they were asked to evaluate the skin appearance, moisturization, smoothness, brightness, texture and to answer about purchase intentions.

Statistical analysis

The non-parametric Friedman test was used for the statistical analysis of experimental data, which showed a non-Gaussian distribution. Differences were considered significant with p value <0.05.

RESULTS

Objective analysis

The results for all parameters determined by biophysical techniques are represented in Figures1, 2, 3 and 4. They suggest the following observations:

- enhancement of stratum corneum hydration with all formulations studied (immediate and long term effects) (Figures 1.I and 2.I);
- TEWL reduction (immediate and long term effects), which may indicate an improved protection of the skin barrier function, only with formulations containing polysaccharides (A, B and C) (Figure 1.II and 2.II) and
- No significant alterations on skin parameters related to viscoelastic / elastic ratio and micro-relief after 2 hours (immediate effects) (Figure 3.I, II and.III) and after 15 and 30 days (long term effects) (Figure 4I, II, and III). However some images (Figure 5) showed a tendency in the improvement on micro-relief parameters that was not considered statistically significant.

Subjective analysis (sensorial properties)

The data obtained by subjective evaluation (Figure 6) allowed the following observations:

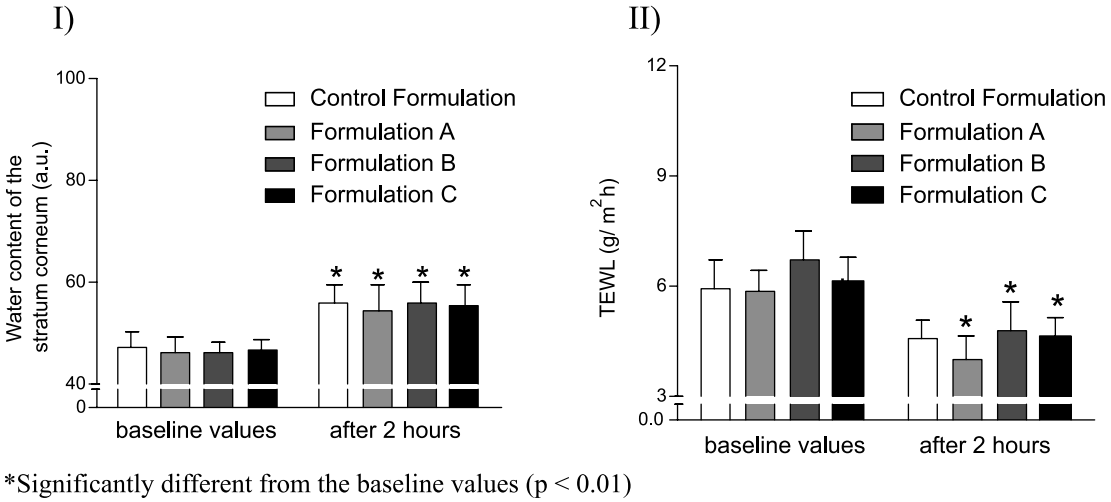

FIGURE 1 - Stratum corneum water content (I) and transepidermal water loss / TEWL (II) on volunteers forearm skin before (baseline values) and 2 hours (immediate effects) after the application of the formulations: control, 10% polysaccharides obtained by biotechnological process (A), 3% hydrolyzed *Myrtus communis* extract (B) and combination of both products (C).

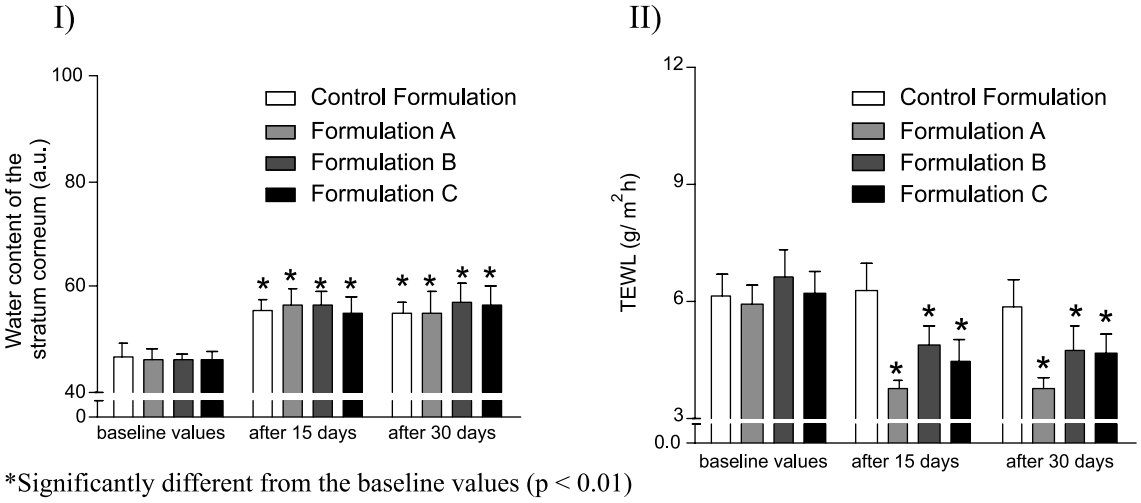

FIGURE 2 - Stratum corneum water content (I) and transepidermal water loss / TEWL (II) on volunteers forearm skin before (baseline values) and 15 and 30 days (long-term effects) after the application of the formulations: control, 10% polysaccharides obtained by biotechnological process (A), 3% hydrolyzed *Myrtus communis* extract (B) and combination of both products (C).

- formulation B presented the highest degree of acceptance, showing the best sensorial attributes regarding spreadability and skin appearance (Figure 6.I), skin feeling just after the application (Figure 6.II) and purchase intention (Figure 6.III) and
- the remaining sensorial attributes were not considered significant; however formulation B presented a tendency to a higher degree of acceptance regarding skin hydration (Figure 6.IV).

DISCUSSION

The present study demonstrated that all formulations studied provoked an enhancement of skin moisture and that only the formulations containing technologically originated polysaccharides or hydrolyzed extracts of Myrtus communis improved skin barrier function.

Thus, polysaccharides-based formulations induced a TEWL reduction which suggests that the daily use of these substances is important to protect the skin barrier function, since immediate effects observed in the short term study were confirmed in long term evaluations. It is known that maintenance of the cutaneous barrier is essential to normal skin and that it improves results of dermatological treatments and maintains skin homeostasis (Dobrev, 2007).

Galacturonans, the main polysaccharides of hydro-

lyzed *Myrtus communis* extract, are polymers formed by units of galacturonic acid that form gels in the presence of water. Enhancement of skin firmness (tensile strength) is related to galacturonans film formation.

Some skin tightening may also be produced by surface deposition of certain film-forming actives, including

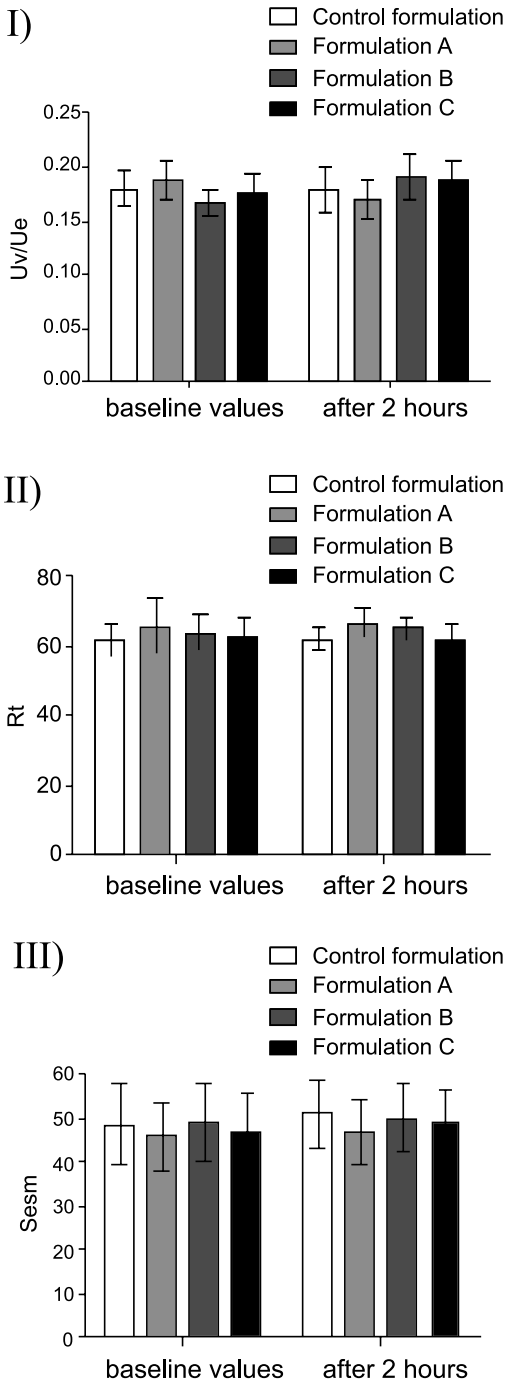

FIGURE 3 - Viscoelastic / elastic distension ratio (Uv/Ue) (I) skin roughness (Rt) (II) and skin smoothness (SE_{sm}) (III) on volunteers forearm skin before (baseline values) and 2 hours (immediate effects) after the application of the formulations: control, 10% polysaccharides obtained by biotechnological process (A), 3% hydrolyzed *Myrtus communis* extract (B) and combination of both products (C).

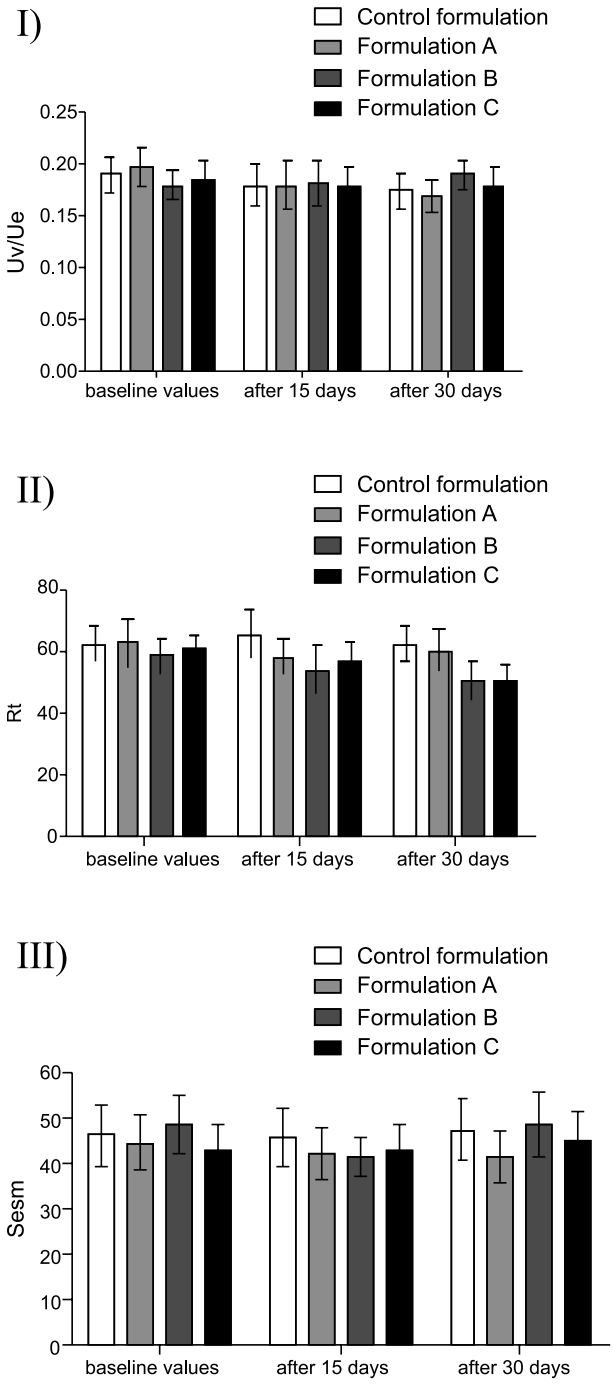

FIGURE 4 - Viscoelastic/elastic distension ratio (Uv/Ue) (I), skin roughness (Rt) (II) and skin smoothness (SE_{sm}) (III) on volunteers forearm skin before (baseline values) and 15 and 30 days (long-term effects) after the application of the formulations: control, 10% polysaccharides obtained by biotechnological process (A), 3% hydrolyzed *Myrtus communis* extract (B) and combination of both products(C).

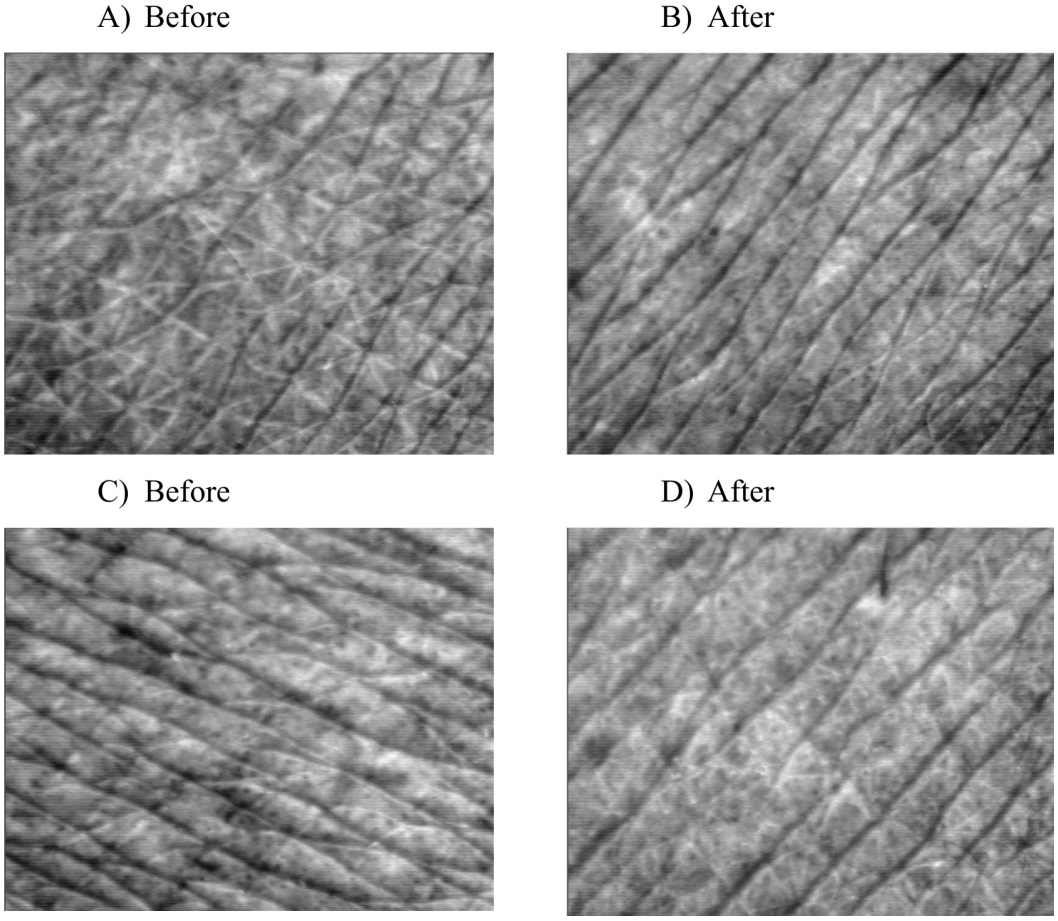

FIGURE 5 - Skin surface evaluation (skin micro-relief). Surface of the skin of a volunteer who applied formulation B before **(A)** and after **(B)** 30 days of application and of a volunteer who applied formulation C before **(C)** and after **(D)** 30 days of application. Skin surface was evaluated by Visioscan® VC98 software.

proteins, polysaccharides, and polymers (Kligman, Papa, 1965; Gillon *et al.*, 1999).

The moisturizing effects could, in addition, reduce the irritant effects of anti-aging treatments such as the ones with retinoids. These TEWL is enhanced during cell renewal (Rieger, 2000) and the use of moisturizing substances to protect the skin is very important to keep it in good conditions.

The products used in the study did not significantly affect the hydration of deeper skin cell layers as demonstrated by the lack of alterations on the skin viscoelastic/elastic ratio and skin micro-relief (Dobrev, 2000).

The vehicle formulation supplemented with 3% hydrolyzed *M. communis* extract had the highest degree of acceptance, according to the sensorial analysis. It showed the best sensorial profile as well as higher efficacy on subjective evaluation as compared to placebo and formulations with 10% polysaccharides obtained by biotechnological processes combined or not with 3% hydrolyzed *M. communis* extract.

It is considered that clinical results are relevant and confirm other observations concerning efficacy of different moisturizing formulations (Wiedersberg, Leopold, Guy, 2009). Indeed, the immediate and long-term positive effects observed in objective and subjective evaluations in this study confirm that continuous use may improve skin conditions regarding hydration, TEWL and skin barrier function (Dal'belo, Gaspar, Maia Campos, 2006; Dobrev, 2007). The polysaccharides in cosmeceutical products may be helpful for daily skin care, after cosmetic procedures and as adjuvant in dermatological therapy regimens (Kikuchi, Tagami, 2008), since this film forming effect could enhance patient compliance and acceptance of therapy.

In conclusion, this investigation showed the clinical immediate and long-term skin effects of polysaccharides-based formulations, which resulted in TEWL reduction. In addition, the daily use of these substances is very important to protect the skin barrier function, since immediate effects observed in the short term study were confirmed in the long term evaluation.

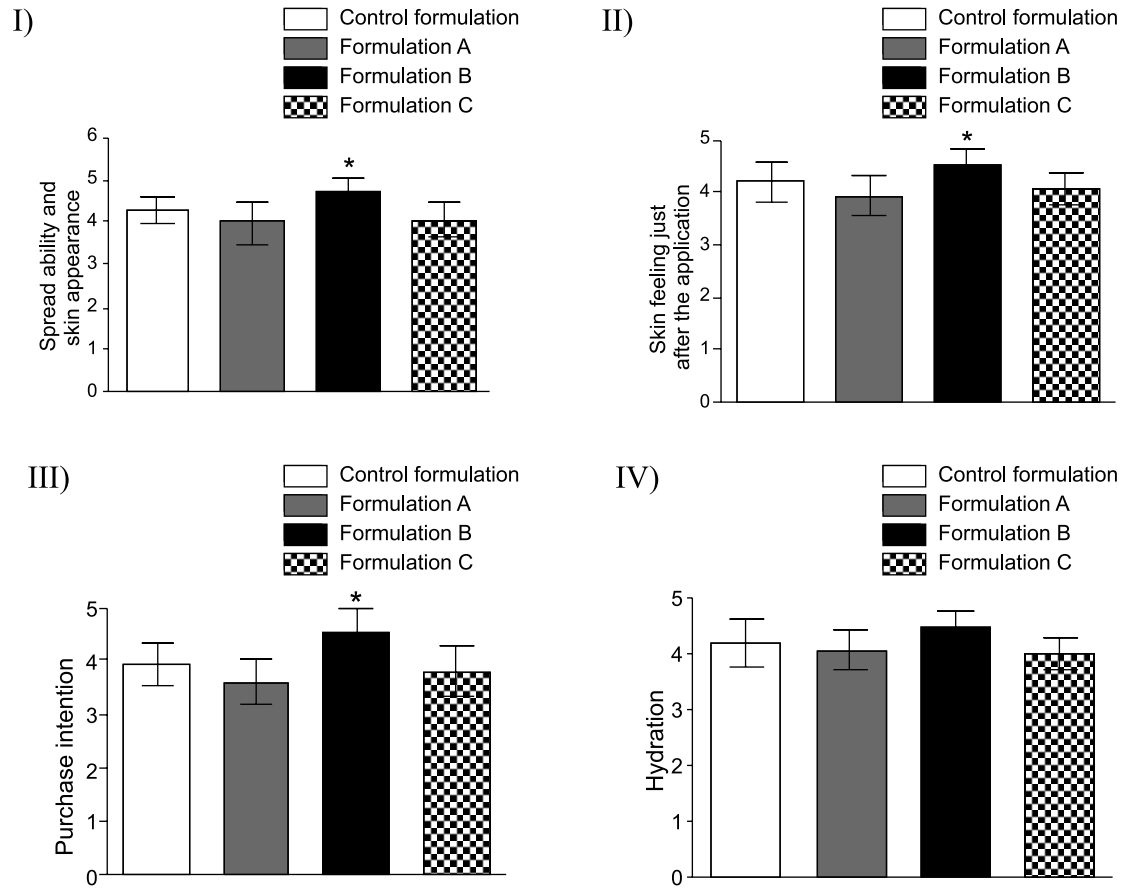

FIGURE 6 - Spreadability and overall skin appearance (I), skin feeling just after the application (II), purchase intention (III) and hydration (IV) after the application of the formulations: control, 10% polysaccharides obtained by biotechnological process (A), 3% hydrolyzed *Myrtus communis* extract (B) and combination of both products (C).

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