

# The antiageing efficacy of donkey milk in synergy with pomegranate extract and UV protection

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## Introduction

Skin ageing is influenced by intrinsic and extrinsic factors that promote the accumulation of physicochemical alterations of the cutaneous structure and function [1,2]. These alterations are revealed as clinical changes, including e.g. wrinkling, dyschromias and elastosis [3].

Donkey milk is highly hydrating due to its protein-rich content, namely casein,  $\alpha$ -lactoalbumin and  $\beta$ -lactoglobulin [4,5]; it also contains traces of lactic acid, lactate and calcium, claimed to have positive effects on aged skin [6,7].

An O/W emulsion for topical application was developed to address signs of skin ageing by a combination of different cosmetic ingredients: donkey milk, pomegranate extract and UV filters (SPF 20). The synergistic efficacy of these raw materials was assessed *in vivo*.

## Methods

*In vivo* efficacy evaluation studies were performed on a selected population of 30 women (45 – 65 years) during 28 days (measurements performed on D0 and D28), namely:

- Standardized photographic images of the front face and both hemifaces were obtained with visible, cross polarized and UV light before and after the treatment (D0 and 28) with the system VISIA-CA (Canfield Scientific, USA);
- 3D images of the skin topography in the periocular area were obtained by a digital fringe projection using DLP® micro mirror displays. A fringe standard was projected on the skin and detected by the CCD camera of the optical system;
- A Phase Shifting Rapid *In vivo* Measurement of Skin system was used (PRIMOS 3D 40x30 mm evaluation area, Gfm, Germany);
- A biomechanical skin evaluation was performed using a Cutometer® dual MPA 580 with a 2 mm probe to assess the firming effect and elasticity recovery;
- Hydration content was obtained by an electrometric system using a Corneometer CM825 connected to a Cutometer dual MPA 580. Measurements were performed in the periocular area. Trans-epidermal water loss (TEWL) was used as an assessment of skin barrier by using a Tewameter® TM 300 on a randomly selected facial area.

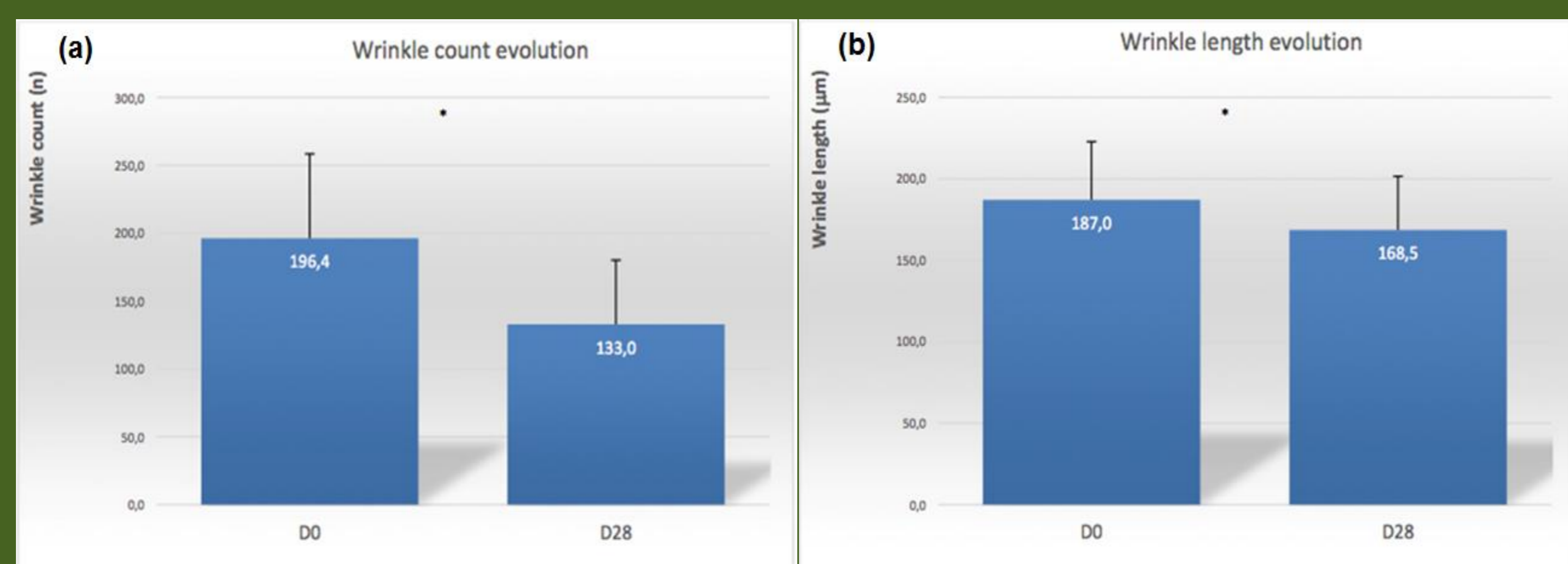
## Results

The topical application of an O/W emulsion with donkey milk, pomegranate extract and a SPF of 20 during 28 days has resulted in a statistically significant decrease in both the wrinkle count and the wrinkle length in the periocular area ( $p < 0.0001$ ). By comparing the relative transformation in relation to D0, this product has induced a 32.9% reduction in wrinkle count, while the wrinkle length has reduced by 9.6%. Figure 2 illustrates the noticeable results associated with the data in Figure 1.

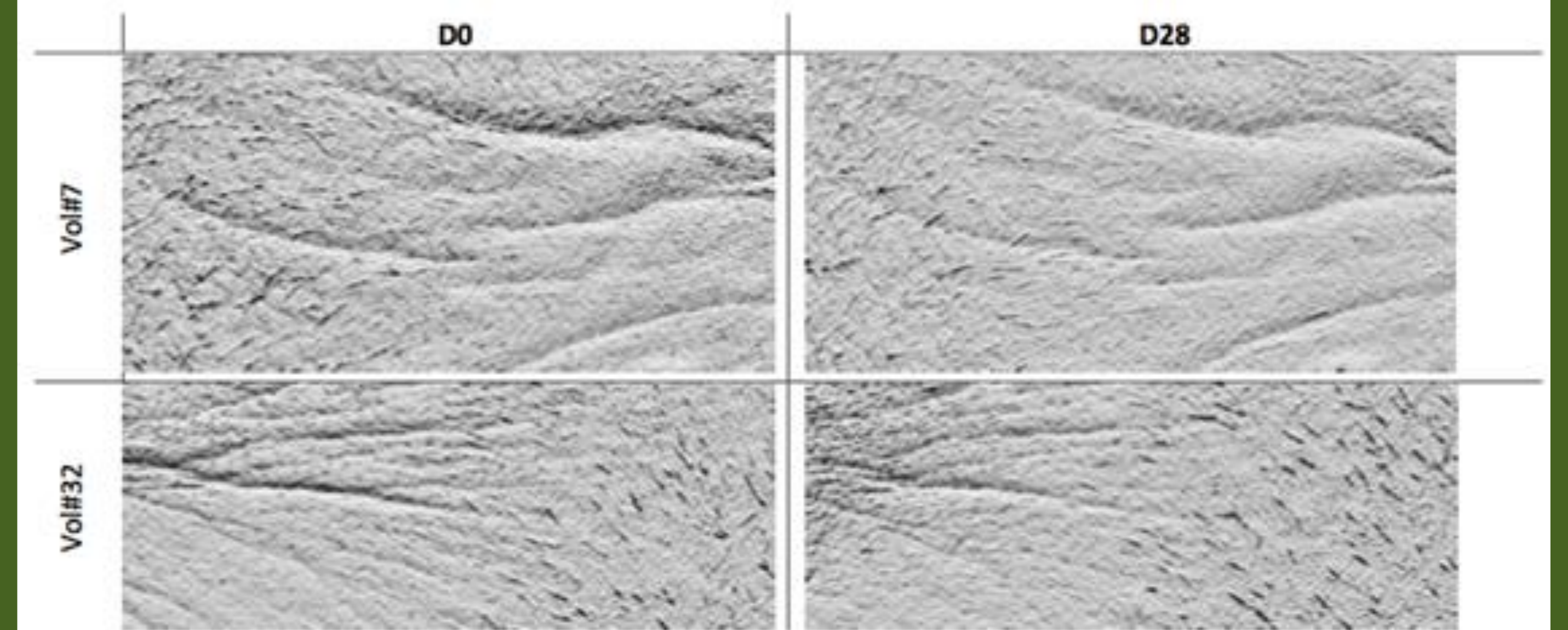
The application of the same product has not resulted in a statistically significant change in skin roughness. By comparing the relative transformation in relation to D0, there has been a 12.0% and 14.7% non-significant increase in the Ra and Rz variables, respectively (data not shown). This suggests a maintenance of the skin smoothness in the measured areas.

A 3.5% and 6.8% reduction in the number of brown spots and the number of UV spots respectively (data not shown), have been obtained after 28 days of product application. There has also been a reduction of the area of UV spots of 6.8% (data not shown). These results have been associated with an increase in the homogeneity of the skin colour; in Figure 3 an example of the increase in skin colour homogeneity is shown.

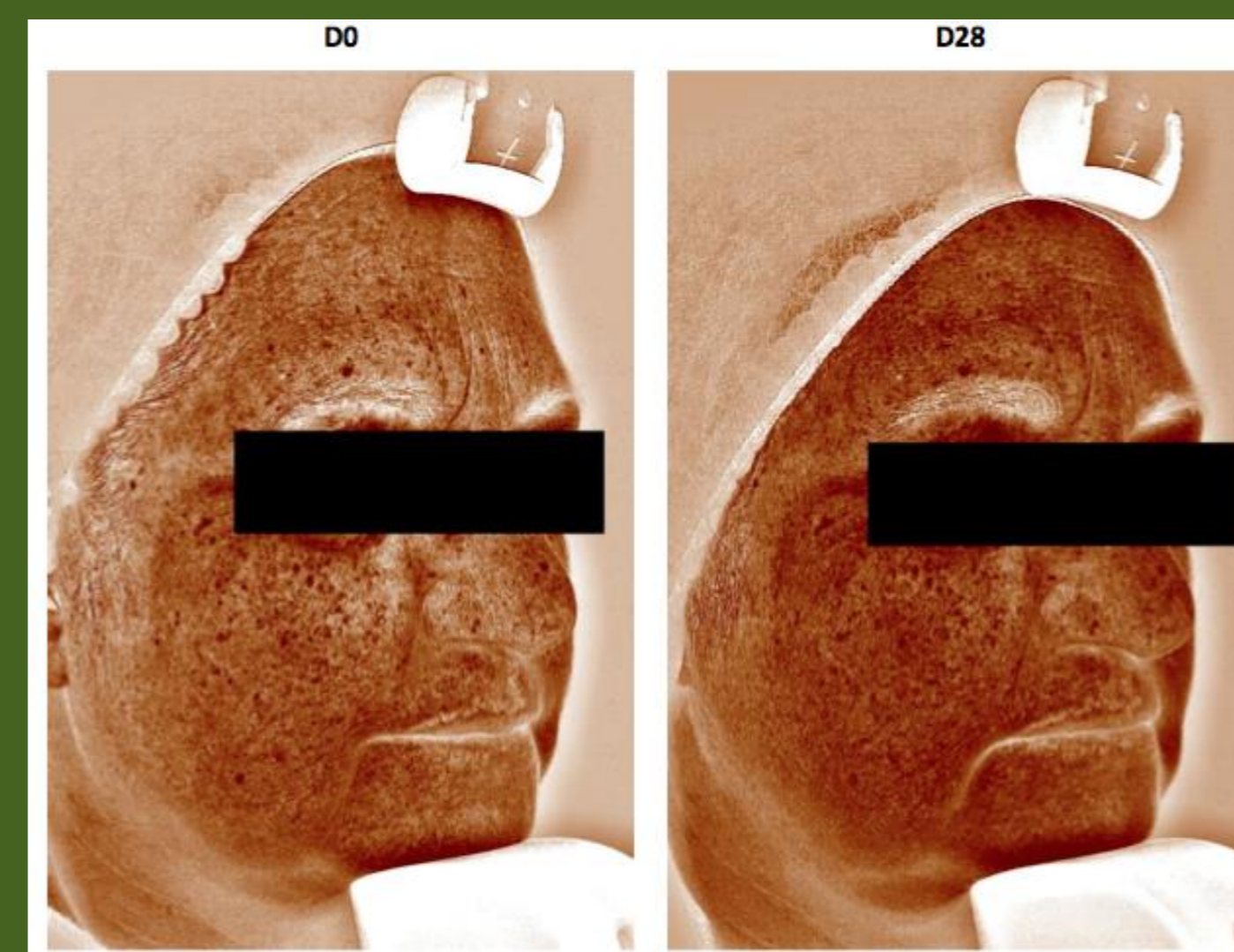
Skin hydration has increased by 11.4%, alongside an increase in TEWL of 21.4%. Skin firmness and elasticity have increased by 9.6% and 16.1% respectively. These results are shown in Table 1.



**Figure 1.** The influence of topical application of an O/W emulsion with donkey milk, pomegranate extract and a SPF of 20 on (a) wrinkle count (D0 to D28) (mean + SD values, n = 32) and (b) wrinkle length evolution (D0 to D28) (mean + SD values, n = 32).



**Figure 2.** Photographs of examples of the evolution of wrinkle reduction (D0 to D28) in two subjects, showing the anti-wrinkle effect of an O/W emulsion with donkey milk, pomegranate extract and a SPF of 20.



**Figure 3.** Photographs of one example of the increase in the homogeneity of the skin colour (D0 to D28) by topical application of an O/W emulsion with donkey milk, pomegranate extract and a SPF of 20.

**Table 1.** Evolution of skin hydration, TEWL, skin firmness and skin elasticity with the topical application of an O/W emulsion with donkey milk, pomegranate extract and a SPF of 20 (D0 to D28) (mean + SD values, n = 32).

	Time	n	Mean	SD	p-value
Skin hydration (AU)	D0	32	68.9	9.7	< 0.0001
	D28	32	76.1	8.1	
TEWL (g/h.m <sup>2</sup> )	D0	32	7.9	2.7	0.0069
	D28	32	9.1	2.8	
Skin Firmness	D0	32	0.261	0.128	0.017
	D28	32	0.204	0.039	
Skin Elasticity	D0	32	0.460	0.107	0.011
	D28	32	0.521	0.130	

## Discussion & Conclusion

The topical application of donkey milk, pomegranate extract and UV filters in an O/W emulsion vehicle has showed promising results in addressing skin ageing; it has been assumed this is due to a synergistic effect.

It is suggested that donkey milk, due to its high whey-protein content, has been responsible for the significant increase in skin hydration [4,5]. The increase in skin hydration was consistent with the results obtained for TEWL, which suggests an increase in the water content of the skin surface. The findings shown in this work are consistent with previous studies that revealed the efficacy of pomegranate extract in improving the signs of skin ageing due to its properties not only as an antioxidant [8], but also for its role in reversing hyperpigmentation by inhibiting tyrosinase activity [9].

Donkey milk may have also contributed, in synergy with the pomegranate extract, to the increase in skin colour homogeneity due to the tyrosinase-inhibiting capacity of  $\alpha$ -lactoalbumin and  $\beta$ -lactoglobulin [10]. The use of pomegranate in skincare has also been associated with the inhibition of photoageing induced by UV radiation [11].

Thus, together with UV filters, whose key role in preventing extrinsic ageing has been extensively reviewed [12–15], it is suggested that this formulation acts both by preventing photoageing and by improving the signs of ageing in photoaged skin.