

Luminescence increased by plant-derived lipophilic active

Skin ageing is commonly revealed by the appearance of wrinkles and loss of tone. Those cutaneous signs of ageing are predominantly caused by an excessive exposure to UV radiation – actinic ageing – and thus more apparent on skin sites exposed to the sun. In addition to an obvious change in surface topography, skin ageing in UV-exposed skin areas is also betrayed by the appearance of age spots that are characterised by a localised hyperpigmentation. It is important to mention that, even though they are associated to ageing by connotative definition, age spots are more related to the effect of UV radiation rather than the chronological ageing itself – intrinsic ageing. This is why age spots are mainly visible in the face, neck, upper chest area and on the dorsal side of hands and outside of forearms. Actinic ageing and intrinsic ageing are closely linked to each other as UV-induced damage occurring from repetitive excessive sun exposure accumulates with time. Cutaneous signs of ageing such as age spots most likely appear from a super imposition of photodamage and chronological ageing.

The etiology of age spots is not yet entirely elucidated. However, recent studies have proposed various pathways contributing to the formation and the visual appearance of age spots. While there is an obvious accumulation/concentration of melanin pigments,¹ the main culprit of age spot formation remains UVB radiation. Indeed, UVB can directly cause an increase in the production of melanogenic growth factors from either stressed keratinocytes² or photoaged fibroblasts.³ Through a more indirect mechanism, UVB can, by an enzymatic-dependent disruption of the dermo-epidermal junction (DEJ), provoke the release of additional pro-melanogenic factors previously embedded and kept silent in the DEJ matrix.⁴ Those cell-derived and matrix-derived growth factors and cytokines will locally stimulate melanocytes encouraging melanin deposition and age spot formation. Such UV-induced synthesis/release of cytokines might explain gene expression study reports showing an



Ribes nigrum.

upregulation of inflammation pathways in age spot areas.^{5,6}

Another pigment, lipofuscin, is known to be generated by an incomplete degradation of oxidised protein and lipids. It accumulates in senescent cells – cells that have reached a state of non-proliferation – and is thus considered as a marker of ageing.⁷ Some authors have proposed an involvement of this so-called ‘age pigment’ in the formation of age spots but experimental demonstrations are still lacking.

Innovacos has developed a line of lipophilic active ingredients called Activoil (patent pending). The main characteristic of that line of actives is the use of a liposoluble matrix enriched with a lipophilic plant-derived extract and protected from oxidation by the addition of two other

specific lipophilic botanical fractions. Activoil Spotless is composed of *Ribes nigrum* (blackcurrant) seed oil and a *Paeonia suffruticosa* root bark super critical CO₂ fluid extract. *Ribes nigrum* seed oil has been selected for its content in biologically active fatty acids namely linoleic, alpha-linolenic and gamma-linolenic forming an omega 6-3-6 combination. Those fatty acids, especially linoleic, are known to inhibit melanogenesis mainly at the tyrosinase level. Known as the ‘plant of healing’, *Paeonia suffruticosa* produces magnificent white flowers – ‘peony’ – and is used in a variety of Asian traditional medicines. Using CO₂ super critical fluid extraction technologies, a lipophilic fraction enriched in paeonol was obtained from the *Paeonia* root bark. Paeonol is known to have anti-inflammatory, antioxidant and

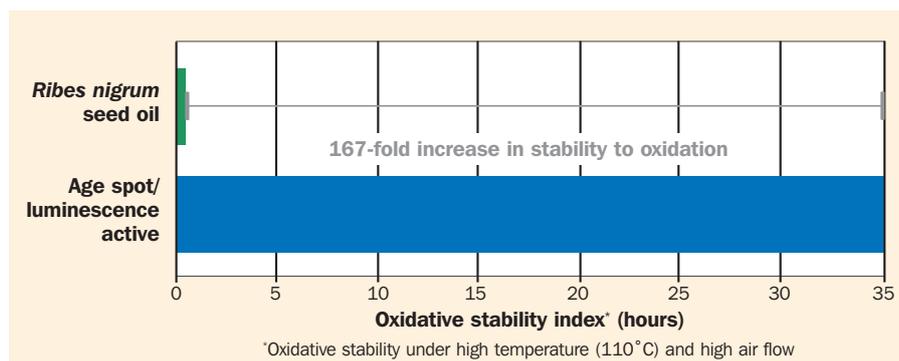


Figure 1: Oxidative stability of the age spot/luminescence active. Rancimat test comparing the oxidative stability of the age spot/luminescence active to that of *Ribes nigrum* seed oil.

anti-melanogenic actions. Activoil Spotless (hereafter referred to as age spot/luminescence active) represents an ideal stabilised complex of lipophilic ingredients to reduce the appearance of age spots and increase skin brightness and luminescence.

The proposed INCI name is: Ribes Nigrum (Black Currant) Seed Oil (and) Octyldodecanol (and) Octyldodecyl Oleate (and) Octyldodecyl Stearoyl Stearate (and) Alcohol (and) Paeonia Suffruticosa Root Extract (and) Rosmarinus Officinalis (Rosemary) Leaf Extract (and) Solidago Virgaurea (Goldenrod) Extract.

Materials and methods

Rancimat test

In this method, a sample of oil is heated under atmospheric pressure, and air is allowed to bubble through the oil at a selected temperature. Under these conditions, a lipoperoxidative reaction occurs and the short-chain volatile acids produced are recovered and measured by an increase in conductivity in distilled water. The time required to produce a sudden increase in conductivity, due to the formation of volatile acids, determines an induction time – also known as the Rancimat time – which can be defined as a measure of the oxidative stability of oil. A Metrohm 743 Rancimat (Herisau, Switzerland) was used in accordance to the method ISO 6886-2006 in our experiments. Rancimat time was measured for the age spot/luminescence active in comparison to standard *Ribes nigrum* (blackcurrant) seed oil. Oxidative stability was measured under 110 °C.

To measure the clinical efficacy of the age spot/luminescence active fifteen healthy subjects aged between 38 and 71 with visible age spots were enrolled. Studies were designed as a placebo-controlled, split-face, clinical protocol. The placebo cream or the same cream formulation containing 2% of the age spot/luminescence active were topically applied twice a day on each side of the face down to the upper chest area according to a randomised scheme. Clinical evaluations of age spot appearance and skin luminescence were measured at Day 0 (baseline) and after 30 and 60 days of product applications.

Evaluation of age spot melanin pigment intensity

Age spot melanin intensity was measured using a Mexameter MX-18 (Courage + Khazaka electronic, GmbH) based on the optical absorption of the pigment. The skin melanin index was computed from the intensity of light absorbed and reflected at specific wavelengths.

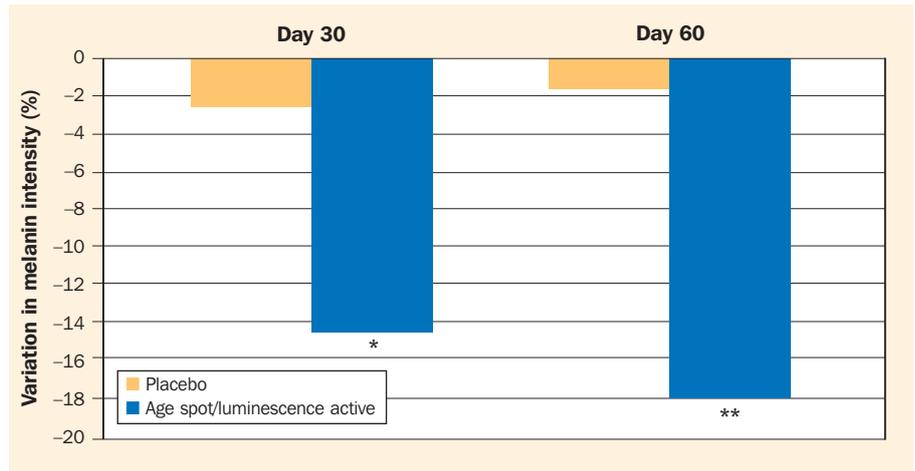


Figure 2: Reduction in the melanin pigment intensity of age spots. Effect of a placebo cream formulation, or the same formulation containing 2% of the age spot/luminescence active, after 30 and 60 days of application. Variation in melanin intensity is shown in % and compared to Day 0 (baseline). *p<0.05 and **p<0.01 vs. placebo.

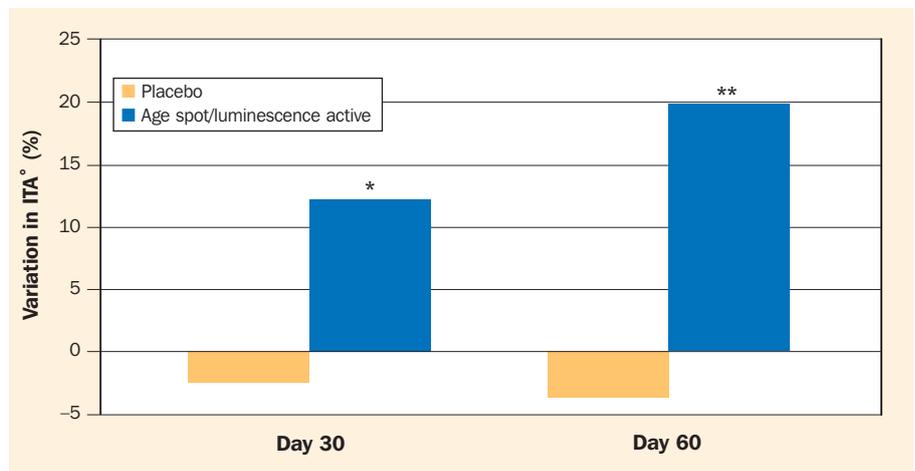


Figure 3: Reduction in the general colour intensity of the age spots. Effect of a placebo cream formulation, or the same formulation containing 2% of the age spot/luminescence active, after 30 and 60 days of application. Variation in ITA° is shown in % and compared to Day 0 (baseline). *p<0.05 and **p<0.01 vs. placebo.

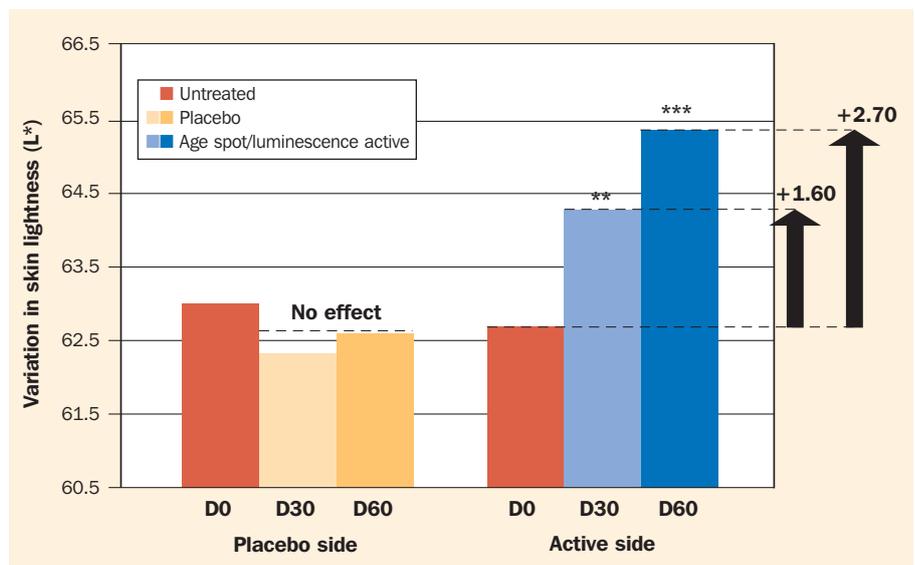


Figure 4: Increase in global skin luminescence. One side of the face was treated with a placebo cream formulation, and the other side with the same formulation containing 2% of the age spot/luminescence active, after 30 and 60 days of application. Variation in skin luminescence is shown in L* units and compared to untreated skin (Day 0). **p<0.01 and ***p<0.001 vs. placebo.

Evaluation of global colour intensity of age spots – individual typology angle (ITA°)

The colour intensity of age spots was evaluated using a spectrophotometer CM700d (Konica Minolta Optics, Japan) in the CIELab colour space. Age spot areas were illuminated with a pulsed xenon arc lamp and values obtained for L^* (luminosity index ranging between a value of 0 to 100) and b^* (colour saturation ranging from +60 'yellow' to -60 'blue') parameters were used to calculate the ITA°. The higher the ITA°, the lighter the age spot skin colour. Unlike the Mexameter, results obtained with the spectrophotometer do not inform about the pigment generating colour.

Evaluation of skin luminescence

The global skin luminescence was evaluated using a spectrophotometer CM700d (Konica Minolta Optics, Japan). Both sides of the face were assessed for luminescence by the measurement of the L^* parameter. Light emitted by the instrument was reflected by the skin surface and collected by 36 photodiodes each with different spectral sensitivity (from 400 nm to 700 nm). The sensitivity of the photodiodes was regulated according to a 'standard observer' simulating the sensitivity of the human eye.

UV-induced skin erythema

Ten healthy subjects were enrolled in this study. On Day 0, baseline skin colour (non-erythematous skin) was measured using a Mexameter MX-18 set with wavelengths corresponding to the spectral absorption peak of haemoglobin. On Day 1, subjects were treated with a placebo cream on one skin area or with the same cream formulation containing 2% of the age spot/luminescence active on another area. Placebo and active cream formulations were applied for 10 consecutive days (from Day 1 to Day 10, inclusively). Two skin areas were left untreated. On Day 11, twenty-four hours after the last application, pre-treated and one untreated skin areas were exposed to 1.5 minimal erythema dose (MED) with UVA+B radiation using a solar simulator to induce redness. UVA+B-induced erythema was measured on Day 12 with the Mexameter MX-18. One skin area was kept untreated and unexposed for reference. All conditions were tested in duplicates.

Statistics

Parametric data were submitted to the bilateral Student's *t*-test for paired data.

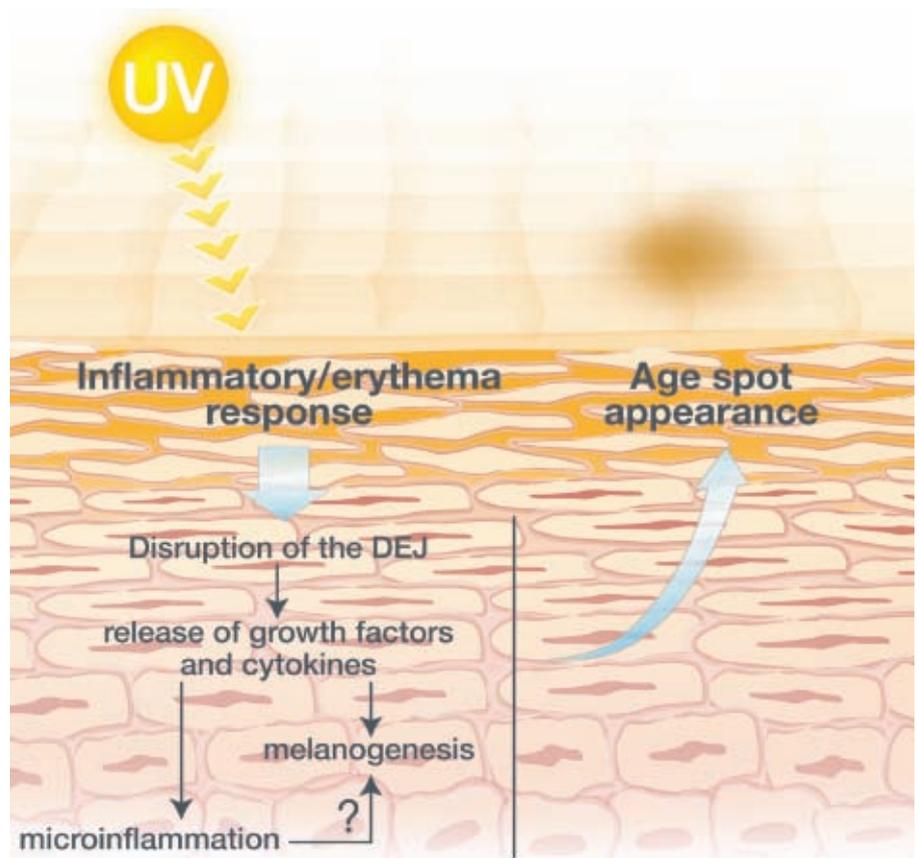


Figure 5: Age spots: an outcome of UV-induced inflammation. UV radiation can generate inflammation leading to skin erythema or micro-inflammation without any visible sign of redness (sub-clinical erythema). In addition to an upregulation of pro-inflammatory pathways, UV can induce melanin synthesis and micro-inflammation through the release of growth factors and cytokines from the dermo-epidermal junction (DEJ) matrix. Ultimately, activation of such pathways can lead to the appearance of age spots.

Results

Despite their biological activity profile, unsaturated fatty acids can be prone to oxidation because of the presence of double bonds. The incorporation of a combination of rosemary leaf extract and solidago extract together with *Ribes nigrum* seed oil and *Paeonia suffruticosa* root bark extract provided an increased stability to oxidation by 167-fold in comparison to normal *Ribes nigrum* seed oil (Fig. 1).

The age spot/luminescence active was then taken through a series of clinical trials to verify its efficacy in reducing the appearance of age spots and improving skin luminescence. At first, melanin pigment intensity was quantified using a Mexameter adjusted to specific wavelength readings. Secondly, we used a spectrophotometer to measure the individual typology angle (ITA°) that also informs on the colour intensity of the age spots but without being specific to any physiological pigment. An increase in ITA° (higher angle of light reflection) indicates a lightening of the age spot. Both Mexameter and spectrophotometer measurements were made directly on age spots. The topical application of the active cream formulation containing the age

spot/luminescence active demonstrated a significant reduction in the melanin pigment intensity of age spots of 14.4% and 18.1% after 30 and 60 days, respectively (Fig. 2). A positive effect was observed for more than 86% of the subjects. The side of the face to which the placebo was applied revealed no improvement (Fig. 2). ITA° measurements revealed a significant parallel reduction in the general colour intensity of the age spots by 12.4% and 20.1% after 30 and 60 days, respectively (Fig. 3). It is worth noting that the placebo produced a decrease, although non-significant, in ITA° measurement revealing a slight darkening action. Results obtained using the Mexameter and the spectrophotometer strongly suggest that the reduction in the appearance of the age spots is predominantly due to a reduction in melanin pigment intensity – although it cannot be ruled out at this point that other pigments such as lipofuscin may also be present.

In a third study, the spectrophotometer was again used to verify the effect of the age spot/luminescence active on global skin luminescence (also referred to as brightness, lightness or luminosity) through

the measurement of the parameter L^* . It is generally accepted that a change in one L^* unit is visually perceptible. Treatment of the skin with the placebo for 30 or 60 days brought no change in the parameter L^* in comparison to the reading made at baseline on Day 0 (Fig. 4). However, the side of the face treated with the active formulation demonstrated a significant increase in parameter L^* by 1.60 and 2.70 units after 30 and 60 days, respectively (Fig 4). The 'active-treated' side of the face showed an L^* improvement in 93% of the subjects. In addition to provide a significant positive effect on skin luminescence, the age spot/luminescence active action could also be interpreted as an anti-ageing one. Indeed, a well-designed study recently showed that the skin luminescence, expressed as L^* , progressively decreases with age. The authors of that study calculated an average loss of 1.3 L^* unit for every decade especially between 20 and 60 years of age.⁸ Therefore, a gain in 1.60 and 2.70 L^* units obtained with the age spot/luminescence active could be considered as a 'youth gain' of approximately 10 years and 20 years, respectively, in terms of skin luminescence and brightness.

Finally, as UV and micro-inflammation are thought to be important etiological factors in the formation and appearance of age spots, we sought to determine the effect of the age spot/luminescence active on UV-induced skin erythema. In addition to provoke cutaneous redness, UV also favours melanin synthesis and protein and lipid oxidation that ultimately may lead to lipofuscin accumulation (Fig. 5). When applied as a preventive regimen, a formulation containing 2% of the age spot/luminescence active significantly reduced by approximately 50% the level of skin redness (inflammation) when compared to untreated skin or placebo treatment (Fig. 6). Pre-treatment with the placebo formulation did not provide any significant anti-inflammatory effect when compared to untreated skin.

Conclusion

In addition to wrinkles and sagging skin, age spots are also considered hallmarks of ageing. UV-induced damage and its accumulation with time underly the formation and the appearance of age spots. Indeed, a time-dependent progression of the histological morphology of age spot lesions has recently been described using reflectance confocal microscopy.⁹ The involvement of UV as a causal agent in the formation of age spots also appears to draw in pro-inflammatory pathways.^{3,10} As per its clinically demonstrated reduction of UV-induced

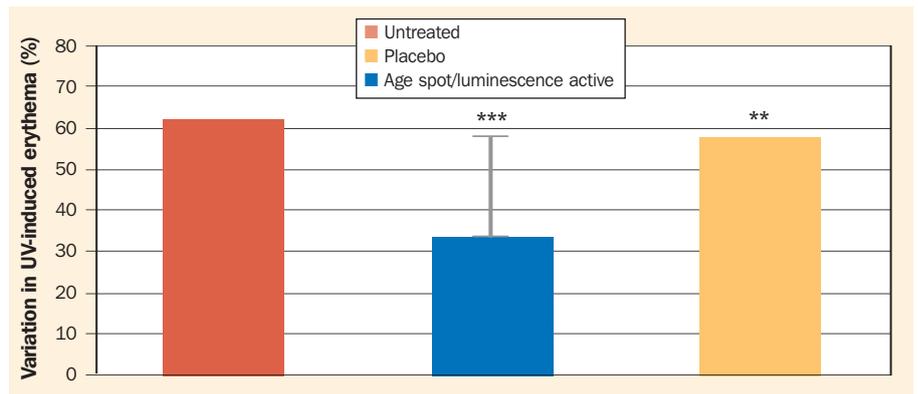


Figure 6: Effect of the age spot/luminescence active on UV-induced skin erythema. Various skin areas were treated with a placebo cream formulation, with the same formulation containing 2% of the age spot/luminescence active for 10 consecutive days, or left untreated. Upon formulation applications, all skin areas were exposed to UVA+B and UV-induced erythema was measured 24 hour after UV stress. Variation in UV-induced erythema is shown in % compared to an untreated and unexposed skin area. *** $p < 0.001$ vs. placebo and ** $p > 0.05$ vs. untreated skin.

erythema, the age spot/luminescence active (Activoil Spotless) most likely down regulates the inflammatory cascade triggered upon sun exposure that can ultimately participate in age spot formation.

The use of a formulation containing 2% Activoil Spotless succeeded to significantly reduce the appearance of age spots in placebo-controlled, split-face trials and the effect was observed after 30 days. Results obtained with the Mexameter (melanin-specific) were somewhat comparable to those obtained by measuring the ITA^o (not specific to any chromophore) with the spectrophotometer. This observation could reveal that the reduction in age spot appearance is mostly due to a reduction in melanin pigment (decrease in synthesis or an increase in degradation). However, even though the contribution of other pigments such as lipofuscin to age spots formation remains to be demonstrated, it cannot be excluded that Activoil Spotless may simultaneously act on pigments other than melanin when reducing age spot appearance.

The clinical action of Activoil Spotless is not limited to age spots but also extends to skin luminescence. A significant improvement in the spectrophotometry L^* parameter was observed already after 30 days and this action progressed up to Day 60. This increase in the parameter L^* also reveals a unique anti-ageing action for Activoil Spotless that can translate into a 'youth gain' of up to 10 and 20 years in terms of skin luminescence.

Since multiple pathways appear to join their actions in forming age spots, using an ingredient such as Activoil Spotless itself made of a combination of stabilised lipophilic active components emerges as being an ideal cosmeceutical tool in reducing and preventing the appearance of age spots and improving skin luminescence. **PC**

● Activoil™ is a Trademark of Innovacos Corp, US

References

- Unver N, Freyschmidt-Paul P, Hörster S, Wenck H, Stäb F, Blatt T et al. Alterations in the epidermal-dermal melanin axis and factor XIIIa melanophages in senile lentigo and ageing skin. *Br J Dermatol* 2006; **155** (1): 119-28.
- Hattori H, Kawashima M, Ichikawa Y, Imokawa G. The epidermal stem cell factor is over-expressed in lentigo senilis: implication for the mechanism of hyperpigmentation. *J Invest Dermatol* 2004; **122** (5): 1256-65.
- Kovacs D, Cardinali G, Aspite N, Cota C, Luzi F, Bellei B et al. Role of fibroblast-derived growth factors in regulating hyperpigmentation of solar lentigo. *Br J Dermatol* 2010; **163** (5): 1020-7.
- Iriyama S, Ono T, Aoki H, Amano S. Hyperpigmentation in human solar lentigo is promoted by heparanase-induced loss of heparan sulfate chains at the dermal-epidermal junction. *J Dermatol Sci* 2011; **64** (3): 223-8.
- Goyarts E, Muizzuddin N, Maes D, Giacomoni PU. Morphological changes associated with aging: age spots and the microinflammatory model of skin aging. *Ann N Y Acad Sci* 2007; **1119**: 32-9.
- Aoki H, Moro O, Tagami H, Kishimoto J. Gene expression profiling analysis of solar lentigo in relation to immunohistochemical characteristics. *Br J Dermatol* 2007; **156** (6): 1214-23.
- Jung T, Höhn A, Catalgol B, Grune T. Age-related differences in oxidative protein-damage in young and senescent fibroblasts. *Arch Biochem Biophys* 2009; **483** (1): 127-35.
- Zedayko T, Azriel M, Kollias N. Caucasian facial L^* shifts may communicate anti-ageing efficacy. *Int J Cosmet Sci* 2011; **33** (5): 450-4.
- Pollefliet C, Corstjens H, González S, Hellemans L, Declercq L, Yarosh D. Morphological characterisation of Solar Lentiginos by in vivo Reflectance Confocal Microscopy: a longitudinal approach. *Int J Cosmet Sci* 2012 Oct 17. [Epub ahead of print]
- Chen N, Hu Y, Li WH, Eisinger M, Seiberg M, Lin CB. The role of keratinocyte growth factor in melanogenesis: a possible mechanism for the initiation of solar lentiginos. *Exp Dermatol* 2010; **19** (10): 865-72.