

Technical Bulletin
AarhusKarlshamn
Lipids for Care



Functional Lipids
Lipex[®] Canola-U
Lipex[®] Shea-U

Natural Vegetable Functional

Functional Lipids – Lipex® Canola-U and Lipex® Shea-U for cosmetic applications

Introduction

Natural lipids are a diverse group of organic compounds found in living organisms, known to impart structural, modulatory as well as regulatory roles within the human body.

The dominating lipids of vegetable oils are the triglycerides. The non-glyceride fraction consists of a mixture of minor lipid components, also known as the unsaponifiable part of the oil, including tocopherols, phytosterols, triterpenic alcohols, aliphatic alcohols, hydrocarbons and waxes. Each of these lipid components have specific properties which depend on their respective, precise structure.

The Lipex® functional lipids, Lipex® Canola-U and Lipex® Shea-U, are derived from purified Canola oil and Shea butter respectively. Lipid minor components are enriched by gentle processing to obtain a high content of natural bioactive components. Lipex® Shea-U and Lipex® Canola-U are intended for use primarily in cosmetic formulations as active ingredients in daily-use skin and hair care formulations.

This technical bulletin presents the chemical, physical and functional properties of Lipex® Canola-U and Lipex® Shea-U, and gives some examples of applications. In the final part of the bulletin, references are given to published reports on functional properties of tocopherols, phytosterols and triterpenes in dermal applications.

Chemical composition

Lipex® Shea-U

Lipex® Shea-U is a liquid shea butter with a high content of natural functional lipids. In a gentle process, the unique cinnamic acid esters of triterpene alcohols are enriched to a high content in the oil, while also the phytosterol content is increased. Dominating triterpene alcohols are α -amyirin, butyrospermol, lupeol, β -amyirin and parkeol. The most characteristic phytosterols of Lipex® Shea-U are stigmasterol, α -spinasterol and avenasterol.

Lipex® Canola-U

Lipex® Canola-U is derived from Canola oil by gentle processing to obtain an enriched content of phytosterols in combination with a high content of natural tocopherols. Dominating phytosterols are β -sitosterol, campesterol and brassicasterol. The tocopherol content is characterised by mainly γ - and α -tocopherols.

A typical composition of Lipex® Shea-U and Lipex® Canola-U is shown in Table 1.

Table 1: Chemical composition of Lipex® Shea-U and Lipex® Canola-U, given as typical values, in weight -%

	Lipex® Shea-U typical analysis	Lipex® Canola-U typical analysis
Triglycerides	65	90
Mono/diglycerides	10	5
Free fatty acids	0.3	0.3
Unsaponifiables, total	22	3
Triterpene cinnamates	20	-
Phytosterols	0.3	2.3
Tocopherols	0.06	0.4
Fatty acid composition		
C16:0	5	2
C18:0	10	4
C18:1	66	83
C18:2	14	8

Contaminants and minor components

The Lipex® functional lipids are manufactured from carefully selected raw materials with processing conditions optimised to minimise undesirable contaminants and breakdown products. For further details see separate product documentation.

Chemical properties

Hydrolytic stability

Since the major components of the Lipex® functional lipids are esters of fatty acids, extreme pH values should be avoided to minimise hydrolysis. The products are stable in the range from pH 6 to 8.

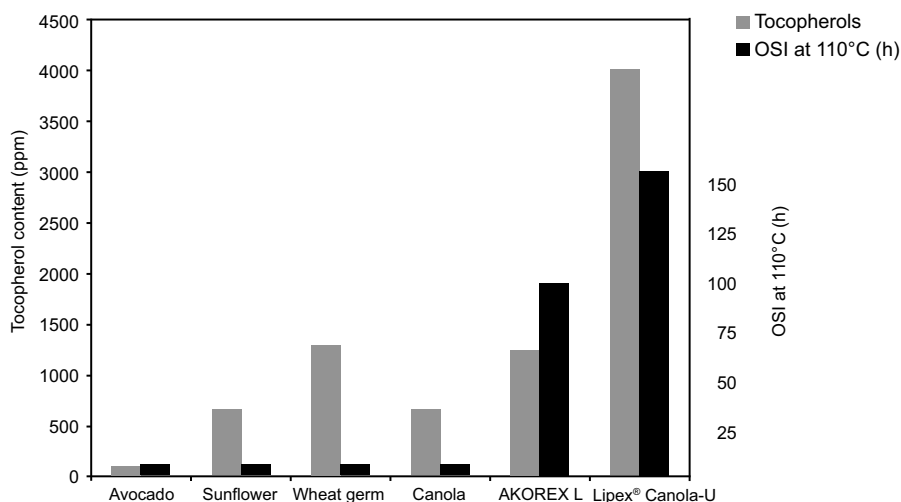
Oxidation stability

The oxidation stability of vegetable oils is determined by individual differences with regard to the ratio of polyunsaturated and monounsaturated fatty acids and the content of natural antioxidants. Typical contents of natural tocopherols and OSI (Oil Stability Index) values for some common vegetable oils are illustrated in Figure 1.

Lipex® Canola-U presents a unique, high content of tocopherols, mainly γ - and α -tocopherols, both known to offer good protection against oxidative breakdown. The tocopherols in combination with a high content of monounsaturated fatty acids result in excellent oxidation stability, OSI value of 160 hours at 110 C.

Lipex® Shea-U presents an OSI value of 15 hours at 110 C. When stored in small open bottles, in the dark at 20 C, the peroxide value is kept below PV 2 during one year for both Lipex® Shea-U and Lipex® Canola-U, indicating a long shelf life for both oils.

Figure 1: Relative oxidation stability versus tocopherol content in various vegetable oils.



Nomenclature

The nomenclature and classification of Lipex® Canola-U and Lipex® Shea-U according to various sources are shown in Table 2.

Table 2: Nomenclature and classification

	Lipex® Shea-U	Lipex® Canola-U
Chemical name (IUPAC)	Triglyceride	Triglyceride
CAS number	194043.92-0	120962-03-0
EINECS/ELINCS	270-311-6	273-313-5
INCI name (EU)	Butyrospermum Parkii	Canola
INCI name (U.S)	Butyrospermum Parkii (Shea butter)	Canola Oil

Physical properties

Appearance

The Lipex® functional lipids are semisolid oils at room temperature, with a typical melting point of 13 C and 17 C for Lipex® Canola-U and Lipex® Shea-U respectively. The temperature at which the oil starts to form crystals and becomes cloudy – the cloud point – is 7 C for Lipex® Canola-U and –5 C for Lipex® Shea-U.

Solubility properties

AarhusKarlshamn functional lipids are soluble/dispersible in polar solvents such as ethanol, and soluble in common vegetable triglyceride oils. The solubility in water is low.

Compatibility

Lipex® Canola-U and Lipex® Shea-U are compatible with many other commonly used lipophilic raw materials. Due to the typical, slightly polar properties of the functional lipids, these can be favourably formulated with other triglyceride oils and fats as well as with esters.

General physical properties

Some general physical properties of Lipex® Shea-U and Lipex® Canola-U are summarised in Table 3.

Table 3: Physical properties of Lipex® Shea-U and Lipex® Canola-U, given as typical values.

Property	Lipex® Shea-U	Lipex® Canola-U	Method
Colour, red	Max 5.0	Max 3.5	Lovibond 51/4"
Density (g/cm ³ , @20 C)	0.95	0.91	IUPAC 2.010
Viscosity 20 C (m Pas)	312	115	Rotational viscometry
Melting point (C)	17	13	Differential scanning Calorimetry
Cloud point (C)	-5	7	AOCS Cc9a-47

Functional properties of Lipex® Shea-U and Lipex® Canola-U

The natural content and composition of minor lipid components in vegetable oils are unique for each raw material. The functionality of Lipex® Shea-U and Lipex® Canola-U is explained by their respective composition of the minor lipid components gently enriched and protected in the original oil.

Lipex® Canola-U presents a unique combination of natural tocopherols and phytosterols enriched from Canola oil. The phytosterols represent the dominating minor lipid components and differ with regard to composition from other common vegetable oils by containing the additional brassicasterol. The biological activity with regard to photo-protecting and anti-inflammatory properties has been demonstrated by in vivo and in vitro tests.

Lipex® Shea-U is characterised by a high content of minor lipid components enriched from Shea butter. The non-glyceridic part of the oil is dominated by triterpene esters, mainly cinnamic fatty acid esters, and by phytosterols. Anti-inflammatory and fibroblast proliferating properties are indicated by performed in vitro tests, and UV-B absorbing properties are shown in combination with a commercial sunscreen.

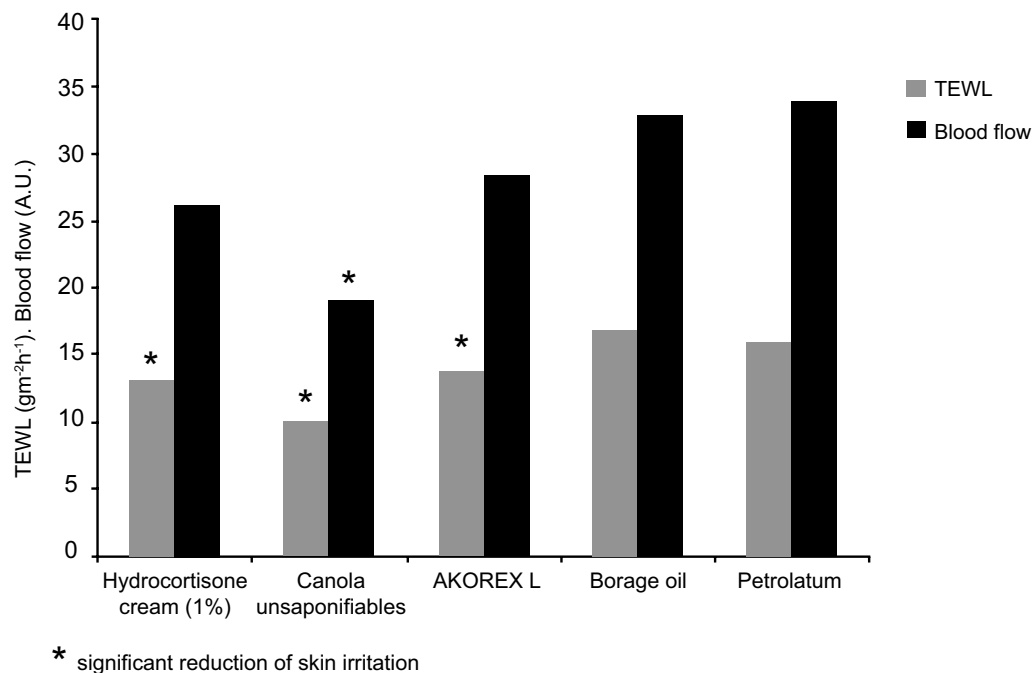
The bioactivity of shea butter, related to its unique high content of triterpene esters, has been known for ages by the inhabitants of West Africa. Shea butter was used on skin and hair to protect and moisturise, and also on the skin for healing small afflictions.

Anti-inflammatory effect of Lipex® Canola-U and Lipex® Shea-U

In vivo evaluation

An anti-inflammatory effect on surfactant-irritated skin of the minor lipid components of Canola oil has been shown in a clinical study including 21 healthy humans (1). Parameters assessed were visible signs of irritation, objectively measured cutaneous blood flow and transepidermal water loss (TEWL). This study showed a significant reduction of skin irritation with regard to all three assayed parameters from the Canola unsaponifiable fraction, comparable to and even more pronounced than the results from a hydrocortisone cream (Hydrocortisone 1%). No significant reduction was obtained with any of the other lipids included in this test apart from pure Akorex L (a Canola derived oil from AarhusKarlshamn, with a slightly enriched content of tocopherols and sterols). The effect on TEWL and blood flow of both Canola fractions, Canola unsaponifiables and Akorex L, is illustrated in Figure 2.

Figure 2: Effect on TEWL and superficial skin blood flow of some substances on surfactant irritated human skin



***In vitro* evaluation**

There is increasing evidence that epidermal keratinocytes play an active role in the initiation of primary contact irritancy and contact hypersensitivity reaction in the skin through the synthesis and release of soluble pro-inflammatory cytokines. It was therefore of interest to study the protective effect of pure Lipex® Canola-U and Lipex® Shea-U on IL-8 and IL-1a production by chemically stimulated keratinocytes. The *in vitro* study was conducted by BIO-HC Conseil Recherche France, an independent test laboratory. The protective effect of both oils was studied in normal human epidermal keratinocytes exposed to a non-sensitising contact irritant (Croton oil) by measuring cytokine production after treatment with irritant stress. The method was based on the evaluation of intercellular IL-1a production and secreted IL-8 in human keratinocyte cultures. The results are summarised in Figure 3.

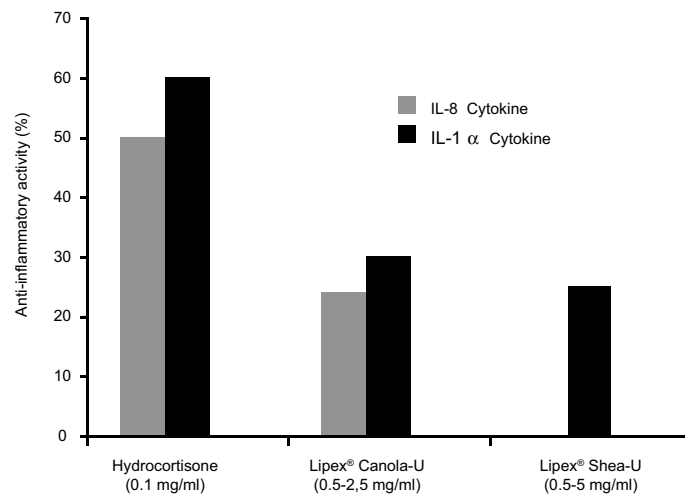
Under the experimental conditions Croton oil induced substantial increases in IL-8 and IL-1a cytokine production by 50 and 3 times, respectively. An obvious reduction of cytokines was detected from pure Hydrocortisone 21-hemisuccinate sodium treated cultures, included as a positive control.

Lipex® Canola-U decreased the inflammatory response induced by Croton oil by reducing both IL-8 and IL-1a production by some 25% and 30% respectively, at concentrations between 0.5 and 2.5 mg/ml, suggesting an anti-inflammatory potential. These results further confirm the shown anti-inflammatory effects of Canola derived products, evaluated by the described *in vivo* test.

Lipex® Shea-U decreased the inflammatory response induced by Croton oil by reducing IL-1a cytokine production by some 25%, at concentrations between 0.5 and 5.0 mg/ml, whereas no significant reduction of IL-8 was observed.

The results of the presented study correspond with reported anti-inflammatory properties of sterols and triterpenes and allow us to conclude that both Lipex® Canola-U and Lipex® Shea-U offer potentials as anti-inflammatory ingredients and a natural protection against environmental irritants.

Figure 3: Anti-inflammatory activity of Lipex® Canola-U and Lipex® Shea-U in comparison with Hydrocortisone



Fibroblast proliferating properties of Lipex® Shea-U

A significant effect on fibroblast proliferation has been demonstrated for Lipex® Shea-U, illustrated in Figure 4. The study was conducted by BIOalternatives France, an independent test laboratory. Due to the lipophilic nature of the evaluated material, human fibroblasts were incubated in an epidermis conditioned medium, followed by evaluation of 3H-thymidine incorporation. Lipex® Shea-U was initially topically applied to reconstructed epidermis, and after 24 h of incubation the culture medium was transferred to NHDF monolayer cultures. Lipex® Shea-U (at 75 w/w % solubilised in Akosun, a high oleic sunflower oil from AarhusKarlshamn) significantly increased fibroblast growth by 180% of control ($p < 0.01$).

These results, in accordance with reported keratinocyte and fibroblast proliferating activities of triterpenes and their esters, further substantiate the potential use of Lipex® Shea-U as a bioactive ingredient for skin care applications.

Figure 4: In-vitro evaluation of fibroblast proliferating properties from Lipex® Shea-U

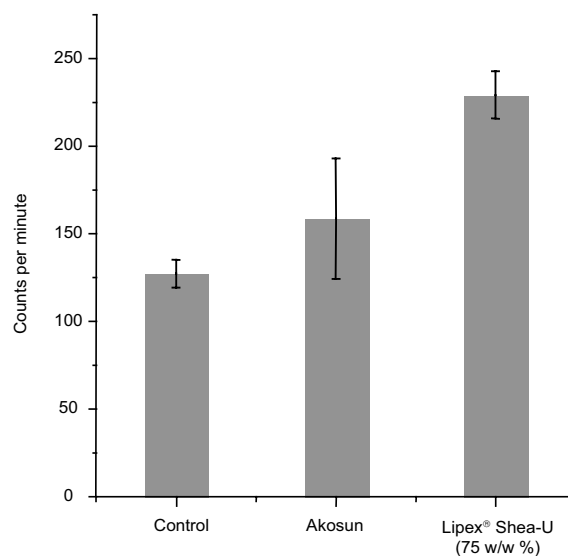


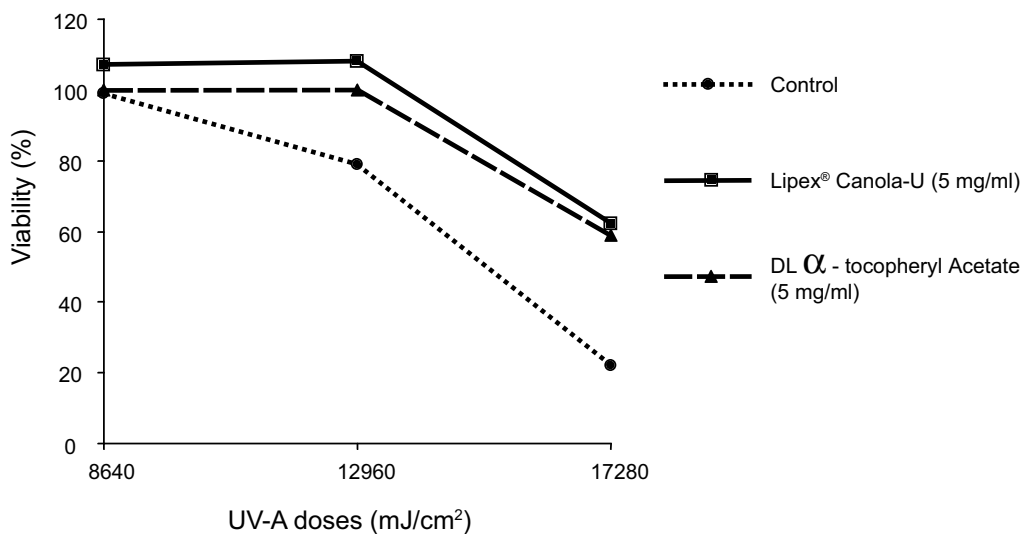
Photo-protecting effect of Lipex® Canola-U

The protective effect of Lipex® Canola-U against free radicals generated by UV irradiation on normal human dermal fibroblasts has been evaluated in a study conducted by BIO-HC Conseil Recherche France. The method used is based on the evaluation of UV induced cytotoxicity on control cells and on cells treated with different concentrations of Lipex® Canola-U, and pure DL- α -tocopheryl acetate included as a positive reference. The photo-protective activity of the products against UV-induced cellular damage was measured by comparing the ID50 UV dose (UV dose inducing 50% cell death) in controlled and treated cultures.

The cell cultures were exposed to each test substance for 48 hours before UV exposure (immediate treatment), followed by the exposure of various UV doses in the presence of each test product. After UV radiation the test compound was added to the cell cultures, which were then incubated for an additional 24 hours (curative treatment). Finally, the cell viability was evaluated by Neutral Red Uptake assay.

In the UV irradiation study, a very good protective activity against cytotoxicity at UV-A doses up to 17000 mJ/cm² was shown for Lipex® Canola-U. These results are comparable with the results obtained under the same conditions for the reference substance DL α -tocopheryl acetate. The results are summarised in Figure 5.

Figure 5: Photo-protecting effect of Lipex® Canola-U in comparison with DL α -tocopheryl acetate



Toxicological properties

In vitro evaluation of cytotoxicity

A cytotoxicity test has been performed on both normal human dermal fibroblasts and normal human epidermal keratinocytes exposed to pure Lipex® Canola-U and Lipex® Shea-U. No cytotoxic effect (100% cell viability) was observed after 48 hours of incubation in neither of the two assays. Cell viability was assessed by the Neutral Red Uptake (NRU) test. The NRU assay can be used to score cell injury as well as to determine the number of remaining cells after toxic insult. These results show no cytotoxic potential of Lipex® Canola-U and Lipex® Shea-U.

In vitro evaluation of phototoxicity

Lipex® Shea-U has been evaluated with regard to potential phototoxicity. The pure oil was diluted at 25 w/w % in Akosun (high oleic sunflower oil), 20 ml of each test compound was topically applied onto reconstructed human epidermis. A control with Akosun tested pure was used and the phototoxic reference compound used in this assay was chlorpromazine (at 40 mg/ml). The cell cultures were irradiated with a total UVA dose of 6 J/cm². Results were expressed as % of viability (evaluated by MTT assay) respectively to the untreated/non irradiated and untreated/irradiated controls. Under these experimental conditions Lipex® Shea-U was found neither cytotoxic nor phototoxic for the epidermis.

In vivo evaluation of skin irritation

Both pure shea and canola oil fractions have been evaluated by a clinical single patch test on 21 humans (1). The degree of irritation after 48 hours of exposure was assayed by visual and instrumental evaluation, objectively quantified by measuring the TEWL and superficial blood flow. No significant skin irritation was observed among the evaluated lipids.

Applications

The presented evaluations of Lipex® Canola-U and Lipex® Shea-U demonstrated several possible benefits to the skin of safe daily-use skin applications.

Lipex® Canola-U is a unique vegetable oil due to its high oxidation stability, shown photo-protecting and anti-inflammatory properties. The high content of natural tocopherols, known to offer good activity as free radical scavengers and as a quencher of singlet oxygen, offers a biochemical protection of epidermal cells as well as of other active ingredients in a formulation.

Natural tocopherols in combination with phytosterols in Lipex® Canola-U add additional benefits to the product due to their potentially enhanced reduction of photo-damage. Both tocopherols and phytosterols are also known to offer membrane stabilising effects resulting in an improved water-binding capacity of the skin.

The demonstrated anti-inflammatory effect together with a potential repairing effect of Lipex® Canola-U is of potent interest for caring skin applications for safe and non-irritant products. Typical formulations are facial creams for daily use and body care/sun care lotions, treating dry, sensitive and stressed skin conditions. Lipex® Canola-U finds additional applications within hair care, particularly in creams and balsam, hair masks and treatments for sensitive scalps.

Lipex® Shea-U presents a high content of minor lipid components, protected in the shea oil to be easily incorporated in caring cosmetic formulations. Dominating triterpene esters offer caring and moisturising properties to the formulation resulting in excellent skin softness and suppleness. Lipex® Shea-U is recommended for selective daily-use skin/body, personal care and hair care formulations.

Besides providing emollient and moisturising characteristics to the formulation, the incorporation of Lipex® Shea-U together with a physical or chemical sunscreen offers a slight additional UV-B absorbing effect. This makes Lipex® Shea-U a good emollient for sunscreen and facial care formulations.

The high content of triterpene esters in Lipex® Shea-U also adds potential anti-inflammatory, collagen regenerating and keratinocyte proliferating properties to the formulation. Properties of significant interest for dry and sensitive skin care and for delaying the visible signs of ageing.

Lipex® Canola-U and Lipex® Shea-U may be used to formulate both o/w and w/o emulsions at a typical concentration of 1-50% of the oil phase. Emulsifier systems with an HLB value of 6-7, developed for vegetable oils and other polar oils, are recommended.

Functional properties of tocopherols, phytosterols and triterpenes in dermal applications

Tocopherols, phytosterols and triterpenes have been investigated with regard to bioactivity in several scientific studies and are well described in the literature. Below, references are given to published studies of their functionalities, a summary given in Table 4.

Tocopherols

Natural tocopherols are the most important lipid soluble antioxidants and essential nutrients for humans and animals. Tocopherols are relatively heat-stable and non-toxic substances used in food products as well as in cosmetic and pharmaceutical applications. Tocopherols act by a free radical scavenging mechanism inhibiting lipid peroxidation in biological cell membranes and cell organelles, but also act as a quencher of excited oxygen atoms (singlet oxygen) which are part of numerous photo-oxidation reactions (2, 3, 4).

Vitamin E is the generic term for all substances possessing vitamin E activity. The natural δ -tocopherols have been proven to show significantly higher biological activity than the synthetic dl-tocopherols. The two most important tocopherols in topical applications are generally agreed to be the δ - α and δ - γ -tocopherols, (2, 5). The skin's limited capacity to cleave esterified forms of vitamin E is proposed to explain the lower ability of vitamin E-acetate or -succinate to prevent UV-induced skin damage, (6, 7).

UV radiation damage to the human skin is related to both short-term and long-term effects. Topical applications of natural α - and γ -tocopherols prior to irradiation have been demonstrated in several in vivo studies to reduce UV-induced skin damage such as erythema, oedema, skin sensitivity and skin wrinkling (6, 7, 9, 23, 24). Research has further demonstrated, in vitro, that tocopherols may protect epidermal cells from the deleterious effects of specifically UV-A irradiation (10).

Tocopherols in combination with other antioxidants and synergists such as phospholipids, ascorbic acid, β -carotene and citric acid are shown to have beneficial effects on the antioxidant activity, (2, 5). For a vitamin E and C (L-ascorbic acid) in combination with a commercial UV A sunscreen (oxybenzone) a synergistic effect against photo damage has been demonstrated (8).

Tocopherols are also shown to have a physicochemical, stabilising effect in biological membranes. The proposed interaction with membrane phospholipids may have functional consequences such as the inhibition of polyunsaturated fatty acid (PUFA) oxidation, reduction of the membrane permeability and prevention of phospholipid degradation (11, 12, 13).

The protection of cell membranes is shown to exert beneficial effects on the water-binding and moisturisation properties of the skin (14).

Topical application of tocopherols is proved to show anti-inflammatory effects on various inflammatory skin diseases such as dermatitis, as well as on immune and inflammatory responses in rheumatic diseases (15, 16). Beneficial effects in wound healing by vitamin E have been further demonstrated by several investigators (14).

Phytosterols

Phytosterols are regarded as non-toxic substances, common in a vegetable diet (17). The chemical structures of phytosterols are similar to cholesterol but can only be synthesised by plants.

The natural content and composition of phytosterols in vegetable oils are characteristic for each raw material. Phytosterols occur mainly in the free form or as esters of fatty acids. The main phytosterols found in vegetable oils are β -sitosterol, campesterol, stigmasterol, D5-avenasterol and D7-stigmasterol. Depending on their structure, sterols and their derivatives – such as hormones and vitamin precursors – impart various important biological functions within the human body.

Sterols have been demonstrated to have a structural role, similar to cholesterol, by interacting with the skin lamellar lipid layers. Increased skin moisturisation has been demonstrated for topical phytosterols and cholesterol (18, 19). In a recent study the *in vitro* effect of plant sterols were investigated with regard to their uptake and membrane lipid fluidity in human keratinocytes (25). It was concluded that the incorporation of cholesterol, sitosterol and stigmasterol resulted in an increased content of oleic acid (C18:1) in the plasma membrane. Whereas cholesterol and sitosterol had no significant effect on the membrane fluidity, the presence of stigmasterol increased the fluidity.

Phytosterols have been shown to impart both anti-inflammatory and wound healing effects on damaged skin (1, 18, 20, 21). The antiphlogistic effect is claimed to be similar to those of corticosteroid. The action mechanism, however, is proposed to differ from that of cortisone. Research has demonstrated that some phytosterols exert an influence on the arachidonic acid cascade by reducing the level of leucotrienes, important mediators of inflammatory reactions, such as atopic dermatitis, eczema, acne and phytoerythema. Improved skin conditions were shown from a topical phytosterol-containing emulsion (1% phytosterols) with regard to decreased squamation and erythema in patients suffering from neurodermatitis (18).

An additional protective effect against UV-B and UV-A radiation has been reported from binary combinations of an antioxidant (tocopherol) with an anti-inflammatory agent (hydrocortisone) (22).

Triterpenes

Being widespread vegetable lipids, triterpenes represent a large group of compounds with physiological activities including inhibition of tumour promotion, inflammation and lipid peroxidation.

Ten different triterpenes representing the oleanane (including β -amyirin), ursane (including α -amyirin) and lupane (including lupeol) series have been evaluated by *in vivo* models of inflammation (26). The aim of the study was to investigate a possible relationship between their chemical structure and pharmacological properties. It was concluded that all triterpenes were remarkably active against the edema produced by TPA (12-o-tetradecanonylphorbol acetate), while only some of the triterpenes, for example α -amyirin, significantly reduced carrageenan and EPP-induced edemas.

In addition to their anti-inflammatory properties, triterpenes – belonging to the ursane and lupane series – have shown skin repair *in vitro* activities (27, 28). Lupeol esters were investigated in an *in vitro* model of human skin keratinocytes. The results showed a good differentiation of keratinocytes and a well-formed stratum corneum was observed (28). Oleanolic and ursolic acids are as well reported to show a protective effect against lipid peroxidation (29).

In conclusion, scientific research – including a large number of in vivo and in vitro tests – has confirmed that natural tocopherols, phytosterols and triterpenes may repair and protect the skin as well protecting cell membranes from environmental insults caused by various sources, such as UV-radiation, chemical pollutants, unfavourable climatic conditions and irritants – all of which are aspects of importance for caring cosmetics.

Table 4: Functional properties of tocopherols, phytosterols and triterpenes in dermal applications.

Properties	Evaluation	Reference
Antioxidant		
Tocopherols	Antioxidant activity in vitro. Biological activity on topical application. Effects of other antioxidants and synergists.	2, 5
Triterpenes	Oleanane and ursane series, antioxidant potency	29
Photoprotecting		
Tocopherols	Protection of cell membranes against light-induced oxidative damages.	2, 3, 4
	Reduction of UV-B and UV-A induced skin damage.	8, 9, 10, 23, 24
	Activity of α -tocopheryl esters.	6, 7
	Binary combinations of antioxidants and anti-inflammatory agents, additional photoprotecting activities	22
Membrane stabilising		
Tocopherols	Reduced phospholipid peroxidation and PUFA oxidation. Physicochemical stabilisation, reduced permeability and increased moisturisation.	11, 12, 13, 14
Phytosterols	Interacting with lamellar lipids, in membranes. Effect on water binding and moisturisation.	18, 19
Anti-inflammatory		
Tocopherols	Effects on dermatitis, inflammatory and immune responses	1, 15, 16
Phytosterols	Anti-inflammatory effect, similar to cortisone. Improved skin conditions such as squamation, erythema, photo-erythema, eczema, psoriasis, acne.	1, 18, 20, 21
Triterpenes	Anti-inflammatory activity of natural triterpenes	26, 28
Skin repair		
Tocopherols and Phytosterols	Healing effects on damaged skin.	14, 18
Triterpenes	Keratinocyte proliferation and stimulated collagen I synthesis.	27, 28

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The data in this publication are given as examples to illustrate the properties and applications of Lipex® Canola-U and Lipex® Shea-U. Current specifications, toxicological profiles and regulatory aspects are available from AarhusKarlshamn on request.

Lipex® is a registered trademark for natural, vegetable and functional lipids used by AarhusKarlshamn.

March 2006

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