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FEATURE

Cosmetic emollients with high stability against photo-oxidation

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Summary

Natural antioxidants and minor lipids are of increasing interest for protecting against photo-oxidation in cosmetic and skin care applications. They also offer extended shelf-life to vegetable oil based formulations as well as protection of skin cell constituents such as proteins, lipids and DNA. This study demonstrates the photo-protective effect of fractionated rapeseed oil enriched in natural tocopherols and phytosterols. The fractionated rapeseed oil investigated is shown to lower the oxidation sensitivity of natural oils rich in polyunsaturated fatty acids and offers protection of skin cells while exposed to UV-radiation.

Introduction

Tocopherols are important antioxidants frequently used in both food and skin care applications. In vegetable oils their main function is to protect polyunsaturated fatty acids against oxidation, properties of importance also for the shelf-life of the oil during storage. In the human skin they are part of the natural defence system with α-tocopherol being the predominant lipid-soluble antioxidant in human stratum corneum. The tocopherols protect cellular components such as DNA, proteins and lipids against free radicals and reactive oxygen species caused by UV radiation, pro-oxidative environments and air pollutants.

Table 1. Typical tocopherol and phytosterol composition of standard rapeseed oil and a partially-hydrogenated and fractionated low-erucic rapeseed oil (FCO; fractionated canola oil) in parts per million (ppm).

	FCO	Rapeseed oil
Tocopherols		
α-Tocopherol	300	240
γ-Tocopherol	600	360
δ-Tocopherol	10	10
Phytosterols		
β-Sitosterol	3400	2900
Campesterol	2400	2300
Brassicasterol	700	500
Others	300	300

Many common natural oils such as rapeseed oil, sunflower oil and soybean oil are rich in polyunsaturated fatty acids, mainly linoleic (18:2*n*–6) and α-linolenic (18:3*n*–3) acids. These natural oils are of significant nutritional importance and are also desirable emollients for skin care applications. However, the drawback of the unsaturated oils is their high sensitivity to oxidation and many of them present limited shelf-life during storage as well as in application.

In addition, natural oils are good sources for tocopherols and phytosterols, components offering both antioxidant activity and bioactivity for skin care applications. By careful processing these minor lipids can be retained and even enriched during the purification of vegetable oils (1).

There are two important reasons why we should consider alternative routes to deliver photo-protective antioxidants to the skin. The first is that skin cells contain components that are sensitive to photo-induced oxidation and the second is the common practice of formulating skin care products with polyunsaturated vegetable oils. The use of fractionated natural oils is already known within the cosmetic industry and the anti-inflammatory effect of minor lipids from fractionated rapeseed oil was evaluated in an early clinical study by Lodén and colleagues (2). Also, Alander *et al.* (3) have reported the anti-inflammatory properties of fractionated rapeseed oil rich in tocopherols and phytosterols evaluated in a human skin cell test.

This paper describes a study we conducted to demonstrate the antioxidant and photo-protecting effects of the natural tocopherols and phytosterols in a partially-hydrogenated and fractionated low-erucic

Table 2. Typical fatty acid composition (%) of investigated oils. FCO = fractionated canola oil (partially-hydrogenated and fractionated low-erucic rapeseed oil); MCT = medium-chain triglyceride (caprylic/capric triacylglycerols).

	Fractionated canola oil (FCO)	Soybean oil	Passion flower seed oil	Gold of pleasure oil	Medium-chain triglycerides (MCT)
8:0	–	–	–	–	58
10:0	–	–	–	–	38
16:0	3	11	11	5	–
18:0	6	4	3	2	–
18:1	81	23	15	13	–
18:2	6	53	70	16	–
18:3	–	8	–	36	–
20:1	–	–	–	15	–

rapeseed oil (fractionated canola oil, FCO; International Nomenclature of Cosmetics Ingredients name INCI: Canola, trade name: 'Akorex L'). The oil is characterized by a high content of monounsaturated fatty acids in combination with elevated levels of α- and γ-tocopherols, and phytosterols. The minor lipid composition of FCO is given in **Table 1**. The typical low content of polyunsaturated fatty acids in combination with the tocopherols in FCO results in a liquid oil with high stability to oxidation (OSI >100 hours at 110°C). Common vegetable oils rich in polyunsaturated fatty acids show typical OSI values of < 8 hours at 110°C.

FCO was combined with four different vegetable oils: soybean oil, passion flower seed oil, gold of pleasure (camelina) oil and medium-chain triglycerides (MCT; caprylic/capric triacylglycerols) representing various degrees of unsaturation. Passion flower seed oil has the highest content of polyunsaturated fatty acids at 70%, gold of pleasure 56% and soybean oil 61%, while MCT is an inert fully-saturated liquid oil. The typical fatty acid compositions are shown in **Table 2**. We also compared the antioxidant activity of a commercial α-tocopherol (dl-α-tocopherol, DSM Nutritional Products) with that of FCO.

This study did not distinguish between the antioxidant effect of tocopherols and phytosterols or between the effects of α- and γ-tocopherols. Several authors have extensively investigated and reported the importance of different tocopherols as antioxidants in vegetable oils at various concentrations, under different oxidation conditions such as temperature, UV exposure and availability of oxygen, and also with differing types of lipid substrates. In addition, it has been shown that when α-tocopherol has been depleted, γ- and δ-tocopherols can also give substantial antioxidant activity. For example, Lampi *et al.* (4) report higher antioxidant activity from γ-tocopherol, compared to α-tocopherol, when used at levels above 100 ppm while studying the oxidation of rapeseed oil at 40°C in the dark.

α-Tocopherol is the predominant tocopherol in living tissues and regarded as the most potent isomer in terms of antioxidant effect while γ- and δ-tocopherols have been seen as less active. In the human skin, α-tocopherol is an important part of the natural antioxidant system protecting cell membrane lipids from oxidation damage. However, after exposure to UV radiation the enzymatic and non-enzymatic antioxidant capacity of the skin is decreased significantly, indicating that the cutaneous defence system is challenged by photo-oxidation.

Our understanding of the intrinsic role of the tocopherol isomers in the skin and living tissues is still unclear and under investigation by several groups. Jiang *et al.* (5) show evidence of *in vivo* anti-inflammatory activity of γ-tocopherol but not α-tocopherol. The activity of γ-tocopherol is shown to be similar to that of COX-2 inhibitors, non-steroidal anti-inflammatory drugs that are effective in attenuating inflammatory response and beneficial for inflammation-associated diseases, and thus important for human disease prevention and therapy.

Protective effect on soybean oil

In order to demonstrate the protective effect of FCO on PUFA (polyunsaturated fatty acids), an *in vitro* experiment was carried out. Combinations of soybean oil (rich in linoleic and linolenic acid) with either FCO (protective emollient) or MCT (inert emollient) were subjected to medium-intensity simulated daylight from a fluorescent tube for 8 hours at 35°C (skin temperature) with free access of oxygen. The level of

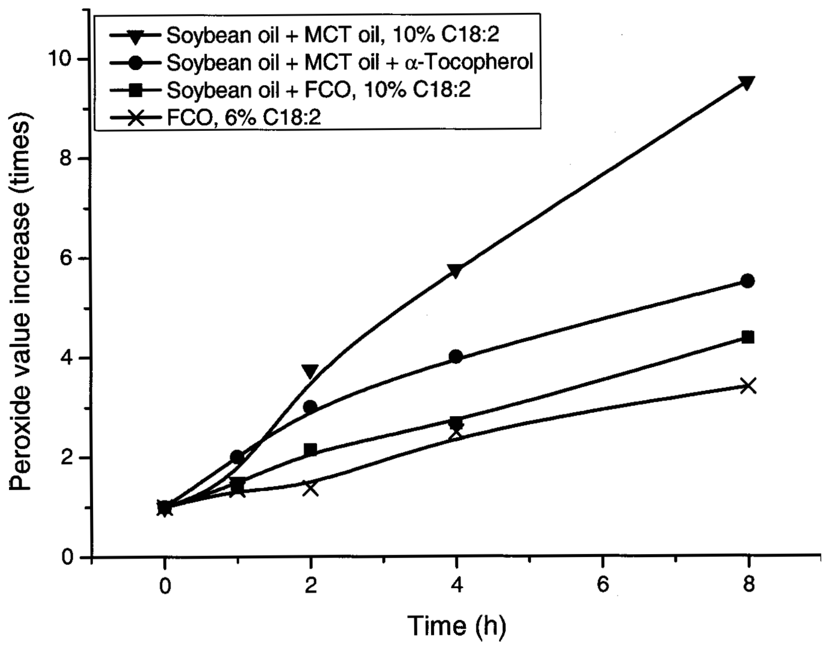

Figure 1. Photo-oxidation of emollient blends. The photo-oxidation rate is given as the number of times the initial peroxide value is increased as a function of time. FCO = fractionated canola oil (a partially-hydrogenated and fractionated low-erucic rapeseed oil); MCT = medium chain triglyceride (caprylic/capric triacylglycerols).

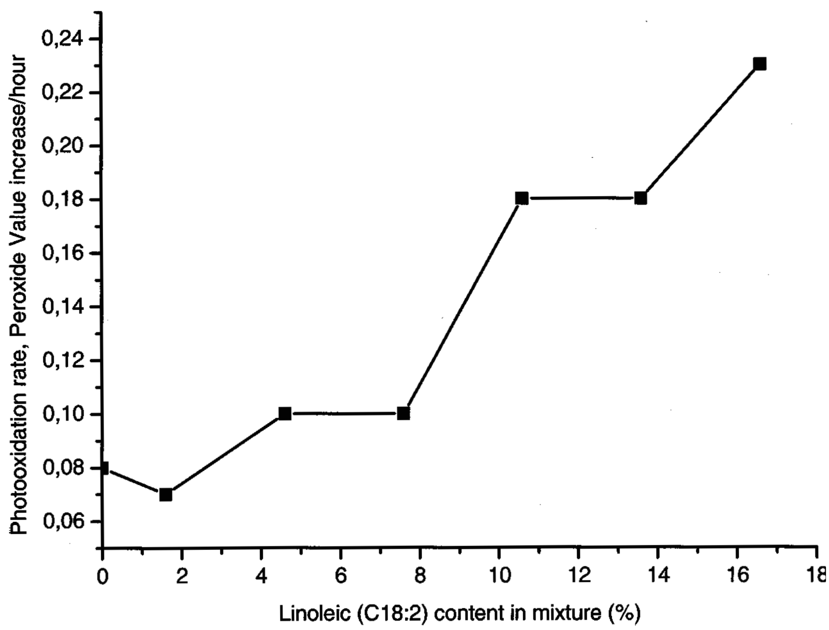

Figure 2. Photo-oxidation rate as a function of linoleic acid content for emollient blends. The photo-oxidation rate at body temperature and mild UV radiation increases rapidly when the linoleic acid content exceeds 10% in the mixture. Each mixture contains 20% FCO and the linoleic acid content is adjusted by the addition of passionflower oil to MCT oil.

PUFA in the emollient mixture was standardized to 10% linoleic and approximately 1% linolenic acid by mixing appropriate amounts of the two emollients with soybean oil.

Also, the antioxidant effect of commercial α-tocopherol was compared to that of the natural tocopherols in FCO. The peroxide value, measuring the formation of primary oxidation products, was determined after 1, 2, 4 and 8 hours of irradiation.

The peroxide value in pure FCO with approximately 6% of linoleic acid increased by about 2 units compared to the original value during the 8 hour test (**Figure 1**). The mixture of soybean oil and MCT containing 10% linoleic acid showed a large increase of about 8 units. When soybean oil was mixed with FCO the peroxide value increased by about 1 unit compared to pure FCO, thus demonstrating the protective effect of the tocopherols. The increase in peroxide value was about 4 units compared to the original value when we added commercial α-tocopherols to the soybean oil and MCT mixture at antioxidant concentrations corresponding to those in the blend of FCO and soybean oil.

The test clearly demonstrates that FCO offered a more efficient protection against oxidation than did the α-tocopherol. Observed differences could be explained by differences in antioxidant activity due to different tocopherol compositions or different antioxidant potential between the two sources of antioxidants.

Protective effect on cosmetic oils rich in PUFA

A further test to demonstrate the antioxidant properties was performed using blends of FCO with passionflower seed oil (PFO; INCI: *Passiflora incarnata*) and with gold of pleasure oil (GPO; INCI: *Camelina sativa*). Mixtures containing FCO (20% w/w), PFO and MCT were made in order to increase the level of linoleic acid from 0.2 to 17%. The minimum level of linoleic (0.2%) is obtained by mixing 20% FCO in MCT with no PFO added. In this mixture the linolenic acid is also 0%.

In a second set of trials, the content of linoleic acid was held constant at 8% while increasing the level of linolenic acid from 0 to 10% by mixing FCO, PFO and GPO with MCT. The minimum level of linolenic acid (series 2) is obtained by mixing 12% PFO, 20% FCO and 68% MCT. In this mixture the linolenic acid is 0% and the linoleic acid approximately 8%. The photo-oxidative stress test was carried out as described above. The peroxide value increased linearly with time and the photo-oxidation rate was determined as the slope of the peroxide value versus time during the first 6 hours of irradiation.

The effect of 20% FCO in mixtures with PFO and GPO is shown in **Figures 2** and **3**, respectively. There is a rapid increase in oxidation rate when the total linoleic acid content exceeds about 8%, corresponding to approximately 10% of PFO in the mixture. Similarly, a rapid increase in oxidation rate occurs at about 5% of linolenic acid, corresponding to 10% of GPO in the mixture. In the latter case, the mixture also contains about 8% of linoleic acid which also contributes strongly to the oxidative behaviour. As a comparison, the photo-oxidation rates of the original oils in peroxide value units/hour are PFO 0.47, GPO 0.52 and FCO 0.08. These results show clearly the beneficial effect of FCO on protecting polyunsaturated oils from photo-oxidation.

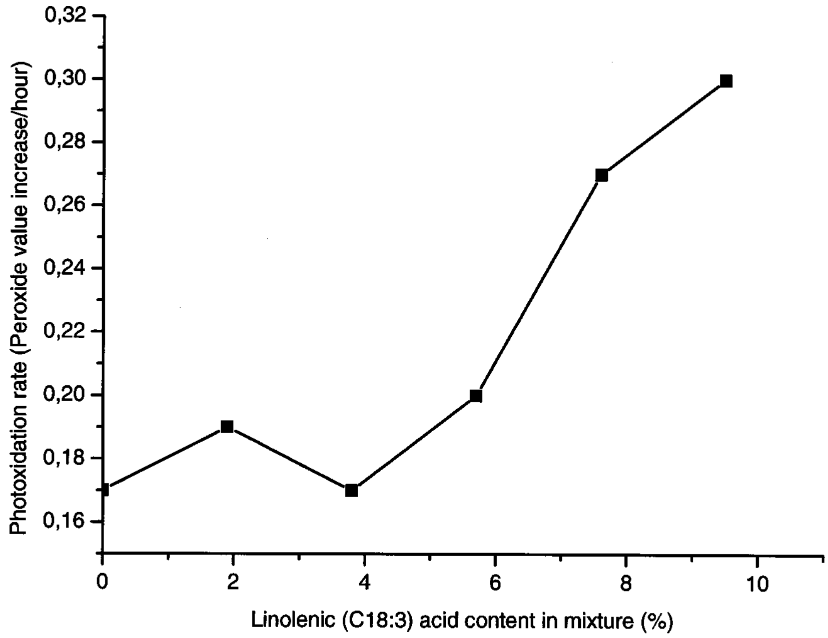

Figure 3. Effect of linolenic acid content on the photo-oxidation rate of emollient blends. Photo-oxidation rates increase rapidly above 6% linolenic acid. Linoleic acid is kept constant at 8% by adjusting the proportions of passionflower, gold of pleasure and MCT oils. FCO content in mixture is 20%.

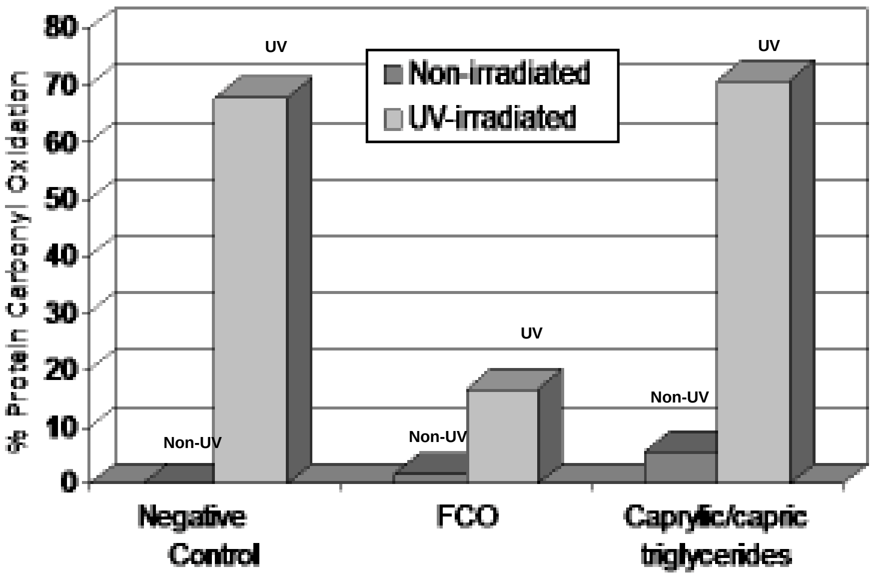

Figure 4. Protective effect on protein carbonyl oxidation of human skin cells *in vitro.* Protein carbonyl oxidation is measured in the keratinocyte cultures by reaction with DNPH (dinitrophenylhydrazine). FCO gives a significant protection against protein oxidation in the cultures subjected to photo-oxidative stress in comparison to caprylic/capric triglycerides (MCT) and the negative control.

Photo-protection demonstrated by human skin cell test

The protective effects were further investigated in an in-vitro test using human skin cells. Normal, human-derived epidermal keratinocytes ('EpiDerm') were treated with FCO and irradiated with simulated solar UV radiation (60 minutes at 1.7 mW/cm^2 and room temperature). Two endpoints were used for estimating the efficacy of FCO: protein oxidation measured by carbonyl formation 2 hours after irradiation and lactate dehydrogenase (LDH) release 24 hours after irradiation. LDH release measures the integrity of the cell membranes and cell viability after a challenge.

The protective effect already seen in the photo-oxidation tests described above was confirmed in this study, expressed as the reduction of protein carbonyl formation after an oxidative stress caused by UV radiation. FCO, as an "active" emollient gives a clear advantage over the inert MCT which shows no inhibition of protein oxidation (**Figure 4**). Furthermore, cell viability expressed as LDH release after irradiation was clearly increased by the FCO treatment while no protection was seen with the MCT.

Conclusions

The results from this study clearly show that fractionated rapeseed (canola) oil (FCO) offers protection against photo-oxidation of natural oils rich in PUFA. Addition of FCO will provide sufficient tocopherols to prevent oxidation of PUFA as long as the proportion of PUFA in the mixture doe not exceed 3–4%. A human skin cell test also shows protection of proteins and cell membranes against UV-induced oxidation. The observed protective effects are most probably associated with the high content in this 'active' emollient of a combination of natural tocopherols in their free dl-form and phytosterols.

Several studies show that skin damage induced by free radicals can be limited by supplementing the skin with antioxidants such as α-tocopherol and thereby strengthening its antioxidant capacity (5). However, the bioavailability of topically applied vitamin E, commonly used in skin care applications in the esterified form (such as α-tocopherol acetate), is reported to be low or zero. A study by Alberts *et al.* (6) showed significant skin absorption of α-tocopherol acetate but no bioconversion to the unesterified active form within the skin. They concluded that the cleavage capacity of human skin is extremely low or absent. Hence, it can be assumed that esterified tocopherols offer less photoprotective effect than unesterified tocopherols when applied topically. Consequently, the FCO containing natural unesterified tocopherols which we used in our studies may be bioavailable and capable of photoprotection in dermal applications.

References

1. Alander, J. *et al.* (2005) European patent, EP 1084215.
2. Lodén, M. *et al.* (1996) *Br. J. Dermatol.*, 134, 215–220.
3. Alander, J. *et al.* (2005) *Cosmetics & Toiletries*, 120, 73–79.
4. Lampi, A-M. *et al.* (1999) *J. Am. Oil Chem. Soc.*, 76, 749–755.
5. Jiang, Q. *et al.* (2003) *The FASEB Journal*, 17, 816–822.
6. Dreher, F. *et al.* (2001) *Curr. Probl. Dermatol.*, 29, 157–164.
7. Alberts, D.S. *et al.* (1996) *Nutrition and Cancer*, 26, 193–201.