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Lingonberry boosts hydration with anti-ageing benefits

ABSTRACT

Lingonberry seed oil produced via supercritical CO_2-extraction is exceptionally high in essential α-linolenic (18:3n-3) and linoleic (18:2n-6) acids, and in γ-tocotrienol with strong antioxidant activity. The effect of orally administered lingonberry seed oil on the skin was studied in 30 women who consumed 2 g of lingonberry seed oil daily as capsules for three months. Skin hydration, elasticity, roughness, echogenicity (reflecting skin density), and the colour of hyper-chromatic skin spots were measured instrumentally at the beginning of, during, and at the end of the intervention. Over the course of the lingonberry seed oil intervention, there was a significant ($p<0.05$) improvement in skin hydration, elasticity, and density, and the roughness of the skin decreased from baseline to end. In conclusion, this study suggests that regular intake of CO_2-extracted lingonberry seed oil improves the skin's hydration and induces skin changes that can be described as anti-ageing.

Lingonberry (*Vaccinium vitis-idaea*) is a nutritious berry that is widely abundant and harvested in wild form in the Nordic countries. In recent years, it has gained a reputation as a health-promoting superfruit. Lingonberries are used in several ways in Scandinavian cuisine: as a side dish, garnish or components of desserts. Lingonberries are rich in vitamins C and E and in polyphenols including anthocyanins, proanthocyanidins and flavonols.[1] Seeds containing ≈30% oil by dry weight, represent about 1.5% of lingonberries' fresh weight.[2] The fatty acid composition of lingonberry seed oil is exceptional. Essential α-linolenic (ALA, 18:3n-3) and linoleic (LA, 18:2n-6) acids commonly represent about 80% of all fatty acids in the CO_2-extracted oil ([3] and unpublished results of analyses carried out at Aromtech Ltd). They are present in an LA to ALA ratio of about 0.8:1. Western diets, generally are deficient in n-3 fatty acids, and their n-6 to n-3 ratio of ≈15:1 is much greater than the nutritionally preferable level, between 4:1 and 1:1.[4] This makes lingonberry seed oil nutritionally interesting in its potential as an ingredient to balance the ratio of essential fatty acids in the diet. Both LA and ALA are known to contribute to skin health via their effects on the inflammatory cascades, with LA being particularly important, as a component of ceramides in the skin.[5]

Another interesting characteristic of lingonberry seed oil is its high content of γ-tocotrienol. Tocotrienols differ from tocopherols, the most common forms of vitamin E in the Western diet, in the structure of the phytyl side chain attached to the chroman head. In tocopherols, the side chain is saturated, whereas in tocotrienols the phytyl side chain has three double bonds (Fig. 1). The natural γ-tocotrienol content in the lingonberry seed oil extracted via the gentle method of supercritical CO_2-extraction is generally 100-120 mg/100 g oil ([3] and unpublished results from Aromtech Ltd). Tocotrienols are effective antioxidants. Several studies report tocotrienols as having even higher antioxidant/anti-ageing potential than tocopherols do. Recently the unique biological activities of various forms of vitamin E, beyond the antioxidant potential, have been recognised.[6]

We aimed to investigate the effect of supplementation with CO_2-extracted lingonberry seed oil on the characteristics and wellbeing of skin in healthy women. The main interest was in skin hydration and measures that describe changes occuring in ageing skin. Previously, a lightening effect of topical application of CO_2-extracted lingonberry seed oil to age spots was observed (patent EP1909918). With this in mind, we also sought to determine whether oral lingonberry seed oil would have effects on hyper-chromatic skin spots on the face.

Subjects and methods

The study was carried at the Institute of Skin and Product Evaluation (ISPE) in Milan during spring 2012 in accordance with the Declaration of Helsinki. All participants gave their written informed consent before participation. In total, 30 healthy women 35 to 65 years of age were recruited. All subjects had hyper-chromatic skin spots on their face. They were asked to avoid exposure to UV radiation and tanning beds during the intervention. During the intervention, and for a wash-out period of one week before the study, the participants were asked to wash the face according to their current skin care regimen and then apply only the base cream supplied to them. They were asked not to change any food supplements they were taking, only to add the study product. Women on medication with potential to affect the results, or who had a skin disorder were not included in the study. During the intervention, one participant dropped out for of personal reasons not related to the study product or procedure.

Each day during the intervention period of three months, the participants took 2 g (4 capsules) of lingonberry seed oil produced via supercritical CO_2-extraction by Aromtech Ltd (Tornio, Finland). Instrumental measurements of their skin were performed at the beginning of the study, after 1.5 months, and at three months – when the intervention ended. Skin hydration was measured with a Corneometer CM 825 (Courage & Khazaka). The device measures capacitance, and the result is expressed in corneometric units (c.u.), this instrument's arbitrary units. Increase in c.u. indicates an increase in the skin's hydration. Skin elasticity was assessed with a cutometer (Cutometer MPA 580; Courage & Khazaka), which measures deformation of the skin sucked into the opening of a measurement probe. The results are expressed as maximal deformation of the skin (parameter R0; decrease indicates improvement in elasticity), overall elasticity of the skin (parameter R2; increase indicates improvement in elasticity), and viscoelastic ratio (parameter R6). Skin wrinkles were analysed in terms of skin roughness (Ra, average roughness of the profile; Rz average maximum roughness of the profile) by means of negative imprints of the skin surface (skin replicas), which were analysed by image processing software (Quantiline, from Monaderm), with a reduction in roughness indicated by a decrease in the Ra and/or Rz parameter. Skin density was assessed through ultrasound scanning (Ultrasound Scanner Dermascan C Ver. 3, Cortex Technology). The intensity of the sound waves or echoes reflected by tissue is a function of tissue density, with increased density indicating improvement in dermal structure. The results of the measurements of skin echogenicity/density are expressed as percentages. The colour of hyper-chromatic skin spots was evaluated by a dual-channel reflecting colorimeter with intergrated microcomputer, and a xenon light source in the measuring head (Chroma Meter CR-400, Minolta). The colour is rated in terms of parameters L^*, reflecting changes in pigmentation intensity, and ITA°, which expresses the melanin index and is inversely proportional to pigmentation intensity. Increase in the L^* and ITA° values reflects whitening of skin spots.

The instrumental measurements were carried out under controlled humidity and temperature conditions on the face of the participants. Participants were asked not to wash the face for three hours before the measurement and not to apply any product for 12 hours before each study visit. In addition to the measurements taken on the face, hydration was measured on the volar forearm (10 cm from the elbow bone). The skin colour measurements were performed for a selected hyper-chromatic spot. At the end of the study, participants were asked to complete a self-assessment questionnaire on the treatment.

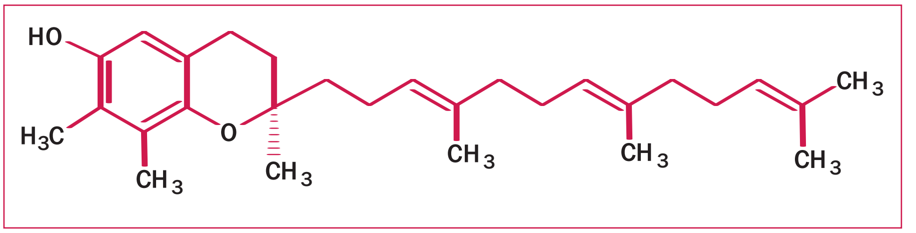

Figure 1: *γ-Tocotrienol*

Mean values, standard deviations, and variations were calculated for each set of values. According to the results of normality tests (Kolmogorov-Smirnov test, Lilliefors test and Shapiro-Wilk test), the instrumental data (at 0 months = beginning of the study, 1.5 months, and 3 months = end of the study) were statistically compared by means of repeated-measures ANOVA and Bonferroni testing for parametric data. A significance threshold of 0.05 was used throughout.

Results

Skin hydration increased significantly ($p<0.05$) from the beginning of the study to 1.5 months and to the end both on the face and on the forearm of the participants (Fig. 2). The results indicate clear hydration effect of lingonberry seed oil on the skin.

Skin elasticity increased significantly from baseline to the end of the intervention when judged via the parameter of maximal deformation (R0) or via the parameter of overall elasticity (R2), and also from the baseline to 1.5 months when assessed in terms of the parameter of maximal deformation (R0) (Fig. 3). There were no significant changes in the viscoelastic ratio during the intervention (results are not shown).

Skin roughness decreased from the baseline level after 1.5 months ($p<0.05$ for parameter Ra) and from the baseline to the end of the study ($p<0.05$ for parameters Rz and Ra), indicating a significant beneficial effect of lingonberry seed oil supplementation on facial wrinkles (Fig. 4).

Skin echogenicity increased significantly ($p<0.05$) from the start of the study to 1.5 months and the end (3 months). This indicates that lingonberry seed oil intake improves the skin's density (Fig. 5).

The changes in the parameters of skin colour in the course of the study were not statistically significant ($p>0.05$), even though there was a slight trend toward whitening of the hyper-chromatic skin spots. The value of the L^* parameter increased from a mean of 59.39 (±1.95) at baseline to 59.53 (±1.82) at the end of the study ($p>0.05$), while the ITA° parameter increased from the mean value of 26.7 (±5.3) at baseline to 27.4 (±4.9) at the end ($p>0.05$).

Subjective evaluation of the lingonberry seed oil capsules at the end of the study was positive and indicated that the participants felt they had benefitted from the capsules. Ninety per cent of participants reported feeling that the treatment was very or fairly effective in making the skin more hydrated, 86% felt the treatment to be very or fairly effective in making the skin more elastic, 62% felt it to be very or fairly effective in redensifying

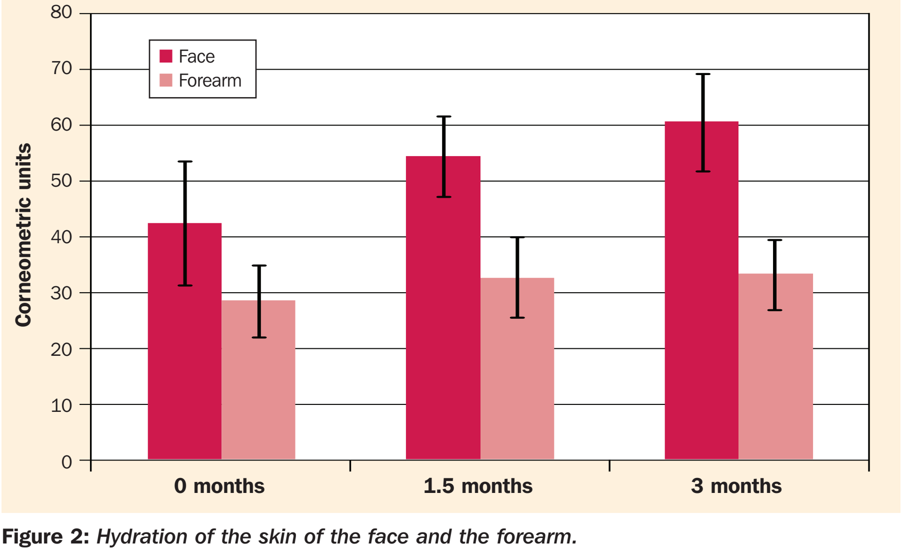

Figure 2: ***Hydration of the skin of the face and the forearm.***
A significant (p<0.05) increase in corneometric units from baseline to 1.5 months and three months was observed both in face and in the forearm. The results show a hydration effect on the skin through intake of lingonberry seed oil.

the skin, 55% felt the treatment to be very or fairly effective in reducing wrinkles and fine lines, 90% felt that it was very or fairly effective in improving skin smoothness, and 34% felt the product was very or fairly effective in whitening the hyper-chromatic skin spots. In all, 93% of participants found the treatment as a whole either very good or fairly good.

Discussion

It is most likely that the beneficial effects observed in this study were due to the synergy of the components of lingonberry seed oil: the high content of ALA and LA in a nutritionally favourable ratio in combination with the effects of γ-tocotrienol, the main form of vitamin E in lingonberry seed oil.

An adequate supply of ALA and LA is essential for healthy skin. They cannot be synthesised by the body and must be obtained from the diet. LA is a component of ceramides, which are part of the extracellular lipid matrix forming the *stratum corneum* permeability barrier. When the intake of essential polyunsaturated fatty acids from the diet is low, they are replaced by other fatty acids in the structural lipids of the skin, resulting in abnormal *stratum corneum* permeability and increased transepidermal water loss.[5] ALA and LA are important for the skin's wellbeing as precursors of the fatty acids of longer chain length and higher unsaturation, which can, in turn, be converted into eicosanoids, regulating inflammation. A deficient epidermal barrier and abnormalities in the conversion of ALA and LA to their derivates are associated with skin disorders such as atopic dermatitis and psoriasis, characterised by dry and inflamed skin. The role of n-3 and n-6 fatty acids in the wellbeing of the skin is, accordingly, more than just that of a structural component. As the parent molecules in the metabolic reaction chains leading to eicosanoids, they contribute to regulation of inflammation in the skin.[5]

The damage caused by reactive oxygen species and oxidative stress is central in the process of skin ageing. Reactive oxygen species affect both in chronological ageing and that caused by extrinsic factors. As the antioxidant mechanisms of the body deteriorate with age, oxidative damage to cell components increases. Reactive oxygen species induce increased expression of proteolytic enzymes such as matrix metalloproteinases (MMPs) and elastase, which hydrolyse proteins of the extracellular matrix. Degradation of collagen and elastic fibres lead to reduced mechanical tension of the skin and facilitates formation of wrinkles.[7] The central importance of reactive oxygen species points to the great importance of antioxidants in protection of the skin against signs of ageing.

Lingonberry seed oil has an exceptional antioxidant profile, owing to its high content of γ-tocotrienol. Tocopherols and tocotrienols can quench free radicals by donating their phenolic hydrogen and an electron so can, via this mechanism, protect other compounds against oxidative damage. Several studies report that tocotrienols can show even higher antioxidant activity than tocopherols.[6] Unlike tocopherols, tocotrienols have an unsaturated side chain, which facilitates their better permeation of membrane bilayers.[8] It was recently found that γ-tocotrienol was effective in protecting against detrimental effects induced by squalene hydroperoxide in keranocytes.[9] Squalene is the main component of sebum, the secretion from the sebaceous gland that covers skin's surface. Squalene is oxidised by UV light to squalene hydroperoxide, which is associated with detrimental skin changes, including acne and sunburn. Oxidised squalene can also induce activation of transcription factors regulating expression of inflammatory cytokines and thus promote inflammation.[9] The excellent potential of γ-tocotrienol in downregulating inflammation is supported by several studies.[8]

Conclusions

The results of this study indicate that regular intake of CO_2-extracted lingonberry seed oil increases skin hydration, improves the skin's elasticity and density, and has beneficial effects on wrinkles as assessed by instrumental measurements. Regular

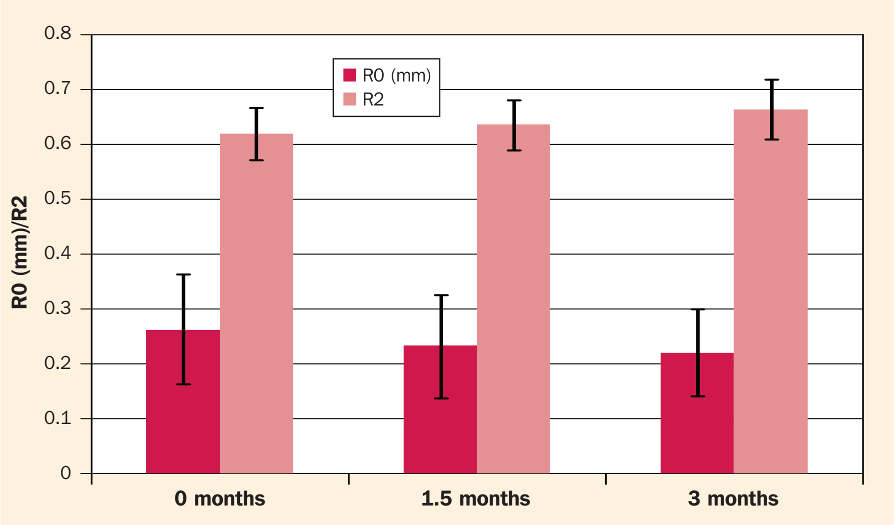

Figure 3: ***Elasticity of the skin.***
A significant (p<0.05) decrease in R0 from baseline to 1.5 months and from baseline to end, and a significant increase in R2 from baseline to end were observed. This result indicates improvement in skin elasticity with the tested intake of lingonberry seed oil.

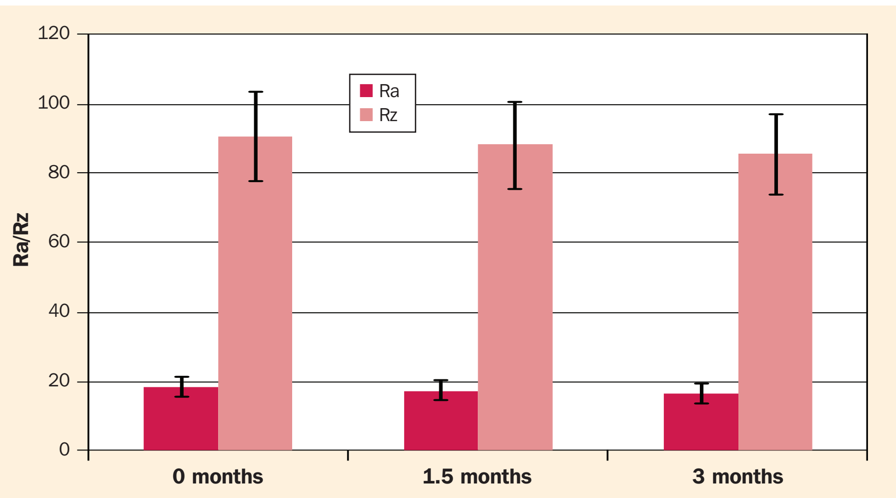

Figure 4: *Roughness of the skin.*
A significant (p<0.05) decrease from baseline to three months was observed for both average roughness (Ra) and average maximum roughness (Rz) of the skin of the face. For average roughness, a significant decrease was seen already at 1.5 months. The results indicate beneficial effects of lingonberry seed oil supplementation on facial wrinkles.

intake of lingonberry seed oil capsules and the treatment as a whole was found to be effective and tolerated well by the participants in the study. The beneficial effects are most likely mediated by the synergy of the combination of bioactive compounds in the oil: the high α-linolenic and linoleic acids and γ-tocotrienol. PC

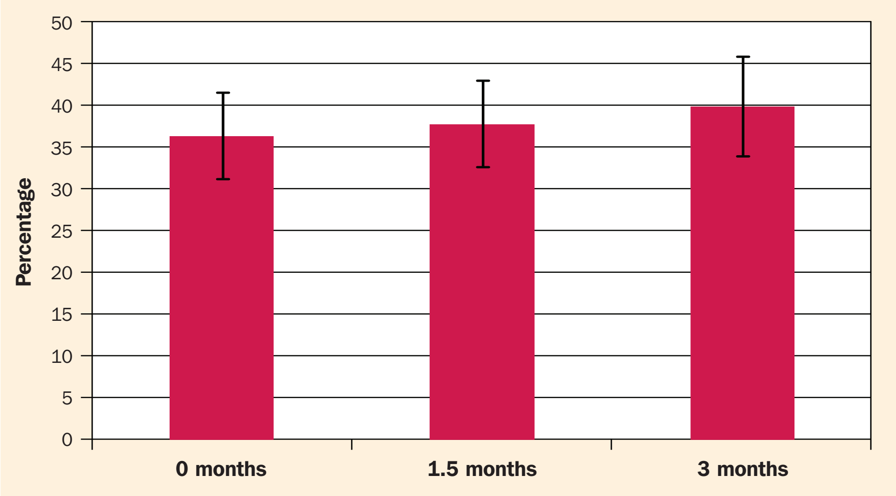

Figure 5: *Echogenicity of the skin.*
A significant (p<0.05) increase in echogenicity from the beginning of the study to 1.5 months and to the end, at three months, took place during the lingonberry seed oil supplementation. This indicates an increase in skin density due to the lingonberry seed oil.

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