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A matrix assisted laser desorption ionization time-of-flight mass spectrometry investigation to assess the composition of cod liver oil based products which displayed a different in vivo allergenic power

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ABSTRACT

Cod liver oil is a well-known "nutraceutical", which contains a wide range of substances, including triacylglycerols (TAGs), mono- and di-acylglycerols, free fatty acids, vitamins and $n-3$ polyunsaturated fatty acids. Topically applied, cod liver oil contributes to faster wound healing and improvement in skin quality. We recently reported a case of allergic contact dermatitis to cod liver oil contained in a topical ointment, in whom the patch test reaction with the ointment containing cod liver oil at a concentration of 40% was stronger than the reaction induced by a pure cod liver oil at the same concentration. We hypothesized that the different reactivity could be explained by differences in composition of the two products. In order to verify this hypothesis, we assessed the composition of those products using a matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The results obtained showed that the spectra of the ointment and of the cod liver oil samples were very similar, even if a major number of peaks were observable in the higher mass range of the spectra relevant to the analysis of the ointment sample, that have been assigned to higher molecular weight TAGs. Our results suggest that the different reactivity to the two products could be due to differences in the amount of contained TAGs. TAGs may favor the penetration of the allergen(s) or may be the direct culprit substances, taking into account that TAGs have been reported to have sensitizing properties.

1. Introduction

Fish is considered an important source of essential fatty acids, which are fundamental from a physiological viewpoint (Belitz and Grosch, 1999). Cod liver oil (CLO), in particular, is a well-known "nutraceutical" and is widely consumed especially in Northern European countries. This food contains a wide range of major compounds, i.e. triacylglycerols (TAGs), mono- and di-acylglycerols, free fatty acids, and minor compounds, i.e. an abundant amount of specific vitamins (A, D and E) and $n-3$ polyunsaturated fatty acids (commonly known as omega-3 fatty acids), especially eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), and, due to this, is widely used as a dietary supplement (Brustad et al., 2004). The cod family (*Gadus sp.*) is one of the largest families of fish, found in all oceans of the world. There are more than 90 species of cod, of which 40 are commercially available as oils. Even in the same waters, different species of the *Gadus* family can have a great variation in fatty acid profile (Wetzel, 2005) and vitamin A and D content.

Topically applied, cod liver oil contributes to faster wound healing and improvement in skin quality (Terkelsen et al., 2000). However, we recently reported a case of allergic contact dermatitis to cod liver oil contained in a topical ointment (Foti et al., 2007). The main allergen was unknown. The negative patch tests with vitamins A and D led us to exclude the role of these vitamins as responsible for the allergic reactions. Moreover we observed that the reaction with the topical ointment containing cod liver oil at a concentration of 40%, white wax and petrolatum, was really stronger (vesicular-bullous) (Fig. 1A) compared with the reaction induced by a pure cod liver oil (Fig. 1B). We hypothesized that the reactions could be due to TAGs, that have been reported (Laube et al., 2002, 2003) to have sensitizing properties, and that the different reactivity to the patch test could be explained by the different compositions of the two products, in particular in the TAGs content. In order to verify this hypothesis, we assessed the composition of those products using a matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), a technique that has emerged (Asbury

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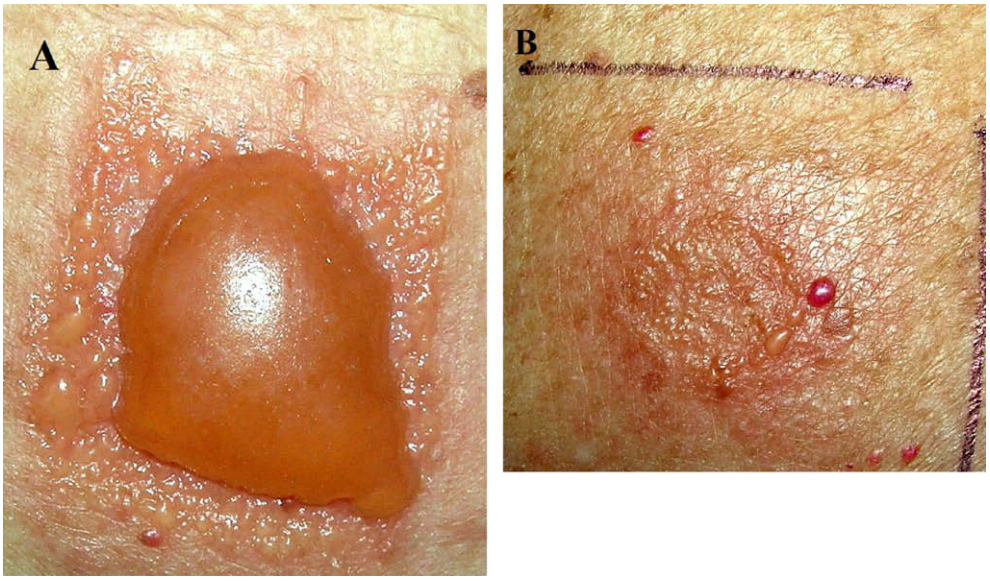

Fig. 1. (A) Vesico-bullous reaction to patch test with the cod liver oil based ointment; (B) vesicular reaction to patch test with pure cod liver oil.

et al., 1999; Ayorinde et al., 1999a,b,c; Ayorinde et al., 2000; Hlongwane et al., 2001; Jakab et al., 2002; Robins and Limbach, 2003; Al-Saad et al., 2003; Schiller et al., 2002a,b; Calvano et al., 2005, 2007) as a useful tool for different oils characterization, offering the advantages of fast and easy sample preparation without the need for analyte-derivatization.

2. Experimental

2.1. Chemicals

All solvents used (Sigma–Aldrich, St. Louis, MO, USA) were HPLC grade and were used without further purification. The matrices

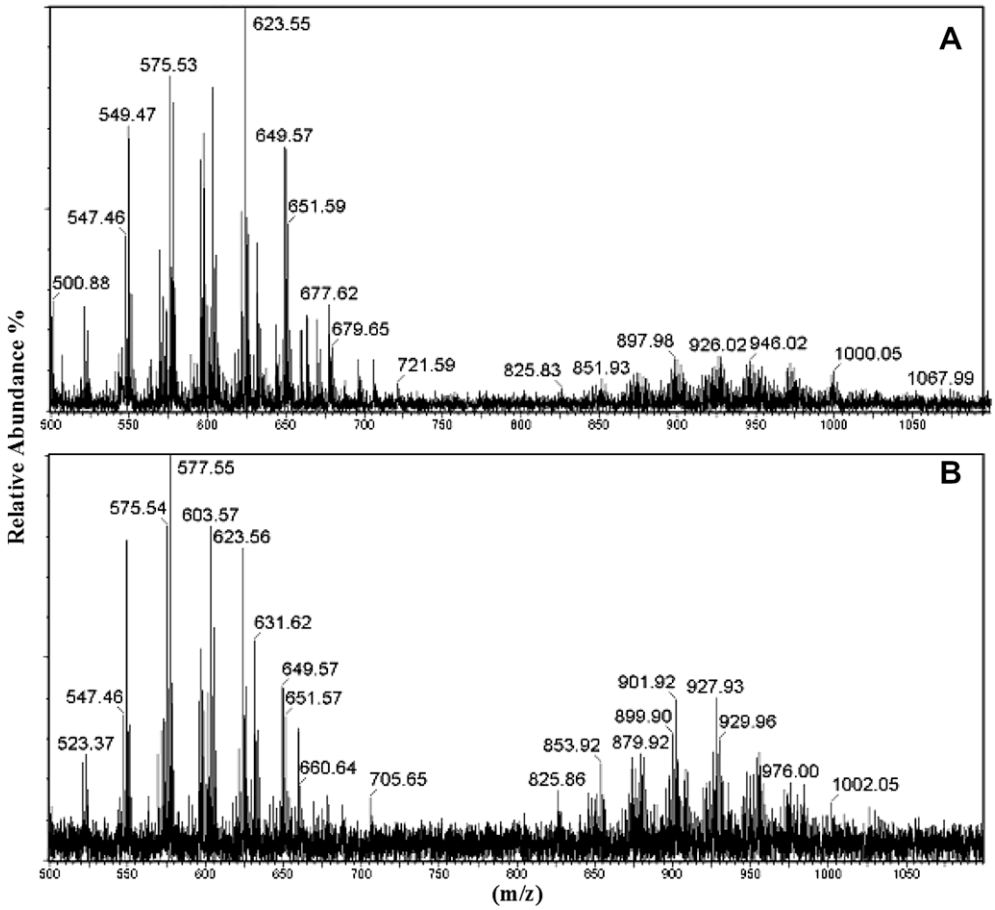

Fig. 2. MALDI-TOF-MS spectra obtained in the analysis of (A) a sample of the cod liver oil based ointment responsible for the reported allergic contact dermatitis, and (B) a sample of pure cod liver oil, both dissolved in pentane as described in the experimental section.