Applications
Fish Oil

Stability of fish oil against oxidation using the CL approach.

Introduction

Fish oil is recommended for a healthy diet because it contains the omega-3 fatty acids, eicosapentaenoic acid (EPA, 20:5(n-3)), and docosahexaenoic acid (DHA, 22:6(-3)), precursors to eicosanoids that reduce inflammation and produce anti-cancer effects throughout the body. Fish do not actually produce omega-3 fatty acids, but instead accumulate them by either consuming micro-algae that produce these fatty acids, as is the case with fish like herring and sardines, or, as is the case with fatty predatory fish, by eating prey fish that have accumulated omega-3 fatty acids from microalgae. Such fatty predatory fish like mackerel, lake trout, flounder, albacore tuna and salmon may be high in omega-3 fatty acids, but due to their position at the top of the food chain, these species can also accumulate toxic substances. For this reason, the FDA recommends limiting consumption of certain (predatory) fish species (e.g. albacore tuna, shark, and swordfish) due to high levels of toxic contaminants such as mercury, dioxin, PCBs and chlordane. There are vegetarian, DHA Omega-3 products made from algae available if toxic contaminants are of concern.

Some experts believe that taking fish oil (in any form) can help regulate cholesterol in the body, because fish oil has high levels of omega-3 fatty acids. The regulation occurs through effects of the EPA and DHA constituents on peroxisome proliferator-activated receptor alpha (PPAR).

Dietary DHA may reduce the risk of heart disease by reducing the level of blood triglycerides in humans. Low levels of DHA result in reduction of brain serotonin levels and have been associated with ADHD, Alzheimer's disease, and depression, among other diseases, and there is mounting evidence that DHA supplementation may be effective in combating such diseases.

The oxidation problem

The shelf life of food products and nutraceuticals is often limited by oxidative reactions. Food products that are rich in oils or fats are particularly prone to oxidation even in ambient atmosphere – they turn rancid. These changes lead to sensorial distortions (taste, odor, color) and ultimately, food products become inedible.

The food industry verifies on a daily basis the sensorial quality of ingredients. Because they are particularly sensitive, fats and oils are routinely tested for peroxide content and volatile compounds that arise during storage (commonly measured by the rancimat method). These methods allow an objective assessment of the products' current condition without relying on subjective sensorial impressions of the person carrying out the testing. However, these methods are unsuitable for the prediction of oxidative reactions. For this task, the chemiluminescence technology of ACL Instruments provides a valuable alternative.

The chemiluminescence associated with lipid peroxidation has found a widespread use in monitoring the oxidative stress. The main characteristics of this excited-state generation are typical for oxidation of unsaturated organic substances. Thus, the violet-green chemiluminescence from triplet carbonyls has been found in the oxidation of lipids. The formation of these excited species has been discussed in terms of both the Russel mechanism of the alkylperoxy-radical recombination and the intervention of dioxetane intermediates in the oxidation process. In polyun-saturated fatty acids, it is believed dioxetane intermediates may be formed by the cyclisation of alkylperoxy radicals produced during the peroxidation process or by the one-electron oxidation of polyunsaturated fatty acid derived hydroperoxides.

CL of fish oil oxidation

Below, the results of testing the stability of fish oil against oxidation, including the quantification of kinetic parameters, are reported. Fish oil from small deep-sea fish originating from the clean waters around Antarctica was tested. The samples tested are commercial supplements protected by Vitamin E (0.34% wt) and with an EPA:DHA ratio of 25.7%:12.2% (wt:wt), the total content of unsaturated fatty acids is 62.5% wt. The samples are pharmaceutical grade, purified with a patented CO_2 distillation process (hexane free) to remove heavy metals, dioxins and pesticides.

The data acquisition of fish oil oxidation was performed under isothermal conditions from 60°C to 80°C (Fig. 1) in synthetic air with a single channel basic instrument configuration.

ACL Instruments AG
Innovative Solutions for the
Characterization and Prediction of
Long Term Oxidation Reactions.

Industriestrasse 11
Postfach 10
3210 Kerzers
Switzerland

www.aclinstruments.com
welcome@aclinstruments.com
T +41 (0)31 755 46 66
F +41 (0)31 755 76 37

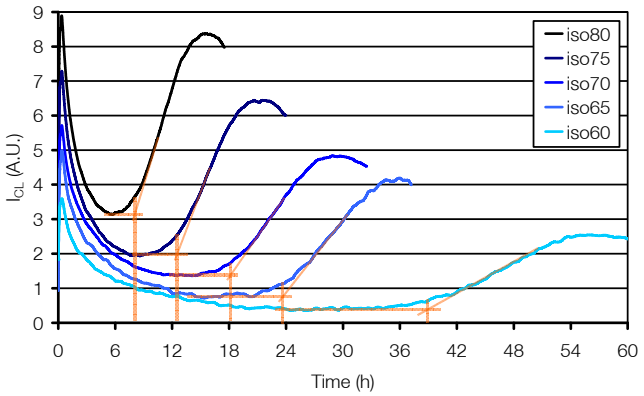

Fig. 1: Example of fish oil oxidation characterised using the chemiluminescence method at different isothermal temperature conditions (60..80°C, ΔT = 5°C) with synthetic air.

The end of antioxidant effectiveness results in characteristic change in the CL curves: refer to the oxidation onsets (vertical orange lines in Fig. 1). Kinetic parameters were calculated applying simple Arrhenius linearisation (Fig. 2) for the times up to the end of antioxidant activity (activation energy in the event of oxidation onset) and by applying differential isoconversional analysis (Fig. 3: activation energy as a function of the oxidation process).

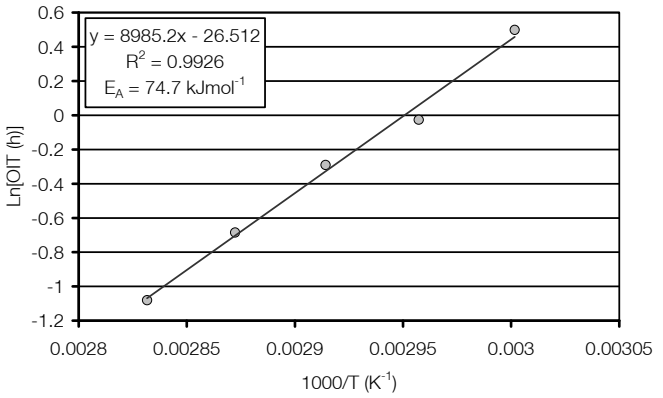

Fig. 2: Arrhenius plot depicting the parameterisation of the oxidation onset events by linear regression. The resulting activation energy refers only to the oxidation onset event (not for the whole oxidation reaction).

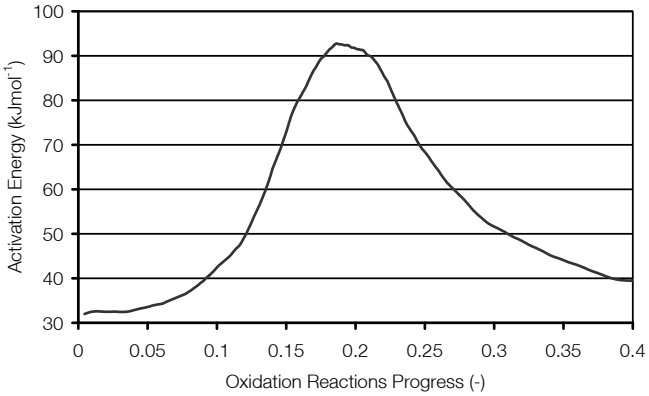

Fig. 3: Activation energy as a function of oxidation reaction progress resulting from differential isoconversional analysis.

Differential isoconversional kinetic analysis was performed using the AKTS-Thermokinetics software V3.00 (www.akts.com/).

Prediction of fish oil oxidation

The kinetic parameters of the oxidation process, calculated from the chemiluminescence's signals by means of the differential isoconversional method of Friedman, are subsequently applied for the simulation of the fish oil oxidation under different storage temperature profiles to assess the life time of fish oil products and supplements for any individual storage and/or application temperature conditions:

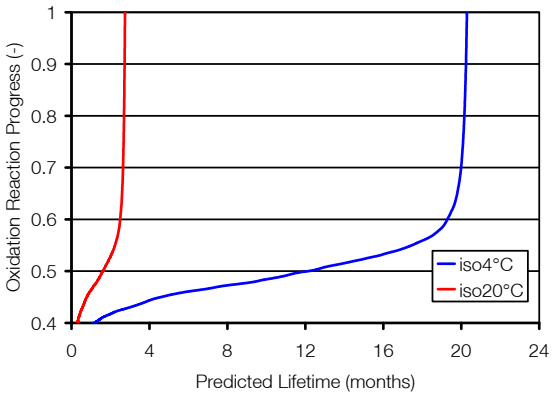

Fig. 4: Prediction of fish oil lifetime against oxidation when kept in the refrigerator (4°C, blue curve) and when kept at 20°C (red curve).