

Analysis of Ethoxyquin and its Metabolites in Salmon Using QuEChERS

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Introduction

Ethoxyquin (EQ) is a quinoline-based antioxidant, but it is still listed as a fungicide with pre- and post-harvest applications, mainly on pears, to reduce scald. It is also used in paprika powder and curcuma to reduce color loss due to oxidation of carotenoids.

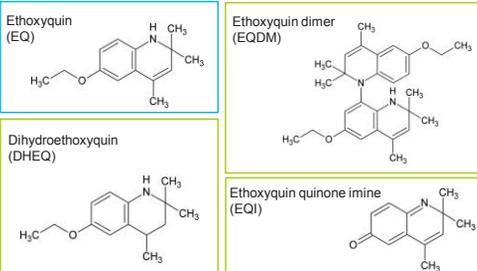
There are also numerous feed-related applications with indirect food relevance such as the use in fish feed to prevent lipid-oxidation.

Legal aspects

EQ is an **approved feed additive** in this field and is listed in the EU register on feed additives (Reg. 1831/2003/EC) as E324. It has a permissible level of 150 mg/kg in **fish feed** (Reg. 2316/1998/EC). **Fish** is not yet regulated by Reg. 396/2005/EC, so no MRLs exist at this time.

EQ and Metabolites

EQ degrades to a multitude of metabolites but only a few of them are commercially available as standards: Ethoxyquin-dimer (EQDM), 2,2,4-Trimethyl-quinolinon (EQI) and Dihydroethoxyquin (DHEQ). Some of these metabolites also exhibit antioxidant properties themselves. **EQDM is the most prominent metabolite in salmon** and as it is more stable than EQ it may serve as a **good indicator** of salmon fed with EQ-containing feed.



Analytical method

Our studies mainly focused on salmon, and extractions were done by the QuEChERS method using 5 g homogenate to reduce the influence of lipids on the recoveries. As in a previous study on pears (see analytical observation on Ethoxyquin, EURL-SRM website) **the impact of ascorbic acid (AA) was checked by adding it:**

- prior to cryogenic milling,
- prior to extraction by QuEChERS,
- to the final extract (no protection during milling and extraction)

The extracts were measured via LC-MS/MS (ESI-positive mode).

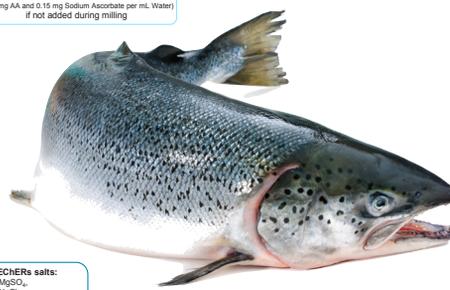
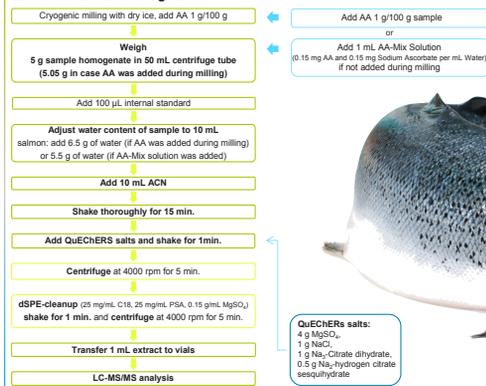
Method details: www.eurl-pesticides.eu



Summary

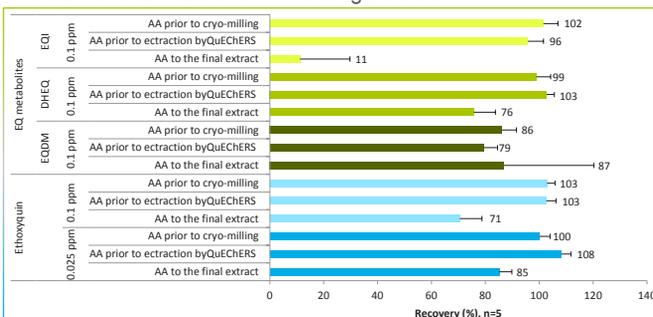
- On **wild salmon**, addition of AA showed a strong protective effect on EQI and a weaker effect on EQ and DHEQ. EQDM seemed unaffected.
- In **farmed salmon** the impact of AA on EQ extraction yields was minimal, but AA addition prior or during QuEChERS extraction is still recommended to be on the safe side.
- All tested samples of **farmed salmon** showed EQ and EQDM residues. EQDM levels were higher.

Extraction method at a glance:



Results

The recoveries for EQ and its metabolites depending on different variations of AA addition to wild salmon blank are shown in the figure below.



In recovery experiments on wild Atlantic salmon AA showed a **strong protective effect on EQI** (losses w/o AA ca 90%). **Protection of EQ and DHEQ was weaker** (losses w/o AA ca 25%). **EQDM was more stable** and not notably affected by the addition of AA.

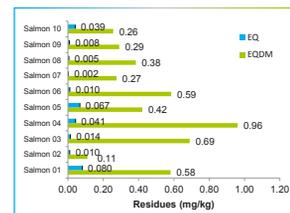
In contrast, the impact of AA on EQ extraction yields from farmed salmon was minimal. We assume that this is due to the high levels of other antioxidants added to fish feed and accumulating in farmed fish. To be on the safe side, addition of AA prior to QuEChERS extraction or even during cryogenic milling is still recommended.

Using AA in the extraction step, validation of EQ, EQDM, DHEQ and QI in wild salmon showed satisfactory recoveries and RSDs. Partitioning of EQDM (LogK_{ow} 6.2) into the lipid phase during QuEChERS resulted in lower recoveries.

Analysis of Samples from the Market

Several salmon samples and shrimp samples from the market were analyzed. All **farmed salmon** samples contained both EQ and EQDM, but not EQI and DHEQ. The concentrations of EQDM were significantly higher than those of EQ.

None of the **wild salmon** and **shrimps** samples contained EQ or its metabolites.



EPRW 2016



Baden-Württemberg