Quantification of wax ester content in escolar

Dr. Andreas Miller

The Bavarian Health and Food Safety Authority (LGL) analyses and assesses food samples in the framework of the official food control. The HPTLC method presented here has been developed in collaboration between Dr. Andreas Miller, LGL, and Prof. Dr. Gertrud Morlock of the Justus Liebig University Giessen.

1

Left: filets of escolar ("Buttermakrele"; Lepidocybium flavobrunneum); right: gunnel ("Butterfisch", Pholidae)

Introduction

The fish species escolar (*Lepidocybium flavobrunneum*) is offered to the consumer as filets for roasting/barbecue as well as ready-to-eat smoked fish filets. Due to the high fish oil content (ca. 18–21 %) escolar exhibits a very juicy and delicious flesh. However, this fish oil consists of indigestive wax esters (> 90 %) which can lead to acute gastro-intestinal symptoms when products of this fish species are consumed.

The wax esters are carboxylic esters consisting of long-chain fatty acids esterified to fatty alcohols. Gastro-intestinal symptoms include cramps and oily diarrhea from the undigested wax esters. Individuals differ in their sensitivity to the consumption of escolar [1]. According to European legislation, food business operators have to inform the consumer about the risk of developing gastro-intestinal symptoms when offering escolar or other fish species belonging to the family *Gempylidae* [2]. Nevertheless, escolar is frequently offered without this mandated information or it is put on the market under a wrong trade name. Especially in sushi restaurants, escolar is offered not under its correct German trade name "Buttermakrele" but as "Butterfisch". However, "Butterfisch" is the German trade name for *Peprilus spp., Poronotus spp.* and *Psenopsis spp.* These fish species do not contain indigestible wax esters and therefore do not require a warning.

A cost-efficient and reliable HPTLC method with uncomplicated sample preparation has been developed for the rapid control of this fish species by determination of the indigestible wax esters [3]. The fish sample is simply homogenized and extracted. Quantification is performed after selective derivatization with the Rhodamine B reagent and subsequent fluorescence measurement.

Sample preparation

Homogenized fish (0.5 g) was mixed with 3 mL *n*-hexane for 2 min using a vortex. After centrifugation the supernatant was collected in a 10 mL volume flask. This extraction process was performed three times and the volume flask containing the three extracts was filled-up with *n*-hexane.

Standard solution

Stearyl stearate and oleyl oleate (100 mg each) were dissolved together in *n*-hexane and filled up ad 10 mL. This stock solution was diluted 1:10 with *n*-hexane (1 µg/µL).

Layer

HPTLC plates silica gel 60 (Merck), 20 x 10 cm

Sample application

Bandwise with Automatic TLC Sampler (ATS 4), band length 6.0 mm, distance from the lower edge 8.0 mm, distance from the left side 13.0 mm, application volume 2.0–15.0 µL for standard solution and 2.0 µL for extracts

Chromatography

In the twin-trough chamber with *n*-hexane – toluene 7:3 after 10 min pre-saturation (using wetted filter paper), migration distance 60 mm (ca. 13 min).

Postchromatographic derivatization

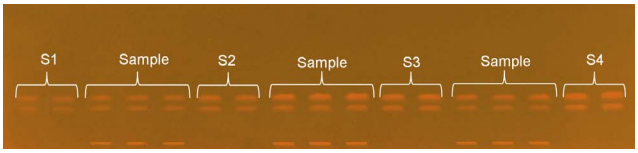
Dipping in aqueous Rhodamine B reagent (0.025 %) using the Chromatogram Immersion Device; detection can be improved by using the 5-fold more sensitive Rhodamine 6G reagent [4].

Documentation and densitometry

With TLC Visualizer under UV 366 nm, densitometry using TLC Scanner 4 and winCATS, fluorescence detection at 366/>400 nm (Hg lamp)

Results and discussion

In preliminary tests gas chromatography (GC) had been used to analyze sample extracts. GC separated the class of wax esters into several individual peaks. However, signal intensity in the flame ionization detector (FID) was influenced by the structure of the individual wax ester: oleyl oleate exhibited ca. 1.8-fold larger peak area compared to stearyl stearate. Therefore, quantification of the wax ester content by GC-FID would only be possible, if individual wax ester peaks in the sample are identified and each wax ester is quantified individually. Using the described HPTLC method oleyl oleate (hR_F 30) and stearyl stearate (hR_F 40) exhibited nearly identical signal intensities. Slightly different chemical structures and chain lengths have little effect on the derivatization with Rhodamine B reagent and consequently on the signal intensities [4]. Therefore, quantification of the wax ester content is possible although analytical standards may not be available for each individual ester. The content of wax esters can be quantified via stearyl stearate or oleyl oleate. After derivatization with Rhodamine B reagent wax esters exhibit an orange fluorescence at UV 366 nm. The mean recovery rate (104 % ± 3 %) was determined by analyzing salmon samples spiked to contain 20% wax ester (3-fold analysis on one day and repetition on another day).



2

HPTLC chromatogram under UV 366 nm of wax esters in salmon samples spiked to contain 20 % wax esters (n = 3) and standard solutions (S1–S4) of oleyl oleate (hR_F 30) and stearyl stearate (hR_F 40) after derivatization with Rhodamine B reagent

The three-fold repeated analysis of an escolar sample revealed wax ester contents of 18–22 %. This was in accordance to literature [1]. The repeatability of each analysis was below 5 % and the mean laboratory precision was 3 %. In the framework of official food control this HPTLC method enables the economic analysis of routine samples due to its high sample throughput (parallel analysis of up to 20 samples). This rapid HPTLC method may help to identify the cause of gastro-intestinal symptoms occurring after fish consumption.

Thanks to Dr. Iris Kraemer and Dr. Ingrid Huber (identification of fish species) as well as to Dr. Ulrich Schwank (GC).

[1] The EFSA Journal 92 (2004) 1

[2] Verordnung (EG) Nr. 853/2004

[3] G.E. Morlock, L. Winheim, A. Miller, in submission

[4] H. Jork, W. Funk, W. Fischer, H. Wimmer, Dünnschicht-Chromatographie, VCH Verlagsgesellschaft, Weinheim 1989

Further information is available on request from the authors.

Contact: Dr. Andreas Miller, Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Veterinärstr. 2, 85764 Oberschleissheim, Germany, andreas.miller@lgl.bayern.de and Prof. Dr. Gertrud Morlock, Justus Liebig University Giessen, 35392 Giessen, Germany, gertrud.morlock@uni-giessen.de