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ORIGINAL PAPER

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# Liquid chromatographic quantification of synthetic colorants in fish roe and caviar

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**Abstract** According to the directive 94/36/EC of the European Union (EU), quantities of synthetic colorants added to foods are restricted by upper limits and, therefore, reliable methods for their quantification have to be established. Approved colorants, defined by so-called E numbers, are permitted for dying fish roe (commonly named caviar). We developed a chromatographic method for the quantitation of colorants in roe. The recovery rates of 14 synthetic food colorants from solid materials ($Al_2O_3$, XAD-2, anion exchangers, and polyamide-6) were tested, and polyamide powder was selected as adsorbent for quantitative determination of colorants in fish roe. The most effective sample preparation comprises extraction of colorants from roe with 1 M aqueous ammonia, defatting of the solution with *n*-hexane, adjustment of pH 2 of the extract, adsorption of dyes on the polyamide and desorption with a mixture of aqueous ammonia (25%) and methanol (1:9 v/v). The isolated colorants were analyzed by RP-HPLC with diode-array detection. In several caviars, the maximum of individual colorants regulated by EU were exceeded or colorants declared on food labels were not detected.

**Keywords** RP-HPLC · Sample preparation · Adsorbents · Food analysis · Colorants (dyes) · Fish roe

## Introduction

Caviar is defined as a food product made from fish eggs (ovaries), commonly called roe. Genuine caviar represents exclusively the roe of the sturgeon (family *Acipenseridae*). The three main types of caviar are the pale silver-gray to grayish white beluga (from *Huso huso*), the gray to brownish gray osetra (from *Acipenser gueldenstaedti*, *Acipenser persicus*, or *Acipenser nudiventris*) and the gray sevruga (from *Acipenser stellatus*). These caviars, if salted, are always labeled 'malossol' [1].

Since genuine caviar has always been a precious delicacy, and wild sturgeons became exceedingly rare owing to overfishing together with poaching, change of the habitat, and environmental pollution, the roe of other fishes is intensively used for similar products due to egg size, texture, and flavor. Such products are eaten principally as garnish or spread, as with hors d'oevres. In most countries, the name of these products has to be combined with the name of the fish, e.g. red trout caviar (from *Salmo trutta*), lumpfish/lumpsucker caviar from *Cyclopterus lumpus* represents tiny, hard, black eggs, and pale orange to deep red salmon caviar from *Onchorhynchus* spp. (e.g. *Onchorhynchus keta*, *Onchorhynchus masou*) [1, 2]. In Europe, the name of a country has to be added resulting in products named 'Deutscher Kaviar' from roe of lumpfish. 'Capelin Caviar' from Iceland and other countries is roe from the fish capelin (*Mallotus villosus*).

In order to increase the acceptance by the consumer and to standardize the products, fish roe is frequently colorized with single or mixed synthetic food colorants resulting in black, red, or orange appearance of the products. For colorizing caviar products only approved colorants are allowed and their presence must be stated on the food label by name or a coded abbreviation (E number) in order to be unequally identified.

Since directives of the European Union regulate application fields and maximum quantities of individual food colorants, it has been stressed that reliable methods for their quantitative determination have to be established [3]. This is not an easy task, as colorants stick tightly to complex food matrices, the components of which also interfere with chemical analyses.

For coloring fish roe, synthetic organic colorants are used preferably. According to directive 94/36/EU of the

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European Union, addition of maximal 300 mg of an approved food colorant per kilogram fish roe is allowed (with the exception of E 123, Amaranth, were 30 mg/kg roe are permitted). The major colorant groups used for colorizing fish roe are the azo dyes with one or two azo groups in the molecule connecting two or three sulfonated aromatic ring systems. The following azo dyes are permitted in the European Union (E numbers in parentheses; for structures see Fig. 1): Tartrazine (E 102), Sunset Yellow FCF (E 110), Azorubine (E 122), Amaranth (E 123), Cochineal Red (E 124), Red 2G (E 128), Allura Red AC (E 129), Brilliant Black BN (E 151), Brown FK (E 154) and Brown HT (E 155) [4, 5]. The group of triarylmethane colorants is represented by Patent Blue V (E 131), Brilliant Blue FCF (E

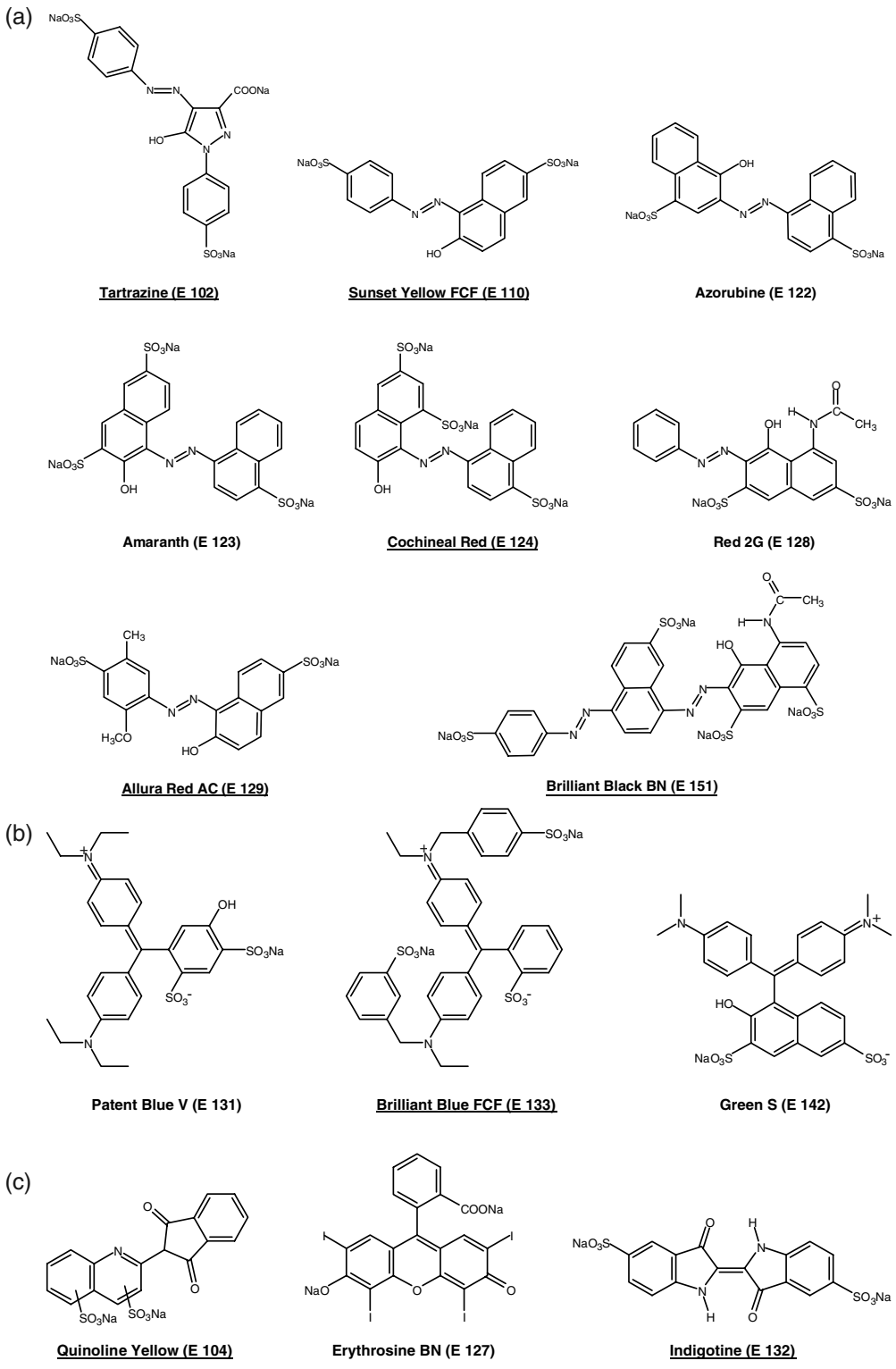


**Fig. 1** Chemical structures, common names and E-numbers of frequently used synthetic food colorants belonging to the groups of (**a**) azo dyes, (**b**) triphenyl(aryl)methane dyes, and (**c**) quinophthalone, xanthene and indigo dyes. Names of dyes underlined have been detected in colorized fish roes

133), and Green S (E 142). These dyes contain three substituted aryl residues covalently linked to the central carbon atom. Other classes of food colorants are chinophthalon- (Quinoline Yellow, E 104), xanthene- (Erythrosine BS, E 127), and indigo dyes (Indigotine, E 132). Notably, for the colorization of fish roe frequently mixtures of some of the synthetic dyes mentioned earlier are used. A bright black color is achieved by mixing e.g. E 104, E 110, E 132, and E 151.

For analysis, food colorants usually have to be extracted quantitatively from solid food matrices. Depending on the kind of food and chemical structures of colorants used, simple extraction with water [6], ion-pair extraction [7, 8], or adsorption on solid materials such as wool threads [9], alumina [10], octadecylsilyl silica [2], or polyamide [2, 11–13] have been described. In the latter cases desorption of colorants using mixtures of solvents makes possible their analysis.

Quantitative analysis of colorants resulting from these procedures can be performed along various routes. Spectrophotometry allows simultaneous quantitative analyses of mixtures of colorants having similar absorption spectra [14–16]. The use of high-performance liquid chromatography (HPLC) was also reported for quantification of colorants. Ion-exchange HPLC [17], ion-pair reversed-phase chromatography [18–20] or reversed-phase chromatography [18, 21–24] have been described. Recently, capillary zone electrophoresis [25, 26] and micellar electrokinetic capillary chromatography [27, 28] have been shown to be suitable for analysis of colorants.

In previous work, we had developed a method for the complete liquid chromatographic separation and diode-array detection of standard mixtures of 14 most frequently used synthetic colorants [24]. In continuation of this work here we present data on testing various materials for their suitability on adsorbing food colorants. Here we present a reliable method for the effective isolation of colorants added to fish roe using polyamide powder as adsorbent and, after desorption, for their HPLC separation, diode-array detection and characterization.

## Materials and methods

### Chemicals, solvents, and adsorbents

Sodium hydroxide (NaOH), potassium hydroxide (KOH), acetic acid (AcOH), aqueous ammonia (25% w/v), and hydrochloric acid (32% w/v) were purchased from Carl Roth. Acetonitrile (MeCN), methanol (MeOH), *n*-hexane, and sodium acetate (NaOAc) trihydrate were from Merck (Darmstadt, Germany). Dowex MWA-1 (free base, particle size 35–75 mesh), Dowex 1X8 ($Cl^-$-form, particle size 200–400 mesh), and acidic alumina (type 504 C acidic) were purchased from Fluka and XAD-2 adsorber (particle size 0.3–1.0 mm) was purchased from Serva (Heidelberg, Germany). Various batches of polyamide-6 powder for column chromatography were purchased from Carl Roth (particle size 0.05–0.16 mm, named "polyamide A"), Fluka (product no. 02395, named "polyamide B") and Macherey-Nagel, Düren, Germany (product SC 6, particle size 0.05–0.16 mm, designated as "polyamide C"). Demineralized and doubly distilled water from a quartz distill was used exclusively.

Standard solutions of individual food colorants were prepared by dissolving 10 mg of crude colorants in 1 ml water. Multicomponent standard mixtures were prepared therefrom by mixing appropriate amounts and dilution with water. Quantities of pure dyestuffs in crude colorants (containing anorganic salts) had been determined as described previously [24].

### Sources of colorants

Tartrazine (E 102), Quinoline Yellow (E 104), Allura Red (E 129), and Brilliant Blue FCF (E 133) were donations from Cosnaderm Company (Ladenburg, Germany); Azorubine (E 122), Amaranth (E 123), Cochineal Red (E 124), Erythrosine (E 127), Patent Blue V (E 131), and Brilliant Black (E 151) were supplied by BASF (Ludwigshafen, Germany); Brilliant Black BN (E 151) and Green S (E 142) were purchased from Sigma (Steinheim, Germany); Red 2G (Azophloxine, E 128) was from Fluka (Neu-Ulm, Germany), and Sunset Yellow (E 110) was from Carl Roth (Karlsruhe, Germany). For structures and common names of these compounds see Fig. 1.

### Instruments and chromatography

For HPLC a HP 1100 series comprising a Model G1322A degasser, G1311A pump with low-pressure gradient-former, G1313A auto sampler, G1316A column thermostat, G1314A diode-array detector (DAD) with 13 µl flow cell, and software HP ChemStation for LC (Rev. A.04.02) were used (all from Agilent, Waldbronn, Germany or Palo Alto, CA, USA).

For chromatography, a Purospher RP18e column (125 mm × 4 mm internal diameter, particle size 5 µm; from Merck, Darmstadt, Germany) and a binary gradient consisting of 100 mM sodium acetate buffer (pH 7.0) and acetonitrile were used at a column temperature of 50 °C. The sodium acetate buffer (100 mM) was prepared by dissolving NaOAc × $3H_2O$ (13.6 g) in 900 ml water and titration to pH 7.0 by addition of 0.1 M HCl. The buffer was filled up to 1 l and filtered through a membrane (regenerated cellulose RC55, 0.45 µm, Schleicher and Schüll, Dassel, Germany). Details of gradient program and detection parameters are described in [24]. The colorants were determined at wavelengths 430 nm (E 102, E 104), 486 nm (E 110), 520 nm (E 122, E 123, E 124, E 127, E 128, E 129), and 608 nm (E 131, E 132, E 133, E 142, E 151).

### Determination of recovery rates of colorants from adsorbents and ion-exchangers

Recovery rates of colorants were determined in two experiments.

*Experiment* 1. Amounts of crude colorants (2.5 mg) in water (10 ml) were prepared, aliquots (20 µl) injected directly into an HPLC instrument and resulting peak areas recorded at maximum absorbance.

*Experiment* 2. Solutions of crude colorants (2.5 mg) in water (10 ml) were adjusted to pH 2 by addition of 1 M HCl. In separate experiments, 2 g of adsorbents (XAD-2, polyamide (various batches), or aluminium oxide were added and the mixtures gently stirred at 40 °C for 20 min. Then solids were removed by filtration. The treatment of filtrates with the solid phase was repeated until the filtrate was colorless or no further adsorption took place. Adsorbents were combined and desorption of colorants was accomplished with a mixture of MeOH and aqueous ammonia (25% w/v) in the ratio 9/1 (v/v). The colored eluate was concentrated in vacuo, a final volume of 10 ml adjusted by addition of water and 20 µl aliquots were analyzed using RP-HPLC [24]. Recovery rates (RR) were calculated from the corresponding peak areas (A) of experiment 2 ($A_2$) and experiment 1 ($A_1$) according to the equation

$$RR(\%) = 100\,(A_2/A_1)$$

For determination of the standard recovery ranges after anion-exchange chromatography, the activated Dowex 1X8 or Dowex MWA was filled into a glass column (3 cm length × 1 cm inner diameter) and conditioned with 1 M ammonia solution. The colorant standards were dissolved in 200 µl 1 M ammonia solution and applied onto the column. Then the column was washed with water until the effluent was neutral. Colorants were eluted using 5 M HCl until the eluate was colorless. The eluate was concentrated using a vacuum evaporator and transferred into a volumetric flask and made up to 10 ml. Aliquots of 20 µl were analyzed with RP-HPLC.

### Sources of fish roes and caviar

Foods were commercial products purchased from retail outlets. For details of samples and manufacturers declaration on labels see Table 1.

### Analysis of fish roes and caviar

To samples of colorized fish roes and caviar (5 g) amounts of 1 M aqueous ammonia (20 ml) were added. The mixtures were sonificated for 10 min, centrifuged at 3,500×*g*, and the aqueous layer was collected. The extraction step was repeated until the resulting aqueous layer was colorless. The combined aqueous phase was defatted by three-fold treatment with *n*-hexane (10 ml) and adjusted to pH 2 by addition of 1 M HCl. Then polyamide (2 g) was added and the mixture stirred gently at 40 °C for 20 min. Solids were removed by filtration and the procedure was repeated until the solution was colorless. Colorants were desorbed using a mixture of ammonia solution (25%) and methanol in the ratio (1/9 v/v). The colored liquid was concentrated by a vacuum evaporator and analyzed using RP-HPLC [24].

## Results and discussion

### Selection of adsorbent

The recovery rates of food colorant standards following food treatment with alumina, XAD, ion exchangers and polyamide powder are presented in Table 2.

For testing recoveries from $Al_2O_3$ as adsorbent selected colorant standards were used. The test of recovery rates

**Table 1** Characterization and colorants declared for caviar samples A–Q

| Sample (color) | Characterization | Colorants declared |
|---|---|---|
| A (gray) | Sevruga Malossol caviar (Caspian caviar) from sturgeon (*Acipenser sturio* L.) | No colorants declared |
| B (orange) | Caviar from roes of trout (*Salmo trutta* L.), not colorized | No colorants declared |
| C (black) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 110, E 151 |
| D (black) | Icelandic caviar from roes of Capelin (*Mallotus villosus* Muller) | E 102, E 110, E 151 |
| E (black) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 102, E 110, E 151 |
| F (black) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 102, E 110, E 151 |
| G (black) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 102, E 110, E 151 |
| H (dark blue) | Caviar from roes of trout (*Salmo trutta* L.) | E 102, E 129, E 133 |
| I (black) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 104, E 110, E 132, E 151 |
| J (black) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 104, E 110, E 132, E 151 |
| K (black) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 104, E 110, E 132, E 151 |
| L (black) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 104, E 110, E 132, E 151 |
| M (black) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 104, E 110, E 132, E 151 |
| N (black) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 104, E 110, E 132, E 151 |
| O (black) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 104, E 110, E 132, E 151 |
| P (red) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 124 |
| Q (green) | German caviar from roes of lumpfish (*Cyclopterus lumpus* L.) | E 104, E 122, E 142 |

**Table 2** Recovery rates (%) for standards of food colorants treated with anion exchangers and adsorbents (average of two independent experiments)

| E number[a] | Dowex MWA-1 | Dowex 1X8 | $Al_2O_3$ (acidic) | XAD | Polyamide A | Polyamide B | Polyamide C |
|---|---|---|---|---|---|---|---|
| 102 | 60.5 | 27.8 | 1.7 | 32.3 | 102.7 | 89.6 | 88.9 |
| 104 | 25. 9 | 38.9 | 58.1 | 61.9 | 104.6 | 96.6 | 93.3 |
| 110 | 72.8 | 40.8 | 34.9 | 44.0 | 96.8 | 87.1 | 86.0 |
| 122 | * | * | 35.0 | * | 96.1 | 86.7 | 77.4 |
| 123 | 16.9 | 31.9 | 0.2 | 13. 9 | 105.0 | 77.9 | 78.2 |
| 124 | * | * | 0.4 | * | 92.5 | 80.7 | 82.8 |
| 127 | 57.8 | 64.1 | * | * | 93.0 | 61.7 | 70.7 |
| 128 | * | * | 17.1 | * | 89.8 | 77.1 | 88.3 |
| 129 | * | * | 18.6 | * | 96.8 | 81.2 | 93.2 |
| 131 | * | * | 3.1 | * | 50.8 | 76.4 | 92.7 |
| 132 | 64.0 | 37.0 | 1.3 | 11.9 | 93.9 | 49.8 | 83.6 |
| 133 | 58.5 | 30.2 | 7.4 | 14.4 | 93.5 | 88.1 | 80.6 |
| 142 | * | * | * | * | 44.2 | 85.9 | 58.0 |
| 151 | 62.6 | 79.3 | 1.0 | * | 109.8 | 114.3 | 85.4 |

"Polyamide A" from Carl Roth Company; "polyamide B" from Fluka; "polyamide C" from Macherey & Nagel. Relative standard deviations between thee experiments range from 2.8 to 15.9% (ion exchangers), 2.2 to 16.3% ($Al_2O_3$ and XAD), and 1.7 to 11.4% (polyamide A, B, and C)

[a]For common names and structures see Fig. 1

*Recovery rates not determined

on XAD, Dowex MWA-1 and Dowex 1X8 was performed with characteristic examples of colorant standards: E 102 (yellow azo dye), E 104 (yellow chinophthalon dye), E 110 (orange azo dye), E 123 (red azo dye), E 127 (red xanthene dye, recovery tests not performed at $Al_2O_3$ and XAD), E 132 (blue indigo dye), E 133 (blue triphenylmethane dye) and E 151 (black azo dye, recovery tests not performed with XAD).

Highest recovery rates of colorants were found using sample treatment with polyamide, with the exceptions of E 127 (Erythrosine) and E 132 (Indigotine). Notably, the recovery rates of the colorants from polyamide depend on the manufacturers for the adsorbent as well as on batches supplied by the manufacturer (Table 2). In particular, colorants E 123, E127, E131, E132, and E 142 give varying recoveries. Therefore, determination of recovery rates of each polyamide batch used is prerequisite for quantification.

Because of the complex matrix of caviar, isolation of colorants using selective solid adsorbents or ion exchangers before HPLC analysis is necessary. Otherwise caviar constituents would decrease the life time of the analytical HPLC columns drastically. In order to optimize the isolation of the colorants from food, treatment with adsorbents of different chemical structures and ion exchangers as well as various elution systems were tested. Alumina is an anorganic oxide, XAD represents polystyrene which is crosslinked with divinylbenzene, polyamide-6 is polycaprolactam (perlon, nylon-6), whereas Dowex MWA and Dowex 1X8 represent weakly basic and strongly basic anion exchangers, respectively.

The use of alumina [10] and polyamide [2, 11–13] for isolation of synthetic colorants has been described in the literature. However, no reports related to the aim of our work came to our attention of the use of XAD or ion exchangers for the isolation of synthetic food colorants. In order to evaluate their suitability they were included in this study.

In this work, the polyamide supplied by Carl Roth Company ("polyamide A") was selected as adsorbent. This polyamide provided highest recovery rates for most colorants (exceptions are E 151, E 142, and E 131). It was observed that the pH after acidification plays an important role for the kinetics of adsorbance of colorants on polyamide. Adsorbance starts below pH 8, and is most complete at pH < 2.5 [13]. Thus, pH 2 of the analyte was adjusted before adding polyamide. Best results for desorption of the colorants were obtained with a mixture of MeOH and 25% aqueous ammonia (9/1, v/v).

## Confidence of characterization of colorants

Using polyamide as selective colorant adsorbent, disturbing matrix components of fish roe could be removed. It was observed that the resulting peaks in HPLC were better resolved than those without the use of polyamide. This enables more precise integration and, thus, quantification of peak areas.

The use of an HPLC method with diode-array detection for determination of the colorants allows the identification of the colorants by comparison of the retention times as well as absorption spectra using wavelengths of 430 nm for yellow, 486 nm for orange, 520 nm for red, and 608 nm for blue, green and black colorants. Identity of colorants can be further confirmed by applying DAD software and special algorithms to spectra. A chromatogram of a standard mixture of the colorants investigated is shown in [24].

The quantification of colorant concentrations often causes problems owing to the presence of sodium salts in standards. Our colorant standards had different grades of purity, ranging from 66.0 to 99.0% with the exception of the colorant E 131 (48.3%). The grades of purity of colorants can differ considerably depending on the supplier [24]. Further, some colorants are mixtures of chemical congeners. The colorants E 142 (two peaks) and E 104 (four or five peaks, depending on manufacturer) are showing more than one signal in HPLC. The multicomponent colorant Quinoline Yellow (E 104) consists of bissulfonated and monosulfonated components. Here, for quantification the sum of the peak-areas was used. This

fact makes an accurate quantification difficult, in particular for E 104. Notably, treatment of fish roes with polyamide caused elution of two well—separated and narrow peaks of E 151, whereas omitting of treatment resulted in elution of a single broad peak of this colorant.

## Results of the analysis of fish roes and caviar

Analyses of the Caspian caviar and roes of other fishes provided the following results (Table 3). All quantities are based on wet material and are calculated in milligrams of the pure dye in 1 kg of the ready-to-serve food. The calculation of the concentrations of the colorants was performed with the external standard method. All calibration curves were calculated as described in [24]. The final concentrations ($c_{corr}$) resulted from concentrations calculated from the calibration curves ($c_{calc}$) corrected with the recovery rate (RR) of standards:

$$c_{corr} = 100(c_{calc}/RR)$$

For the genuine Sevruga Malossol caviar (sample A), representing the most expensive caviar, no colorant was declared on the label. Indeed, neither the analysis after sample treatment with polyamide, nor comparative analysis without polyamide indicated the presence of any synthetic food colorants. Analogously, in trout caviar (sample B) colorants were neither declared nor detected.

**Table 3** Concentrations (milligram of the pure dye in 1 kg of the ready-to-serve food) of colorants in fish roe and caviar samples

| Sample | Concentration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | E 102 | E 104 | E 110 | E 124 | E 129 | E 132 | E 133 | E 151 |
| A[a] | | | | | | | | |
| B[a] | | | | | | | | |
| C | n.d. | | 368 ± 19 | | | | | 322±19 |
| D | 76 ± 4 | | 354 ± 17 | | | | | 591±26 |
| E | 140 ± 8 | | 45 ± 8 | | | | | 545±17 |
| F | 76 ± 6 | | 47 ± 7 | | | | | 177±12 |
| G | 199 ± 9 | | 26 ± 3 | | | | | 357±18 |
| H | | | | | 531±16 | | 223±23 | |
| I | | 156 ± 12 | 133 ± 10 | | | n.d. | | 470±22 |
| J | | 138 ± 11 | 61 ± 7 | | | n.d. | | 368±18 |
| K | | 94 ± 11 | 128 ± 6 | | | n.d. | | 600±36 |
| L | | 13 ± 3 | 22 ± 1 | | | n.d. | | 156±13 |
| M | | 19 ± 3 | 34 ± 2 | | | n.d. | | 219±16 |
| N | | 45 ± 6 | 68 ± 5 | | | 0.3±0.1 | | 485±11 |
| O | | 319 ± 14 | 78 ± 5 | | | 0.4±0.1 | | 472±13 |
| P | | | | 1548±66 | | | | |
| Q | | see text | | | | | | |

Data are average values of double analyses after sample treatment with polyamide with deviations of the single results

[a]No colorant declared; n.d., declared but not detected

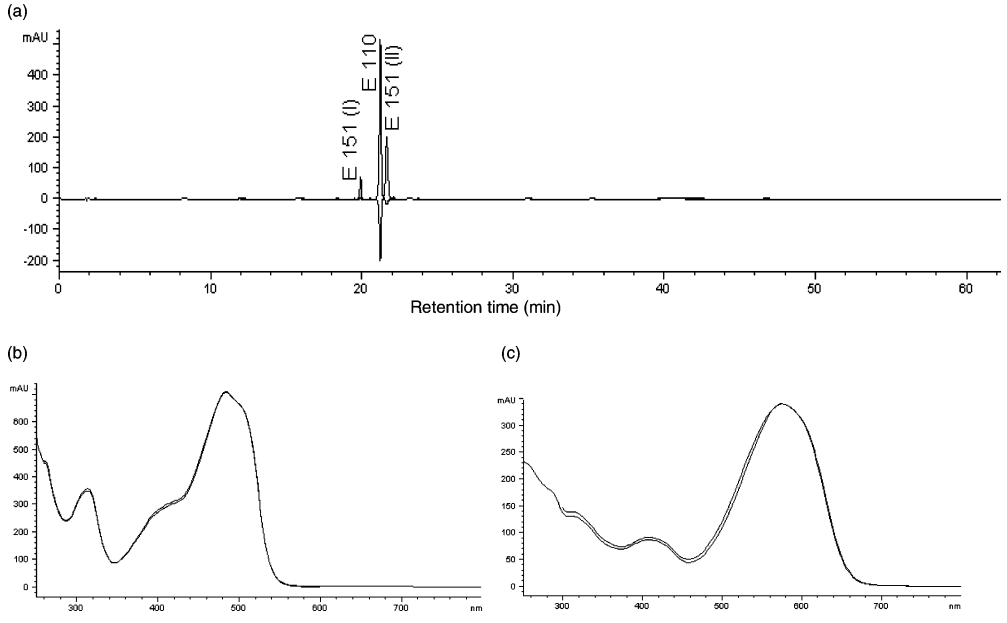


**Fig. 2** (**a**) HPLC of black colorized fish roe (sample C) recorded at 486 and 608 nm; (**b**) superimposed spectra of the peak eluting at 20.7 min and of standard colorant E 110 (Sunset Yellow FCF); (**c**) superimposed spectra of the peak eluting at 21.3 min of standard colorant E 151 Brilliant Black BN), *upper curve* represents the standard colorant

In German caviar from lumpfish (sample C) the colorants E 110 and E 151 were declared and detected. In Fig. 2 the resulting chromatogram and comparisons of spectra of the peaks with colorant standards are shown. After sample treatment with polyamide, presence and quantities of E 110 and E 151 were determined to 368 and 322 mg/kg. These colorant concentrations exceeded the upper limits of 300 mg/kg of the European Union reglementation.

For Capelin caviar (sample D), colorants E 102, E 110, and E 151 were declared. Whereas 354 mg/kg (E 110) and 591 mg/kg (E 151) were detected and their maximum allowed concentrations are exceeded, colorant E 102 was not analyzed in this sample. This indicates either false declaration or very low contents (<0.05 mg/kg).

In the samples E, F, and G (German caviars from lumpfish) the colorants E 102, E 110, and E 151 were declared and determined. The quantities ranged from 76 to 140 mg/kg (E 102), 26 to 47 mg/kg (E 110), and 177 to 545 mg/kg (E 151). Nevertheless, the content of E 151 in the samples E and G exceeded the upper limits of EU reglementations.

For German caviar from trout (sample H), colorants E 102, E 129, and E 133 were declared and detected. Quantities of 199 mg/kg (E 102), 531 mg/kg (E 129), and 223 mg/kg (E 133) were analyzed. The colorant E 129 exceeded the German legal limit of 300 mg/kg. A second analysis of this sample omitting sample preparation with polyamide adsorption was performed. The colorant contents analyzed were 194 mg/kg (E 102; 199 mg/kg with polyamide adsorption), 541 mg/kg (E 129; 531 mg/kg with polyamide adsorption), and 259 mg/kg (E 133; 223 mg/kg with polyamide adsorption). The data resulting from using polyamide for sample preparations are comparable to those where polyamide had been omitted. Peaks resulting from sample work-up without treatment with polyamide are broad and less precise to integrate. Further, life time of HPLC columns are drastically shortened. Therefore, sample preparation with polyamide is recommended for analyzing colorants in fish roe.

For the samples I–O (German caviars from lumpfish) the colorants E 104, E 110, E 132, and E 151 were declared. The contents of colorants ranged from 13 to 319 mg/kg (E 104), 22 to 133 mg/kg (E 110), n.d. to 0.4 mg/kg (E 132), and 156 to 600 mg/kg (E 151). In the samples I, J, K, N, and O, the concentration of E 151 exceeded the maximum limits allowed by the EU for colored products of fish roe (300 mg/kg). Only the colorant contents of the caviars L and M were within the legal concentrations. Notably, in samples I–M, the declared colorant Indigotine (E 132) could not be detected. This indicates either false declaration or complete decomposition of this colorant known to be relatively unstable [5, 29]. In sample J, a second analysis omitting sample preparation with polyamide adsorption was performed. The colorant quantities omitting polyamide were 114 mg/kg (E 104 compared to 138 mg/kg with polyamide adsorption), 56 mg/kg (E 110; 61 mg/kg with polyamide adsorption), and 368 mg/kg (E 151; 368 mg/kg with polyamide adsorption). The data using polyamide for sample preparations are comparable to those obtained without polyamide adsorption. The colorant E 132 could not be analyzed after treatment with or without polyamide sample preparation.

For the red colored German caviar from lumpfish (sample P) only the colorant E 124 was declared and detected. However, quantities of 1,548 mg/kg for E 124 by far exceeded the maximum allowed concentration (300 mg/kg) (see Fig. 3).

The analysis of the green colored German caviar from lumpfish (sample Q) resulted in 44 mg/kg of E 104, 59 mg/kg of E 122, and 4.7 mg/kg of E 142. In this caviar, no colorant exceeded the maximum allowed concentrations.

It is also worth noting in this context that the black appearance of certain caviars is not the result of a single dye but by mixing colorants E 102 (yellow), E 110 (yellow orange), and E 151 (dark blue). The dark blue color of sample H is achieved by mixing E 102 (yellow), E 129 (red), and E 133 (blue); see Table 2.

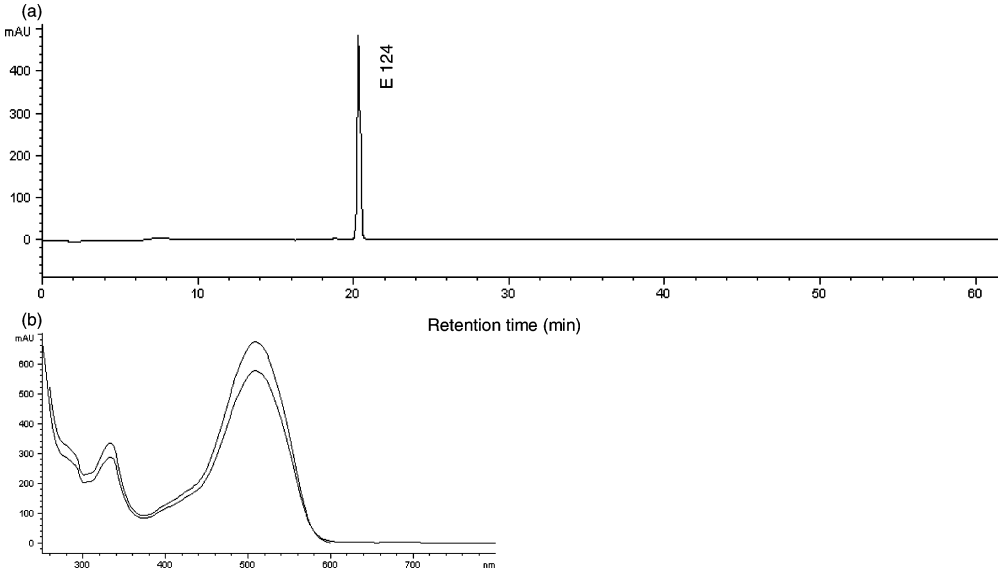


**Fig. 3** (**a**) HPLC of red colorized fish roe (sample P) recorded at 520 nm; (**b**) comparison of spectra of the peak at 20.2 min with standard colorant E 124 (Cochineal Red), *upper curve* represents the standard colorant

## Conclusions

For the quantification of synthetic dyes used for colorizing fish roe, adsorption on polyamide followed by desorption, HPLC separation of colorants and measurements of peak areas using a diode-array detector was found to be highly suitable. It was recognized that recoveries of colorants depend to some extend on batches of polyamides supplied by various manufacturers.

Amounts of pure colorants (100%) in standards have to be determined and taken into account for accurate quantification.

Applying HP ChemStation software to absorption spectra resulting from DAD data assures confidence of colorant identification. Peak areas resulting from chemical congeners of certain colorants have to be summed up for quantification.

From the data presented it is evident that some manufacturers have problems to colorize fish roe in accordance with EU declarations.

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