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Substitution of high-priced fish with low-priced species: Adulteration of common sole in German restaurants

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ABSTRACT

High-priced fish and seafood prepared as restaurant dish is especially prone to fraudulent substitution due to the lack of morphologic characters and less stringent labelling requirements. Common sole (*Solea solea*) is considered one of the most valuable and tasty fish species on the European markets and fraudulent substitutions of common sole in restaurant stores have been reported by official German food authorities in the past (from 2005 to 2011). The aim of the present study was to assess the substitution of common sole prepared as dishes in restaurants and to identify species used as substitutes for common sole. Furthermore, it was investigated whether substitution takes place at the level of the restaurants or earlier within the food supply chain. Altogether, 47 common sole dishes were ordered incognito in 24 restaurants and 98 whole fish specimens or fish fillets were purchased from 35 different shops (including wholesale dealers and speciality markets). Species identities were determined by cytochrome *b* gene sequencing and isoelectric focusing of sarcoplasmic proteins, which are official German methods for fish species identification afforded by §64 of the German Food and Feed Code (LFGB). 50% of the restaurant samples were shown to be substituted by species of lower commercial values, such as catfish species (*Pangasidae*), Senegalese tonguesole (*Cynoglossus senegalensis*) or Portuguese sole (*Synaptura lusitanica*). As almost all samples from the retail were shown to be authentic – only one sample was identified as lemon sole (*Microstomus kitt*) – we presume that fraudulent substitution is performed by restaurant owners and staff rather than by persons responsible for fishing, manufacturing or retail.

1. Introduction

The widespread use of DNA based analytical techniques has resulted in a variety of European and non-European studies targeting mislabelling and substitution of seafood species (e.g. Bénard-Capelle et al., 2015; Carvalho, Palhares, Drummond, & Frigo, 2015; Cutarelli et al., 2014; Espiñeira, González-Lavín, Vieites, & Santaclara, 2008; Griffiths et al., 2013; Herrero, Lago, Vieites, & Espiñeira, 2012; Pappalardo & Ferrito, 2015; Stamatis et al., 2015; Warner, Timme, Lowell, & Hirshfield, 2013). However, most of these studies deal with products from supermarkets and other retail forms whereas fish and seafood is rarely sampled directly from restaurants. Nevertheless, prepared fish and seafood dishes from restaurants are especially prone to fraudulent substitution as morphologic characteristics for fish and seafood species identification are generally lacking. For example, in a Canadian study of Hanner, Becker, Ivanova, and Steinke (2011) mislabelling of fish and seafood from restaurants and takeaways was found to be significantly greater than that of market fillets. Moreover, labelling requirements are less stringent for prepared fish and seafood (e.g. scientific name not mandatory) and control actions by official food control authorities directed towards species authenticity normally focus on samples taken from the storages of the restaurants rather than directly on the prepared dishes.

Common sole (*Solea solea*, Linnaeus, 1758) represents one of the most valuable flatfish species found on the European markets. In Germany consumption of common sole amounted to 649 tonnes in 2013 (Barz & Zimmermann, 2014) and prices range from 20 to 60 EUR per kg fresh whole fish and even more for sole fillets. This species is served in restaurants as whole fish or fish fillet and is considered a very tasty fish with a rather fine and firm texture and high nutritive value. The costs of sole dishes depend on the fish size and catch prices and are usually much higher for whole fish dishes (about 22–40 EUR per dish) compared to dishes prepared with sole

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fillets (about 12–25 EUR per dish). Common sole is especially appreciated by the consumers who are willing to pay appropriate prices for it (Rehbein, Müller-Hohe, & Hanel, 2009).

Common sole belongs to a large family (*Soleidae*) with more than 170 species belonging to 32 genera (Froese & Pauly, 2014). Whereas *S. solea* is found in the Eastern Atlantic southward from Trondheim Fjord to Senegal, the North Sea and Western Baltic and the Mediterranean Sea (Froese & Pauly, 2014), other *Soleidae* species are found in almost all oceans around the world. Shared morphologic characteristics and intraspecies variability often impede an unambiguous species identification of specimens (Rehbein et al., 2009). Especially two other *Solea* species (*Solea senegalensis* and *S. aegyptiaca*) are morphologically very similar to common sole and can be confounded accidently or adulterated deliberately (Boukouvala et al., 2012). Nevertheless, the list of German commercial designations for fish and seafood species (provided by the Federal Office for Agriculture and Food) clearly constitutes that in Germany the name "Seezunge" is exclusively permitted for *S. solea* (Bundesanstalt für Landwirtschaft und Ernährung, 2014).

High exploitation levels (Oceana, 2013) and increased worldwide consumption of common sole have resulted in a shortage of supply and increasing prices (Herrero et al., 2012; Rehbein, 2008) which is also true for a lot of other North Atlantic flatfish species (Rehbein et al., 2009). In the last years fish from other seas (e.g. the South Atlantic or North Pacific) increasingly appeared on the German market, examples being arrow tooth flounder (*Atheresthes stomias*) and yellowfin sole (*Limanda aspera*) from the North Pacific Ocean (Rehbein, 2008) or *Cynoglossus* species from tropical and subtropical areas (Rehbein et al., 2009).

In German food control laboratories several adulterated flatfish samples from restaurants and canteen kitchens have been discovered during the last years (Rehbein et al., 2009). In the year 2011, common sole authenticity was subject to a priority programme of the official German food surveillance and was sampled from the stocks of restaurant kitchens in different processing types. The outcome of the survey was devastating as 32.4% of all samples were shown to be adulterated (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, 2013).

The aim of the present study was to investigate the degree of substitution of prepared common sole dishes in German restaurants and to identify commonly used substitute species for common sole. Samples were collected in 24 restaurants in four German cities, either as whole fish or as fish fillet dishes. Additionally fresh sole products where purchased from the local retail to examine whether a putative adulteration of common sole takes place on the level of the restaurants or on the level of retail. In contrast to the modus operandi of official food control actions, all samples were taken incognito by people presenting themselves as normal guests and consumers.

Furthermore, this study should show whether existing official methods, which are afforded by §64 of the German Foods, Consumer Goods and Feedstuffs Code (LFGB) and have been validated by ring trials (11.00–6 *Detection of fish species of native muscle by means of isoelectric focusing (PAGIF)*, and 10.00–12 Fish species identification in raw fish and fish products by means of sequence analysis of cytochrome *b* sequences) prove their effectiveness when being applied to identification of flat fishes, such as common sole and related species.

Retail samples were screened with isoelectric focusing of sarcoplasmic proteins (IEF), which is an extremely fast and low cost method, and results were confirmed by sequencing of a fragment of mitochondrial cytochrome *b* (cytb) gene. Restaurant samples were directly analysed with sequencing of cytb gene or, in case of unsuccessful cytb amplification, other gene markers such as Cytochrome oxidase I (COI) or 16S rRNA. However, as gene sequencing is a method, which takes comparatively long time, in particular for laboratories lacking appropriate sequencing facilities, it was tested, whether authentication of processed common sole restaurant samples is also feasible with IEF.

2. Material and methods

2.1. Sample collection

All samples were purchased or ordered, respectively, without informing the retailers and restaurant staff about the purpose of the purchase (undercover investigation).

Restaurants with sole on the menu were identified mainly by internet searches and expensive as well as cheaper restaurants were selected to represent different types of quality standards.

A total of 47 common sole dishes were ordered at 24 restaurants in four German cities (Hamburg, Bremen, Frankfurt and Berlin) (see Table 1) in the year 2013. Usually, dishes from one restaurant represented identical compositions but in some cases the composition of side dishes and the price varied. A part of the fish from each plate was collected and frozen at −20 °C until further use.

Fresh and frozen common soles samples – either whole fish specimens or fish fillets – were purchased at 35 different fishmongers, supermarkets, speciality markets, home delivery services and whole sale dealers in the same cities (see Table 2). When possible, more than only one fish specimen or fillet was bought at each shop resulting in a total number of 98 sub-samples. Samples were hold on ice and frozen at −20 °C as soon as possible until further use.

Common sole reference specimens for IEF pattern comparison had been caught during a research cruise in the Celtic and Irish Seas in the year 2007, identified morphologically and preserved frozen at −20 °C.

2.2. DNA extraction, PCR and sequencing

Total DNA was isolated from 50 to 100 mg of fish muscle with CTAB as described in Rehbein (2005), and DNA concentrations were measured as fluorescence enhancement upon binding of the dye Hoechst 33258 (Downs & Wilfinger, 1983) with calf thymus DNA as a DNA standard.

Amplification of an approximately 413 bp fragment of the mitochondrial cytochrome *b* (cytb) gene was conducted with primers L14735 (5′-AAAAACCACCGTTGTTATTCAACTA-3′) and H15149AD (5′-GCICCTCARAATGAYATTTGTCCTCA3-') (Wolf, Burgener, Hubner, & Luthy, 2000). Both primers featured additional M13 tails at the 5'-end, which served as annealing sites for sequencing primers during sequencing reactions (in case of L14735: CCAGGGTTTTCCCAGTCACG; in case of H15149AD: CGGATAACAATTTCACACAGG). PCR reactions were performed in volumes of 20 μl containing 10 μl HotStarTaq *Plus* Master Mix (Qiagen, Hilden, Germany), 10 pmol of forward and reverse primer (Biometra, Göttingen, Germany), each, a total concentration of 2 mM $MgCl_2$ and about 20 ng extracted DNA. Reactions were preheated for 5 min at 95 °C, followed by 35 cycles of 60 s at 95 °C, 60 s at 50 °C and 60 s at 72 °C. Reactions were finished after a final extension step of 10 min at 72 °C.

In case of two samples an approximately 654 bp fragment of the mitochondrial cytochrome oxidase I (COI) gene was amplified with the primers FishF2 (5′-TCGACTAATCATAAAGATATCGGCAC-3′) and FishR2 (5′-ACTTCAGGGTGACCGAAGAACAGAA-3′) (Ward, Zemlak, Innes, Last, & Hebert, 2005). Tails, reaction mixtures and temperature profile were the same as for cytb amplification with the exception of 1.5 mM $MgCl_2$ and 54 °C annealing temperature.