

Simultaneous detection of allergenic fish, cephalopods and shellfish in food by multiplex ligation-dependent probe amplification

Claudia Unterberger · Florian Luber · Anja Demmel ·
Karola Grünwald · Ingrid Huber · Karl-Heinz Engel ·
Ulrich Busch

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Abstract People suffering from food allergy rely on correct food labelling as the ingestion of minimal amounts of the respective allergen can trigger severe allergenic reactions. Probes for the detection of DNA from allergenic fish, shellfish and cephalopod species in food using multiplex ligation-dependent probe amplification were developed. The specificity and the sensitivity of the detection system were investigated. The limit of detection was 20 mg kg⁻¹ for scallop, fish and bivalve species and 100 mg kg⁻¹ for cephalopod, gastropod and crustacean species using self-prepared sushi spiked with the analytes in different concentration levels. The analysis of 10 commercial food samples demonstrates the applicability of the developed method and its suitability for food quality control. Therefore, the method can be used to monitor the compliance with labelling rules regarding food allergens.

Keywords Fish · Seafood · Food allergen · Detection · Multiplex ligation-dependent probe amplification

Introduction

Seafood allergy is very common in Asian countries and paralleling an increasing consumption of seafood, it has become an important issue worldwide. Increasing seafood consumption has led to a rising number of reports of seafood-induced allergenic reactions ranging from oral allergy syndromes to anaphylaxis [1]. The term seafood comprises edible species of the phyla *Mollusca*, *Arthropoda* and *Chordata*. The two *Mollusca* classes *Gastropoda* and *Bivalvia* are usually referred together with *Crustacea* as shellfish. Crustacean shellfish allergy is very common, while molluscan shellfish allergy does not appear to occur as frequently [2].

Even the uptake of minimal amounts of seafood allergens can trigger allergic symptoms in patients within minutes [3]. For these individuals, the only possibility to prevent allergenic reactions is to avoid the intake of the respective allergen. Therefore, patients depend on correct and reliable food labelling. According to Annex II of Directive 1169/2011/EC, crustaceans, fish and molluscs have to be labelled if included as ingredients. Food allergens can also be found in foods resulting from “cross contacts” in consequence of transport and food production circumstances. In this case, labelling is not mandatory, but producers often use terms like “may contain traces of...” due to liability issues. Therefore, sensitive methods for the monitoring of the correct labelling of seafood are needed.

Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) techniques are prevalently used for the detection of allergens in fish, shellfish and cephalopods [6–15]. As tropomyosin has been reported to be the most common allergen in crustaceans and molluscs [4–6], various ELISA methods have been developed for the detection of this muscle protein [7–10]. In fish, the

C. Unterberger · F. Luber · A. Demmel · K. Grünwald · I. Huber ·
U. Busch (✉)
Bayerisches Landesamt für Gesundheit und
Lebensmittelsicherheit LGL, Veterinärstraße 2,
85764 Oberschleißheim, Germany
e-mail: Ulrich.Busch@lgl.bayern.de

F. Luber · K.-H. Engel
Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische
Universität München, Maximus-von-Imhof-Forum 2,
85350 Freising-Weihenstephan, Germany

muscle protein parvalbumin has been identified as major allergen. It is targeted by ELISA method for the detection of fish in food [11]. As the structure of proteins is affected by industrial food processing conditions, ELISA methods have proved to not be applicable to the analysis of highly processed foods [12]. In recent years, DNA-based methods have been used for the detection of allergens in food. They are fast, sensitive and allow the detection of more than one allergen in one assay. Various PCR-based systems for the detection of crustacean [13, 14], shrimps [15], octopus [16] and fish [17] have been described in the literature.

The ligation-dependent probe amplification (LPA), developed in the field clinical diagnostics [18], has recently proved to be a reliable method in food analysis for the detection and relative quantification of DNA from GMOs [19, 20], for the detection of DNA from foods, causing allergic reactions [21, 22], or for the detection and relative quantification of apricot as an indicator for the quality of marzipan products [23]. This technique allows the simultaneous detection of different targets in one assay using one single primer pair: two probes comprising universal primer binding sites are hybridising next to each other on their target sequence. After the ligation of the two probes, the resulting oligonucleotides are amplified using the universal primer pair. One of the primers carries a fluorescence dye. The probes are chosen in a way that each allergen-specific amplicon has a unique length. Therefore, following their separation by size using capillary gel electrophoresis, the amplified products can be assigned to their respective allergens after the determination of the lengths using a size standard. Compared to traditional multiplex PCR systems, the LPA system provides a cheap flexible systems that can be extended by adding further hybridisation probes for a higher level of multiplexing than it is possible with common PCR methods. In addition, it is characterised by high specificity and reproducibility. It can be used for analysing a large set of analytes in only one assay therefore reducing the sample material and reagents needed.

This study describes the development and evaluation of a LPA-based multiplex screening system for the detection of DNA from fish, shellfish and cephalopod species in food for the monitoring of food allergen labelling.

Materials and methods

Materials

Sample material

Fish, shellfish and cephalopod species included in the study (Tables 1 and 3) were purchased at local stores. Snails were

Table 1 Species used for specificity tests of each single probe

Crustaceans	Molluscs	Fish
Crawfish	Abalone	Anchovy
Edible crab	African snail	Anglerfish
Giant river prawn	Cockles	Char
Giant tiger prawn	Common octopus	Codfish
Langoustine	Common cuttlefish	Mackerel
Lobster (American)	European squid	Pangasius
Lobster (European)	Green -shell mussel	Plaice
Pink shrimp	Blue mussel	Saithe
Shrimp (freshwater)	Oyster	Salmon
Shrimp (saltwater)	Scallop	Sardine
Spiny lobster	Sea shell	Seabass
Whiteleg shrimp	Snail 1	Sea bream
	Snail 2	Swordfish
	Snail 3	Tilapia
	Snail 4	Trout
	Snail 5	Tuna
	Venus clam	Victoria perch
		Whitefish
		Wolffish

obtained from a local restaurant. The analysed food samples were purchased in a local store.

Oligonucleotides

Multiplex ligation-dependent probe amplification (MLPA) probes were obtained from Metabion (Martinsried, Germany). The optimised probe mix used in the MLPA contained: 13.3 μ M of bivalvia- and gastropod-specific oligonucleotides, 1.3 nM of crustacean- and fish-specific oligonucleotides, 0.2 nM of cephalopod-specific oligonucleotides and 6.6 pM of pecten-specific oligonucleotides.

Preparation of spiked sushi

Sushi matrix was prepared by mixing 2,500 g rice, 358.91 g avocados, 4.22 g algae, 5 g pepper and 10.38 g salt. All ingredients were homogenised using a food processor (type Thermomix 21/2-1, Vorwerk Deutschland Stiftung & Co. KG, Wuppertal, Germany) at level 5 (about 3,500 U/min) for 30 min at RT.

Allergenic paste was made by mixing 2.72 g tilapia, 2.75 g blue mussel, 2.72 g tiger prawn, 2.7 g scallop, 2.73 common octopus and 2.73 g African snail in the food processor as described above.

An initial amount of sushi matrix containing 1,000 mg of each allergen per kg was produced by mixing 0.5 g allergenic paste with 499.5 g sushi matrix in the food processor at level 2 for 45 min at RT, with changing

stirring directions. The resulting allergen-spiked sushi matrix (100 g) was mixed using the food processor with 150 g sushi matrix for preparing sushi matrix containing 400 mg of each allergen per kg. Serial dilutions of mixtures containing 100, 40, 20, 10 and 5 mg of each allergen per kg were prepared by serially diluting each dilution with unspiked sushi material and mixing as described. Each dilution was homogenised using the food processor at level 2 for 45 min at RT.

Methods

DNA extraction

DNA extraction was performed using a modified CTAB extraction method [DIN EN ISO 21571] [24]. The homogenised food samples (2 g) were mixed with 10 mL CTAB buffer [2 % (w/v) cetyltrimethylammoniumbromide, 1.4 M NaCl, 0.1 M 2-amino-2-hydroxymethyl-propane-1.3-diol (TRIS), 20 mM ethylenediaminetetraacetic acid (EDTA) at pH 8] and 30 µL of proteinase K solution (>600 mAU/mL) (Qiagen, Hilden, Germany) and incubated at 65 °C on a platform shaker (Heidolph Instruments, Schwabach, Germany) over night. After centrifugation at 7.200 *g* for 10 min, 1,000 µL of the supernatant were added to 750 µL chloroform/isoamylalcohol (Ready-Red; MP Biomedicals, Illkirch, France). After centrifugation at 20,000×*g* for 15 min, 750 µL supernatant was added to 750 µL isopropanol (≥99 %). After 30-min incubation at room temperature (RT) and centrifugation for 15 min at 20,000×*g*, the supernatant was carefully removed and the pellet washed with 500 µL ethanol (98 %). The sample was centrifuged at 20,000×*g* for 5 min, the supernatant was removed, and the pellet was dissolved in 100 µL HPLC-grade H₂O (LGC Promochem GmbH, Wesel, Germany). DNA extracts were purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Dilution was done with 50 µL elution buffer. DNA concentration was measured fluorometrically on a GENious plus reader (Tecan, Männedorf, Switzerland) using PicoGreen ds DNA reagent (Invitrogen, Karlsruhe, Germany). Sample DNA was normalised to 20 ng/µL for use in MLPA amplification.

MLPA reactions were performed using SALSA MLPA reagent kit EK5-Fam (MRC Holland, Amsterdam, the Netherlands). All steps were performed in a 96-Primus advanced thermocycler (Peqlab, Erlangen, Germany) with heated lid according to the kit manufacturer's instructions using thin walled PCR Tubes. Five microlitre sample DNA (20 ng/µL) was denaturated for 5 min at 95 °C. After cooling to 25 °C, 1.5 µL SALSA MLPA buffer and 1.5 µL probe mix were added for probe hybridisation. After heating to 95 °C for 1 min, the mixture was incubated at 60 °C

for 16 h. For probe ligation, 32 µL Ligase-65 master mix were added at 54 °C within 3 min containing 25 µL dH₂O, 3 µL ligase buffer A, 3 µL ligase buffer B and 1 µL SALSA ligase-65. In the following 12 min, the hybridised oligonucleotides were ligated at 54 °C. After heating to 98 °C for 5 min, the reaction mix was cooled to 4 °C. For the amplification of the ligated oligonucleotides, 10 µL polymerase master mix containing 7.5 µL dH₂O, 2 µL SALSA PCR primer and 0.5 µL SALSA polymerase was added at 60 °C and the amplification was performed with the following program: 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 60 s followed by a final extension step of 20 min at 72 °C. To reveal possible contaminations, a no template control (LK) and a PCR control (PK) were included in each experiment and Abalone DNA was included as a positive control. Each oligonucleotide pair was tested once. Specificity tests of the combined probes were done in two replicates. The limit of detection (LOD) was determined from three experiments with two replicates each. DNA was extracted for each single experiment.

Fragment length analysis

Fragment length analysis was performed on an ABI PRISM 3130 genetic analyser using capillaries (4 × 36 cm) and the POP-7 performance optimised polymer (Applied Biosystems, Foster City, CA). MLPA reaction products were diluted 1:40 with HPLC-grade H₂O (LGC Promochem GmbH, Wesel, Germany). One microlitre of the dilution was mixed with 15 µL HiDi-formamide (Applied Biosystems, Foster City, CA) containing 0.15 µL 500-LIZ size standard (Applied Bioscience, Foster City, CA).

After denaturation for 2 min at 94 °C, samples were injected for capillary electrophoresis with the following settings: oven temperature, 60 °C; pre-run time, 180 s; injection voltage, 1.2 kV; injection time, 16 s; run voltage, 15.0 kV and run time 1,200 s. Peak pattern evaluation was done using Gene Mapper3 software (Applied Bioscience, Foster City, CA). Only signal peaks of 100 relative fluorescence units (RFUs) and higher were evaluated as positive.

Results and discussion

Design of a MLPA-based detection system for shellfish

MLPA probes are composed of a left probe oligonucleotide (LPO) and a right probe oligonucleotide (RPO). The LPO consists of a 5' forward primer binding sequence and a target-specific hybridising sequence at the 3' end. The RPO consists of a 5' target-specific hybridising sequence and the reverse primer binding sequence at the 3' end. Optionally,

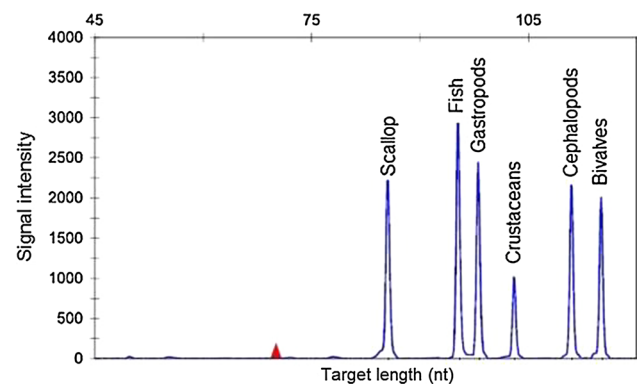
Table 2 Sequences of probes used in the developed MLPA system for the detection of fish, shellfish and cephalopods

Target	GenBank accession No./reference	LPO (5'-3')	RPO (5'-3')	Amplicon size	Detected length
Scallop	AY070153.1	FPB -GGAACAGTCGCTCTC CGTGAA	Pho -ATCCGTAGGTACCA GAAGAGACCG- RPB	88	85.5
Fish	AB211366.1	FPB -gtgtgtTCATTGACCAGG ACAAGAGCGG	Pho -CTTCATTGAGGAGG AGGAGCTGAggtg- RPB	96	95
Gastropods	NC_011193.1	FPB -gtgtgtgtCTACGTGATC TGAGTTCAGACCGG	Pho -CGTGAGCCAG GTCAGTTTCTATCTT- RPB	100	98
Crustaceans	AB270630.1	FPB -gtgtgtgtTCTGTGCAGA AGCTCCAGAAAGGAG	Pho -GTCGACAGGCTCGA AGACGAActgtgtgt- RPB	104	103
Cephalopods	AB218917.1	FPB -gcgcgtgtgtgttcgGAGGC CTCCCAAAGAGAGGACA	Pho -GCTATGAAGAGACTA TTCGGGATCTGACgtgtgt- RPB	112	111
Bivalves	AY267745.1	FPB -tgtgtgtgtCATAGATACA GGCCAGGAACAGTCGCTCT	Pho -TCGTGAGATCAGAAG ATACCAGActgtgtgtgt- RPB	116	115

Sequences common to all probes: forward primer binding sequence (FPB): 5'-**GGGTTCCCTAAGGGTTGGA**-3' and reverse primer binding sequence: (RPB): 5'-**TCTAGATTGGATCTTGCTGGCAC**-3'. Target-specific hybridising sequences are written in upper case letters, and stuffer sequences are written in small form letters

a stuffer sequence is included between the primer binding sequences and the specific hybridising sequences of the LPO and/or the RPO, respectively. Only if the two oligonucleotides (LPO and RPO) hybridise next to each other on their target sequence, they are ligated. The resulting probe is then amplified by a universal primer pair that also amplifies ligated probes targeting other sequences. One of the primers is labelled with a fluorescence dye, which is included in the amplification products during the PCR. The stuffer sequences are used to give each probe and hereby each amplification product a unique characteristic length. Therefore, the amplification products can be separated by their size and detected using capillary electrophoresis and fluorescence detection.

Probe target sequences were chosen by searching published PCR methods for the detection of the DNA of the respective allergens. Subsequently, sequence alignments of the target allergen and closely related species were done using HUSAR software (DKFZ, Heidelberg, Germany). Regions identified as specific for the allergenic species group were used to design probes according to the guidelines provided by MRC Holland [25]. Designed probes were first tested in silico for their specificity by aligning the complete hybridising sequence against all sequences available online within the NCBI GenBank using the basic local alignment search tool (BLAST). In a second approach, each probe (LPO and RPO oligonucleotides) was tested for its target specificity using DNA from fish, shellfish and cephalopod species (Table 1). Only probes that gave single peaks with the expected size for their respective target sequence (data not shown) were included in the detection system (Table 2). The specificity of each probe was 100 % for the tested species. Probe concentrations were optimised to give peaks of similar intensities. For example,

**Fig. 1** Electropherogram of the developed MLPA system, using a mixture of shrimp, octopus, blue mussel, scallop, snail and tilapia DNA as template. All six species can be detected in one experiment

lowering the concentration of the scallop probe resulted in an increased intensity of the gastropod-specific peak. This ensures the detection of gastropod DNA even if present in low amounts.

Due to the lack of a common sequence for all of the *Mollusca* species, individual probes were designed for *Gastropoda* and *Bivalvia*. The probe specific for *Bivalvia* failed to detect scallop (*Pecten jacobaeus*), a frequently consumed mussel; therefore, an additional probe was designed. Gene Bank accession numbers and detailed information on the probes are summarised in Table 2. The performance of the LPA system was tested using DNA from all target species. All species could be detected as each single probe resulted in a specific peak with the respective nucleotide length. No unspecific signals were observed. The simultaneous detection of representatives of all target allergens is shown in Fig. 1.

Differences between expected and observed amplicon sizes have been described before [21, 22] and pose no problem as long as the size of each amplicon representing a specific target is reproducible. Low peaks adjacent to the specific peaks are due to the injection rate of the sequencer.

Specificity of the developed MLPA system

As target group specificity of each single probe has been extensively tested among fish, shellfish and cephalopod species, the functionality of the combined probes was approved on an assortment of already tested species. No difference in specificity has been observed if probes are used alone or in combination. As the system was developed for food analyses, specificity tests were focused on DNA from possible food ingredients (Table 3).

The specificity was restricted as the *Gastropoda* probe set gave a positive signal with DNA from the *Apiaceae* species fennel and carrot as well as with DNA from radish belonging to the *Brassicaceae* family. The crustacean-specific probe set detected fennel and anise (*Apiaceae*). Repeating BLAST search of the hybridising sequence of the *Gastropoda*-specific probe set with *Apiacea* species revealed a sequence homology of 11 nucleotides (nts) of the LPO and 12 nts of the RPO with carrot DNA. Sequence homology of the crustacean-specific probe revealed 13 nts sequence homology of the LPO and 13 nts of the RPO with carrot DNA, but in the specificity tests, no peaks using DNA extracted from carrots were observed.

The reason why the *Gastropoda*-specific probe set gives a signal with carrot DNA remains unresolved, because neither the genome of carrot nor that of *Gastropoda* are completely known; therefore, sequence homologies cannot be totally excluded. Unspecific binding of the probes seems to become more likely the longer the hybridisation time is, since preliminary experiments employing shorter hybridisation times resulted in increased specificity (data not shown). These observed cross-reactivities need to be kept in mind when analysing food samples. If the *Gastropoda* or the crustacean probe gives a positive signal and the presence of fennel, carrot, anise and/or radish cannot be ruled out, alternative methods for the confirmation of the presence/absence of these species need to be applied, for example a conventional PCR for the detection of DNA from *Apiaceae* species [26] real-time PCR methods for the specific detection of the different relevant species, e.g. carrot DNA [27] or other methods for species identification. Ultimately, the development of more specific probe sets for *Gastropoda* and *Crustaceae* would be preferable. This is challenging due to the number of species in these groups and the high sequence variety between the species, but it should be a focus of ongoing work in the area of food allergen detection using MLPA.

Sensitivity of the MLPA system

As there was no suitable reference material available containing one representative of each species group, sensitivity

Table 3 Species used to determine the specificity of the whole system

Crustaceans	Fish	Plants			
Crawfish	Mackerel	Algae	Fennel	Spelt	
Edible crab	Pangasius	Almond	Fenugreek	Thyme	
Giant tiger prawn	Plaice	Anise	Garlic	Tomato	
Langoustine	Salmon	Apple	Green pea	Triticale	
Lobster (european)	Sardine	Apricot	Kamut	Walnut	
Pink shrimp	Trout	Avocado	Leeks	Wheat	
	Tuna	Barley	Lens		
		Black pepper	Lupine		
Molluscs	Microorganism	Caraway	Maize		
Abalone	<i>Enterobacter</i> sp. s U 180	Carrot	Mustard (yellow)	Milk	
African snail	<i>Pseudomonas</i> sp. U 230	Cashew	Nutmeg		
Common octopus		Cayenne pepper	Oat		
European squid	Animals	Celery	Onion		
Mytilus mussels	Beef	Chervil	Peanut		
Oyster	Chicken	Clove	Peppers		
Scallop	Goat	Coconut	Pistachio		
Snail 1	Lamb	Coriander	Radish		
Venus clam	Ostrich	Cucumber	Rice		
	Pork	Cumin	Rye		
	Turkey	Dill	Soya		

tests were performed using self-made sushi. A sushi matrix was prepared and spiked with a mixture of tilapia, blue mussel, shrimp, scallop, common octopus and African snail, resulting in concentrations of 100, 40, 20, 10 and 5 mg of every respective allergen and per kg sushi matrix. For testing the sensitivity and determining the limit of detection (LOD), three DNA extractions of each spiking level were examined in two replicates per extraction using the MLPA system. The results are summarised in Table 4. The LOD is defined as the lowest spiking level for which

all results are positive. The LOD of the probe sets specific for scallop, fish and *Bivalvia* was determined to be 20 mg/kg. For the probe sets of cephalopods, gastropods and crustaceans, the LOD is 100 mg/kg. Considering the results of the 5 mg/kg and 10 mg/kg sushi material for the probe set specific for bivalves, a lower LOD could be assumed, due to the fact, that 6 positive results for 5 mg/kg and only one negative result for 10 mg/kg are obtained. Similar to these results, the probe set specific for gastropods may also has a lower LOD, as for the 20 mg/kg spiked sushi material, all

Table 4 Determination of the LOD by examining different spiking levels of self-made sushi with the MLPA system (A: replicate 1; B: replicate 2)

	Extract	Spiking level replicate									
		5 ppm		10 ppm		20 ppm		40 ppm		100 ppm	
		A	B	A	B	A	B	A	B	A	B
Scallop	1	+	+	+	+	+	+	+	+	+	+
	2	–	–	+	–	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+	+
Fish	1	–	+	+	–	+	+	+	+	+	+
	2	+	–	+	–	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+	+
Gastropods	1	+	+	+	+	+	+	+	+	+	+
	2	–	–	+	–	+	+	–	+	+	+
	3	+	+	+	+	+	+	+	+	+	+
Cephalopods	1	+	+	–	–	+	+	+	+	+	+
	2	–	–	+	–	–	+	–	–	+	+
	3	+	+	+	–	+	+	+	+	+	+
Bivalves	1	+	+	–	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+	+
Crustaceans	1	+	+	–	–	+	+	+	+	+	+
	2	–	–	+	–	–	+	–	+	+	+
	3	+	+	+	+	+	+	+	+	+	+

Table 5 Commercial foods tested with the MLPA system

Figure	Product	Declared fish and shellfish allergenic ingredients	Detected species
A	Filet of herring in cream	Filet of herring	Fish
B	Bouillabaisse	Alaska Pollock (<i>Theragra chalcogramma</i>), fish stock Mussels (<i>Mytilus spp.</i>) Prawn (<i>Penaeus spp.</i>), crustacean stock	Fish Bivalves Crustaceans
C	Cream of crab	Crab (<i>Cancer pagurus</i>) Dorschrogen (<i>Gadus morhua</i>)	Crustaceans Fish
D	Crabs in garlic oil and herbs	Prawn (<i>Penaeus monodon</i>)	Crustaceans
E	Pangasius filet with mango	Pangasius	Fish
F	Mussels in tomato dressing	Mussels (<i>Mytilus edulis</i>)	Bivalves
G	Tuna paté	Tuna and other fish	Fish
H	Herring in curry sauce	Herring (<i>Clupea harengus</i>)	Fish
I	Seafood salad	Squid, Octopus Mussels	Cephalopods Bivalves
J	Sardine paste	Sardine and other fish	Fish

six replicates gave a positive signal and only one replicate of the 40 mg/kg material is negative. The LOD of the probe sets for scallop, fish, cephalopods and crustaceans could be unambiguously deduced from the depicted results.

Analysis of commercial foods

To evaluate the system for routine analysis, commercial food samples from local stores were examined for the presence of DNA from fish and seafood allergens. Table 5 lists the allergenic ingredients declared on the labels of the respective products. Using the developed MLPA system, all declared ingredients were detected in all samples. The results are summarised in Table 5. The corresponding electropherograms are shown in Fig. 2. Regarding the results of the examined bouillabaisse, cream of crab and seafood salad, the advantage of the multiplex LPA becomes

obvious. The use of probes of different lengths that are separated according to their size enables the detection of several allergenic ingredients using only one PCR reaction. In the food sample “cream of crab”, crustaceans and fish were detected, while the presence of cephalopods and bivalves was confirmed in the sample “seafood salad”. Three ingredients (fish, crustaceans and bivalves) were detected in the bouillabaisse. These examples illustrate the multiplex character of the developed MLPA method. The detection of several allergenic ingredients in parallel in only one experiment saves both reagents and sample DNA extract.

Conclusion

A MLPA system for the simultaneous detection of the allergenic species scallop, fish, bivalvia, cephalopoda,

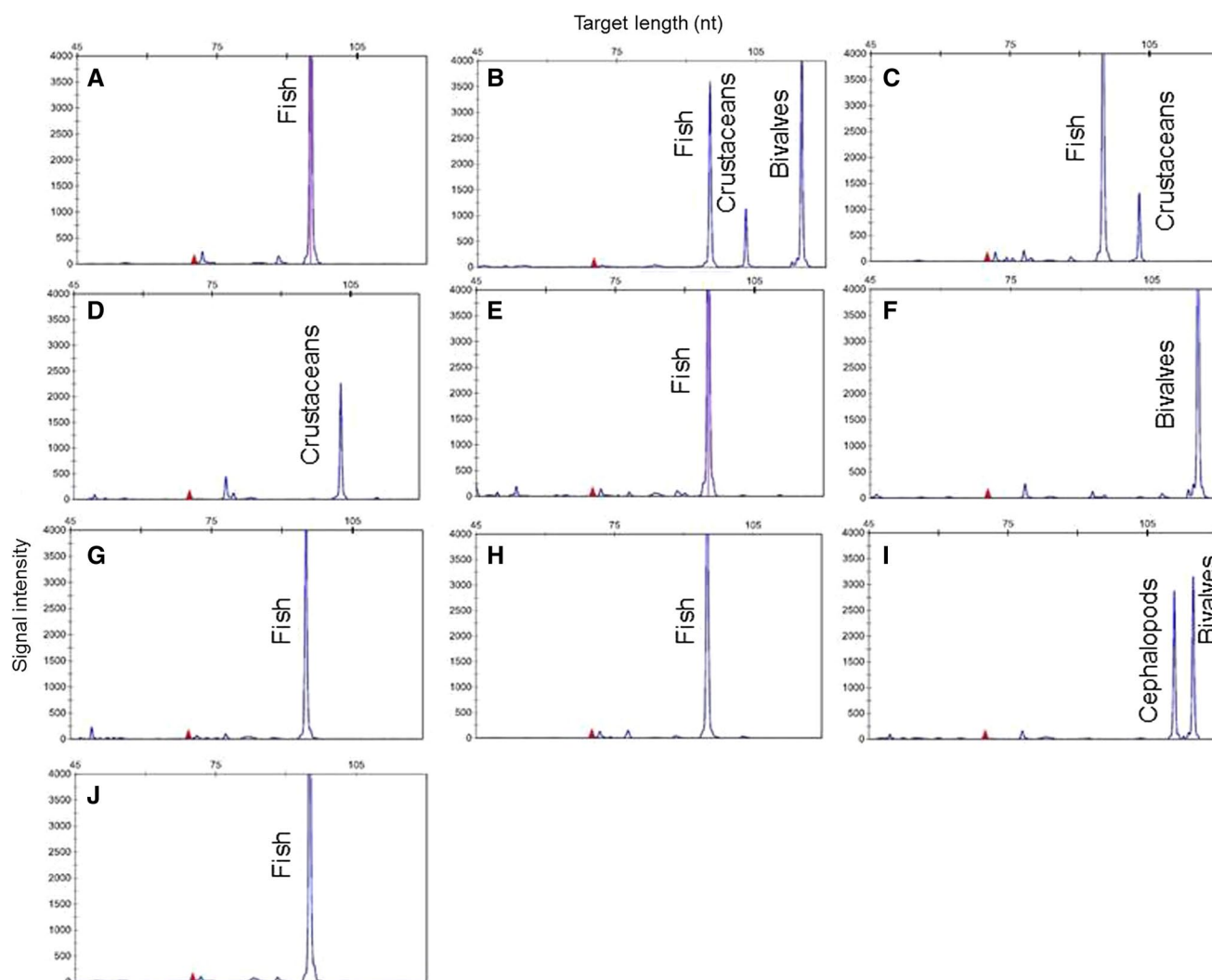


Fig. 2 Electropherogram of commercial food samples (Table 5). Each allergenic ingredient was detected by the MLPA system. Amplificates were diluted 1:100

gastropoda and crustacean in food was developed. The specificity and the sensitivity of the detection system were investigated. Using self-prepared sushi spiked with the analytes at different concentration levels, the limit of detection was determined to be 20 mg kg⁻¹ for scallop, fish and bivalvia species and 100 mg kg⁻¹ for cephalopod, gastropod and crustacean species. The applicability of the developed method was demonstrated through the analysis of 10 commercial food samples. Therefore, the method can be used to monitor the compliance with labelling rules regarding food allergens.

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Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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