Food Chemistry 119 (2010) 75–80

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of processing on detection and quantification of the parvalbumin gene in Atlantic salmon (*Salmo salar*)

Sabine Hildebrandt, Eric A.E. Garber *

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Regulatory Science, Division of Bioanalytical Chemistry, HFS-716, 5100 Paint Branch Parkway, College Park, MD 20740, USA

ARTICLE INFO

Article history:
Received 14 October 2008
Received in revised form 22 May 2009
Accepted 28 May 2009

Keywords:
Parvalbumin
Salmon
PCR
Baking
Processing
Pressure cooking

ABSTRACT

Consumption of Atlantic salmon is a common cause of fish allergies with parvalbumin (*Sal s1*) being the major allergen. The presence of DNA encoding *Sal s1* indicates the presence of Atlantic salmon in food. Using real-time polymerase chain reaction (PCR), the effects of food processing on the ability to detect and quantify the *Sal s1* gene were determined. The method was specific for salmon and did not cross-react with 53 other species. Baking and pressure cooking caused a 5–100-fold decrease in detectable copies of the *Sal s1* gene. Despite a 98% reduction in detectable copies following pressure cooking for 60 min, the relative standard deviation (RSD) between replicates was 20% and the response was 100-fold grater than the lowest copy number of *Sal s1* reliably detected by the assay. Despite efforts to develop a quantitative assay, the PCR assay was qualitative. It is impossible to predict the effects of food matrices not included in this study, some of which may affect the reliability of the assay. Analyses of raw and pressure cooked salmon using a commercial PCR kit indicated comparable results to the PCR assay.

Published by Elsevier Ltd.

1. Introduction

Fish, peanuts, shellfish, and tree nuts account for the majority of food anaphylactic reactions in the United States of America (Sampson, 2000). Consumption of Atlantic salmon (*Salmo salar*) is one of the most common causes of fish allergies with parvalbumin (*Sal s1*) recognised as the major allergenic protein (Elasyed & Bennich, 1975; Elsayed & Apold, 1983). Reliable detection and quantification methods for allergenic foods are necessary to ensure compliance with food labelling regulations and ensure consumer protection. However, no commercial methods exist for the quantification of fish and detection can be problematic because numerous antigens have been detected in fish (Aukrust, Apold, Elsayed, & Aas, 1978; Aukrust, Grimmer, & Aas, 1978; Dory et al., 1998; James, Helm, Burks, & Lehrer, 1997). In addition, allergic individuals cross-react differently to different species of fish (de Martino et al., 1990; Dory et al., 1998; Hansen, Bindslev-Jensen, Stahl Skov, & Poulsen, 1997; Pascual, Martin Esteban, & Crespo, 1992; Sten et al., 2004).

Real-time polymerase chain reaction (PCR) provides a sensitive tool for the specific detection of DNA markers in allergenic foods. However, it does not detect the allergen. Therefore, the detection of fish by PCR does not necessarily indicate the presence of allergenic protein (Lindstrøm, Van Do, Hordvik, Endresen, & Elsayed, 1996). DNA-based methods offer many advantages over protein-based methodologies. These include ease of extraction and insensitivity to factors that may induce conformational changes in the protein (i.e., food processing and preparation) that inhibit its detection. This is a concern since during processing a protein may become denatured making it undetectable by enzyme-linked immunosorbent assay (ELISA) but still able to generate an allergenic peptide when digested. Processing of food can also cause DNA degradation and affect nucleic acid-based detection of analytes (Hrnčírová, Bergerová, & Siekel, 2008; José Chapela et al., 2007; Masataka, Tomohiro, & Katsumi, 2004; Peano, Samson, Palmieri, Gulli, & Marmiroli, 2004; Bauer, Weller, Hammes, & Hertel, 2003).

Common preparations of Atlantic salmon fillets include baking and canning, raw salmon is used for products such as sushi. Most baking procedures utilise an oven temperature of approximately 190 °C and baking times ranging from 10 to 40 min. Canning of salmon requires pressure heating the fish in air-tight jars, cans, or pouches to a temperature that destroys contaminating microorganisms such as *Clostridium botulinum*. Typical canning conditions for a 6 oz can of salmon are 121.1 °C and 20 psi (psi) for 35 min (Manual on fish canning, 1988).

Using real-time PCR, the effects of food processing on the ability to detect and quantify the *Sal s1* gene were examined along with the ability to detect salmon spiked into another fish homogenate.

* Corresponding author. Tel.: +1 301 436 2224; fax: +1 301 436 2332.
E-mail address: Eric.Garber@fda.hhs.gov (E.A.E. Garber).

0308-8146/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2009.05.074

2. Materials and methods

2.1. Atlantic salmon

Raw fillets of farm raised Chilean Atlantic salmon (*S. salar*) were obtained from a local market. Replicate fillet portions of 20 g (approximately 8 × 3 × 1 cm) were baked in a laboratory oven for 10, 20, 30, and 40 min at 190 °C. Four portions were prepared for each time period. Pressure cooking in an autoclave was used to imitate canning. Replicate fillet portions of 170 g were pressure cooked in cans using typical canning conditions (121 °C at 20 psi) for 10, 21, 35, and 60 min using an MK II autoclave (Consolidated Stills and Sterilizers, Boston, MA, USA). Three portions were prepared for each time period. The masses of the fillet portions were measured before and after the heat treatments to determine the loss of moisture content of the meat due to processing.

2.2. DNA extraction and quantitation

Test samples of the raw (n = 6), baked (n = 4 for each time period), and pressure cooked (n = 3 for each time period) salmon portions were prepared by homogenising in a laboratory blender (Waring, Torrington, CT, USA). DNA was extracted from 25 mg of test portions using the Qiagen DNeasy® Blood and Tissue kit (Valencia, CA, USA) following the spin-column protocol for the analysis of muscle tissue and analysed without further dilution.

The amount of total DNA extracted was determined using a ND-100 Spectrophotometer (NanoDrop, Wilmington, DE, USA) and its software for DNA quantification. The amount of DNA measured was corrected for changes in the mass of the meat due to processing (see Section 2.1). Baking for 10 and 40 min typically resulted in losses of mass of 10% and 30%, respectively. Pressure cooking typically resulted in a reduction in mass of <12% after 60 min while 21 min or less resulted in no loss. The data presented have been accordingly corrected and are expressed per gram wet weight of unprocessed raw salmon. Each processed sample was analysed simultaneously with a raw fillet sample.

2.3. Detection and quantification of the Sal s1 gene

The presence and copy number of the *Sal s1* gene were determined using real-time PCR (iQ5, BioRad, Hercules, CA, USA) employing 5′ ATGGCCTGTGCCCATCTGTGC 3′ (IFF 156) and 5′ GGACTTCGAGGCAAAGCCAAT 3′ (IFF 157) as primers according to a procedure developed by Rehbein and Kress (2005) with the following modifications. The PCR assay contained 3.2 μL of undiluted DNA extract (according to Section 2.2), 500 nM of each primer, and 10 μL of SYBR Green Mix (Thermo Scientific, UK) in a total volume of 20 μL. Raw fish samples typically included 15 ng/μL of total DNA per extract. The PCR samples were heated at 95 °C for 15 min, followed by 40 cycles of 1 min/95 °C, 30 s/61 °C, and 30 s/72 °C. The initial copy number of the *Sal s1* gene in processed salmon was determined by comparing the observed cycle threshold (Ct) value with a standard curve prepared using DNA from unprocessed salmon. The number of copies in the DNA extract used to prepare the standard curve was determined based on one copy per 3.1 pg of haploid genome (Gregory, 2008).

2.4. Cross-reactivity

Fifty-four fish species representing 30 families and 10 orders were extracted as described in Section 2.2 and analysed as described in Section 2.3. All species were extracted and determined in triplicate, the results were compared to that of Atlantic salmon.

2.5. Comparison of the PCR assay to a commercially available PCR-test kit

The results of the PCR assay were compared with results from a commercial test kit, SureFood® Allergen Fish real-time PCR-test kit, manufactured by Congen Biotechnologie GmbH (2005) and distributed by R-Biopharm (Marshall, MI, USA). The commercial test kit was designed for the qualitative detection of fish with no information provided regarding its use for quantitative measurements. Though the commercial test kit was designed for the qualitative detection of fish, the quantitative performance was also evaluated. Further, the performance of the test kit with extracts prepared using the SureFood® PREP Allergen kit (distributed by R-Biopharm) was compared to extracts prepared using the Qiagen DNeasy® Blood and Tissue kit (see Section 2.2). Both extraction kits recommend cell lysis times of 1 h or more and use of spin columns to purify the nucleic acids. Since lysis was not complete in 1 h, extracts were lysed for 1, 6, and 16 h and the results compared.

2.6. Detection of the Sal s1 gene in fish

Raw fillets of farm raised Chilean Atlantic salmon (*S. salar*) and Atlantic croaker were obtained from a local market. The fillets (approximately 300 g each) were homogenised separately in a blender. The Atlantic croaker was spiked with Atlantic salmon homogenate to achieve concentrations of 0%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%, and 10%. The spiked samples were homogenised again and the DNA extracted as described above. The amount of *Sal s1* copies g^{-1} food was determined as described in Section 2.3. Seven replicates of each level of spiked material were extracted and the *Sal s1* content determined using the standard curve (see Section 2.3).

3. Results

3.1. Effects of processing on DNA content

The amounts of total DNA from raw and processed Atlantic salmon fillets are given in Table 1. Spectrophotometric analysis of raw salmon indicated s total DNA content of 674 (RSD = 9%, n = 6) μg g^{-1} wet weight fillet. Only 63–70% of the total DNA was measured after baking of salmon fillets at 190 °C for 10, 20, and 30 min. Baking for 40 min resulted in being able to measure only 33% (RSD = 30%, n = 4) of the total DNA. Pressure cooking of salmon at 121 °C and 20 psi resulted in a less extractable total DNA than baking. After 10 and 21 min at 121 °C and 20 psi, an average of 59% (n = 6) of the total DNA could be measured. Pressure cooking for 35 and 60 min resulted in decreases to 29% (RSD = 38%, n = 3) and 11% (RSD = 27%, n = 3), respectively, of the original amount of DNA.

3.2. Determination of copy number of Sal s1

A standard curve was prepared from DNA extracted from Atlantic salmon at 3×10^4, 3×10^3, 3×10^2, 3×10^1, and 3×10^0 copies of the *Sal s1* gene. A standard curve with an amplification efficiency of 97.9% and the corresponding PCR curves are shown in Fig. 1 with a plot of the Ct value versus initial copy number prior to amplification. The assay was able to reliably detect 30 copies of the target sequence in a PCR reaction, an amount equivalent to 3.8×10^4 copies g^{-1} wet weight of raw Atlantic salmon.

Table 1 presents the effects of processing on the *Sal s1* gene copy number. Raw fillets of Atlantic salmon contained 1.2×10^8 (RSD = 17%, n = 6), copies g^{-1} wet weight. Baking of Atlantic salmon fillets at 190 °C for 10–30 min decreased the copy number by 50–53% to 6×10^7 (RSD = 33%, n = 4) copies g^{-1} salmon fillet,

Table 1
Effects of processing Atlantic salmon on the detection of total DNA and the *Sal s1* gene.[a]

Preparation (min)	DNA content[b]		Copy number of *Sal s1*[c]			Number of replicates
	(%)	RSD (%)	($\times 10^7$)	(%)	RSD (%)	
Raw	100	9	12	100	17	6
Baked (10)	70	14	6	47	33	4
Baked (20)	63	19	6	48	33	4
Baked (30)	63	22	6	50	33	4
Baked (40)	33	30	1.6	13	25	4
Pressure cooked (10)	59	22	6	54	33	3
Pressure cooked (21)	58	24	6	50	33	3
Pressure cooked (35)	29	38	5	40	20	3
Pressure cooked (60)	11	27	0.3	2	20	3

[a] Atlantic salmon fillets were analysed either raw, after baking at 190 °C, or after pressure cooking at 121 °C and 20 psi for the listed time periods. Each processed sample was analysed simultaneously with a raw fillet sample.
[b] The content of total DNA relative to the amount of total DNA in raw salmon fillets. The DNA was extracted using the DNeasy® Blood and Tissue kit (Qiagen, USA) and quantified using a ND-100 Spectrophotometer (Nanodrop, USA). Data are presented as the means and relative standard deviations (RSD).
[c] *Sal s1* gene copy number and percent change relative to raw salmon fillet samples expressed per gram wet weight. The copy numbers were measured by real-time PCR using IFF 156 and IFF 157 as primers. Data are presented as the means and relative standard deviations (RSD).

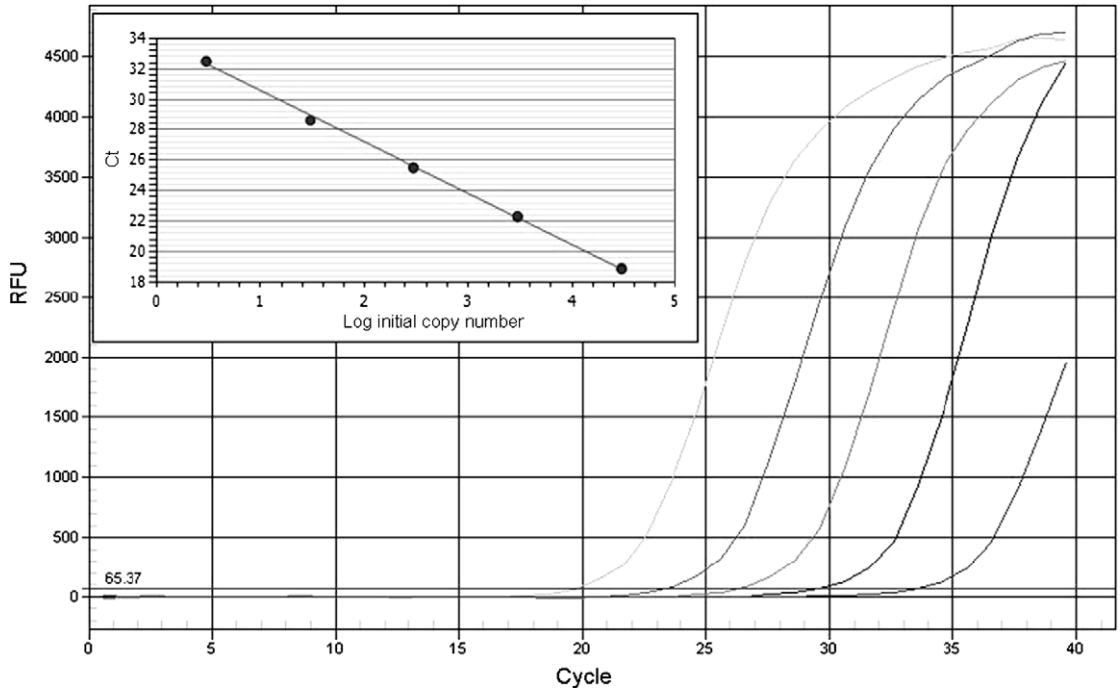

Fig. 1. Standard curve for the detection of the *Sal s1* gene. Standards containing 3×10^4, 3×10^3, 3×10^2, 3×10^1, and 3×10^0 copies of the *Sal s1* gene (amplification efficiency = 97.9%, $R^2 = 0.999$, and slope = −3.373) were analysed by real-time PCR and plotted as the relative fluorescence units (RFU) after subtraction of the background fluorescence versus the number of PCR cycles. The insert is a plot of the log of the initial copy number of the *Sal s1* gene versus the Ct value. SYBR green was used as the fluorophore for monitoring and quantifying the amplification reaction.

whereas baking for 40 min decreased the copy number by 87% to 2×10^7 (RSD = 25%, $n = 4$) copies g^{-1} salmon fillet. A similar pattern was observed with pressure cooking. Pressure cooking (121 °C at 20 psi) for 10–35 min decreased the copy number by 46–60%, whereas pressure cooking for 60 min resulted in a 98% decrease in copy number to 3×10^6 (RSD = 20%, $n = 3$) copies g^{-1} salmon fillet. The low relative standard deviation values (RSD) associated with greatly decreased copy numbers (20% for a 98% decrease in copy number) reflects the large dynamic range of the PCR assay.

3.3. Cross-reactivity testing

The cross-reactivity of the *Sal s1* DNA assay was assessed using filets from 54 different species of fish (Table 2). Only Sockeye (Pacific) salmon generated a measurable response. Specifically, Sockeye yielded an average copy number of $10 \times 10^7\ g^{-1}$ fish compared to 12×10^7 for Atlantic salmon, a cross-reactivity of 83%. All other species indicated copy numbers of less than 3.8×10^4 copies g^{-1} fish, the lowest amount the assay was able to reliably detect using DNA from Atlantic salmon.

3.4. Comparison of the PCR assay to a commercially available test kit

The results of detection and quantitation of the *Sal s1* gene using the real-time PCR assay was compared to the results obtained using a commercial test kit for fish. Though the test kit was designed for the qualitative detection of fish, the quantitative performance of the test kit was also evaluated. Extracts of raw and pressure cooked (121 °C, 20 psi, 60 min) Atlantic salmon displayed comparable copy numbers irrespective of whether the commercial test kit or the PCR assay was used to amplify the extracted DNA.

Table 2
Cross-reactivity of fish species.[a]

Common name	Scientific name	PCR-test result	Common name	Scientific name	PCR-test result
Atlantic salmon	*Salmo salar*	12×10^7 copies g^{-1} fish	Ladyfish	*Elopssaurus*	<
Vermillion Snapper	*Rhomboplites aurorubens*	<[b]	Longtail Wrasse	*Hemanthisa leptus*	<
Red Snapper	*Lutjanus campechanus*	<	Bermuda Chub	*Kyohosus sectatrix*	<
Lane Snapper	*Lutjanus synagris*	<	Nile Tilapia	*Oreochromis nilotica*	<
Yellow Tail Snapper	*Ocyurus chrysurus*	<	Barrelfish	*Hyperoglyphe bythites*	<
Grey Snapper	*Lutjanus griseus*	<	Bluefish	*Pomatomus saltatrix*	<
Dog Snapper	*Lutjanus jocu*	<	Cobia	*Rachycentron canadum*	<
Yellow Fin Tuna	*Thunnus albacares*	<	Pomfret	*Brama dussumiere*	<
Black Fin Tuna	*Thunnus atlanticus*	<	Spadefish	*Chaetodipterus faber*	<
King Mackerel	*Scomberomorus cavalla*	<	Goldenfaud Tilefish	*Caulolatilus chrysops*	<
Spanish Mackerel	*Scomberomorus maculatus*	<	Triple Tail	*Lobotes surinamensis*	<
Bonito	*Euthynnus alletteratus*	<	Sockeye Salmon	*Oncorhynchus nerka*	10×10^7 copies g^{-1} fish
Wahoo	*Acanthocybium solandri*	<	Grey Triggerfish	*Balistes capriscus*	<
Atlantic Croaker	*Micropogonias undulatus*	<	Spotted Triggerfish	*Canthidermis maculata*	<
Sand Sea Trout	*Cynoscion arenarius*	<	Puffer	*Lagocephalus laevigatus*	<
Spot	*Leiostumus xanthurus*	<	Gulf Flounder	*Paralichthys albigutta*	<
Speckled Trout	*Cynoscion nebulosus*	<	Southern Flounder	*Paralichthys lethostigma*	<
Hybrid Striped Bass	*Morone saxatilis* × *chrysops*	<	Red Hake	*Urophycis chuss*	<
White Perch	*Morone americana*	<	Silver Hake	*Merluccius bilinearis*	<
Striped Bass	*Morone saxatilis*	<	Hardhead Catfish	*Ariopsis felix*	<
Pompano	*Trachinotus carolinus*	<	Gafftop Sail Catfish	*Bagre marinus*	<
Almaco Jack	*Seriola rivoliana*	<	Hammerhead Shark	*Sphyrna tiburo*	<
Blue Runner	*Caranx crysos*	<	Bull Shark	*Carcharhinus leucas*	<
Spottail Pinfish	*Diplodus holbrooki*	<	Atlantic Stingray	*Dasyatis sabina*	<
White Bone Porgy	*Calamus leucosteus*	<	Southern Stingray	*Dasyatis americana*	<
Red Porgy	*Pagrus pagrus*	<	Tarpon	*Megalops*	<
Black Sea Bass	*Centropristis striata*	<	Ground Mullet	*Menticirrhus americanus*	<
Gag Grouper	*Mycteroperca microlepis*	<			

[a] All fish samples, except Yellowfin Tuna, Blackfin Tuna, Sockeye salmon, Wahoo, Speckled Trout, and Tarpon were authenticated by the Smithsonian Institution (Washington, DC).
[b] Less than *Sal s1* copy number reliably detected using DNA from Atlantic salmon, 3.8×10^4 copies g^{-1} wet weight.

This was true irrespective of whether the lysis was done for 6 or 16 h, though the efficiency of lysis and the detectable copy number were greater with the longer extraction period. The extraction kit recommended for use with the commercial test kit displayed similar extraction efficiencies to the procedure used with the PCR assay, though a slight difference was observed between the two extraction procedures with raw salmon fillets extracted for only 6 h (data not shown).

3.5. Detection of the Sal s1 gene in fish

The detection of *Sal s1* in Atlantic croaker spiked with 0–10% (g/g) Atlantic salmon is summarised in Table 3. In absence of Atlantic salmon, the response generated was equivalent to 3.2×10^3 (RSD = 39%, $n = 7$) copies of the *Sal s1* gene g^{-1}. Fish spiked with 0.1% or less Atlantic salmon generated responses comparable to the background response observed with pure Atlantic croaker. The lowest amount of Atlantic salmon spiked into Atlantic croaker that generated a response significantly different from background was 0.5%. The response measured for 0.5% Atlantic salmon in Atlantic croaker was equivalent to 2.8×10^4 copies g^{-1} wet weight, an amount comparable to lowest number of *Sal s1* copies (3.8×10^4) reliably detected using DNA standards. The low recoveries and the lack of a 1:1 correlation between spike level and detectable *Sal s1* copies may reflect lack of homogeneity in the extracts, the large concentration range analysed, and possible matrix effects. These results make use of the assay for the quantification of Atlantic salmon problematic. Despite these quantitative limitations, all of the responses measured were considerably above background with the lowest response measured for a sample containing 0.5% Atlantic salmon being more than fivefold greater than the background response.

4. Discussion

Using primers developed by Rehbein and Kress (2005), it was possible to detect the *Sal s1* gene in Atlantic salmon and confirm a copy number of 1.2×10^8 genes g^{-1} wet weight.

The assay was able to detect 30 copies of the *Sal s1* gene per PCR reaction mixture, which is equivalent to 3.8×10^4 copies per gram of wet weight salmon filet. When mixed with other fish the lowest amount of salmon filet that could be detected was 0.5% or 5 mg

Table 3
Detection of the *Sal s1* gene in fish spiked with Atlantic salmon.

No	Content of Atlantic salmon (%)	PCR-test result (copies g^{-1} fish)	RSD (%)	Recovery (%)	Number of replicates
0	0	3.2×10^3	39	–	7
1	0.005	5.7×10^3	72	100%	7
2	0.01	8.4×10^2	39	7%	7
3	0.05	4.3×10^3	60	8%	7
4	0.1	2.4×10^3	39	2%	7
5	0.5	2.8×10^4	20	4%	7
6	1	7.0×10^4	51	6%	7
7	5	1.9×10^6	87	32%	7
8	10	7.5×10^7	99	625%	7

salmon in 1 g of non-salmon fish. Though a lowest observed adverse effect level (LOAEL) for individuals with fish allergies has not been established for Atlantic salmon, values ranging from 5 mg to 6000 mg have been published for other fish (Helbling et al., 1999; Taylor et al., 2002). Thus, the detection of 0.5% salmon in a mixture with other fish, an amount equivalent to 500 mg in a 100 g serving, is within the range reported for the LOAEL.

Use of the method to quantify the amount of salmon present in a sample was problematic due to the large RSD values associated with the analyses. This limitation in quantification, though important does not reduce the utility of the method for the qualitative detection of salmon. The detection of 7.5×10^7 copies of *Sal s1* in croaker with an RSD of 99% is equivalent to a lower limit of 7.5×10^6 copies, which is >200-times the copies of *Sal s1* the assay reliably detects.

Baking and pressure cooking of the salmon fillets resulted in decreases in the detectable copy numbers of the *Sal s1* gene, which varied with the heating time. A decrease of approximately 50% in copy number was observed within 10 min of heating. Heating for an additional 20 min resulted in either no significant change or a slight decrease in detectable copy number. Further baking, for a total of 40 min, decreased the copy number to 13% of its original level while pressure cooking for a total of 60 min decreased the detectable copy number to 2%. This discontinuity in the detectable copy number of the *Sal s1* gene with time of heating could reflect multiple isoforms of the *Sal s1* gene. Zebra fish contain a minimum of nine isoforms of the *Sal s1* gene (Friedberg, 2005). Electrophoretic analysis of Atlantic salmon genomic DNA using another set of parvalbumin specific primers, IFF232 and IFF233 (Swaboda et al., 2002), indicated multiple isoforms of the *Sal s1* gene (data not shown). However, the primers used in this study were specific for one size band of DNA only. Thus, it seems doubtful that multiple isoforms are the cause of the discontinuity observed with heating, unless two isoforms had similar lengths not distinguishable in the electrophoretic gels.

Seven of the eight heat treatments applied to Atlantic salmon displayed a reduction in total DNA that was less than the decrease in detectable *Sal s1* gene copy number. A possible explanation for this discrepancy is that the processing damaged the *Sal s1* gene resulting in detectable, but not amplifiable DNA. Such a situation may result from breakage of the DNA at a site located between the two primer binding sites.

A double-blind, placebo-controlled, oral food challenge (DBPCFC) and IgE binding studies indicate a 98–99% reduction in the allergenicity of canned fish (Bernhisel-Broadbent, Strause, & Sampson, 1992). Though this reduction is due to the effects of canning on proteins and not DNA damage, it does indicate the possible utility of nucleic acid-based detection methods as an indicator of changes in allergenicity for canning methods. Obviously more research is necessary to better quantify the effects of processing on the allergenic proteins and the DNA before nucleic acid-based detection methods can be used as biomarkers for food processing.

The primers used in this study displayed no cross-reactivity with 53 other species of fish. Only Sockeye salmon (*Oncorhynchus nerka*) could also be detected with a cross-reactivity of 83% to Atlantic salmon. Inasmuch as the DNA sequence of the parvalbumin gene of Sockeye salmon is not known, all that can be concluded is that the primers designed based on the sequence of the Atlantic salmon gene were homologous to the Sockeye salmon gene.

5. Conclusions

Real-time PCR was used in an attempt to quantify and measure the effects of baking and pressure cooking on the detectable copy number of the gene encoding parvalbumin, the major allergen in salmon. Though the utility of this salmon specific PCR assay for quantitative purposes is questionable, the assay was comparable in sensitivity to a commercial test kit that was not salmon specific and cross-reacted with >35 different fish. Further research is needed to determine the effects of food matrices on the performance of this salmon specific assay.

Acknowledgements

Gratitude is expressed to Jonathan Deeds, Ph.D. (FDA) for providing the fish filets used in the cross-reactivity studies. Appreciation is also expressed to Anthony D. Hitchins, Ph.D. (FDA), Narjol Gonzalez-Escalona, Ph.D. (FDA), and Steven R. Monday, Ph.D. (FDA) for advice and guidance.

References

Aukrust, L., Grimmer, Ø., & Aas, K. (1978). Demonstration of distinct allergens by means of immunological methods. *International Archives of Allergy and Applied Immunology, 57*, 183–192.

Aukrust, L., Apold, J., Elsayed, S., & Aas, K. (1978). Crossed immunoelectrophoretic and crossed radioimmunoelectrophoretic studies employing a model allergen from codfish. *International Archives of Allergy and Applied Immunology, 57*, 253–262.

Bauer, T., Weller, P., Hammes, W. P., & Hertel, C. (2003). The effect of processing parameters on DNA degradation in food. *European Food Research and Technology, 217*, 338–343.

Bernhisel-Broadbent, J., Strause, D., & Sampson, H. (1992). Fish hypersensitivity. II: Clinical relevance of altered fish allergenicity caused by various preparation methods. *Journal of Allergy and Clinical Immunology, 90*, 622–629.

Congen Biotechnology GmbH (2005). *Instruction manual for SureFood® Allergen Fish real-time PCR kit for the qualitative detection of DNA from fish*. Version: S_311_FishRT_1.1, 9-10.

de Martino, M., Novembre, E., Galli, L., de Marco, A., Botarelli, P., Marano, E., et al. (1990). Allergy to different fish species in cod-allergic children: *In vivo* and *in vitro* studies. *Journal of Allergy and Clinical Immunology, 86*, 909–914.

Dory, D., Chopin, C., Aimone-Gastin, I., Guerin, L., Saintr-Laudy, J., Moneret-Vautrin, D. A., et al. (1998). Recognition of an extensive range of IgE-reactive proteins in cod extract. *Allergy, 53*, 42–50.

Elasyed, S., & Bennich, H. (1975). The primary structure of allergen M from cod. *Scandinavian Journal of Immunology, 4*, 203–208.

Elsayed, S., & Apold, J. (1983). Immunochemical analysis of cod fish allergen M: Locations of the immunoglobulin binding sites as demonstrated by the native and synthetic peptides. *Allergy, 38*, 449–459.

Friedberg, F. (2005). Parvalbumin isoforms in zebrafish. *Molecular Biology Reports, 32*, 167–175.

Gregory, T. R. (2008). *Anomal genome size database*. <http://www.genomesize.com>.

Helbling, A., Haydel, R., Jr., McCants, M. L., Musman, J. J., El-Dahr, J., & Lehrer, S. B. (1999). Fish allergy: Is cross-reactivity among fish species relevant? Double-blind placebo-controlled food challenge studies of fish allergic adults. *Annals of Allergy, Asthma, and Immunology, 83*, 517–523.

Hansen, T. K., Bindslev-Jensen, C., Stahl Skov, P., & Poulsen, L. K. (1997). Codfish allergy in adults: IgE cross-reactivity among fish species. *Annals of Allergy, Asthma, and Immunology, 87*, 187–194.

Hrnčířová, Z., Bergerová, E., & Siekel, P. (2008). Effects of technological treatment on DNA degradation in selected food matrices of plant origin. *Journal of Food and Nutrition Research, 47*, 23–28.

James, J. M., Helm, R. M., Burks, A. W., & Lehrer, S. B. (1997). Comparison of pediatric and adult IgE antibody binding to fish proteins. *Annals of Allergy, Asthma, and Immunology, 79*, 131–137.

José Chapela, M., Sotelo, C. G., Pérez-Martín, R. I., Ángel Pardo, M., Pérez-Villareal, B., Gilardi, P., et al. (2007). Comparison of DNA extraction methods from muscle of canned tuna for species identification. *Food Control, 18*, 1211–1215.

Lindstrøm, C. D. V., Van Do, T., Hordvik, I., Endresen, C., & Elsayed, S. (1996). Cloning of two distinct cDNAs encoding parvalbumin, the major allergen of Atlantic salmon (*Salmo salar*). *Scandinavian Journal of Immunology, 44*, 335–344.

Manual on fish canning (1988). *FAO fisheries technical paper – 285, Food and Agriculture Organization of the United Nations*, Rome. M-47 ISBN 92-5-102726-9.

Masataka, U., Tomohiro, N., & Katsumi, T. (2004). Thermostability of DNA from wheat in heated products. *Food Preservation Science, 30*, 195–198.

Peano, C., Samson, M. C., Palmieri, L., Gulli, M., & Marmiroli, N. (2004). Qualitative and quantitative evaluation of the genomic DNA extracted from GMO and non-GMO Foodstuffs with four different extraction methods. *Journal of Agricultural and Food Chemistry, 52*, 6962–6968.

Pascual, C., Martin Esteban, M., & Crespo, J. F. (1992). Fish allergy: Evaluation of the importance of cross-reactivity. *The Journal of Pediatrics, 121*, S29–S34.

Rehbein, H., & Kress, G. (2005). Detection of short mRNA sequences in fishery products. *Deutsche Lebensmittel-Rundschau, 101*, 333–337.

Sampson, H. A. (2000). Food anaphylaxis. *British Medical Bulletin, 56*, 925–935.

Sten, E., Hansen, T. K., Stahl Skov, P., Andersen, S. B., Torp, A. M., Bindslev-Jensen, U., et al. (2004). Cross-reactivity to eel, eelpout and ocean pout in codfish-allergic patients. *Allergy, 59*, 1173–1180.

Swaboda, I., Bugajska-Schretter, A., Verdino, P., Keller, W., Sperr, W. R., Valent, P., et al. (2002). Recombinant carp parvalbumin, the major cross-reactive fish allergen: A tool for diagnosis and therapy of fish allergy. *The Journal of Immunology, 168*, 4576–4584.

Taylor, S. L., Hefle, S. L., Bindslev-Jensen, C., Bock, S. A., Burks, A. W., Jr., Christie, L., et al. (2002). Factors affecting the determination of threshold doses for allergenic foods: How much is too much? *Journal of Allergy and Clinical Immunology, 109*, 24–30.