ORIGINAL ARTICLE

https://doi.org/10.21708/avb.2023.18.3.11967

Fraud detection on canned tuna marketed in Brazil

Detecção de fraude em conservas de atum comercializadas no Brasil

Yete Gambarini Ferri [1], Danilo Alves de França [1], Murilo Piologo Ciciliato [1], João Pessoa Araújo Júnior [1], Marianna Vaz Rodrigues [1*]

ABSTRACT: Tuna provides a healthy diet, it's rich on omega 3, high biologic value proteins, vitamins, and other nutrientes. The most popular presentation is in cans. Due to the fish's high price however, frauds were reported taking advantage over other species with similar taste but lower cost on fish used for this purpose, reducing production cost, a commonly fact observed in tuna cans. Use of species not declared in the product label implies on fraud. Nowadays Brazilian legislation forecasts detection of fraud in food by molecular analysis in routine inspection. Thus, the study aimed to detect economic fraud through species substitution in canned tuna by means of conventional PCR technique associated with real-time PCR (qPCR). Among 47 analyzed samples merely 7 (14,89%) contained only tuna, 9 had low and 25 had high concentration of another fish, and 7 did not amplified tuna's DNA, meaning there weren't any tuna. The results show that economic fraud via non declared species exists with high percentage, showing that is important to install survaillance active, once consummer is being mistaking and this practice is crime according to Brazilian legislation. Another point is that qPCR technique can be used to detect this kind of crime, which present high sensitivity, specificity, fast execution and low cost.

KEYWORDS: forensic genetics; qPCR; economic fraud; fish.

RESUMO: O atum proporciona uma dieta saudável, pois é rico em ômega 3, proteínas de alto valor biológico, vitaminas e outros nutrientes. A apresentação mais popular é em latas. No entanto, devido ao alto preço do peixe, foram relatadas fraudes que se aproveitaram de outras espécies com sabor semelhante, mas com custo mais baixo, no peixe utilizado para esse fim, reduzindo o custo de produção, fato comumente observado em latas de atum. O uso de espécies não declaradas no rótulo do produto implica em fraude. Atualmente, a legislação brasileira prevê a detecção de fraudes em alimentos por meio de análise molecular na inspeção de rotina. Assim, o estudo teve como objetivo detectar fraude econômica por substituição de espécies em atum enlatado por meio da técnica de PCR convencional associada à PCR em tempo real (qPCR). Das 47 amostras analisadas, apenas 7 (14,89%) continham apenas atum, 9 tinham baixa e 25 tinham alta concentração de outro peixe e 7 não amplificaram o DNA do atum, ou seja, não havia atum. Os resultados mostram que a fraude econômica por meio de espécies não declaradas existe em alta porcentagem, mostrando que é importante instalar uma vigilância ativa, uma vez que o consumidor está sendo enganado e essa prática é crime de acordo com a legislação brasileira. Outro ponto é que a técnica de qPCR pode ser utilizada para detectar esse tipo de crime, pois apresenta alta sensibilidade, especificidade, rapidez de execução e baixo custo.

PALAVRAS-CHAVE: genética forense; qPCR; fraude econômica; peixes.

INTRODUCTION

Bony fish from the Thunnus genus, popularly known as tunas, belong to the Actinopterygii class, Perciformes order and Scombridae family (South, 1845). Species belonging to this genus are: *Thunnus alalunga*, *T. albacares*, *T. atlanticus*, *T. maccoyii*, *T. obesus*, *T. orientalis*, *T. thynnus* and *T. tonggol* (Froese; Pauly, 2019).

The albacore (*T. alalunga*), is one of the most marketed species of tuna (FAO, 2018a); it is also easily found in areas of contact between hot and cold water (Froese; Pauly, 2019). This fish species have worldwide distribution and have been reported on several continents such as: Africa, Asia, Europe, North America, Oceania and South America, therefore it could be considered an endemic specie, native or introduced (Schultz, 2000; Fishbase, 2020). Due to the European Union regulation 1536/92, the term white tuna includes exclusively *Thunnus alalunga*, the light tuna refers to *T. albacares* and the word tuna includes any fish from Thunnus genus or similar species (e.g., *Katsuwonus pelamis*) (EUR-Lex, 1992).

[1]Universidade Estadual Paulista, Botucatu/SP, Brasil
*Corresponding author: mvazrodrigues@gmail.com
Received: 08/01/2023. Accepted: 10/17/2023

In Brazil, canned process uses tunna and sardine, but fraud practice is most common in tuna canned. Thus, this process is defined as attainment of whole tuna, filled in airtight and sterilized containers to prevent microorganism's proliferation and maintain the products integrity until the expiration date. For processing of tuna-like products in Brazil, the Ministry of Agriculture, Livestock and Supply (MAPA) published the Normative Instruction 46 from December 15, 2011, in which the technical identity and quality regulation is standardized for tuna and bonito conserves. Among the specifications, there is a list of fish considered tuna for conserve (*T. alalunga*, *T. albacares*, *T. atlanticus*, *T. obesus*, *T. maccoyii*, *T. thynnus*, *T. Tonggol*, *Katsuwonus pelamis*, *Euthynnus affinis*, *Euthynnus alleteratus*, *Euthynnus lineatus*) (Brasil, 2011; FAO, 2018b). Accordingly, economic fraud occurs through substitution of non-declared species. Besides being a procedure not based in brazilian legislation, it causes economic losses to the customer, in violation of the consumer law.

In Brazil, the nº 9.013 decree was published in march 29, 2017, for the new Inspection Regulation for Products of Animal Origin (RIISPOA) by the Ministry of Agriculture, Livestock and Supply (MAPA). The decree includes in the inspection routine performing of molecular biology analyzes, such as DNA testing, in addition to more severe penalties for fraud (Brasil, 2017; França *et al.* 2022). Thus, the present work aimed detect economic fraud through species substitution in pickled tuna by means of conventional PCR technique associated with real-time PCR (qPCR).

MATERIAL AND METHODS

In January 2015, samples were collected randomly to the present study. This stage was made by health surveillance agents in three supermarket chains in the municipality of Botucatu (SP, Brazil). The samples were made up of canned tuna from seven trademarks with different form of presentation: solid, piece, grated, in oil, natural, light and tomato sauce, totaling 47 samples. Thus, one sample was considered one canned tuna product market of one presentation (solid, piece, grated, in oil, natural, light or tomato sauce). These were delivered by the inspectors with duly identified seals.

Fresh tuna (*T. albacares*) that had been previously identified as a positive control was gathered whole in order to standardize PCR. The negative control in the reaction was made using nucleic acid free water. The pre-treatment of the samples was carried out as described by Chapella *et al.* (2007).

DNA extraction was performed with the Wizard® SV Genomic DNA Purification System (Promega®), according to manufacturer's instructions. After the procedures of oil removal, the remaining contents were frozen individually in order to reproduce the steps if necessary. To verify the extraction method efficiency, DNA quantity and quality were evaluated through spectrophotometry by NanoDrop 2000C spectrophotometer (Thermo Fischer Scientific Inc., Waltham, MA, EUA), using as purity parameters of the extracted samples, the ratios of 260/280 and 260/230.

For the qPCR reaction mix preparation was used: the Gotaq qPCR Master Mix 1X (Promega®), 10pmol/µl from each primer (target for tuna with primers CB-TALB-150-F and CB-TALB-150-R) (Chuang; Chen; Shiao, 2012) and as endogenous control were used activators for the Beta-actin fish gene S (5' ATC-CTG-ACA-GAG-GGT-GGC-TA 3') and Beta-actin fish gene R (5' CAT-CTC-CTG-CTC-GAA-GTC-C 3') designed in this study (Table 1), from the genetic sequencing described by He *et al.*, 2014, 1,5% of DMSO (v/v) and nucleic acid free water for final reaction volume adjustments. The qPCR reaction was performed in thermal cycler 7500 real time PCR (Thermo Fisher Scientific, Inc.) system in the following conditions: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute, by the end the dissociation curve was added. With the results in hand, the cT (threshold cycle) was determined for each sample.

For conventional PCR the same concentrations of primer were applied, but only the tuna-specific pair of primers (CB-TALB-150-F e CB-TALB-150-R) (Chuang; Chen; Shiao, 2012) were aplied in the mix with DMSO. The GoTaq Green Master Mix (Promega®) enzyme was used in 1X concentration, as recommended by the manufacturer. Visualization of the amplified product was performed through electrophoresis in 1.5% agarose gel with the intercalant SyberSafe (Thermo Fisher Scientific, Inc.) followed by exposure in ultraviolet light. Reaction condition for conventional PCR were: initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of 94 °C for 1 minute, 55 °C for 1 minute and 72 °C for 1 minute, with a final extension of 72 °C for 5 minutes.

There was amplification trough qPCR reaction of the positive control and the 47 samples using the pair of initiators for the Beta-actin gene as well as using the tuna-specific pair of primers. When evaluated by conventional PCR, only 7 samples did not amplify.

Table 1. List of oligonucleotides used for PCR and qPCR techniques for tuna fraud.

Primer	Sequence	Size (bp)	Reference
CB-TALB-150-F	5' CCC-CTA-GTA-TGG-GCG-ACA-GA 3'	63	Chuang; Chen; Shiao, 2012
CB-TALB-150-R	5' CGT-TCC-CTT-GCG-GTA-CTT-TC 3'	63	Chuang; Chen; Shiao, 2012
Beta-actin S	5' ATC-CTG-ACA-GAG-GGT-GGC-TA 3'	109	Designed in this study from the sequence: KJ126772.1 (He *et al.*, 2014)
Beta-actin R	5' CAT-CTC-CTG-CTC-GAA-GTC-C 3'	109	Designed in this study from the sequence: KJ126772.1 (He *et al.*, 2014)

For qPCR results interpretation, samples with Threshold cycle (cT) difference between reaction with Beta-actin primer and tuna-specific primer, close to fresh tuna (6.5-8) were considered without fraud (green) Samples with smaller differences than fresh tuna were considered to have a low concentration of another fish, with a difference of 4-6.5 cT (yellow) or with a high concentration of another fish, that is, a difference of 0-4 cT (red) as shown in Table 2.

Of the 47 samples tested by qPCR, only 7 were considered to contain only tuna, 9 were considered to have a low

Table 2. Differences in threshold cycle between different canned tuna samples with Beta-actin primer and specific primer.

Beta-Actin	Threshold cycle	Cb-talb	Beta-Actin	Threshold cycle
Sample 1	28,900	Sample 1	27,521	1,379
Sample 2	29,064	Sample 2	24,436	4,628
Sample 3	33,804	Sample 3	30,289	3,515
Sample 4	30,621	Sample 4	30,505	0,116
Sample 5	30,279	Sample 5	28,422	1,857
Sample 6	32,267	Sample 6	27,399	4,868
Sample 7	27,449	Sample 7	20,881	6,568
Sample 8	28,585	Sample 8	26,965	1,620
Sample 9	25,813	Sample 9	19,288	6,525
Sample 10	33,278	Sample 10	29,608	3,670
Sample 11	30,425	Sample 11	29,893	0,532
Sample 12	28,465	Sample 12	25,358	3,107
Sample 13	29,202	Sample 13	26,577	2,625
Sample 14	27,705	Sample 14	24,837	2,868
Sample 15	28,390	Sample 15	25,451	2,939
Sample 16	24,952	Sample 16	20,871	4,081
Sample 17	27,235	Sample 17	20,346	6,889
Sample 18	28,395	Sample 18	24,685	3,710
Sample 19	30,641	Sample 19	24,472	6,169
Sample 20	33,457	Sample 20	26,426	7,031
Sample 21	27,842	Sample 21	26,417	1,425
Sample 22	30,500	Sample 22	25,887	4,613
Sample 23	33,128	Sample 23	27,128	6,000
Sample 24	31,754	Sample 24	30,078	1,676
Sample 25	32,472	Sample 25	29,740	2,732
Sample 26	28,929	Sample 26	25,718	3,211
Sample 27	25,81	Sample 27	23,491	2,327
Sample 28	35,95	Sample 28	33,85	2,1
Sample 29	29,44	Sample 29	29,74	-0,3
Sample 30	27,78	Sample 30	0	*
Sample 31	31,26	Sample 31	0	*
Sample 32	29,18	Sample 32	34,22	5,04
Sample 33	34,27	Sample 33	39,41	5,14
Sample 34	25,49	Sample 34	0	*
Sample 35	27,42	Sample 35	0	*
Sample 36	32,80	Sample 36	0	*
Sample 37	33,21	Sample 37	0	*
Sample 38	26,91	Sample 38	28,51	-1,6
Sample 39	29,09	Sample 39	24,50	4,59
Sample 40	30,15	Sample 40	27,02	3,13
Sample 41	27,87	Sample 41	28,26	-0,39
Sample 42	27,53	Sample 42	19,79	7,74
Sample 43	30,92	Sample 43	22,17	8,75
Sample 44	31,32	Sample 44	30,13	1,19
Sample 45	27,21	Sample 45	26,48	0,73
Sample 46	25,81	Sample 46	26,64	-0,83
Sample 47	26,66	Sample 47	18,15	8,53
Fresh tuna	16,774	Atum fresco	08,888	**7,886**

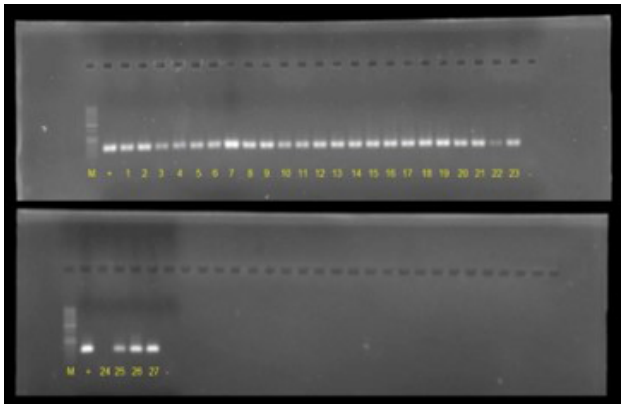

Figure 1. Product amplification of 100 bp. M: molecular marker. 1-27: canned tuna. +: positive control. -: negative control.

concentration of another fish and 25 were considered to have a high concentration of another fish (Figure 1). It is noticed that, in samples 24, 30, 31, 34, 35, 36, 36 there was no amplification by PCT, however they were considered with a high concentration of another fish by qPCR. Thus, by combining the two techniques, it was possible to state that these samples were not 100% tuna.

DISCUSSION

It is considered that, in view of the results obtained in the present study, 63% of the samples contained a high concentration of other fish and 22% were partially defrauded, that is, 85 % of the total samples showed a high rate of economic fraud, through substitution from more expensive species to cheaper species, but with a high sale price, damaging the Brazilian consumer protection code. Only 15 % of the samples contained 100% tuna.

Although some samples have a low 260/280 ratio, all were amplified by qPCR, showing that the process was efficient and that there was no inhibitory gene in the reaction. There was amplification of the positive control and of the 47 preserved samples using the pair of primers for the endogenous Beta-actin gene, as well as using the pair of specific primers for tuna. These data demonstrate that the techniques were properly standardized and allowed the analysis of the preserved samples. Although there was no amplification by traditional PCR in samples 24, 30, 31, 34, 35, 36, 37, the results obtained by qPCR prove the high concentration of another fish, justified by the latter technique being more sensitive.

In a study by Bojolly *et al*. (2017), two different methods based on qPCR (standard and endogenous curve) were developed for the identification and quantification of two very similar genetically types of canned tuna, being *T. obesus* and *T. Albacares*. Among the studied samples of canned tuna, the tuna species *T. alalunga*, *T. obesus*, *Katsuwonus pelamis* and *T. albacares* were labeled. Although there is a difference in standardization of the methodology based on qPCR, the results suggested that there was also the presence of different species in the cans or the incorrect identification by the industries.

As described by Liu *et al*. (2016), five species of high-value tuna (*T. maccoyii*, *T. obesus*, *T. albacares*, *T. alalunga* and *Katsuwonus pelamis*) could be identified using the qPCR technique, in canned products, corroborating with the present work. Thus, it is considered that the molecular method for identifying canned tuna species must be fast and reliable, in order to ensure better management in fishing and consumer rights.

Under Brazilian legislation, only the tuna species *T. alalunga*, *T. albacares*, *T. atlanticus*, *T. obesus*, *T. maccoyii*, *T. thynnus*, *T. tonggol*, may be processed for sale, therefore, tuna's meat is considered as a mandatory ingredient. In addition, other species can only be included in the technical report with authorization from MAPA (Brasil, 2011). Therefore, if species other than those described in the legislation are not declared on the label, an economic fraud process has occurred. In Brazil, MAPA included in the RIISPOA (Regulation of Industrial and Sanitary Inspection of Products of Animal Origin) the use of molecular tests for inspection purposes, assisting in this crime practice. However, currently the main challenge is the standardization of molecular methods for this type of test (Brasil, 2017).

In view of the need to standardize the molecular method for detecting fraud in canned tuna and that the marketed product must have credibility to guarantee consumer rights and food safety (Barbosa, 2015, França *et al*. 2022), the applied methodology developed in the present study can contribute as an important tool for the detection of commercial fraud in canned tuna.

CONCLUSIONS

The results show that economic fraud via non declared species exists with high percentage, showing that is important to install survaillance active, once consummer is being mistaking and this practice is crime according to Brazilian legislation. Another point is that qPCR technique can be used to detect this kind of crime, which present high sensitivity, specificity, fast execution and low cost.

REFERENCES

BARBOSA, J. M. Fraudação na comercialização do pescado. **Actapesca**, v. 3, n. 2, p. 89-99, 2015.

BOJOLLY, D. *et al*. Development of a qPCR Method for the Identification and Quantification of Two Closely Related Tuna Species, Bigeye Tuna (Thunnus obesus) and Yellowfin Tuna (Thunnus albacares), in Canned Tuna. **Journal of Agricultural and Food Chemistry**, v. 65, n. 4, p. 913-920, 2017.

BRASIL. Regulamento técnico de identidade e qualidade para conservas de atuns e bonitos. **Instrução Normativa 46 de 2011**.

BRASIL. Regulamento Da Inspeção Industrial e Sanitária de Produtos de Origem Animal (RIISPOA). **Instrução Normativa n° 18, de 25 de maio de 2017**. Aprovado Pelo Decreto N° 30.691, de 29/03/52, Alterado Pelo Decreto N° 1.255, de 25/06/62, Alterado Pelo Decreto N° 1.812.

CHAPELA, M. J. *et al*. Comparison of DNA extraction methods from muscle of canned tuna for species identification. **Food Control**, v. 18, n. 10, p. 1211-1215, 2007.

CHUANG, P. S.; CHEN, M. I.; SHIAO, J. C. Identification of tuna species by a real-time polymerase chain reaction technique. **Food Chemistry**, v. 133, n. 3, p. 1055-1061, 2012.

EUR-Lex. Laying down common marketing standards for preserved tuna and bonito. **Council Regulation (EEC) No 1536/92 of 9 June 1992**. Disponível em: https:/eur-lex.europa.eu/legal-content/PT/TXT/?uri=CELEX:31992R1536. Accessed: June 30, 2022.

FAO. The State of World Fisheries and Aquaculture - **Meeting the sustainable development goals**. In FAO. 2018a. Disponível em: https:/www.fao.org/family-farming/detail/en/c/1145050/. Accessed: July 01, 2022.

FAO. Standard for canned tuna and bonito: **CXS 70-981**. 2018b, p.2. Disponível em: https:/www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B70-1981%252FCXS_070e.pdf. Accessed: July 01, 2022.

FISHBASE. Albacore - Tunnus alalunga (Bonaterre, 1788): **Country List**, 2020. Disponível em: https:/www.fishbase.se/Country/CountryList.php?id=142&genusname=Thunnus&speciesname=alalunga&lang=english. Accessed July 02, 2022.

FRANÇA, D. A. *et al*. Avaliação da qualidade do pescado comercializado na região de Botucatu, São Paulo. **Veterinária e Zootecnia**, v. 29, p. 001-009, 2022.

FROESE, R.; PAULY, D. Search FishBase. **World Wide Web Electronic Publication**, 2019. Disponível em: https:/fishbase.se/search.php. Accessed: July 02, 2022.

HE, A. Y. *et al*. Identification, characterization and nutritional regulation of two isoforms of acyl-coenzyme A oxidase 1 gene in Nile tilapia (Oreochromis niloticus). **Gene**, v. 545, n. 1, p. 30-35, 2014.

LIU, S. *et al*. Identification of five highly priced tuna species by quantitative real-time polymerase chain reaction. **Mitochondrial DNA Part A**, v. 27, n. 5, p. 3270-3279, 2016.

SCHULTZ, K. **Fishing encyclopedia**: worldwide angling guide. Foster City, California: IDG Books Worldwide, Inc. 1916 p. DOI/ISBN: 0-02-862057-7. 2000.

SOUTH, J. F. Thunnus. In: **Encyclopedia Metropolitana**; or, Universal Dictionary of Knowledge. v. 25, p. 620-622, 1845.