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Research Article

Investigation of the Impact of Can-filling Medium on the DNA Quality of Canned Tuna Sold in Supermarkets

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ABSTRACT

Canned tuna is one of the most commonly consumed food products globally. Due to its high profitability and the increasing demand for it, fraudulent canned tuna products have become a serious problem. The traceability of fish species in packaged material and, in the case of highly processed forms, in canned products, has become impossible; therefore, canned tuna is on the list of the top ten food items affected by fraud. These fraudulent actions cause not only unfair trade in the commercial market and fishing industry, but also cause health damage (such as allergies and poisoning) to the public. Complex food matrices also affect the extracted DNA quality when the main food products are served with another medium. Brine solutions, different kind of oil, and several types of sauce are used as filling medium in the canned tuna production process. These filling medium can cause contamination depending on whether they include oil, salt or other ingredients during DNA extraction from main products. DNA-based protocols have become popular due to their higher reliability rate compared to other protocols. This research investigates the potential impact of can-filling medium on DNA quality, which is a key factor for food traceability research. With this aim, canned tuna from various brands in different can-filling medium such as olive oil, sunflower oil and different kinds of sauces, were obtained from a Turkish supermarket. The quality properties, such as yield and purity, affected the traceability analyses. This study was designed to investigate the potential effect of the filling medium on DNA quality. The results revealed that different kinds of sauce utilization as a can-filling medium cause a reduction in the DNA quality of canned tuna compared to other canned tuna samples that contain olive oil and sunflower oil. The purity of extracted DNA in canned tuna where olive oil was used was found to be relatively higher than other tuna groups with different can-filling medium. Melting curve analyses revealed that sunflower oil causes relatively lower degradation than olive oil and different types of sauce used as filling medium. These results could be beneficial for further seafood traceability research, especially in complex matrices.

Keywords: Canned tuna, filling medium, DNA quality, DNA yield, traceability

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INTRODUCTION

Due to its health benefits, seafood consumption has increased dramatically over recent years and has reached around 20 kg per capita. Seafood products are considered one of the most traded food items (Asche, Bellemare, Roheim, Smith, & Tveteras, 2015; FAO, 2018). The rising trend of seafood consumption and the reduction of fish stocks have caused a significant increase in fraudulent actions in the seafood industry (Tamm, Schiller, & Hanner, 2016). Fish species are considered the third-highest risk group for fraudulent actions among other foods (Reilly, 2018). The most common types of fraudulent actions in seafood production can be classified as intentional species substitution, species adulteration and mislabelling (Fox, Mitchell, Dean, Elliott, & Campbell, 2018). Substitution or intentional mislabelling of a species

generally appear when replacing the high-value species with a cheaper or less-desirable species for illegal economic gain. In addition, unintentional mislabelling may arise because of misidentification or doubts in the naming of closely related species along the seafood supply chain (Barendse et al., 2019). All these fraudulent actions not only cause unfair financial gain and pose a serious threat to public health but may also have ecological impacts, such as affecting biodiversity and further fisheries activities (Pardo & Jimenez, 2020).

Tuna species, a large group of important fishes that belong to members of Scombridae family, are classified into three genera (Katsuwonus, Sarda, and Euthynnus) and these species have different economic and ecological value (Abdullah & Rehbein, 2015). The over-consumption of the most desirable species, challenges regarding raw material sustainability for the tuna canning industry and mostly unregulated economic incentives could cause an increase in substitution and mislabelling in the canned tuna industry (Sotelo et al., 2018). Fraudulent actions in the tuna industry differ depending on the country, local market demand, consumer preference and regional tuna catch annually. Gordoa, Carreras, Sanz, & Viñas (2017) highlighted that the mislabelling and substitution rate changed from 37% to 48% in the Spanish tuna processing chain, and the mislabelling of tuna products rate was found to be much higher (95%) in Brussels restaurants (Europe, 2015). These results revealed that the importancet of monitoring of traceability in tuna products globally. While reaching the higher-yield DNA of unprocessed raw material is relatively easy, it becomes a complex problem with DNA extracted from processed and mixed seafood due to thermal treatment, acidic application and pressure. In the canned tuna industry, several can-filling mediums consisting of sauce, spices and other ingredients are used for increasing consumer acceptance and product variability. Despite their benefits, the filling medium decrease the quality and yield of DNA due to the variations in thermal conductivity and acidity. These cause degradation of DNA from canned tuna. Sunflower oil, olive oil and brine solutions are the most commonly used can filling mediums in the canning industry. Sunflower oil offers more palatable tuna with a relatively lower cost, and olive oil extends shelf-life by retarding the oxidation of tuna and leads to more acceptable colorimetric characteristics (Boughattas, Le Fur,& Karoui, 2019). Recently, usage of various sauces, spices and slices of vegetables as filling medium has become more common due to increasing consumer preference. These can-filling mediums seem to benefit the consumer, however, different spices have not only been used as flavouring and colouring agents in the food items, but also in fraudulent actions such as masking undesirable colour and rancid taste (Julien-David & Marcic, 2020). In light of increasing fraudulent actions in the seafood industry, usage of these risky components in canned tuna products should be controlled through authorized methods. The quality and yield of DNA from food items does not depend on the initial material characterization. DNA extraction technique is another essential factor impacting DNA quality. In addition to several confirmed chemical, lyses and enzymatic methods, different commercial kits have also been used for the DNA extraction process (Barbosa, Nogueira, Gadanho & Chaves, 2016; Sajali et al., 2018).

MATERIAL AND METHODS

Twenty-one commercial canned tuna products (Table 1) obtained from Turkish supermarkets were purchased in April 2021 and analysed. The 21 products were chosen to represent all the main cannery brands available on the Turkish market (12) with various can-filling media (e.g., oils, spices and/or sauces). Commercial samples representing the three product categories considered in this study (canned tuna with sunflower oil (SO), canned tuna in olive oil (OO) and canned tuna with sauces (SA) including different ingredients such as pepper, tomato or mustard, etc.) were extracted with the same protocol. Commercial canned tuna samples were dried with sterile filter paper to eliminate the oils, spices and sauces, and then 15-g samples of tuna were transferred into 50 mL Falcon tubes and stored at -80° C. DNA was extracted from all samples with the same extraction methods: 20-mg sample, 250 µl Buffer ATL and 20 µl Proteinase K were mixed. Thermal processing was applied to the mixture until the mixture was completely lysed. The lysed mixture was centrifuged at 12000 g for 30 seconds and then the supernatant was directly transferred to the sterile tube. An extractions buffer (250 µl) was added to the spin column and heated at 56 °C for 10 minutes in order to reach a better yield of DNA. Then 250 µl of a binding buffer (BF) was added and the total mixture was vigorously mixed in a vortex for 15 seconds. Afterward, the mixture was applied to the mini-spin columns for binding DNA. Then the spin column was washed with AW1(650 µl) and AW2 (500 µl) and centrifuged. Finally, pre-heated buffer AE (200 µL) was used for the elution of the purified DNA and then thr purified DNA was stored at -20°C until further analysis.

Determination of DNA quality and amplificability

The quality of DNA from canned tuna in terms of concentration, purity and presence of any contaminants was investigated with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Pittsburgh, PA, USA). The amplificability of the gDNA was then determined by amplifying a 655 bp region from the COI which was targeted teleo primer (COI F: 5′ TCGACTAATCATAAAGATATCGGCAC 3′ and COI R: 3′ ACTTCAGGGTGACCGAAGAATCAGAA 5′) (Ward, Zemlak, Innes, Last, & Hebert, 2005). The reaction mixtures were prepared as follows: 2 µL of template gDNA, 2 µL of forward and reverse primer, 10 µL Master Mix (Thermo Scientific™ Maxima SYBR Green/ROX qPCR Master Mix (2X) and 6 µl DNA free water. The PCR was run and analysed on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) according to the following cycling protocol: denaturation at 95°C for 2 minutes, 35 cycles of 30 s at 94 °C, 30 s at 53 °C, and 60 s at 72 °C, and the final extension at 72°C for 10 minutes. Generally, real-time PCR and melting curve analysis (MCA) are performed in combination to better understand the kinetics of denaturation and offer detailed knowledge about the following sequence, which differs depending on main food items and different components found in food matrices. The melting curve analysis (MCA) was carried out between 65°C and 95°C.

Statistical analysis

For comparison of the yield, purity and contamination level of DNA extracted from all commercial canned tuna samples (SO, OO, SA), a two-way cross-classification analysis of variance

Table 1. Canned tuna sample description.

Sample	Fish type[a]	Canning Matrix[b]	Brand[c]	Exp.Date
1	Tuna	Olive oil	Brand 1	22.06.2024
2	Tuna	Tomato sauce	Brand 1	22.12.2024
3	Tuna	Mustard sauce	Brand 1	20.01.2024
4	Tuna	Olive oil	Brand 2	20.01.2026
5	Tuna	Sunflower oil	Brand 2	19.11.2024
6	Tuna	Olive oil	Brand 2	19.11.2025
7	Tuna	Sunflower oil	Brand 3	11.07.2024
8	Tuna	Pepper sauce	Brand 3	11.07.2026
9	Yellowfin tuna	Olive oil	Brand 4	05.12.2025
10	Yellowfin tuna	Sunflower oil	Brand 4	05.12.2026
11	Skipjack tuna	Sunflower oil	Brand 5	15.11.2025
12	Skipjack tuna	Pepper sauce	Brand 5	15.11.2025
13	Tuna	Sunflower oil	Brand 6	22.09.2025
14	Tuna	Olive oil	Brand 6	22.09.2025
15	Skipjack tuna	Sunflower oil	Brand 7	19.11.2024
16	Skipjack tuna	Olive oil	Brand 8	19.11.2025
17	Tuna	Pepper sauce	Brand 8	25.10.2025
18	Tuna	Sunflower oil	Brand 9	25.10.2026
19	Skipjack tuna	Tomato sauce	Brand 10	21.12.2025
20	Skipjack tuna	Tomato-pepper sauce	Brand 11	23.12.2025
21	Yellowfin tuna	Olive oil	Brand 12	19.09.2024

[a] fish variety declared in the product label; [b] canned tuna packaged with different filling medium; [C]For privacy reasons, brands are not reported and are listed numerically.

(ANOVA) was performed. All the statistical significances were determined by SPSS software version 19 (Chicago, Illinois, USA). Statically important differences were evaluated at a level of 5% ($P< 0.05$). All the DNA quality analyses were performed in triplicate assays for each canned tuna sample group.

RESULTS AND DISCUSSION

Determination of DNA quality

The purity of the gDNA is a key parameter that can powerfully affect the success of PCR amplification and sequencing processes and thereof the traceability analyses. The purity of the DNA was calculated with the A260/A280 ratio. Significant differences were observed among the canned tuna samples with different can filling medium (Table 2). While the highest values were obtained in canned tuna with sunflower oil (with 2.26), the other groups (filling medium of olive oil and sauce) have 1.95 and 1.90. The optimal purity value is between 1.8-2.0 (Piskata, Pospisilova, & Borilova, 2017). These differences could be related to the thermal integrity of sunflower oil and components that differ from sauce. Treatment with different compounds cause differentiation of the purity of DNA, which is accepted as an indicator for the DNA yield. Another key parameter for the molecular analyses performed for food traceability is the A260/A230 ratio, which is accepted as a sign of the presence of organic contaminants (from carbohydrates to salts) in the extracted DNA. There were no significant differences in terms of the presence of contaminants observed in canned tuna products with olive oil and sunflower oil filling medium canned tuna products. The highest contamination rate was determined to be in the tuna soaked in a sauce filling medium. These differences could be explained by treatment with different ingredients present in sauce, which reduces the purity of extracted DNA. As stated by Lucena-Aguilar et al (2016), the optimal value for the presence of any contaminant in extracted DNA ranges from 2.0 to 2.2. The highest contaminant values observed from canned tuna samples with sauce was 2.31. This significant higher value may be related to the presence of more than one medium in the sauces used for canned tuna.

The results of the extracted DNA from canned tuna samples with different can-filling medium are given in Figure 1 and Table 2. Calculation of the DNA yield was performed depending on DNA concentration, initial weighted tuna muscle and the final volume obtained. As stated in Table 2 and Figure 1, significant differences in terms of DNA yield were found among canned tuna with various can-filling medium ($P<0.05$). The highest DNA yield was determined in the SO group (916.1 (ng/ul) and the lowest DNA yield, which was found to be significantly lower than the other groups, was determined in the SA group (211.9 (ng/ul). This is similar to other quality parameters. As a filling medium, sunflower oil and olive oil lead to better DNA yield, DNA purity and the absence of organic contaminants than does sauce. Chapela et al. (2007) performed a comparative study for canned tuna with different filling medium such as vinegar, brine tomato sauce and oil by various extraction techniques; they reported comparatively higher DNA concentrations obtained in tuna in oil groups in all extraction methods. Due to can-filling sauce made with several ingredients that could have different sensitivities to thermal process and acidity, the quality and yield of DNA is not stable. This is consistent with what was highlighted by Chapela et al. (2007)

and Elsanhoty, Ramadan & Jany (2011), who stated that a significant reduction in DNA purity for those canned tuna samples with filling sauce may be related to the acidity causing the hydrolytic degradation mechanism of DNA.

radation of DNA is an important problem that can cause disruptions in species control and protection against food fraud. The thermal process is accepted as the main reason for the DNA degradation. Because of the variation in consumer consumption

Table 2. DNA quality assessment of canned tuna sample.

Canned tuna with can-filling medium	Quality assessment		
	DNA Yield (ug/uL)	Purity (A260/A280)	Chemical Contamination (A260/A230)
SO	916.1±0.03^{c}	2.26±0.10^{b}	2.13±0.11^{b}
OO	578.10±0.01^{c}	1.95±0.16^{ab}	2.19±0.07^{b}
SA	211.9±0.10^{b}	1.90±0.05^{a}	2.31±0.09^{a}

Groups: Canned tuna with sunflower oil (SO), canned tuna in olive oil (OO), canned tuna with sauces (SA).
Values are expressed as average ± standard deviation (n =3).
Values in the same column followed by different numbers show significant difference ($P < 0.05$) between canned tuna groups.

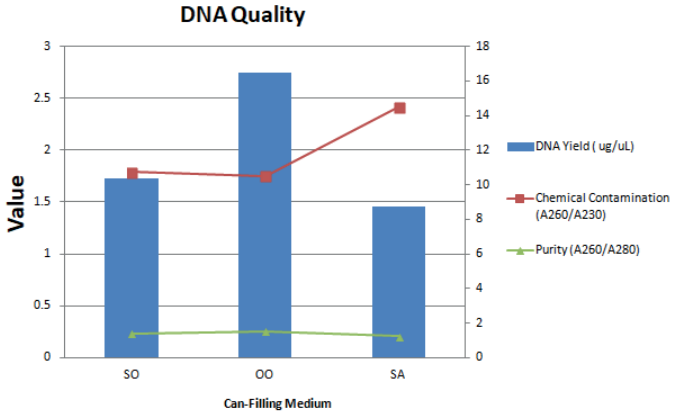

Figure 1. DNA quality variation of canned tuna sample.
Groups: (SO): canned tuna with sunflower oil, (OO): canned tuna in olive oil, (SA): canned tuna with sauces

The quality characteristics of DNA directly impact the amplification, sequencing and therefore the achievement of molecular methods for food authentication, especially in complex food matrices. The canning process includes both thermal treatment and high pressure, which have an impact on the stability of DNA. Different filling medium has also cause variation in DNA yield, quality and organic contaminant presence in extracted DNA, which can directly impact the further step of traceability analyses. The achievements of food traceability research are mainly driven by the extracted DNA quality and purity.

Determination of DNA degradation

DNA degradation among canned tuna samples with different can-filling medium determined by threshold cycle (Ct) value. The statically significant differences in terms of melting curves among the groups are shown in Figure 2. These variations could be explained by the impact of heat treatment on the filling medium during the canning and sterilization process. Ballari and Martin (2013) also reported that the variations in DNA fragmentation and amplificability observed resulted from autoclaving. Regardless of the aim of sequencing in DNA-based methods, the degtendency toward rapidly consumed food and technological improvements, the ready-to-eat food market has grown rapidly and several processed food products are consumed globally. This case also causes challenges in the traceability of food products, which is very important for public health and fair trade. Fraud detection analyses depend on quality, yield and degradation level.

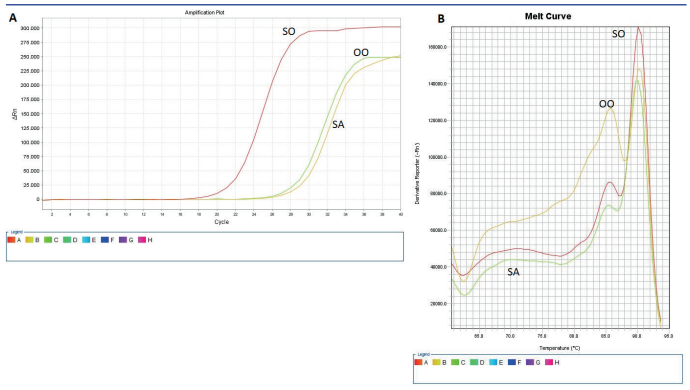

Figure 2. Amplicon plots (A) and Melting Curve (B) of tuna sample with different can-filling medium.
Groups: (SO): canned tuna with sunflower oil, (OO): canned tuna in olive oil, (SA): canned tuna with sauces

CONCLUSION

The effects of can-filling medium on the quality of DNA and the level of DNA degradation in canned tuna were compared with the same extraction methods and the same amplification procedures. In total, 21 different commercial canned tuna products with different can-filling medium examined in terms of DNA quality (DNA yield, DNA purity and presence of contaminants) and DNA degradation. The results revealed that different filling medium caused variance in DNA degradation and quality parameters in canned tuna, which could be related to the thermal integrity of different compounds used as a filling medium, such as oil or sauce ingredients. These findings could be useful for other thermally processed products, especially seafood products

which are highly perishable without acidic or thermal treatment. The results of this research are also valuable for other complex food matrices. As part of the increased demand for well-organized analyses methods for food authentication, better quality and low DNA degradation are accepted as the initial step within molecular-based methods. The results revealed that monitoring of DNA quality and yield are essential for the food traceability research in thefor other seafood , especially in seafood products that have complex food matrices.

Note: This study was presented as an online presentation at the 2nd Aquatic Biotechnology Symposium.

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