Turkish Journal of Agriculture - Food Science and Technology, 9(sp): 2385-2389, 2021
DOI: https://doi.org/10.24925/turjaf.v9isp.2385-2389.4706

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Effect of Industrial and Domestic Processing Techniques On the DNA Degradation of Anchovy[#]

Elif Tuğçe Aksun Tümerkan[1,a,*]

[1]*Department of Food Processing, Vocational School of Technical Sciences, Ankara Yıldırım Beyazıt University, 06760 Ankara, Turkey*
[*]*Corresponding author*

ARTICLE INFO

[#]*This study was presented as an online presentation at the 2nd International Journal of Agriculture - Food Science and Technology (TURJAF 2021) Gazimağusa/Cyprus*

Research Article

Received : 04/11/2021
Accepted : 29/12/2021

Keywords:
Processing techniques
Anchovy
DNA degradation
Species fraud
Food safety

ABSTRACT

Food fraudulent activities have become a serious issue over the world recently. Seafood products have trading and profitable potential in Turkey owing to the abundance of fisheries and other species. While morphological features are commonly used for species identification in raw seafood products, this identification does not meet the correct classification in cryptic species and processed seafood products. Molecular techniques have been utilized for species authentication in processed seafood items successfully. In this study, the effect of different processing techniques on the DNA quality and DNA degradation isolated from raw and processed anchovy was investigated. Anchovy is one of the important species in both fisheries activities and processing and consumption in Turkey. For this aim, DNA was isolated from processed anchovy groups and un-processed anchovy groups as control by the same extraction methods and the quality of DNA was compared among the groups. The most common processing techniques, frying, baking, smoking, roasting, baking and grilling were applied to anchovy. The results revealed that not only different thermal processing but also treatment with acid and salt cause DNA degradation and quality loss of DNA parameters which are essential for authentication of species and traceability for public health.

[a] *etaksun@gmail.com* *https://orcid.org/0000-0003-1993-0569*

Introduction

Within increasing population, the demand of animal-based protein sources has also increased. Seafood product meets this demand owing to its highly nutritional value and biochemical properties (Giusti et al., 2019). Due to these characteristics, seafood is one of the profitable items in food industry. The reported food fraudulent cases have increased globally since 2000s. Food fraudulent is one of the important threats to food security in both plant based and animal-derived food products by misinforming of consumer. Food fraudulent also results in uncontrolled stock management and biodiversity. Mislabelling, species adulteration and species substation are main fraudulent activities in seafood industry, especially in fish species due to increasing market size and highly profitable cases of this industry. Mislabelling generally occurs that labelling of less valued species as more desirable or more valued species. While species identification can be possible in fresh and non-processed species with morphological properties, it becomes difficult in packaged or processed products. This mislabelling or species substation seems just unfair gain, it actually cause to more serious problem to public as allergic symptoms or bioaccumulation of some heavy metals (Shehata et al., 2018).

Similar to the growing market size of seafood globally, consumption of processed products types and exporting of seafood products have increased in Turkey. Besides to fresh consumption of fish species, smoked, marinated or cooked with different techniques fish products commonly consumed in Turkey. The exporting of seafood increased 27 thousand tonnes with 97 million dollar in 2002, this rate reported as 177 thousand tonnes accounting as 952 million dollar in 2018 (Dağtekin et al., Fisheries Sector Policy Document, 2019). Anchovy is one of the most consumed fish species and accounting more than 50% of fisheries activities in Turkey. Annual anchovy fisheries rate increased from 103 thousand tonnes to 171 thousand tonnes between 2016-2020. While anchovy commonly consumed as fresh form in local scale, frozen, smoked and marinated anchovy exported frequently (Karslı.,2021). While fraudulent in seafood this issue has researched with 4500 product test in more than 50 publications globally, there is very limited information about traceability of seafood in Turkey. Two previous research that focused particularly on surimi and squid products obtained from the markets (Keskin and Atar, 2011; Keskin and Atar, 2012). More recently, Koban Baştanlar (2019) investigated the

mislabeling of frozen tilapia fillet purchased from market. Despite there is considerable amount of consumption and export rate of anchovy in Turkey, the traceability of processed anchovy is remaining unclear.

Protein based methods used for determination the traceability of food products formerly; molecular based techniques have become popular more recently (Nagalakshmi et al.,2016). DNA quality and purity are the main factors for the amplification and sequencing steps performing in species identification which has been very important issue in the food traceability and food safety.

This research was aimed to investigation of DNA degradation in anchovy processed with both industrial and domestic scales. For this aim, the DNA was extracted from marinated anchovy, marinated with sauced and spiced anchovy and smoked anchovy representing the industrial processing methods; fried; oven baked and grilled anchovy, representing domestic processing. The degradation level and quality of DNA from all processed anchovy groups compared with un-processed anchovy with Nano drop and Real-time PCR results.

Material and Methods

Products Sampling and Processing At Industrial And Domestic Scale

Anchovy (*Engraulis encrasicolus* L.) purchased from local fishery market anchovy from the Samsun province of the Black Sea region in Turkey March 2021. Anchovy species were caught and kept in polystyrene boxes with ice and transferred in ice to the laboratory immediately. Anchovy were washed in tap water for 4-5 min, and then, the heads of the fish were cut off; internal organs were removed; the fillets filleted by using the tip of a sharp blade prior to all treatments. The total amount was divided into seven groups, raw and prepared for cooking in six different methods. The raw anchovy without cooking was taken as control groups. All the anchovy samples taken from same batch referring to same catching area and catching period to avoiding any variation caused by species differentiation. All the processing method experiments were conducted at least in triplicate.

Frying: 500 gr of the anchovy fillet was added to 1 L of commercially available boiled sunflower oil for 4 min in 2 L stainless steel non-stick deep cooking pot (Tefal, Paris, France). The probe was located in the centre of cooking pot followed by monitoring the cooking temperature reached up to 180°C (Xavier et al.,2018).

Baking: For the baking treatment, an electric oven was preheated to 200 ºC, anchovy fillets placed on a metal tray samples in oiled aluminium dishes were baked uncovered for 20 min. Following to baking process, samples removed and allowed to cool. Yılmaz and Koca, (2020).

Grilling: Anchovy fillets grilled on a gas-operated oven (covered with foil) for 3 min (1.5 min of each side) at 350ºC (Özogul et al.,2009). Each sample was grilled individually in a separate. In all cases, the thermally processed anchovy fillets were permitted to reach at room temperature (24-25 ºC). The thermally processed anchovy were then grounded, homogenized, dried, sieved, labelled, bottled, freeze-dried until DNA extraction processes.

Marinating: Marinating processes were carried out according to the method of Olgunoglu (2007) with a slight modification in a seafood processing plant (Pakyürek, Adana, Turkey). Cleaned anchovy fillets were dipped into solution containing salt (10%), alcohol vinegar (4.5%) and citric acid (0.2%) in the ratio of 9:10 (marinate solution: fish fillets) for marinating practice through 24 hour in a refrigerator (0-4°C). Following to marination process, anchovies were taken off from the solution and filtered for avoiding any solution. After then anchovies were putted in plastic box (200 g), and sun flower oil added in the ratio of 175g: 75g (marinated anchovy fillets: sunflower oil). Plastic boxes were vacuum packaged and stored at 0± 2 ºC for 6 months. For sauced- marinate approach, all the marinating processes were performed same as mentioned above and then anchovies were placed in plastic box (200 g), and sun flower oil and pepper sauce added in the ratio of 170g:70g:10g (marinated anchovy fillets: sunflower oil: pepper sauce) Plastic boxes were vacuum packaged and stored at 0± 2°C for 6 months. Following to 6 months samples were stored at -80°C.

Smoking: process was performed according to the method of Balıkçı (2009) with slight modification in the seafood processing plant (Pakyürek,Adana,Turkey). The anchovy fillets were dipped into a brine solution containing 22% NaCl at a ratio of 1:2 (w/w) for 12 hr. The thermal process applied in two stages: a preliminary drying and cooking period (50 min) at 85°C. After cooling at cold temperature (60 min), the smoked anchovy fillets were dried in the industrial oven to drain off excess water by keeping the samples at 60°C for 70 min. Then, the brined and dried anchovy fillets were transferred to the industrial smoke chamber (Braai Smoker, Bradley, Canada) for smoking treatment. Oak sawdust smoke was used as smoking agents in the smoking chamber at 60°C at 50 min. The smoked products were cooled for an hour, on the stainless steel table and packaged. The smoked anchovy fillets were stored at 80°C for further experiments.

DNA Extraction

Each group of approximately 50 gr raw and processed with industrial and domestic techniques were finely minced and mixed. All samples were pre-treated removing oils, spices and sauces by blotting with sterile filter paper and 15 g of each product were weighed into the sterile 50 mL Falcon tubes. Each DNA sample was isolated from the raw anchovy and processed anchovy s using a DNAeasy Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with minor modifications.

Initially, 20 mg fish sample was weighed out and finely ground with a grinder with liquid nitrogen. Then 250 µl Buffer ATL were mixed and heated incubation wih 20 µl of 0.5 mg/ml proteinase K at 56°C for entirely lysing aim. Then, the lysed mixture was centrifuged at 12000 g for 30 sec. Following to centrifugation, the supernatant transferred to another sterile tube and mixed with 250 µl extraction buffer, then the mixture was heated at 56ººC for 10 minutes. Afterwards, the mixture was mixed with 250 µl binding buffer and vortexed for 15 second and transferred to spin columns. Subsequent to the Extracted DNA introduction, 650 µL of washing buffer 1 (AW1) and washing buffer 2 (AW2) were, respectively added to the spin column for removing any inhibitors which cause to amplification process inhibitors. Elution buffer was pre-

heated at 37°C to facilitating to accessing more DN. Finally, the purified DNA was eluted with 200 µL Buffer AE. The purified DNA was stored at -20°C until for following applications.

Determination of DNA Quality and Amplificability

The purity, quality (in terms of 260/230 and 260/280 ratios) and the concentration of DNA were determined by a NanoDrop 2000c spectrophotometer (Thermo Scientific, Pittsburgh, PA, USA). The integrity was also assessed by gel electrophoresis (1% agarose gel in Tris-Sodium acetate-EDTA (TAE) buffer containing 1× SYBR Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, USA) at 100 V. Finally, the gel was visualized under UV light imaging analyzer (UV Tech. Cambridge, England). The final concentration of isolated DNA was adjusted to 10 ng/lL for use in Real-time PCR. The amplificability of the gDNA was then evaluated by amplifying a 226 bp region from the cytochrome c oxidase I was targeted (CO1) primer (COI F: 5' CACGACGTTGTAAAACGACACYA AICAYAAAGAYATIGGCAC 3' and COI R: 3' GGAT AACAATTTCACACAGGCTTATRTTRTTTATICGIG GRAAIGC 5)' (Shokeralla et al.,2015). The reaction mixtures contained 2 µL of template DNA, 10 µL Master Mix(Thermo Scientific™ Maxima SYBR Green/ROX qPCR Master Mix (2X), 2 µL of each primer, and 6 µl DNA free water. The PCR was conducted using a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with the following program: an initial denaturation step at 95°C for 2 min, 35 cycles of 30 s at 94°C, 30 s at 53°C, and 60 s at 72°C, followed by the final extension at 72°C for 10 min. Following to amplification, the melting curve analysis (MCA) was performed by cooling amplification products at 65°C for 95 s and then heating from 65°C to 95°C.

Statistical Analysis

All statistical analyses were performed using Software IBM SPSS version 16.0. A two-way cross-classification analysis of variance (ANOVA) was performed for determining the differences in terms of yield of DNA and quality parameters of extracted DNA from both raw and processed anchovy sample. Statistically significant differences were considered at a level of 5% ($P<0.05$) All the DNA quality analyses were conducted in triplicate for all anchovy group.

Results and Discussion

DNA Quality of Raw and Processed Anchovy

As given in Figure 1 and Table 1, the results of extracted DNA from raw and processed anchovy samples at the industrial and domestic scale, significant differences were observed. The yield of extracted DNA was determined with initial anchovy muscle weight, observed DNA concentration, and the final volume of DNA–containing. There were significant differences found among raw and processed anchovy sample ($P<0.05$) in terms of DNA yield (Table 1, Figure 1). The highest DNA yield was obtained from raw anchovy (1108 ug/uL) in all the experiment groups. Similar results reported by Tagliavia et al., (2016) who stated the different processing methods cause to degradation when compared the raw form of tested seafood products. Grilling process cause to main loss in terms of DNA yield; grilled anchovy has 10.4 ug/uL DNA yield was not just affected by thermal process, marinating applications regardless containing pepper sauce or not cause to significant reduction of DNA yield. Marinated anchovy without and with pepper sauce sample have 27.6 ug/uL and 20.7 ug/uL., respectively. The most commonly applied processing techniques as smoking, frying and baking of anchovy also reduced the DNA yield significantly. There were no significant differences in these groups. Pollack et al., (2018) reported the similar reduction of DNA yield depending on thermally processed (acid-cooked, canned, boiled, baked, fried, and smoked) various fish species such as salmon, tuna, tilapia, etc).

In marinated anchovy groups, the reduction of DNA could be explained by utilization of acidic solutions during marinating process. Similarly used salt in smoking process could also a significant reduction in terms of DNA yield in anchovy fillets. DNA yield is a key parameter effect to achievements of further steps of molecular analyses. Besides, thermal degradation of DNA from processed meat and fish products, utilized salt, spices and acidic medium also cause to DNA degradation (Spychaj et al., 2021). The DNA yield was determined in optimal limits from other processed anchovy groups.

Another important parameter for the achievements of DNA based analyses is the A 260/A230 ratio, which signed the presence of an organic contaminant such as carbohydrates residual phenol or salt in extracted DNA sample and it should be found between 2.0 and 2.2, ideally (Lucena-Aguilar et al., 2016; Roy et al.,2020). The highest organic material contamination level determined from marinated anchovy sample as 17.6 and the lowest contamination rate was found in roasted groups as (-3.36). While this rate seems below the optimal limit, it still causes to problem in amplification and sequencing process which are the key elements for reliability and. The 260/A230 ratio was found exceed the optimal limit in all the processed anchovy sample in both thermally and non-thermally processed groups. These contaminations could be related to used salt, and brine solution in marinated and smoked groups.

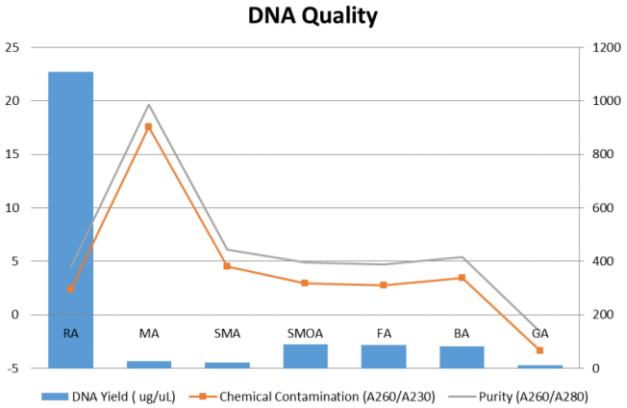


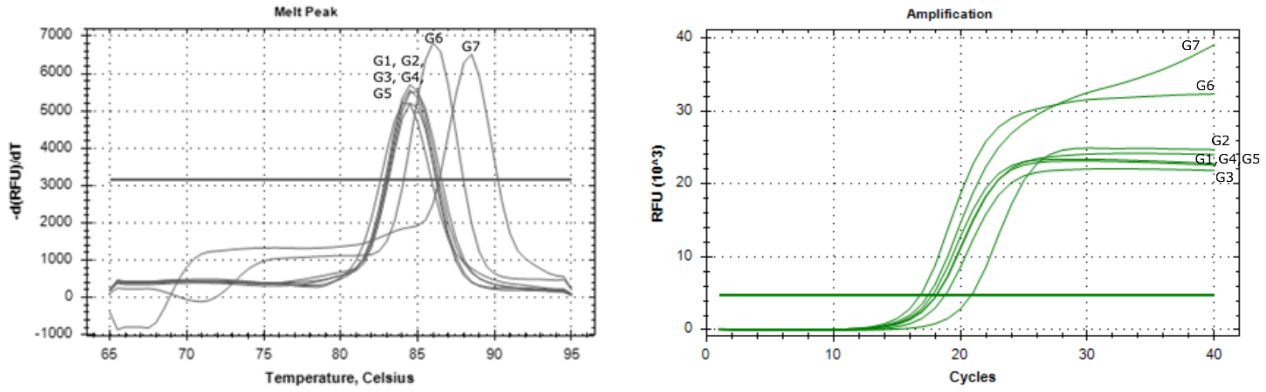
Figure 1: Variation of DNA Quality isolated from raw and processed anchovy samples at the industrial and domestic scale.

Groups: RA: Raw anchovy muscle, MA: marinated anchovy sample, SMA: marinated anchovy containing pepper sauce, SMOA: Smoked anchovy, FA: fried anchovy, BA: oven baked anchovy, GA: grilled anchovy

Table 1. Quality differences among the raw and processed anchovy groups

Anchovy group	Quality assessment		
	DNA Yield (ug/uL)	Purity (A260/A280)	Chemical Contamination (A260/A230)
RA	1108,6±0.12^{d}	2.04±0.06^{c}	2.39±0.07^{b}
MA	27.6±0.18^{b}	2.07±0.03^{c}	17.6±0.06^{c}
SMA	20.7±0.09^{b}	1.58±0.04^{a}	4.55±0.05^{a}
SMOA	89.7±0.13^{c}	1.96±0.02^{b}	2.97±0.03^{b}
FA	86.0±0.22^{c}	1.91±0.03^{b}	2.78±0.04^{b}
BA	81.1±0.20^{c}	1.97±0.03^{b}	3.45±0.05^{b}
GA	10.4,±0.02^{a}	1.76±0.06^{a}	-3.36±0.04^{a}

RA: Raw anchovy muscle, MA: marinated anchovy sample, SMA: marinated anchovy containing pepper sauce, SMOA: Smoked anchovy, FA: fried anchovy, BA: oven baked anchovy, GA: grilled anchovy Data are expressed as mean value ± standard deviation of triplicates. Values followed by different letters indicate significant differences (P<0.05) Values in a same column followed by different numbers indicate significant differences of the parameter with respect to Groups



A B

Figure 2. Melting Curve (A) and Amplicon plots(B) of raw and processed anchovy samples

Groups: G1: Raw anchovy muscle, G2: marinated anchovy sample, G3: marinated anchovy containing pepper sauce, G4: Smoked anchovy, G5: fried anchovy, G6: oven baked anchovy, G7: grilled anchovy

The frying oil could be also result in the organic pollutions in anchovy samples. The detected contaminant level could be related to the releasing of organic compound during thermally processing.

The purity of the extracted DNA is also influence the achievements of PCR amplification and sequencing processes in food traceability analyses. The purity of DNA verified by A260/A280 ratio with absorption spectrum from 260 and 280 nm wavelength measured by using Nanodrop as bio-spectrophotometer. There were some significantly, differences were observed among the raw, processed anchovy at the industrial and domestic processing (Table 1). As expected the lowest DNA purity was obtained from marinated anchovy with pepper sauce (1.58). Used acid, pepper and other indigents could be reducing the purity of extracted DNA. The purity of DNA also found below the optimal limit in roasted anchovy groups. The optimal purity of extracted DNA should be higher than 1.7 and not exceed to 2.0 (Miskoska-Milevska et al.,2011; Piskata et al., 2017). In marinated anchovy groups without pepper sauce addition, DNA purity was detected as exceeding the optimal limit also (2.07). Surprisingly the purity of DNA also found higher than optimal limit in raw anchovy group (2.04).

Differences in DNA Degradation

DNA degradation among to raw, processed anchovy samples in both industrial and domestic scale detected by threshold cycle (Ct) value which is the main issue in the sequencing processes in species identification from food safety application. The amplification results from the different processed anchovy sample illustrated by the software as the threshold cycle (Ct) value, which signified the number of cycles required to create a fluorescent signal greater than a pre-described threshold (Antunez et al., 2012). The variation in threshold cycle (Ct) values given in Figure.2, the main differences observed among in baked and grilled anchovy groups and other treatment groups. Similarly, Sanchiz et al. (2017) reported that thermal processing cause to DNA fragmentation in other food items.

As seen in Figure 2; the significant differences in terms of melting curve values were determined. Due to there were no significant differences in marinated anchovy groups; these differences can be related to thermal processing of raw anchovy. In Similar findings reported that DNA fragmentation and amplificability of DNA resulted by thermal process or variation in pH (Bauer et al.,2003; Besbes et al.,2011).

Stronger signals for the raw anchovy presumably are resulted by higher relative binding of SYBR Green to the longer fragment relatively shorter anchovy -specific amplicons which cause to higher melting peak (Marín et al., 2010). Lee Tan et al. (2019) also highlighted that the thermally processing lead to variance in melting curve.

Conclusion

While the consumer tendency was consumption food items as fresh and less-processed form previously; the processed, packaged and prepared to "ready to eat" products have become more popular than fresh consumption resulted by the changing tendency of consumers. Within the consumer's demand and technological advance; various processed food products have become more popular than fresh product nowadays and therefore the importance of food safety and food traceability concerns have increased. Increasing thermal processing and variations in acidity of food matrix also cause to reduction of DNA quality and DNA fragmentation which are key steps molecular analyses for food traceability and food security.

Conflict of Interest Statement

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Antunez K, Anido M, Garrido-Bailon, E, Botias, C, Zunino, P, Martinez-Salvador, A, Martin-Hernandez, R, Higes, M. 2012. Low prevalence of honeybee virusesin Spain during 2006 and 2007. Research in Veterinary Science, (93):1441–1445.

Balıkçı E. 2009. Tütsülenmiş Uskumru (Scomber scombrus) Marinatlarının (Sade ve Dereotlu) Duyusal, Kimyasal ve Mikrobiyolojik Kalite Parametrelerinin Belirlenmesi. Yüksek Lisans Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Adana.

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

Besbes N, Fattouch S, Sadok S. 2011. Comparison of methods in the recoveryand amplificability of DNA from fresh and processed sardine and anchovymuscle tissues.Food Chemistry, 129(2): 665-671.

Dağtekin M, Üstündağ E, Candemir S. 2019. Su Ürünleri Sektör Politika Belgesi 2019-2023. Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü, Ankara

Giusti A, Tinacci L, Sotelo, CG., Acutis P. L, Ielasi N, Armani A. 2019. Authentication of ready-to-eat anchovy products sold on the Italian market by BLAST analysis of a highly informative cytochrome b gene fragment. Food Control, (97): 50-57.

Karsli B. 2021. Determination of metal content in anchovy (Engraulis encrasicolus) from Turkey, Georgia and Abkhazia coasts of the Black Sea: Evaluation of potential risks associated with human consumption. Marine Pollution Bulletin, (165): 112108.

Keskin E, Atar HH. 2011. İşlenmiş Kalamar Ürünlerinde Sitokrom Oksidaz I Gen Dizileri Kullanılarak Tür Tayini. Gıda, 36(6): 343-348.

Keskin E, Atar HH. 2012. Molecular identification of fish species from surimi-based products labelled as Alaskan pollock. Journal of Applied Ichthyology, (28): 811-814.

Koban Baştanlar E. 2019. Identification of Mislabelling in Frozen Fish Fillets Based on DNA Barcoding Analysis. Marine Science and Technology Bulletin, 8(1): 30–35.

Lee Tan L, Aminah Ahmed S, Kit Ng S, Citartan M, Raabe C. A, Rozhdestvensky T. S, Hock Tang T. 2019. Rapid detection of porcine DNA in processed food samples using a streamlined DNA extraction method combined with the SYBR Green real-time PCR assay. Food Chemistry: 125654.

Lucena-Aguilar G, Sánchez-López AM, Barberán-Aceituno C, Carrillo-Ávila JA, López-Guerrero JA,Aguilar-Quesada R. 2016. DNA Source Selection for Downstream Applications Based on DNA Quality Indicators Analysis. Biopreservation and Biobanking: 14(4): 264-270.

Marín F. García N, Munoz X, Capella G, González CA, Agudo A, Sala N. 2010. Simultaneous genotyping of GSTT1 and GSTM1 null polymorphisms by melting curve analysis in presence of SYBR Green I. The Journal of Molecular Diagnostics, 12(3): 300-304

Miskoska-Milevska EZT. Popovski BR. Dimitrievska KD. Porcu 2011: Isolation of DNA for Fragment Analysis from Tomato Leaves and Seeds. Conference of biotechnology with international participation, Faculty of Agronomy, Ćaćak, 16 (18): 59-64.

Nagalakshmi K, Annam P-K, Venkateshwarlu G, Pathakota,G-B, Lakra, WS. 2016. Mislabeling in Indian seafood: An investigation using DNA barcoding. Food Control, (59): 196–200.

Olgunoğlu Aİ. 2007. Marine edilmiş hamside (Engraulis enchrasicolus L. 1758) duyusal, kimyasal ve mikrobiyolojik değişimler, Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Su Ürünleri Avlama ve İşleme Teknolojisi Anabilim Dalı, Doktora Tezi, Adana, Türkiye,

Ozogul Y, Ozyurt G, &Kuley Boga E. 2009. Effects of cooking and reheating methods on the fatty acid profile of sea bream treated with rosemary extract. Journal of the Science of Food and Agriculture, 89(9): 1481–1489.

Piskata Z, Pospisilova E, Borilova,G. 2017. Comparative study of DNA extraction methods from fresh and processed yellowfin tuna muscle tissue. International Journal of Food Properties, 20(1): 430-443.

Pollack SJ, Kawalek M.D, Williams-Hill DM, HellbergRS. 2018. Evaluation of DNA barcoding methodologies for the identification of fish species in cooked products. Food Control, (84): 297–304.

Roy D, Tomo S, Modi A, Purohit P, Sharma P. 2020. Optimising total RNA quality and quantity by phenol-chloroform extraction method from human visceral adipose tissue: A standardisation study. MethodsX, (7): 101113.

Sanchiz Á, Ballesteros I, Martin A, Rueda J, Pedrosa MM, Dieguez M. del C. … Linacero R.2017. Detection of pistachio allergen coding sequences in food products: A comparison of two real time PCR approaches. Food Control, (75): 262–270.

ShehataH.R,Naaum A,Garduno RA, Hanner R.2018. DNA barcoding as a regulatory tool for seafood authentication in Canada .Food Control, (92):147–153.

Shokralla S, Hellberg RS, Handy SM, King I, Hajibabaei M. 2015. A DNA mini-barcoding system for authentication of processed fish products. Scientific Reports, (5): 15894.

Spychaj A, Goderska K, Fornal E, Montowska M. 2021. A Practical Approach to Identifying Processed White Meat of Guinea Fowl, Rabbit, and Selected Fish Species Using End-Point PCR. International journal of food science, (2021): 7710462.

Tagliavia M, Nicosia A, Salamone M, Biondo G, Bennici C.D, Mazzola S, Cuttitta A.2016. Development of a fast DNA extraction method for sea food and marine species identification, Food Chemistry, (203): 375–378.

Yılmaz VA, Koca I. 2020. Development of gluten-free corn bread enriched with anchovy flour using TOPSIS multi-criteria decision method. International Journal of Gastronomy and Food Science, (22):100281.

Xavier M, Piyadarshini B, Ninan G. Zynudheen AA, Mathew PT, Nair KGR, Joseph AC. 2018. Enrobed Snack Product from Devis's Anchovy (Stolephorus commersonnii) and its Quality Evaluation during Frozen Storage. Journal of Aquatic Food Product Technology, 1–9.