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Selection the Most Suitable Method for DNA Extraction from Muscle of Iran's Canned Tuna

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Abstract. High quality and purity of DNA isolated from canned tuna is essential for species identification. In this study, the efficiency of five different methods for DNA extraction was compared. Method of national standard in Iran, the CTAB precipitation method, Wizard DNA Clean Up system, Nucleo Spin and Genomic Prep were employed. DNA was extracted from two different canned tuna in brine and oil of the same tuna species. Three samples of each type of product were analyzed with the different methods. The quantity and quality of DNA extracted was evaluated using the 260 nm absorbance and ratio A260/A280 by spectrophotometer picodrop. Results showed that the DNA extraction from canned tuna preserved in different liquid media could be optimized by employing a specific DNA extraction method in each case. Best results were obtained with CTAB method for canned tuna in oil and with Wizard method for canned tuna in brine.

Keywords: DNA, DNA Extraction Methods, Species Identification, Canned Tuna PCR

1. Introduction

Tuna species identification in tuna cans has a clear importance since different tuna species have very different commercial values. Several research groups have pursued the development of identification methods to provide authorities, industry and consumers with useful control tools which allow enforcement of labeling regulations and the safeguard of fair trading with these species and their products. DNA has shown to be the most appropriate molecule to reveal species contained in these products, mainly cans [1]. One of the problems to perform most of the proposed method is the quantity and quality of the DNA extracted from tuna. It has been shown that DNA suffers degradation due to the thermal treatments to which the muscle is subjected during the canning process (cooking and sterilization) but also the type of liquid that is added may play a role in this degradation [2]. Tuna cans may present different liquid media, like brine, oil, vinegar or tomato and these may produce differences in the quantity and quality of the extracted DNA. In Iran only Tuna cans in brine and oil are produce and use. Many DNA extraction protocols are available but they have been rarely compared [3]-[5]. The objective of this work is to analyze the effect of the filling media in the quantity and quality of the extracted DNA, and the efficiency of different commercial DNA extraction methods.

2. Materials and Methods

2.1. Sample Pre-Treatment

Five different canned tuna (tuna in brine and tuna in oil) of the same tuna species were studied. They were obtained from the same cannery company. Canning process conditions include a sterilization process of 45 min at 115 ℃. Oil and lipids were removed from canned muscle by soaking in chloroform/methanol/water

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(1:2:0.8) overnight. The defatted muscle was recovered by filtration and stored frozen until DNA was extracted.

2.2. DNA Isolation

DNA was extracted with the following commercial kits: Wizard DNA Clean up System with proteinase K previous digestion, Nucleospin, Genomic Prep, the CTAB precipitation method and method of national standard in Iran.

2.2.1. Wizard method

2 g of dried muscle were mixed with 860 µL of 1 × TNE buffer (10 mM Tris-HCl pH 8, 2 mM EDTA, 150 mM NaCl, 1% SDS), 40 µL of proteinase K (20 mg/ml) and 100 µL of 5 M of guanidinium thiocyanate. Samples were incubated in a thermo shaker at 56 ℃. After 2h, 40 µL of proteinase K (20 mg/ml) were added again and samples were left at 56 ℃ overnight. After digestion DNA was extracted following the manufacturer's instructions.

2.2.2. Nucleospin method

The procedure was performed according to the manufacturer with the following modifications. One gram of dried muscle were mixed with 360 µL of buffer T1 and 50 µL of proteinase K and incubated in a termoshaker at 56 ℃, after 2 h proteinase K was added again and samples were incubated overnight under the conditions described. When lysis is complete, 200 µL of buffer B3 was added to the sample. After vortexing the sample it was incubated at 70 ℃ for 10 min. Two hundred and ten µL of ethanol (96–100%) was added to the sample and vortexed again. The NucleoSpin column was placed into a 2 ml collection tube and the sample was added and centrifuged until it was drawn through the membrane completely. Then the membrane was washed two times with 500 µL of buffer B5 and was be removed completely by performing a centrifugation step. Finally the NucleoSpin column was placed in a clean 1.5 ml microcentrifuge tube and DNA was eluted with 50 µL of buffer BE at 70 ℃.

2.2.3. GenomicPrep method

2 g of dried muscle were mixed with 1 ml of Cell Lysis Solution and heated at 65 ℃ for 60 min. Then 15 µL of proteinase K (20 mg/ml) were added and samples were incubated overnight at 55 ℃. 3 µL of Rnase solution were added, mixed by inversion and incubated at 37 ℃ for 60 min. Samples were cooled and 200 µL of the protein precipitation solution were added, after vortexing the samples for 20 s samples were centrifuged for 3 min. Proteins form a visible pellet. Supernatant was transferred to a 1.5 ml tube that contains 600 µL of isopropanol and mixed by inversion. After centrifugation DNA is visible as a small white pellet and it was washed with 70% ethanol. DNA hydration solution was added to the pellet and DNA was rehydrated overnight at room temperature, after DNA was stored at -20 ℃.

2.2.4. CTAB Method

1g of the sample was mixed with 5 ml of CTAB-Buffer 1 (1.2% w/v hexadecyl trimethyl ammoniumbromide, 10 mM EDTA-Na, 60 mM Tris, 0.8 M NaCl, pH 8), 1 µL of 3 mercaptoethanol 1:2 propanediol (1% v/v) and 25 µL of proteinase K (10 mg/ml). Samples were incubated for 24 h at 65 ℃, and then centrifuged for 10 min at 12,000 rpm. The supernatant was transferred to a new 1.5 ml tube, extracted with 5 ml of chloroform and centrifuged again for 54 min at 12,000 rpm. The upper phase was transferred into a new tube, precipitated with 2 ml of chloroform and centrifuged for 5 min at 12,000 rpm. The supernatant was transferred into a new tube and 2 volumes of the CTAB-Buffer 2 (1% w/v hexadecyl trimethyl ammonium bromide, 10 mM EDTA-Na, 50 mM Tris, pH 8) were added, mixed and centrifuged for 10 min at 12,000 rpm. The supernatant was discarded and the pellet was mixed with 4 ml of CTAB-Buffer 3 (1 M NaCl, 10 mM Tris, 1 mM EDTA-Na, pH 8) and incubated at least 30 min at 65 ℃. After incubation samples were cooled and 4 ml of Isopropanol were added, and let stand for 10 min, and after samples were centrifuged 10 min at 12,000 rpm. Pellet was washed twice with 5 ml of ethanol (70%) before drying in a SpeedVac for 5 minutes. The pellet was dissolved in 100 µL of TE-buffer (1 mM EDTA-Na, 10 mM Tris, pH 8 with NaCl) and frozen at -20 ℃ until needed.

2.2.5. National standard of Iran

2g of the sample was mixed with 10 ml of TEK- Buffer (50 mM Tris, 10 mM EDTA-Na, 1.5% KCl, pH 7.5); Samples were mixed with sacarose buffer then centrifuged for 10 min at 12,000 rpm. The supernatant was transferred to a new 1.5 ml tube then centrifuged for 10 min at 10,000 rpm. Discarded the supernatant and precipitations were washed with EST buffer (100 mM EDTA, 10 mM Tris, 150 mM NaCl, pH 8), resolved in EST buffer then added 18% SDS. Added 5 N NaCl and centrifuged again for 15 min at 10,000 rpm. The upper phase was transferred into a new tube, precipitated with phenol and TEK buffer, vortexed and incubated in room temperature for 5 min then centrifuged for 10 min at 12,000 rpm. The supernatant was transferred into a new tube and 1 volume of the phenol, chloroform, isoamyl alcohol were added, mixed and centrifuged for 10 min at 12,000 rpm. The supernatant was transferred into a new tube and 1 volume of the chloroform, isoamyl alcohol were added, mixed and centrifuged for 10 min at 12,000 rpm. The supernatant was transferred into a new tube, added 3M sodium acetate and 1 volume isopropanol and frozen at -20 ℃ for 2-12 hour. Tubes were centrifuged for 20 min at 14,000 rpm. The supernatant was discarded and the pellet washed with 75% ethanol and centrifuged for 5 min. Finally, the pellet was dissolved in 100 µL of TE-Buffer (1 mM EDTA-Na, 10 mM Tris, pH 8 with NaCl) and frozen at -20 ℃ until needed.

2.3. Quantitation of DNA

All types of products were extracted in triplicate. Extracted DNA was quantified measuring the absorbance of the DNA extracts at 260 nm, checking for protein impurities at 280 nm. The amount of 1 O.D. at 260 nm was equalled to 50 g/ml DNA [1].

2.4. Polymerase Chain Reaction of DNA

The PCR method was employed to evaluate the quality of the extracted DNA. For this purpose, primers were designed aimed at amplifying fragments of increasing size (100, 150, 200, 250, and 300 bp) which mentioned as following [1]. Annealing temperature employed was 55 ℃. Amplification was checked by electrophoresis in 2% agarose gels using TAE buffer with ethidium bromide (0.5 g/ml) [1].

- CB-TALB-100-F: 5‘- GGCAAGCCTCCGAAAAACTC-3‘ and
  CB-TALB-100-R: 5‘- AGGCCAAAGTTTCATCATGCAGA-3‘to amplify a fragment of 100 bp.
- CB-TALB-150-F: 5‘- ATCGCTAACGACGCACTAGTTG-3‘and
  CB-TALB-150-R: 5‘- AGGCTGATTCGACATCAGGG-3 to amplify a fragment of 150 bp.
- CB-TALB-100-F: 5‘- GGCAAGCCTCCGAAAAACTC-3‘ and
  CB-TALB-200-R: 5‘- TGGGCTACTGAGGCGA AGG-3‘to amplify a fragment of 200 bp.
- CB-TALB-250-F: 5‘- CAAGAACCCTAATGGCAAGCC-3‘ and
  CB-TALB-250-R: 5‘- CCGGATGAGTCAACCGAAGTT-3‘to amplify a fragment of 250 bp.
- CB-TALB-250-F: 5‘- CAAGAACCCTAATGGCAAGCC-3‘ and
  CB-TALB-300-R: 5‘-TGGAAGTAGATGCAGATAAAGAAGAAAG-3‘to amplify a fragment of 300 bp.

3. Results and Discussion

Tuna cans are often target for species identification analysis to assure their correct labeling. In these products, proteins are denaturalized due to the temperatures of canning process and DNA must be employed for species identification purposes. However, exposure to heat is known to cause fragmentation of DNA and physical or chemical treatments will cause random breaks in DNA strands and reduce DNA fragment size [6]. The aim of an extraction procedure is to obtain the high quality and quantity of DNA. It should contain as little as possible proteins, RNA or any other PCR inhibitor, also avoiding DNA fragmentation during extraction. Important factors such as; time duration, the expenses and the toxicity of the chemical products employed, should be take into account when a DNA extraction method selected.

The objective of this work was to study whether there are differences in DNA quantity and quality from the same tuna species with different preparations (salted water and oil). Samples of cans of the same size and different preparation were obtained from the same company to get an equivalent thermal treatment. Five

extraction protocols were selected: Wizard DNA Clean Up, Nucleospin, GenomicPrep, CTAB, and method of national standard in Iran.

The Quantity of the extracted DNA was calculated using the 260 nm absorbance and analysis of variance (ANOVA) of DNA yield data were conducted to determine the significance of differences found. The statistically significant differences in both liquid media and extraction methodology were observed (Table 1), whereas the DNA extracted from brine tuna yielded the same amount in all extraction methods and the oil cans produced a significantly higher DNA content using CTAB method.

Table 1: Average (ng DNA/mg tissue) of DNA extracted from canned tuna obtained with the five different DNA extraction methods studied

DNA extraction method	Brine	Oil
Wizard	$75.56 \pm 13.24^{a,y}$	$95.17 \pm 2.13^{a,z}$
Nucleospin	$49.23 \pm 10.02^{a,x}$	$124.36 \pm 6.93^{a,y}$
GenomicPrep	–	$115.26 \pm 15.91^{a,x}$
CTAB	$47.36 \pm 39.23^{a,x}$	$198.79 \pm 20.65^{b,y}$
Standard of Iran	–	$158.74 \pm 17.34^{b,y}$

The type of products, a,b: columns, x, y, z: files.

Table 2: Average of the relation A260/A280 in each type of product and extraction method

DNA extraction method	Brine	Oil
Wizard	1.32 ± 0.01	1.56 ± 0.03
Nucleospin	00.89 ± 0.02	1.01 ± 0.36
GenomicPrep	–	1.36 ± 0.06
CTAB	2.01 ± 0.08	2.02 ± 0.08
Standard of Iran	–	1.96 ± 0.06

The quality of the extracted DNA was also evaluated by obtaining the ratio absorbance 260/280 (Table 2); DNA quantification with A260 method was employed previously by other authors to compare different DNA extraction methods [7]-[9]. Values close to 2 indicate a good DNA extract with little protein contaminants. The CTAB method produced the highest ratio A260/A280 and the lowest values were obtained with the Nucleospin kit. The Wizard was also the method with relatively high values (1.56 ± 0.03).

The quality of the extracted DNA was also considered by using a set of primers amplifying fragments of cytochrome b of increasing size, ranging from 100 bp up to 300 bp (Table 3). The higher DNA quality, in terms of size, was obtained from tuna with oil in four out of five DNA extraction methods; whereas clear fragments up to 300 bp were found.

Table 3: DNA amplification of increasing size obtained with primers designed

DNA extraction method	PCR (bp)	Brine	Oil
Wizard	100	+	+
	150	+	+
	200	+	+
	250	+	+
	300	-	+
Nucleospin	100	+	+
	150	+	+
	200	+	+

	250	+	+
	300	-	-
GenomicPrep	100	+	+
	150	+	+
	200	+	+
	250	+	+
	300	-	-
CTAB	100	+	+
	150	+	+
	200	+	+
	250	+	+
	300	-	-
Standard of Iran	100	+	+
	150	+	+
	200	+	+
	250	+	+
	300	-	-

According to our results, the Wizard DNA Clean Up could be used for DNA extraction of canned tuna in brine and for canned tuna in oil the CTAB and the method of national standard in Iran were more suitable. Besides, as the duration of DNA extraction with Iranian method takes long time, the CTAB method introduced and suggested for canned tuna in oil.

4. References

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