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ASSESSMENT OF DNA QUALITY IN PROCESSED TUNA MUSCLE TISSUES

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ABSTRACT

Authentication of tuna fish products is necessary to assure consumers of accurate labelling of food products. The quality of species specific DNA crucially affects the efficiency of amplification during the subsequent PCR. The problem in DNA detection in canned products lies in the possibility of the fragmentation of DNA during the processing technologies and the use of ingredients (oil, salt, spice), that may inhibit the PCR reaction. In this study three DNA extraction methods were compared: DNeasy Blood and Tissue Kit, DNeasy mericon Food Kit and Chemagic DNA tissue 10 Kit. The quantity and quality of DNA were evaluated by measuring DNA concentration and ratios A260/A280. Several parameters were estimated: the effect of whole and mechanically treated muscle, sterilization procedure used in canned process (high temperature in combination with high pressure) and addition of raw materials. The highest DNA concentrations were observed in non-processed muscle that is not influenced by the sterilization process. Canned whole muscle demonstrated lower DNA yield, and furthermore, the mechanical treatment (canned ground) resulted in lower values of DNA concentration that was registered by using all three types of DNA extraction kits. DNeasy mericon Food Kit produced DNA of higher concentration in non-processed sample, Chemagic DNA tissue 10 Kit delivered higher DNA yields than kits DNeasy Blood and Tissue Kit and DNeasy mericon Food Kit in canned samples, although the purity was lower, but still within the range 1.7 – 2.0. DNA was considered to be satisfactorily pure in all three types of samples and using all three types of DNA isolation. In case of the samples enriched of ingredients and treated with sterilization process as whole or ground muscle Chemagic DNA tissue 10 Kit produced in all samples (whole and ground muscle) the highest values of DNA concentration, but almost all values of A260/A280 were lower than 1.7. Therefore DNeasy mericon Food Kit appears to be a favorite one, in all samples with whole muscle gives higher values of DNA concentrations than DNeasy Blood and Tissue Kit. Addition of ingredients influenced the DNA yield in terms of decreasing in samples containing vinegar and lemon, but some of the ingredients resulted surprisingly in higher yield of DNA. This was not consistent in whole and ground muscle, and the differences were described also among particular kits. The impact of ingredients was not conclusively approved and their importance to the suitability of extracted DNA for PCR amplification is needed to be discussed in further analysis.

Keywords: canned product; fish food; DNA extraction; PCR; *Thunnus albacares*

INTRODUCTION

Fish species identification gains attention due to the commercialization of fish through filleted, salted, smoked or canned fish products. Tuna fish belong among the most economically important fishery resources because are typically used to manufacture canned products, the main format for marketing of these species **(Espineira et al., 2009)**. Different quality and price of tuna species can lead the manufacturers to the tendency to highlight the quality of fish products. From that reason the substitution or mixing of valuable fish by less valuable ones may occur. The Council Regulation (EC) **No. 1536/92** laying down common marketing standards for preserved tuna and bonito states specific rules for the tuna marketing. The species belonging to tuna and bonito are named in the annex of this Regulation. Below is determined, that the trade description on the prepackaging of preserved tuna or bonito shall state the type of fish (tuna or bonito). The identification of tuna and bonito species according to their morphological features is possible only in whole or lightly processed fish. In processed products such as filleted or canned fish the morphological characteristics are removed, hence analytical methods as an important tool for species identification must be used. Analytical methods are focused mainly on protein or DNA molecule, which are extracted from the fish tissues. Due to the protein denaturation caused by heating or canning (high temperature in combination with high pressure) process **(Mackie et al., 1999)**, DNA is more suitable molecular marker for fish species authentication, because it is more resistant to the thermal treatment. Indeed DNA is also degraded into smaller fragments during the thermal process but these are still detectable. **Ram et al. (1996)** claim, that the canning process degrades DNA to fewer than 123 bp in length. Moreover DNA is largely independent of tissue source, age, or sample damage **(Bossier, 1999; Lockley and Bardsley, 2000)**. Nevertheless the fragment size is limited factor for the

subsequent PCR reaction that is based on the selective amplification of specific region of DNA using oligonucleotides **(Lockley and Bardsley, 2000)**. PCR **(Bartlett and Davidson, 1991; Bottero et al., 1997; Dalmasso et al., 2006)** and its modification – PCR-RFLP **(Takeyama et al., 2001, Pardo and Pérez-Villareal, 2004, Lin et al., 2005, Lin and Hwang, 2007)**, PCR-SSCP **(Rehbein et al., 1999; Colombo et al., 2005)**, real-time PCR **(Lopez and Pardo, 2005)** or PCR-ELISA **(Santaclara et al., 2015)** represents crucial approaches available for tuna fish species identification. PCR analysis comprises of DNA extraction from the sample, PCR and electrophoresis, or alternatively other detection system for the final results evaluation. The critical step is extraction of high quality DNA in great enough quantities from the heterogeneous food matrices. In view of the fact, that raw material for the final product manufacture comes under different effect during the manufacturing process (high temperature, high pressure, addition of ingredient, etc.), which considerable influences the quality of DNA **(Chapela et al., 2007, Besbes et al., 2011, Cawthorn et al., 2011)**, it is required for every type of food products to apply and optimize particular DNA isolation procedure. In addition ingredients and other substances presented in food products may work as PCR inhibitors, substances that may negative affect the sensitivity of PCR reaction. Or in another case, the DNA may be stimulated due to the ingredients.

Primary requirement of this study is to find out, how far is DNA influenced by the technological processes using in food industry (mechanical treatment, high temperature, high pressure, addition of ingredients) in model canned samples from the muscle tissue of yellowfin tuna (*Thunnus albacares*) and how the subsequent sample preparation and DNA extraction procedure can affect its qualitative and quantitative parameters.

MATERIAL AND METHODOLOGY

Samples preparation

The samples of tuna fish were prepared from the muscle tissue of yellowfin tuna (*Thunnus albacares*), that was purchased in the Czech market as frozen steak. Its species identity was confirmed via sequencing of the partial sequence of cytochrome *b* gene (Seqme, Hradec Kralove, Czech Republic). Besides non-processed muscle tissue two types of tuna samples that were made under similar conditions used in cans production were prepared - canning of solid piece of muscle (whole) and canning of

Table 1 List of ingredients.

	Raw food	[g]	Whole muscle / Mechanically modified muscle 27 g	
1	Raw muscle	-	42	
2	Sunflower oil	15	42	
3	Olive oil	15	42	
4	Soy sauce	15	42	
5	Brine	5%	42	
6	Alcohol vinegar	10	37	
7	Wine vinegar	10	37	
8	Apple cider vinegar	10	37	
9	Lemon + juice	4,5	31,5	
10	Tomato puree	20	47	Total [g]
11	Chili spice	1	28	
12	Oregano	0,5	27,5	
13	Fresh garlic	2	29	
14	Garlic spice	1	28	
15	Onion	5	32	
16	Corn	10	37	
17	Pea	10	37	
18	Bean	15	42	
19	Carrot	10	37	
20	Tomatoes	10	37	
21	White + green pepper	5+5	37	
22	Black olives	10	37	
23	Fresh chili pepper	5	32	

mechanically processed muscle (ground). Mechanic treatment was provided using the cutter setting in two rotations. Furthermore the sets of the samples comprising whole/ground muscle enriched of the selective ingredients were mixed thoroughly and placed into the autoclavable glass vessels with caps – the amount and composition is described in Table 1. The proportions were assessed according to the real composition described on the packaging of tuna fish products occurring on the commercial market. The samples were subjected to the sterilization in autoclave (Systec V95); sterilization conditions included the temperature 121 °C and pressure 200 kPa for 15 min. These samples were prepared in laboratories of the Department of Meat Hygiene and Technology (University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic).

DNA isolation

The DNA was extracted in duplicate using three commercial available kits based on the column system (DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) - kit A and DNeasy mericon Food (Qiagen) – kit B) and magnetic separation using magnetic particles (Chemagic DNA Tissue 10 Kit (Chemagen, Baesweiler, Germany) – kit C). Pretreatment of the samples 2 - 8 (Table 1) was performed according to **Chapela et al. (2007)**; oil, lipids or other substances were removed from canned muscle by soaking it in the mixture of chloroform/methanol/water (1:2:0.8) overnight. The extraction procedures were performed according to the protocol supplied by the manufacturer. Sample weight was 10 mg in kit A and C, and 200 mg in kit B, proteolysis was carried out overnight in all types of the extraction protocols.

The assessment of DNA quality

The quality of extracted DNA was compared by measurement the concentration and purity using a UV spectrophotometer (NanoDrop™ 1000, Thermo Scientific). DNA extracts were quantified by measuring the absorbance at 260 nm (A260). DNA purities were estimated by calculating the A260/A280 ratios. Samples calculated to have A260/A280 ratios of 1.7 – 2.0 were assumed to be pure samples, free from protein and other contamination. Every sample was measured three times. The instrument calibration was performed using the Elution Buffer. Measurement was done at room temperature and sufficient mixing of all samples.

Species identification via sequencing of cytochrome b gene

For the species identification of yellowfin tuna (*Thunnus albacares*) in frozen fish the amplification of 569 bp fragment of cytochrome *b* gene using primer pair L14735 and BRmod (Espineira et al., 2009) was used. The PCR protocol consisted of initial denaturation step at 95 °C/3 min, following by 35 cycles including denaturation at 95 °C/30 s, annealing at 60 °C/30 s and extension at 72 °C/30 s, and terminated by final extension at 72 °C/3 min.

RESULTS AND DISCUSSION

In canned products DNA is considered to be damaged, exposure to heat, physical or chemical treatment that can affect the quality and quantity of DNA, presumably the fragmentation of DNA molecule. To choose an optimal extraction procedure several factors have to be taken into account. DNA should contain as little as possible proteins, RNA, organic compounds or any other PCR inhibitors. The DNA concentration and purity were determined spectrophotometrically by measuring the DNA absorbance and A260/A280 ratios. The DNA was considered to be satisfactorily pure when the ratios of the A260 to A280 were within the range of 1.7 – 2.0. Contamination of DNA with proteins usually reduces the A260 to A280 ratio to values lower than 1.7 **(Cawthorn et al., 2011)**. High 260/280 purity ratios are not necessarily indicative of a problem. Residual impurities carried over from the DNA extraction procedure, such as phenol or ethanol, are also reported to reduce the A260 to A280 ratio. Furthermore residual chemical contamination from nucleic acid extraction procedures may result in an overestimation of the nucleic acid concentration.

The main task was to find out whether non-processed and processed muscle tissue (from *Thunnus albacares*) has the difference between the concentration and purity of DNA. Another parameter was to follow up the effect of the addition of ingredients mainly used in canned tuna products. And also to evaluate the efficiency of the three commercial kits used for the DNA isolation. The first group of analyzed samples include sample prepared from non-processed muscle without any further technological processes (frozen muscle), sample prepared from whole muscle undergoing the sterilization process and sample prepared from mechanically treated (ground) muscle undergoing the sterilization process. The comparison of the DNA concentration and DNA purity is shown in Figure 1. The highest DNA concentrations were observed in non-processed muscle that is not influenced by the sterilization process. The sample with canned whole muscle demonstrated lower DNA yield, and furthermore, the mechanical treatment resulted in even lower values of DNA concentration that was registered by using all three

Table 2 Group rate of values A260/A280.

Ratio A260/A280	Kit A	Kit A	Kit B	Kit B	Kit C	Kit C
	W	G	W	G	W	G
<1.7	1	4	11	4	22	22
1.7 – 2.0	11	13	12	17	1	1
>2	11	6	0	2	0	0

Note: W – whole, G – ground.

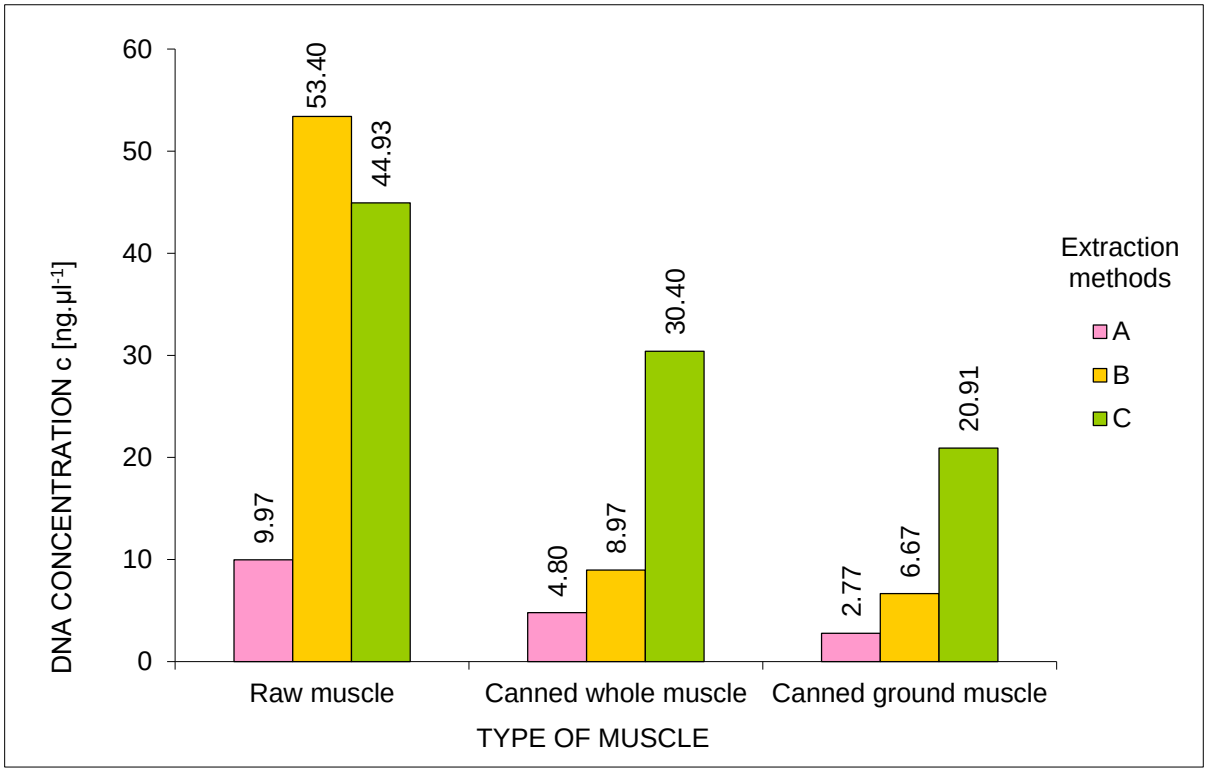

Figure 1 Determination of DNA concentration (type of muscle).

types of DNA extraction kits. Kit B produced DNA of higher concentration in non-processed sample, kit C delivered higher DNA yields than kit A and B, although the purity was lower, but still within the range 1.7 – 2.0. In the case of DNA purities, DNA was considered to be satisfactorily pure in all three types of samples and using all three types of DNA isolation.

The second group consisted of 23 samples prepared from the whole or ground muscle tissue and enriched with the ingredients (22 with ingredients and 1 muscle without ingredients). For the comparison of the samples of whole and ground canned muscle tissue (regardless of the effect of the ingredients) the Wilcoxon matched-pairs signed rank test was used and for the comparison of the efficiency of the particular extraction kits Friedman test + Dunn post-hoc test (non-parametric ANOVA) was used. The frequency values of A260/A280 in groups $A < 1.7$ / $1.7 \leq A \leq 2.0$ / $A > 2.0$ were estimated with χ^2 independence test and Fisher exact test.

Statistically significant differences in DNA concentration between the whole and ground muscle were found in the case of kits A (p <0.01; Wilcoxon test) and B (p <0.05; Wilcoxon test). While in kit A the values of DNA concentrations in most of the samples with whole muscle were lower than in the samples with ground muscle, in kit B it was conversely. In kit C statistically significant difference between whole and ground muscle was not proved. In kit A probably the chemical substances used during the extraction procedure could cause more efficient permeation to the ground muscle in comparison with whole muscle, but this was not observed in sample 1 (whole and ground muscle without ingredients). In case of the samples with whole muscle we managed to prove that among the kits there is statistically significant difference (p <0.01; Friedman test) in DNA concentration. Following testing demonstrated that statistically significant difference is evident between all pairs of kits (p <0.01; Dunn test), while the highest values of DNA concentration is presented with kit C, the lowest in kit A. In case of the samples with ground muscle we managed to prove that among the kits there is statistically significant difference (p <0.01; Friedman test). Following testing showed up that statistically significant difference is only between kit A and C, B and C (p <0.01; Dunn test), while the highest values of DNA concentration is produced by the kit C. Between kit A and B the statistically significant difference was not observed.

Statistically significant difference of A260/A280 between whole and ground muscle was observed only in kit B (p <0.01; Wilcoxon test), while highest values of A260/A280 was reached in samples with ground muscle. In case of the samples with whole muscle we managed to prove that among the kits there is statistically significant difference (p <0.01; Friedman test). Following testing demonstrated that statistically significant difference is evident between all pairs of kits (p <0.01; Dunn test), while the highest values of A260/A280 is presented by kit A, the lowest in kit C (except the samples 1 always under the limit 1.7). In case of the samples with ground muscle we managed to prove that among the kits there is statistically significant difference (p <0.01; Friedman test). Following testing showed up that statistically significant difference is only between kit A and C, B and C (p <0.01; Dunn test), while the lowest values A260/A280 is produced by the kit C. Between kit A and B the statistically significant difference was not observed.

χ^2 independence test confirms that there is association (p <0.01) between the distribution of A260/A280 ratios and kit resp. the type of the sample (whole/ground muscle). In case of the type of the sample the highest statistically significant difference (p <0.05) was detected in kit B (Table 2).

The effect of ingredients mixed together with muscle reveal the differences among particular kits and also among whole and ground muscle. According **to Chapela et al. (2007)** lower amount of DNA can be caused by the presence of brine, this finding could be explain by a washing out effect used in the extraction procedure. The decreasing effect of brine on DNA yield was observed only in kit C. In kits A and B the concentrations of DNA were even higher in comparison with the sample without brine. Other ingredients vinegar and lemon are substances

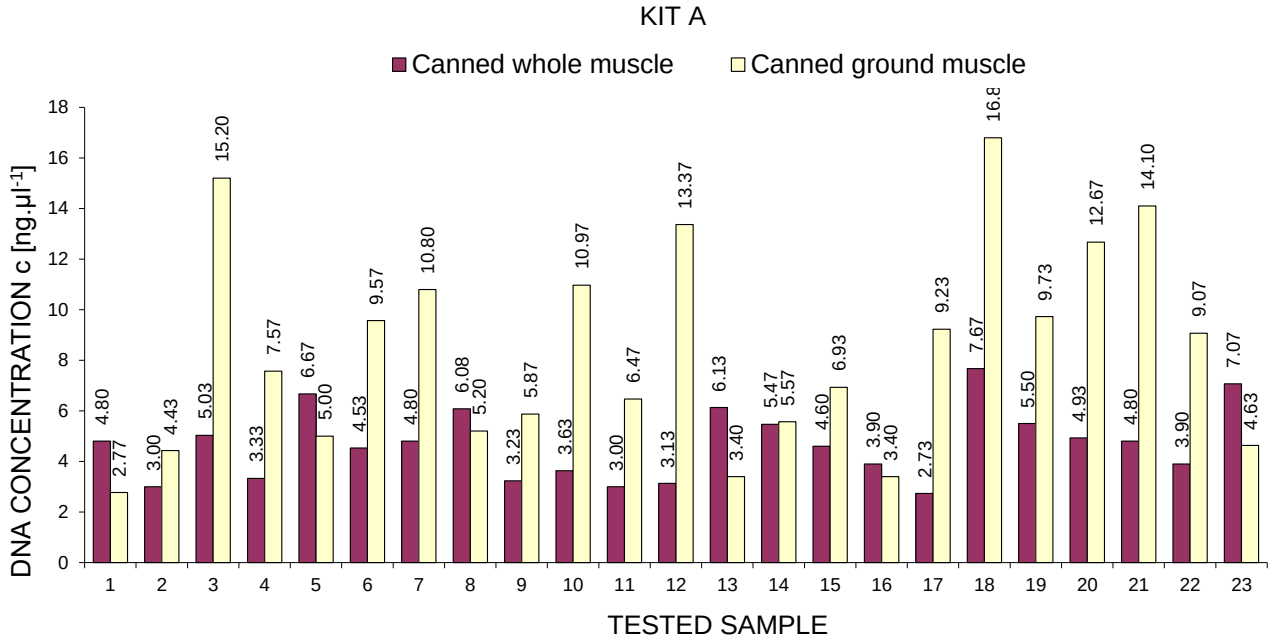

Figure 2 Comparison of DNA concentration of whole and ground muscle determined by kit A.

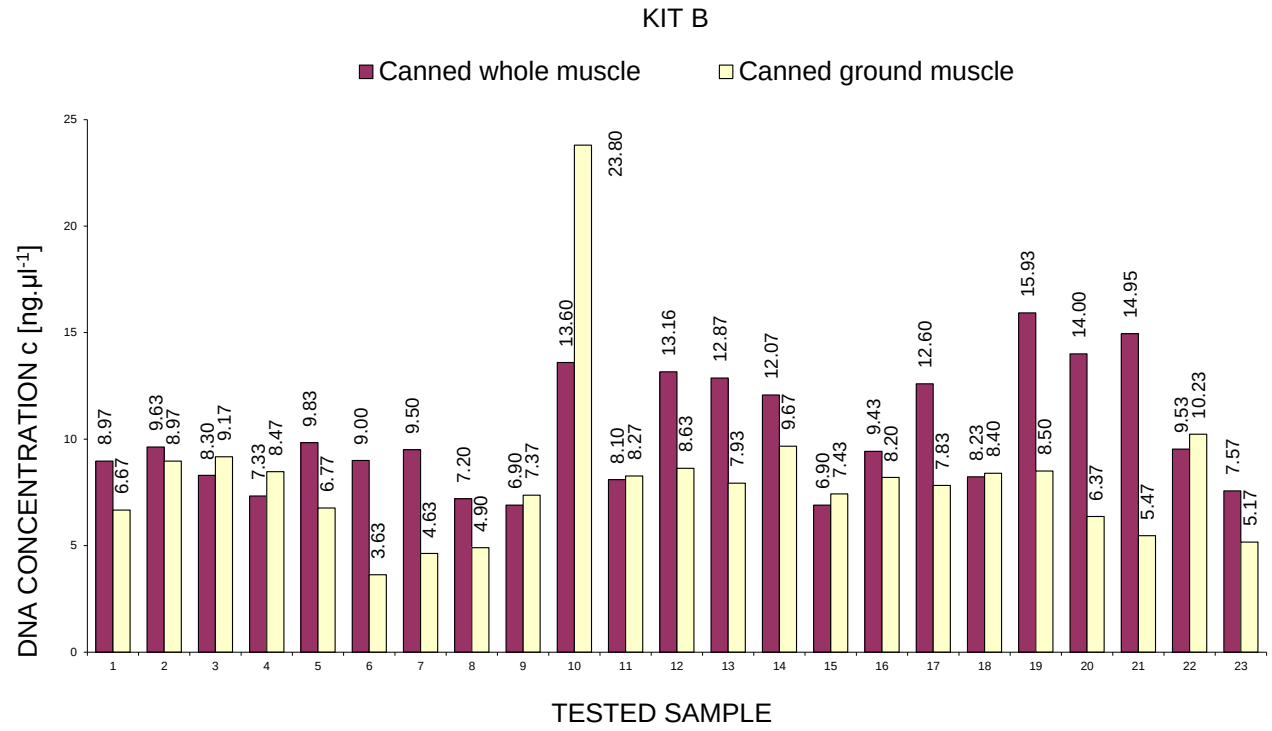

Figure 3 Comparison of DNA concentration of whole and ground muscle determined by kit B.

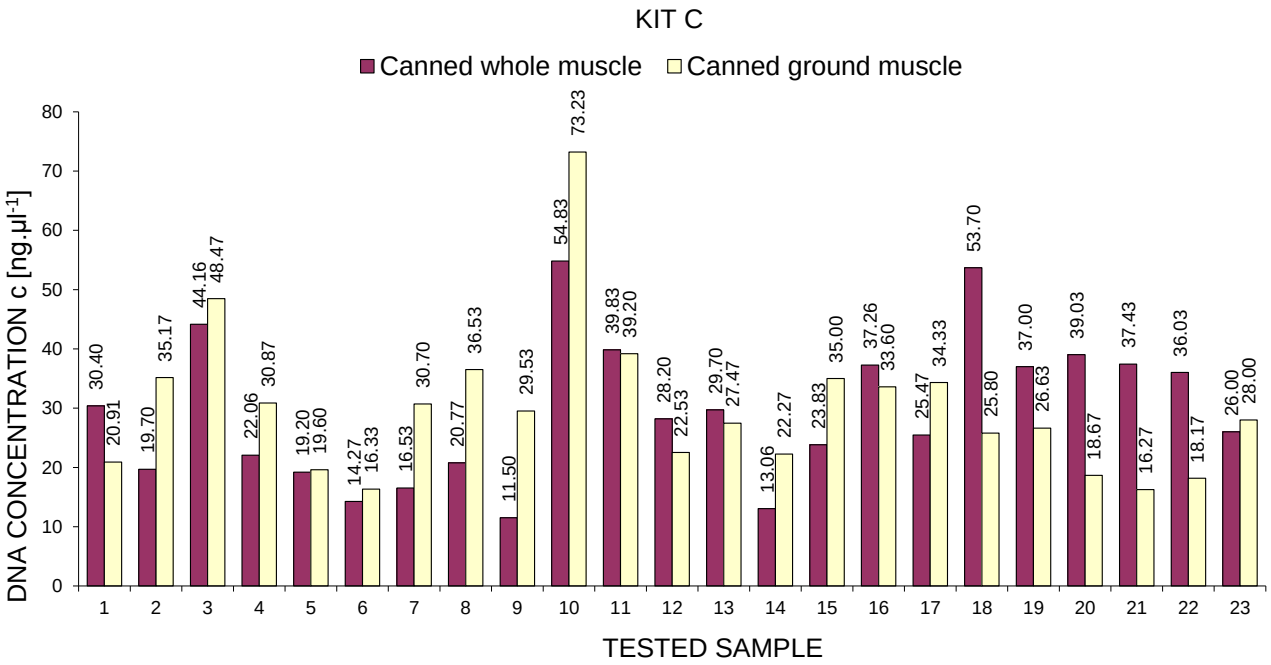

Figure 4 Comparison of DNA concentration of whole and ground muscle determined by kit C.

that are known as a low pH media which could be the reason of DNA degradation **(Bauer et al., 2003)**. In both kit B and C the decreasing effect on DNA yield caused by the presence of vinegar and lemon was observed. In kit A this was observed only in lemon and in case of ground muscle this effect was not demonstrated (Figures 2 – 4). Onion contains quercetin that belongs to the flavonoids and that can inhibit the protein kinase C activity (PKC) and mitogen activated protein kinase 1 (MEK). Therefore it appears to decrease the DNA yield in the samples containing onion **(Lee et al., 2008).** This was observed in whole muscle samples containing onion consistently in all three types of kits. In contrary the presence of ingredients in some samples resulted in better DNA yield. The color extracted from the samples containing particular ingredients (carrots in all three kits, tomato puree, chili, oregano, tomatoes, green pepper or black olives in kit B or C) could cause the higher values of absorbance which could misinterpret obtained results **(Chapela et al., 2007)**. Unexpectedly in kit A the lowest DNA yield was estimated in sample containing pea, the highest value of DNA concentration was assessed in sample containing bean. Although both are legumes their effect was completely contradictory. The quality assessed by the ratios A260/A280 were decreased (A260/A280 <1.7) in samples containing brine and vinegar in both kits A and B, in kit C every sample resulted in ratios lower than 1.7. Although purity ratios are important indicators of sample quality, the best indicator is functionality in the following PCR amplification. There are occasions when the purity ratios are within expected limits, but there is a problem with the sample. Accordingly the presence of ingredients may negative influence the subsequent PCR amplification, when they could inhibit the DNA polymerase activity in PCR **(Di Pinto et al., 2007)** and decrease its sensitivity. The impact of ingredients was not conclusively approved and their connotation to the suitability of extracted DNA for PCR amplification is needed to be discussed in further analysis.

CONCLUSION

The quality of DNA affect the efficiency of amplification during the subsequent PCR reaction. The results of this analysis revealed variability of particular extraction procedures in assessment of DNA quality and quantity in tuna muscle tissue treated with different modifications. The highest DNA concentrations were observed in non-processed muscle, whole canned muscle demonstrated lower DNA yield, and canned ground muscle resulted in even lower values of DNA concentration that was registered by using all three types of DNA extraction kits. Kit B produced DNA of higher concentration in non-processed sample, kit C delivered higher DNA yields in canned whole and ground muscle than kit A and B, although the purity was lower, but still within the range 1.7 – 2.0. In the case of DNA purities, DNA was considered to be satisfactorily pure in all three types of samples and using all three types of DNA isolation. Comparing the parameters of whole and ground canned muscle tissue with the content of ingredients, kit C produced in all samples with whole and ground muscle the highest values of DNA concentration, but almost all values of A260/A280 were lower than 1.7. Kit B in all samples with whole muscle gives higher values of DNA concentrations than kit A, in samples with ground muscle this assumed in almost all samples, so it appears to be a good choice for the DNA isolation from canned whole muscle with ingredients. The effect of ingredients mixed together with muscle reveal the differences in terms of

decreasing but also raising the DNA yield among particular kits and also among whole and ground muscle. Nevertheless the presence of ingredients may negative affect the subsequent PCR amplification, which will be the subject of further comparative analysis.

REFERENCES

Bartlett, S. E., Davidson, W. S. 1991. Identification of *Thunnus* tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome b genes. *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 48, p. 309-317. http://dx.doi.org/10.1139/f91-043

Bauer, T., Weller, P., Hammes, W.P., Hertel, C. (2003). The effect of processing parameters on DNA degradation in food. *European Food Research Technology*, vol. 217, p. 338-343. http://dx.doi.org/10.1007/s00217-003-0743-y

Bossier, P. 1999. Authentication of seafood products by DNA patterns. *Journal of Food Science*, vol. 64, p. 189-193. http://dx.doi.org/10.1111/j.1365-2621.1999.tb15862.x

Bottero, M. T., Dalmasso, A., Cappelletti, M., Secchi, C., Civera, T. 1997. Differentiation of five tuna species by a multiplex primer-extension assay. *Journal of Biotechnology*, vol. 129, no. 3, p. 575-580. http://dx.doi.org/10.1016/j.jbiotec.2007.01.032 PMid:17353060

Besbes, N., Fattouch, S., Sadok, S. 2011. Comparison of methods in the recovery and amplificability of DNA from fresh and processed sardine and anchovy muscle tissue. *Food Chemistry*, vol. 129, p. 665-671. http://dx.doi.org/10.1016/j.foodchem.2011.05.002

Cawthorn, D., Steinman, H. A., Witthuhn, R. C. 2011. Comparative study of different methods for the extraction of DNA from fish species commercially available in South Africa. *Food Control*, vol. 22, p. 231-244. http://dx.doi.org/10.1016/j.foodcont.2010.07.003

Chapela, M. J., Sotelo, C. G., Péez-Martin, R. I., Pardo, M. A., Pérez-Villareal, B., Gilardi, P., Riese, J. 2007. Comparison of DNA extraction methods from muscle of canned tuna for species identification. *Food Control*, vol. 18, p. 1211-1215. http://dx.doi.org/10.5219/189

Colombo, F., Mangiagalli, G., Renon, P. 2005. Identification of tuna species by computer-assisted and cluster analysis of PCR-SSCP electrophoretic patterns. *Food Control*, vol. 16, p. 51-53. http://dx.doi.org/10.1016/j.foodcont.2003.11.006

Dalmasso, A., Civera, T., Bottero, M. T. 2006. Biomolecular approaches for the identification of tuna species. *Veterinary Research Communications*, vol. 30, p. 179-181. http://dx.doi.org/10.1007/s11259-006-0035-7

Di Pinto, A., Forte, V. T., Guastadisegni, M.C., Martino, C., Schena, F.P., Tantillo, G. 2007. A comparison of DNA extraction methods for food analysis. *Food Control*, vol. 18, p. 76-80. http://dx.doi.org/10.1016/j.foodcont.2005.08.011

Espineira, M., Gonzalez-Lavín, N., Vieites, J. M., Santaclara, F. J. 2009. Development of a method for the identification of scombroid and common substitute species in seafood products by FINS. *Food Chemistry*, vol. 117, p. 698-704. http://dx.doi.org/10.1016/j.foodchem.2009.04.087

Lee, K. W., Kang, N. J., Heo, Y. S., Rogozin, E. A., Pugiliese, A., Hwang, M. K., Bowden, G. T., Bode, A. M., Lee, H. J., Dong, Z. 2008. Raf and MEK Protein kinases are direct molecular targets for the chemopreventive effect of quercetin, a major flavonol in red wine. *Cancer Research*, vol. 68, p. 946-955. http://dx.doi.org/10.1158/0008-5472.CAN-07-3140 PMid:18245498

Lin, W. F., Shiau, C. Y., Hwang, D. F. 2005. Identification of four *Thunnus* tuna species using mitochondrial cytochrome b gene sequence and PCR-RFLP analysis. *Journal of Food and Drug Analysis*, vol. 13, p. 382-387.

Lin, W. F. and Hwang, D. F. 2007. Application of PCR-RFLP analysis on species identification of canned tuna. *Food Control*, vol. 18, p. 1050-1057. http://dx.doi.org/10.1016/j.foodcont.2006.07.001

Lockley, A. K.; Bardsley, R. G. 2000. DNA-based methods for food authentication. *Trends in Food Science and Technology*, vol. 11, no. 2, p. 67-77. http://dx.doi.org/10.1016/S0924-2244(00)00049-2

Lopez, I. and Pardo, M. A. 2005. Application of relative quantification TaqMan real-time polymerase chain reaction technology for the identification and quantification of *Thunnus alalunga* and *Thunnus albacares*. *Journal of Agricultural and Food Chemistry*, vol. 53, p. 4554-4560. http://dx.doi.org/10.1021/jf0500841 PMid:15913324

Mackie, I. M., Pryde, S. E., Gonzales-Sotelo, C., Medina, I., Peréz-Martín, R., Quinteiro, J., Rey-Mandez M., Rehbein, H. 1990. Challenges in the identification of species of canned fish. *Trends in Food Science and Technology*, vol. 10, p. 9-14. http://dx.doi.org/10.1016/S0924-2244(99)00013-8

Pardo, M. A., Pérez-Villareal, B. 2004. Identification of commercial canned tuna species by restriction site analysis of mitochondrial DNA products obtained by nested primer PCR. *Food Chemistry*, vol. 86, p. 143-150. http://dx.doi.org/10.1016/j.foodchem.2003.09.024

Ram, J. L., Ram, M. L., Baidoun, F. F. 1996. Authentication of canned tuna and bonito by sequence and restriction site analysis of polymerase chain reaction products of mitochondrial DNA. *Journal of Agricultural and Food Chemistry*, vol. 44, p. 2460-2467. http://dx.doi.org/10.1021/jf950822t

Regulation (EC) No 1536/92 of the European Parliament and of the Council of 9 June 1992 laying down common marketing standards for preserved tuna and bonito. OJ L 163, 17.6.1992, p. 1-4.

Rehbein, H., Mackie, I. M., Pryde, S., Gonzales-Sotelo, C., Medina, I., Perez-Martin, R., Quinteiro, J., Rey-Mendez, M. 1999. Fish species identification in canned tuna by PCR-SSCP: validation by a collaborative study and investigation of intra-species variability of the DNA-patterns. *Food Chemistry*, vol. 64, p. 263-268. http://dx.doi.org/10.1016/S0308-8146(98)00125-3

Santaclara, F. J., Velasco, A., Pérez-Martin, R. I., Quinteiro, J., Rey-Méndez, M., Pardo, M. A., Jimenez, E., Sotelo, C. G. 2015. Development of a multiplex PCR-ELISA method for the genetic authentication of *Thunnus* species and *Katsuwonus pelamis* in food products. *Analytical Methods*, vol. 180, p. 9-16. http://dx.doi.org/10.1016/j.foodchem.2014.11.076

Takeyama, H., Chow, S., Tsuzuki, H., Matsunaga, T. 2001. Mitochondrial DNA sequence variation within and between tuna *Thunnus* species and its application to species identification. *Journal of Fish Biology*, vol. 58, p. 1646-1657. http://dx.doi.org/10.1111/j.1095-8649.2001.tb02319.x

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