Archive of SID

Fraud identification in fishmeal using PCR

Abbas Doosti*, Pejman Abbasi, Sadegh Ghorbani-Dalini

Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran

Correspondening author: Abbas Doosti, E-mail: abbasdoosti@yahoo.com

Abstract

Fishmeal is an important commercial product that is obtained by processing the bones and whole fish. Detection of adulteration in fishmeal with other meats is important for livestock and poultry production and their healthy. The aim of this study was to identification of fraud and adulteration in fishmeal products. 124 fishmeal samples were collected from manufacturers and examined for presence of poultry and ruminants meats. Total DNA was extracted from fishmeal samples and PCR was performed for gene amplification of meat species. Out of 124 fishmeal products examined 9 (7.25%), 4 (3.22%) and 16 (12.9%) samples contaminated with bovine, sheep and chicken, respectively. The PCR is an effective and rapid technique whit high accuracy that can be used to detect and prevent of the fishmeal adulterations.

Key words: Fishmeal, Adulteration, PCR

www.SID.ir

Archive of SID

Introduction

Fishmeal is a commercial product made of fish, bones and fish processed offal. It is a good source of essential amino acids, vitamins, phospholipids, fatty acids and energy (Farajollahi et al., 2009). Fishmeal can be made from almost any type of seafood but is generally manufactured from wild-caught, small marine fish that contain a high percentage of bones and oil, and usually deemed not suitable for direct human consumption (Farajollahi et al., 2009). Most fishmeal and fish oil is manufactured from anchovies, sardines, capelin, and sand eels, and some of the fisheries that target these species are considered to be well-managed (Bellagamba et al., 2003).

The nutrient composition of fishmeal can vary depending on the type and species of fish, the freshness of the fish before processing and the processing methods (Khatoon et al., 2006). High-quality fishmeal normally contains between 60% and 72% crude protein by weight. Fishmeal is a generic term for a nutrient-rich feed ingredient used primarily in diets for domestic animals, sometimes used as a high-quality organic fertilizer (Shi et al., 2009). The vitamin content of fishmeal is highly variable and influenced by several factors, such as origin and composition of the fish, meal processing method, and product freshness (Nagase et al., 2009). The lipids in fishes can be separated into liquid fish oils and solid fats. Although most of the oil usually gets extracted during processing of the fishmeal, the remaining lipid typically represents between 6% and 10% by weight but can range from 4% to 20% (Cozzolino et al., 2009). Fish lipids are highly digestible by all species of animals and are excellent sources of the essential polyunsaturated fatty acids (PUFA) in both the omega-3 and omega-6 families of fatty acids. The majority of the fishmeal produced is included in commercial diets for poultry, swine, dairy cattle, mink and fish (Farajollahi et al., 2009). Worldwide, millions of tons of fishmeal are produced annually. Contrary to recent popular beliefs, most fishmeal and oil are produced from sustainable, managed, and monitored fish stocks, reducing the possibility of over-fishing (Bellagamba et al., 2003). Approximately 4 to 5 tons of whole fish are required to produce 1 ton of dry fishmeal. The quality of fishmeal is often questioned due to adulteration with sheep, bovine and chicken and it is important for economic, safety of poultry and ruminants (Khatoon et al., 2006). Several methods have been developed recently to detect adulteration in fishmeal. Methods have been developed based on electrophoresis, isoelectric focusing, chromatography, DNA hybridization, polymerase chain reaction

www.SID.ir

Archive of SID

(PCR) and enzyme-linked immunosorbent assay (ELISA) for detection of fishmeal fraud (Ong et al., 2007). The purpose of this study was to molecular detection of the rate of adulteration in fishmeal with poultry and ruminants materials in Iran.

Materials and Methods

Fishmeal sample and DNA extraction

A total of 124 samples of fishmeal were collected and examined for presence of poultry and ruminants. Mitochondrial DNA (mtDNA) was extracted from fishmeal samples using DNA extraction kit (Roche applied science, Germany) according to the manufacturer's recommendations. The quality of extracted DNA was checked on agarose gel electrophoresis and quantitation done by UV-spectrophotometry.

Gene amplification

Species-specific oligonucleotide primers reported by Luo et al. (2008) were used for gene amplification (Luo et al., 2008). These primers and amplification fragment length are shown in Table 1. Species-specific DNA segments of bovis, sheep, and chicken were used for amplification and detection of animal derived materials in fishmeal samples. PCR amplification was carried out in a total volume of 25 µl in 0.5 ml tubes containing 1µg of mtDNA, 1µM of each primers, 2mM $Mgcl_2$, 200µM dNTP, 2.5 µl of 10X PCR buffer and 1 unit of *Taq* DNA polymerase (Roche applied science, Germany).

PCR involved an initial denaturation at 94°C for 5 min; followed by 30 cycles at 94°C for 1 min, annealing at 63°C for beef, 59°C for sheep, and 69°C for chicken, and extension at 72°C for 1 min; and a final extension at 72°C for 6 min was done at the end of the amplification. The PCR amplification products (10 µl) were subjected to electrophoresis in a 1% agarose gel in 1X TBE buffer at 80V for 30 min, stained with Ethidium Bromide, and images were obtained in UVIdoc gel documentation systems (UK). The PCR products were identified by 100 bp DNA size marker (Fermentas, Germany).

www.SID.ir

Archive of SID

Results and Discussion

Amplification with species-specific oligonucleotide primers revealed a 271, 274, and 266 bp from bovine, sheep, and chicken genomic DNA, respectively (Figure 1). DNA extraction of fish, poultry, beef and pork were used for positive controls and were also run for each reaction to ensure products obtained were of the correct size. Tubes contained all mixture reaction without DNA was used as negative controls.

PCR reactions for 124 samples of fishmeal were denoted 23 samples (18.54%) contaminated with poultry and ruminants residuals. Out of 124 fishmeal products examined 9 (7.25%), 4 (3.22%) and 16 (12.9%) samples contaminated with bovine, sheep and chicken, respectively.

Some samples were mixed with both or three bovine, sheep and chicken residuals. The detail of the range of poultry and ruminants derived material in fishmeal samples of Iran is shown in Table 2.

Fishmeal is one of the important widely known commercial products. It is also widely used as a food source for variety of purposes such as poultry, pigs, cattle and sheep (Cozzolino et al., 2009). The world-wide supply of fishmeal is presently stable at several million tons a year. Detection of adulteration and quality of fishmeal is important for health of livestock, animal nutrition and economic (Nagase et al., 2009). In addition, determination of the species of origin of the meat components in fishmeal products is an important task in food hygiene, food codex, food control and veterinary forensic medicine (Ayaz et al., 2006). Several methods have been developed to identify fishmeal content. Each method has advantages and disadvantages. The conventional methodology used for the determination of species origin in fishmeal and meat products had been predominantly based on immunosorbent assay (ELISA), immunochemical and electrophorectic analysis of protein. Electrophoresis requires several hours and presents low reproducibility (Ballin et al., 2009). Additionally, through the acquisition of sequence data, DNA can potentially provide more information than type of protein content, due to the degeneracy of the genetic code and the presence of many non-coding regions (Partis et al., 2000). DNA hybridization (Wintero et al., 1990) and PCR methods (Chikuni et al., 1994) have been used for the identification of meats and fishmeal products. PCR is a

www.SID.ir

Archive of SID

helpful technique for fishmeal and meat species identification. The present study is focused on the use of PCR technique for a rapid detection and identification of meat species in fishmeal products of companies in Iran.

The results of this study showed good evidence for molecular markers linked to genetic identification of beef's, sheep's, and chicken's meat in fishmeal products. In current study from a total of 124 fishmeal samples 23 samples (18.54%) contaminated with poultry and ruminants derived materials. The ranges of bovine, sheep and chicken meats in fishmeal samples are 7.25, 3.22, and 12.9, percent, respectively. In Iran beef and sheep meats are abundant and cheaper than other meats, and indicating the possibility of adulteration of companies for economic reasons.

There are many studies for meat and fishmeal adulterations were done. Hsieh et al. (1995) reported that beef or lamb meat was found to be the contaminating species in ground turkey sold in retail markets. The reasons for substituting more expensive meat such as beef and lamb with cheaper meat such as poultry include the use of the unmarketable trimmings from expensive meats and improper cleaning of the grinder between each change of meat species prior to grinding (Hsieh et al., 1995). Meyer et al. detected 0.5% pork in beef using the duplex PCR technique. Their results revealed that PCR was the method of choice for identifying meat species in muscle foods (Meyer et al., 1994). Furthermore, Meyer et al. in 1995 detected 0.01% soy protein in processed meat products using the nested-PCR technique (Meyer et al., 1995). Partis et al. detected 1% pork in beef using RFLP (Partis et al., 2000) whereas Hopwood et al. detected 1% chicken in lamb using PCR (Hopwood et al., 1999). Bellagamba et al. in 2003 were detected mammalian and poultry adulteration in fish meals and their results showed 0.125% beef, 0.125% sheep, 0.125% pig, 0.125% chicken, and 0.5% goat (Bellagamba et al., 2003). The study of Aida et al. in Malaysia showed PCR-RFLP is a potentially reliable technique for detection of pig meat and fat from other animals for Halal authentication (Aida et al., 2005). Khatoon et al. in Pakistan in 2006 were assayed 184 samples of fishmeal for proximate composition, pepsin digestibility, salt, acid insoluble ash and chromium. The results of their study showed a variation in nutrient composition among samples. An inverse relationship was observed between fat, ash, pepsin digestibility, chromium and crude protein contents of fishmeal. All the

www.SID.ir

Archive of SID

samples were adulterated with slightly higher levels of sand and salt than recommended (Khatoon et al., 2006).

Shally et al. were used multiplex PCR technique for detection of meat species via tracing of *cytochrome-b* gene (Jain et al., 2007). Ong et al. in 2007 were used three restriction enzymes in PCR-RFLP using the mitochondrial cytochrome b region to establish a differential diagnosis which detect and discriminate between three meat species and they were showed this technique can be applied to food authentication for the identification of different species of animals in food products (Ong et al., 2007). Luo et al. in 2008 were showed the application of a PCR for detection of beef, sheep, pig, and chicken derived materials in feedstuff and indicated that high sensitivity and specificity of PCR technique with a minimum detection level of 0.1% (Luo et al., 2008). Shi et al. in 2009 showed the feasibility of visible and near infrared reflectance spectroscopy (NIRS) method for the detection of fishmeal adulteration with vegetable meal. The results of this study showed that the NIRS could be used as a method to detect the existence and the content of soybean meal in fishmeal (Shi et al., 2009). Nagase et al. in 2009 were showed authentication of flying-fish-meal content of processed food using PCR-RFLP. They are distinguished between flying fishes and the other fishes by combining amplified DNA fragments with universally designed primers and digesting the PCR products with *Afa*I and *Mfe*I restriction endonucleases (Nagase et al., 2009).

Conclusion

In Iran, fishmeal is being used as a major animal protein source and the results of current study suggested that full screening of fishmeal samples will help to increase the standard of animal feeds.

This study was performed at first time for molecular detection of adulteration in fishmeal that used in Iran. The current study confirms previous findings and showed low adulteration in used fishmeal in Iran. Since, the results of this study might be useful for prevent and control of adulterated and fraud in fishmeal products that used in dairy and poultry industry. So, molecular methods such as PCR were suggested as an effective, rapid, reliable and sensitive technique for the detection of adulteration in fishmeal products used in dairy and poultry industry.

www.SID.ir

Archive of SID

Acknowledgements

The authors thank the all staff of Biotechnology Research Center of Islamic Azad University of Shahrekord Branch in Iran for their sincere support.

References

Aida AA, Che Man YB, Wong CMVL, Raha AR, Son R (2005), Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication, Meat Sci, 69: 47-52.

Ayaz Y, Ayaz ND, Erol I (2006), Detection of species in meat and meat products using enzyme-linked immunosorbent assay, J. Muscle Foods, 17: 214-220.

Ballin NZ, Vogensen FK, Karlsson AH (2009), Species determination – Can we detect and quantify meat adulteration? Meat Sci, 83: 165-174.

Bellagamba F, Valfrè F, Panseri S, Moretti VM (2003), Polymerase chain reaction-based analysis to detect terrestrial animal protein in fish meal, J. Food Prot, 66(4): 682-685.

Chikuni K, Tabata T, Kosugiyama M, Monma M, Saito M (1994), Polymerase chain reaction assay for detection of sheep and goat meats, Meat Sci, 37: 337-345.

Cozzolino D, Restaino E, La Manna A, Fernandez E, Fassio A (2009), Usefulness of near infrared refl ectance (NIR) spectroscopy and chemometrics to discriminate between fishmeal, meat meal and soya meal samples, Cien. Inv. Agr, 36(2): 209-214.

Farajollahi H, Aslaminejad AA, Nassiry MR, Sekhavati MH, Mahdavi1 M, Javadmanesh A (2009), Development and use of quantitative competitive PCR assay for detection of poultry DNA in fish meal, J. Anim. Feed. Sci, 18: 733-742.

Hopwood AJ, Fairbrother KS, Lockley AK, Bardsley RG (1999), An actin gene-related polymerase chain reaction (PCR) test for identification of chicken in meat mixtures, Meat Sci, 53: 227-231.

www.SID.ir

Archive of SID

Hsieh YHP, Woodward BB, Ho SH (1995), Detection of species substitution in raw and cooked meats using immunoassays, J. Food Prot, 58: 555-559.

Jain S, Brahmbhait MN, Rank DN, Joshi CG, Solank JV (2007), Use of cytochrome b gene variability in detecting meat species by multiplex PCR assay, Indian J. Anim. Sci, 77(9): 880-888.

Khatoon S, NQ Hanif, Malik N (2006), Status of fish meal available for poultry rations in Pakistan, Pakistan Vet. J, 26(2): 97-98.

Luo J, Wang J, Bu D, Li D, Wang L, Wei H, Zhou L (2008), Development and application of a PCR approach for detection of beef, sheep, pig, and chicken derived materials in feedstuff, Agr. Sci. China, 7(10): 1260-1266.

Meyer R, Candrian U, Luthy J (1994), Detection of pork in heated meat products by the polymerase chain reaction, J. AOAC Int, 77: 617-622.

Meyer R, Hofelein C, Luthy J, Candrian U (1995), Polymerase chain reaction-restriction fragment length polymorphism analysis: a simple method for species identification in food, J. AOAC Int, 78: 1542-1551.

Nagase M, Maeta K, Aimi T, Suginaka K, Morinaga T (2009), Authentication of flying-fish-meal content of processed food using PCR-RFLP, Fish Sci, 75: 811-816.

Ong SB, Zuraini MI, Jurin WG, Cheah YK, Tunung R, Chai LC, Haryani Y, Ghazali FM, Son R (2007), Meat molecular detection: Sensitivity of polymerase chain reaction-restriction fragment length polymorphism in species differentiation of meat from animal origin, ASEAN Food J, 14(1): 51-59.

Partis L, Croan D, Guo Z, Clark R, Coldham T, Murby J (2000), Evaluation of a DNA fingerprinting method for determining the species origin of meats, Meat Sci, 54: 369-376.

Shi GT, Han LJ, Yang ZL, Liu X (2009), Methods of analyzing soybean meal adulteration in fish meal based on visible and near infrared reflectance spectroscopy, Guang Pu Xue Yu Guang Pu Fen Xi, 29(2): 362-366.

www.SID.ir

Archive of SID

Wintero AK, Thomsen PD, Davies W (1990), A comparison of DNA hybridization, immunodiffusion, countercurrentimmuno electrophoresis and isoelectric focusing for detecting the admixture of pork to beef, Meat Sci, 27: 75-91.

Table 1. Species-specific oligonucleotide primers and expected lengths of amplified segments

Primer name	Primer sequence	Product size
Bovis	F: 5´-GCCATATACTCTCCTTGGTGACA-3´	271 bp
Bovis	R: 5´-GTAGGCTTGGGAATAGTACGA-3´	
Sheep	F: 5´-ATGCTGTGGCTATTGTC-3´	274 bp
Sheep	R: 5´-CCTAGGCATTTGCTTAATTTTA-3´	
Chicken	F: 5´-GGGACACCCTCCCCCTTAATGACA-3´	266 bp
Chicken	R: 5´-GGAGGGCTGGAAGAAGGAGTG-3´	

www.SID.ir

Archive of SID

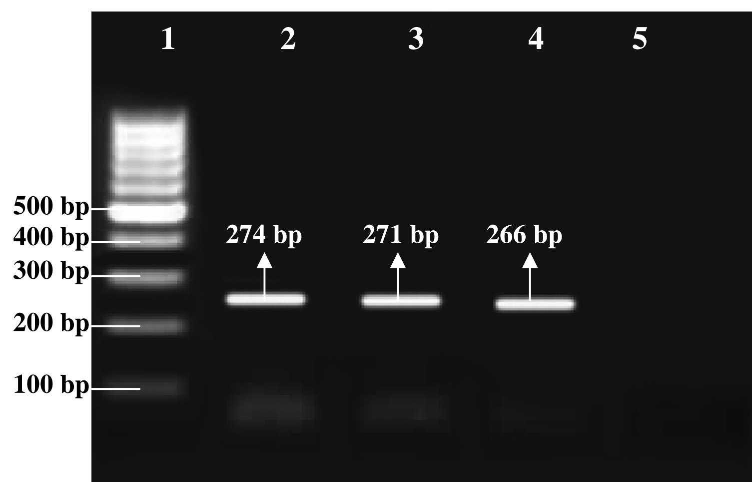

Figure 1.

The electrophoresis of PCR products was generated by species-specific oligonucleotide primers. Line 1 is a 100 bp DNA ladder (Fermentas, Germany). Lines 2-5 are sheep, bovine, and chicken amplified fragments, respectively and line 5 is negative control.

www.SID.ir

Archive of SID

Table 2. The range of poultry and ruminants derived materials in fishmeal samples of Iran.

Poultry and ruminants derived material	Fishmeal samples (percent)
Bovine	9 (7.25%)
Sheep	4 (3.22%)
Chicken	16 (12.9%)
Total	23 (18.54%)

www.SID.ir