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IDENTIFICATION AND CONFIRMATION OF UNKNOWN MEAT USING MICTOCHONDRIAL CYTOCHROME C OXYDASE I (CO-I) MARKER IN DNA BARCODING TECHNOLOGY

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Abstract

Non vegetarian food has expanded market in the world, especially, in those areas where vegetarian foods are available in less quantity. Meat and meat products (cow beef) are obtained by slaughtering animals including cattle. However, the origin of animal species in food links with religious ethics, which are violated by sellers for commercial gain including mismatch in labelling and ingredients and presence of trace amount of restricted animal meat in daily used meat foods. Hence, authentication of the meat based foods comes in role that protects ethics and animal protection. In this review, we have investigated the authentication status of meat (cow beef) based food products using globally used COI gene dependent DNA barcoding technology.

Keywords: Meat and meat products, DNA barcoding technology, COI gene, commercial gain, beef, authentication.

Introduction:

Food is considerably important aspect of life without which survival of organisms is merely impossible. The requirement and types of food materials vary among flora, fauna and microbes depending on their energy demands. There are various types of food products that are essential for humans including vegetarian and non-vegetarian nutritive food substances. The demand of non-vegetarian foods including meat and chicken has been increasing in the globe with increasing population although its consumption is restricted up to limited people. More to the point, meat is extremely favoured by many people in their diet due to its higher nutritive value (V.P. Singh et al., 2014) and its requirement is increasing along with global population, especially, in the developing nations (Delgado, 2003).

In India, *Bos indicus* was majorly used as a source of food including milk and beef which was common practice from many centuries. However, exploitation of this species for beef resulted into its imbalanced and non-sustainable development hampering economic wealth of country, especially, people of rural areas. As a result, government of Maharashtra approved oldest pending bill 'Maharashtra Animal Preservation (Amendment) Act, 1995' in March 4, 2015 under which transporting and slaughtering of the cows and purchasing and possessing their beef in and outside Maharashtra was declared as illegal and person carrying such malpractice was declared as eligible for punishment of imprisonment up to up to five years' or Rs. 10,000 fine or both. Nevertheless, the law makers failed to generate the efficient system for successful implementation of launched act due to unavailability of robust identification system of cut pieces of beef resulting into continued illegal trading of cows and their beef in the state.

DNA Barcoding is a concept in which mitochondrial COI gene (~650bp) is used for identification of biological samples (Hebert, P. D. N. et al., 2003a), for beef specimens in order to put the law in question into operation effectively. This tool is efficient for animal taxonomy since the used gene shows lower intra-species variation than inter-specific divergence (Hebert, P. D. N. et al., 2003b) and is fast, cheaper, with wide range of applicability for food validation (Violeta Fajardo et al., 2010).

Furthermore, COI gene based identification strategy identifies the specimens that are not identified by morphology (Gianni Barcaccia et al., 2016) such as pieces of meat in relation with its source of its origin. Additionally, this method is widely used for identification of both raw (Wang, Y., et al., 2016) and processed food materials. More to the point, DNA barcoding technology is effective for food traceability testing because it includes cost effective molecular analysis, accelerating availability of well developed laboratories along with skilful researchers, freely accessible web-based online databases and elevating number of consumers having awareness of standard food product quality.

Although few organisms are well studied by DNA barcoding, wide range of work is required to generate reference library of less studied groups of organism. In India, very less work has been entertained in the area of DNA based taxonomic identifications of bovine species using their body parts. The identification of species using their common names is more reliable than assigning taxa to them using their scientific name since number of subspecies appear as species (Isaac et al. 2004). In this review, we have analysed success of DNA barcoding in identification of meat products in relation with beef assisting in regulation of protection the species under study and inhibit illegal food trade.

Need of identification of beef meat species:

Identification of animals and their meat products are very important for customers with respect to implications of society, religion, health and economy (Gianni Barcaccia et al., 2016). In case of live stock animals, meat identification system can be implemented due to less number of used molecular markers, cost effectiveness, univocal polymorphisms and less complex statistics (Gianni Barcaccia et al., 2016). In majority of cases, taxonomic complications such as identification of species using their common vernacular names may belong to different taxa are responsible for commercial malpractices (Andrea Galimberti et al., 2013). Therefore, to avoid wrong labelling and commercial scandal, combination of vernacular names, scientific names and DNA barcodes is recommended (Andrea Galimberti et al., 2013).

There is insufficient regulation of species detection in case of food and livestock products due to disappearance of characters required for their identification after processing (Wang, Y., et al., 2016) for example, pieces of beef meat. The mixing of other components in meat has become frequent with their increased prices, worldwide marketing and accelerated manufacturing of high value products from food (Ayaz, Ayaz, & Erol, 2006 Flores-Munguia, Bermudez-Almada and Vazquez-Moreno, 2000, Vandendriessche, 2008). The parameters related with physicochemical, nutritive and fragrance of water buffalo meat is as like as beef from cattle (Kandapeen et al., 2009).

In several nations, mixing cheap meat received from different origin in meat product is widespread malpractice (Nadia Haider et al., 2012). Since such type of crime is not legal causing multiple health, money along with religion related issues (Wang et al., 2010), it has become necessary to identify the components that are used for adulteration to regulate laws related with labelling of food products and to avoid misleading competition (Kesmen, 2010). Moreover, such type of activity is required for securing standards of nation and guarding choices of consumers (Singh Y. et al., 2007).

Need of meat constituent identification:

The infections like BSE and avian flu has caused by meat products has resulted in accelerated perception among people for its quality and animal of origin. These circumstances have made identification of constituents of meat products (Andrea Galimberti et al., 2013).

Meat adulteration:

Consumers, researchers and meat industry are having major concern regarding the food safety and authenticity, and adulteration is being conducted in meat products because these are commercially important (Aly Farag El Sheikha). The cases of meat adulteration are frequently practiced for commercial gain which is against health ethics of consumers. Although Nakyinsige, et al. (2012) claimed that as the meat is sold as fresh, probabilities of its adulteration are rare, the misleading labels were detected with rates of near about 20-70% in diverse meat products like sausage, ground meats, meat balls, deli meats as well as dried meats in South Africa (Ayaz, 2006; Cawthorn D.-M. et al., 2013, D'Amato et al., 2013; Flores-Munguia M. E., 2000, Ozpinar H., 2013).

This view was supported by Food Safety Authority of Ireland (FSAI) (2013) which stated that testing of beef burgers, products of ground beef and salami detected contamination of 37% horsemeat and 85% of pork meat. In addition, despite the regulations by government,

adulteration was found in products of ground meat with wrong labelling pattern in 16.6% of examined products (Hsieh Y.-H. P. et al.1995). Shockingly, the food adulteration activities are also reported. For example, In Italy, adulteration of bovine milk in water buffalo milk for making cheese was detected by Central Inspectorate for Repression of Frauds of the Italian Ministry of Agricultural and Forestry Policy (Gianni Barcaccia et al., 2016). The response of meat customers varies according to countries and their awareness of the meat product quality (Gellynck, X. et al., 2015).

The essential standpoints of minimum requirements for meat authentication may be recall of product, awareness of individual responsibility and whole traceability of the chain of meat (Meuwissen, M.P.M. et al., 2013, Gellynck, X. et al., 2015). Charles A. Quinto (2016) found that the meat product of *Bos taurus*, a domestic cattle were labelled as bison and yak in US market. Furthermore, the Ayaz et al., (2006) observed that 22.0% meat products were belonging to poultry, deer and horse when these were labelled as beef meat in Turkey. This food fraud might interfere with public health as well as creates religious and cultural issues since some species are strictly banned by particular religions for their use as a food.

Substitution of high quality meat by low quality meat is common malpractice in many nations in the globe. For example, horse beef was substituted by horse meat in UK and Kangaroo meat was replaced by beef in Australia (V.P. Singh et al., 2014). The composition of meat mixture may not be detected clearly as physical look, colour along with texture and aroma of meat products get altered after processing (Flores-Munguia et al., 2000).

Reasons of food frauds:

The reasons of frauds in food are based on commercial profit with less investment. Similarly, according to Cawthorn D.M. et al. (2013), Everstine K. et al. (2013), Hsieh et al. (1995), Spink J. and Moyer D. C. (2011), food frauds occur due to low quality traceability, cross contamination, improper cleaning of equipments that are used for different species and attempt to gain economic profit (Cawthorn D.-M. et al., 2013, Everstine K. et al., 2013, Hsieh et al., 1995, Spink J. and Moyer D. C., 2011). Unfortunately, the processed as well as ready to consume foods are available in more quantity that makes the species identification process challenging leading to food adulteration and frauds (Gianni Barcaccia et al. 2016). It is, especially, true for game meats due to their increased prices as compared to pork or beef pieces (Gianni Barcaccia et al. 2016).

Solution of food frauds:

Since production and supply of meat are long lasting processes, there is need of authentic traceability systems for it. Researchers developed molecular techniques to support species identification in the meat products. Manel, et al. (2002) expected emerging methods of molecular identification that these should act as safe guard of not only customers but also producers from food misleads and animals from their exploitation more than limits or their unauthorized dealings.

DNA barcoding, the method in which the mitochondrial COI gene (~650bp) is used for identification of biological samples (Hebert, P. D. N. et al., 2003a) is efficient for animal taxonomy since this gene shows lower intra-species variation than inter-specific divergence (Hebert, P. D. N. et al., 2003b). Moreover, this technique is fast, cheaper, and has wide range of applicability for food validation (Violeta Fajardo et al., 2010). Both domestic and game

meat species can be identified by using mitochondrial as well as nuclear gene sequences (Fajardo et al., 2008a).

Moreover, mitochondrial DNA is preferred over nuclear DNA for species identification because, mtDNA genes are present in many copy number per cell as compared to nuclear DNA, rapid rate of evolution than nuclear DNA leading to more sequence variation aiding identification species that are associated with their phylogenetic relationship (Girish et al., 2005). However, recently, the short DNA fragment analysis techniques has been outdated and is replaced by whole genome sequencing for more accurate study of species in question.

The DNA barcoding technology is reliable method for standardization of food products and may be precise pathway to generate quality assurance of the product in question not only transformers but also customers. Moreover, this technique requires more comprehensive and elaborative work a by scientists and industries dealing with food manufacturing chain and examination of origin of food so that, this method can be applied on broader scale and would assist in getting simple and less expensive solutions from organizations (Gianni Barcaccia et al., 2016). The authentic food traceability operations have entered in the doors of scientific research due to which, many analytical methods are developed to solve the problem (Bottero & Dalmasso, 2011; Fajardo V. et al., 2010; Hellberg & Morrisey, 2011; Mafra I. et al., 2008).

Methods for traceability of meat specimens:

Different methods are developed for traceability of meat species, for example, PCR-RFLP, species-specific PCR and PCR sequencing (Mane et al., 2006; Teletchea et al., 2005) using mitochondrial molecular markers rather than nuclear ones (Andrea Galimberti et al., 2013). Moreover, various methods including DNA hybridization, species-specific polymerase chain reaction (PCR) primers, restriction fragment length polymorphism (RFLP) analysis, single strand conformational polymorphism (SSCP) analysis, random amplified polymorphic DNA (RAPD) analysis, and PCR product sequencing can be used for verification of many types of meats ranging from fish and livestock to game animal species Lockley and Bardsley (2000) which can, however, give false positive results in the analysis (Eugene H.K. Wong and Robert H. Hanner, 2008).

Yanyi Pan et al. (2020) reported the food frauds with 100% success and found that 50% food products belonging to beef was mislabeled (table 1), for commercial gain (table 1). The modulation beefsteak was labeled as having beef ingredients but, in fact, it contained fish (0.2%) and chicken (<0.1%) with pork (0.2%) (Yanyi Pan et al. 2020) intentionally or by contamination due to inadequate training of workers in food processing industries. However, the unwanted components were in less concentration but it is not ethical with religious point of view. The food ingredients which are labeled on the food packets is recommended to be provided to consumers and not other components. Thus, NGS helps to check purity of the beef products.

Table 1 Both detected as well as labeled ingredients investigated in commercial food products. (Yanyi Pan et al. 2020)

Sample name	Labeled ingredients	Detected ingredients	Detected species
Beef tendon balls	Beef, Pork	Beef (66.2%) Pork (29.4%)	*Bos taurus*, *Bubalus bubalis Sus scrofa*
Modulation Beefsteak	Beef	Beef (99.5%), *Pork* (0.2%), Fish (0.2%), Chicken (<0.1%)	*B. taurus*, *B. bubalis, S. scrofa, P. hypophthalmus, G. gallus*

Applicability of cox1 gene:

Although cox1 gene sequences for mammalian species that are used as food are available in very less quantity in BOLD and GenBank databases than cyt b genes (Andrea Galimberti et al., 2013), it can be used for analyzing traceability of mammalian meat products reliably (Cai et al., 2011; Francis et al., 2010; Luo et al., 2011). In addition, techniques of molecular diagnosis can bypass the morphological taxonomic identifications (Eugene H.K. Wong and Robert H. Hanner 2008).

COI gene is suitable for meat species identification. Ward et al. (2005) and Hadjibabaei et al. (2007) tested authenticity of DNA barcoding technology using different groups of animals and stated that many species (>94%) had discrete DNA barcodes with lower variation within the species than divergence among the species and higher variation from closely associated taxa.

Drawbacks of meat consumption:

There is a problem of Bovine spongiform encephalopathy (BSE) related with bovine originated foods that lowered the use of beef in Europe (Gianni Barcaccia et al., 2016) though the bush meat has increased interest by local and international societies for food and traditional as well as modern medicines (Alves and Rosa 2005).

Analytical methods of meat species:

The analysis of meat authentication is based on their protein and DNA study (Violeta Fajardo et al., 2010) and the protein based analytical protocols assisting in diagnosis of meat species origin include electrophoresis (Montowska & Pospiech, 2007), chromatography (Chou et al., 2007), and spectroscopy (Ellis D. I. et al., 2005). In contrast, these techniques cannot work precisely in case of heated material as soluble proteins get dissolved due to heat and in immunoassay procedure, antigen-antibody reactions suffer from cross-reactions of antigen and antibodies belonging to closely related species (Ayaz, Ayaz, & Erol, 2006).

However, the use of DNA based methods can overcome this problem as it is highly stable with longer shelf life and its availability in all organisms (Fajardo et al., 2010). Polymerase Chain Reaction method, one of the DNA based methods, is easy to handle, fast, has capacity to work with small amount of DNA along with specificity provides platform to track substances of animal origin in food products (Mafra, Ferreira, & Oliveira, 2008; Tobe & Linacre, 2008).

Applications of DNA barcoding:

The halal meat can be authenticated by DNA barcoding technology (Aly Farag El Sheikha et al. 2017). Wong and Hanner (2008) could successfully used DNA barcoding technology for detecting white tuna meat mixed in tilapia's meat. Similarly, this tool can be used for identification of meat of *Bos taurus* and *Redunca arundinum* meat (Dalton D.L., Kotze A., 2011), chicken meat samples (Dawnay et al., 2007), raw bovine, chicken, turkey, sheep, pig, camel and donkey meat (Haider et al. 2012) and slaughter house animals (Dawn E. Kane and Rosalee S. Hellberg 2015), fresh and processed meat as well (Gianni Barcaccia et al., 2016). Furthermore, this method identifies the specimens that are not identified by morphology (Gianni Barcaccia et al., 2016) such as pieces of meat in relation with its source of its origin. In addition, this method is widely used for identification of raw food materials (Wang, Y., et al., 2016).

More to the point, DNA barcoding technology is effective for food traceability testing because it includes cost effective molecular analysis, accelerating availability of well developed laboratories along with skilful researchers, freely accessible web-based online databases and elevating number of consumers having awareness of higher standards food product quality. Although few organisms are well studied by DNA barcoding, wide range of work is required to generate reference library of less studied groups of organism. In coming days, this method would become regular test in various areas with particular emphasis to quality assessment and traceability of food products (Andrea Galimberti et al., 2013).

Now a day, authentication of food is regularly performed by methods that work on DNA based analysis and has been widely used for certification of meat products (Lockley & Bardsley, 2000). DNA sequences of various molecular markers are used for identification of diverse meat species (Bartlett and Davidson, 1991; Forrest and Carnegie, 1994; Matsunaga T. et al., 1998; Unseld M., 1995). According to Iwobi et al. (2011), identification of meat samples belonging to chicken or turkey specimens present in the mixture of pork and beef is complex in the case where their availability is <0.5% due to dominance of pork and beef. Meat product of *Bos taurus* or zebu cattle (*Bos indicus*) was mislabelled as yak burger in USA (Dawn E. Kane and Rosalee S. Hellberg (2015), which may be reported with help of the technique under study.

Quality DNA barcodes of meat specimens are obtained if these are not exposed with low pH, UV rays and humidity and processed them immediately on the same day of their deposition at our centre (Teletchea et al., 2005). With mini-barcode, the species that are edible including mammals can be identified with reliability, and use of diverse DNA markers in combination should be entertained to differentiate between species which are closely linked and for reduction of competitive efficiency impact (Yanyi Pan et al. 2020). Consumers are cautious about their food safety and authenticity indicating growing value of DNA barcoding [Sardina, M.T. et al. 2015, Dimauro, C. et al. 2015, Mateus, J.C. et al. 2015, Ng, J. et al. 2015].

DNA barcoding is used for tracking deliberate or accidental food replacements connected with the mislabeling of foods in addition with commercial frauds, and genetic identification of the processed meats along with prevention of food piracy (Gianni Barcaccia et al. 2016). Mitochondrial DNA are selected as molecular markers to identify animals because of its haploid nature, presence of high copy number, unavailability of introns, less

recombination and maternal inheritance (Gianni Barcaccia et al. 2016) leading to its use in beef identification to prevent their illegal trade. Moreover, other reasons of its use are availability of universal primers for amplification of 648 bp gene belonging to the diverse phyla and rate of nucleotide substitution aiding differentiation among species that are closely related species (Gianni Barcaccia et al. 2016).

Standard genetic identification system is used for identification of meat products is vital for consumers because of health, social, economic as well as religious aspects (Gianni Barcaccia et al. 2016). Species of meat from food products can be identified by DNA barcoding promisingly and mini-barcodes are useful for identification of species in the processed products (Rosalee S. et al. 2017) along with use of COI gene, D loop and rRNA genes for food identification (Jia, X. *et al.*2015). Karabasanavar, N., et al. 2017, Wang, J. F., et al. 2011). However, there is a need to have improved set of primers for its application (S. et al. 2017). DNA barcoding is an efficient tool for forensic investigation as well as conservation of wild life and there is a need of barcode database of species in order to solve offenses in relation with wild life (Vikas Kumar et al. 2017).

DNA meta-barcoding is implemented for detection of both multiple as well as unknown species with high-throughput sequencing (Staats, M. *et al.* 2016). Like COI gene, CYTB gene is also used for getting phylogenetic information (Teletchea, F., et al. 2005), which may be used with support by mPCR or PCR-RFLP for identification of meat ingredients of about 20 species (Matsunaga, T. *et al.* 1998, Matsunaga, T. *et al.*1999, Wolf, C. et al,1999, Partis, L. *et al.* 2000, Tobe, S. S. & Linacre, A. M..,2008) and is used for identification of meat components (Yinan Zhang et al. 2020). Furthermore, primers CB1-5 as well as CB3A were used to identify cattle through RFLP-PCR (Bravi, C. M. *et al.* 2004) and similar methods were used to identify cows (Murugaiah, C. *et al.* 2009).

Restriction fragment length polymorphism present in the genes of mitochondrial genes were studied for beef (*Bos taurus*) (Murugaiah, C. *et al.* 2009). Six meats were analyzed with respect to cyt b genes with PCR products with 359 bp size (Murugaiah, C. *et al.* 2009). *BstU*I, *Mse*I, *BsaJ*I, *Alu*I, *Rsa*I are the enzymes (restriction endonucleases) that were used to make differences among meats (Murugaiah, C. *et al.* 2009). The differences among cyt b gene were confirmed by PCR-RFLP success (Murugaiah, C. *et al.* 2009). The identification of DNA of various mammals present in the form of mixture was performed by Tillmar et al. (2013) using 454 GS Junior Sequencer using mitochondrial 16S rRNA as the molecular marker.

D-loop (Jia, X. *et al.* 2015), 16S rDNA (Kitano, T., et al 2012,), , tRNA (Wolf, C. et al. 1999), 12S rDNA are the DNA fragments that are also used for species identification even if *CYTB* gives more advantages (Yinan Zhang et al. 2020).

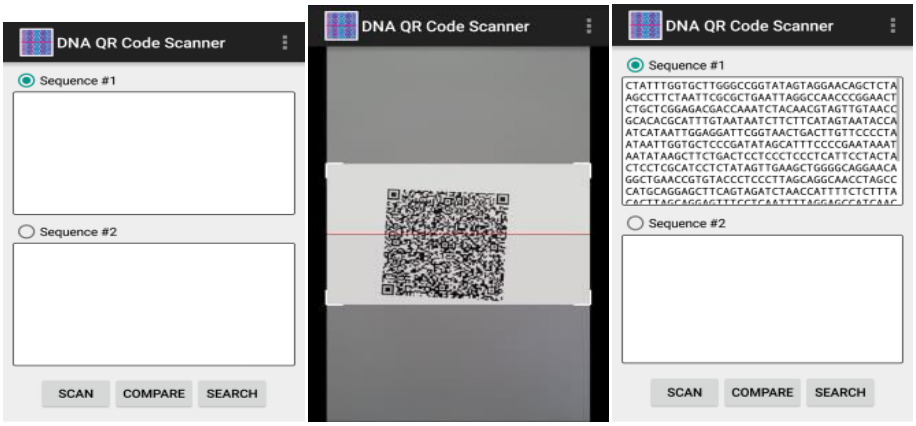

Figure 1: Working of DNA QR Scanner (Naulia, T. 2015).

DNA QR Code:

Identification and authentication of ingredients of meat products from unlawful substitutions should be promoted (Pascal G, Mahe S. 2001). Species of meat products belonging to animals that are closely related can be distinguished by DNA barcoding (Spychaj A et al. 2009). Additionally, it is used to identify the species of meat which is available either individually or in the mixture which is complex (Tillmar AO et al. 2013).

In order to develop a program that encodes the sequences of DNA, the library (in java) of open source QR code was adapted (ZXing et al. 2014). An application starts and then camera of device captures QR code of DNA after which the QR code is decoded in the form of DNA sequence (figure 1) Naulia, T. (2015). As well sequence alignment is performed (figure 2a and 2b) to study matching characteristics of DNA sequence to identify the meat species under study (figure 3).

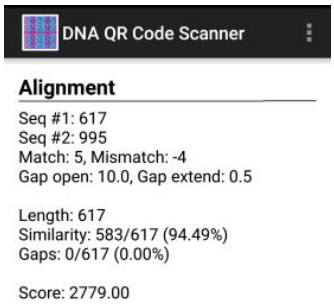

Figure 2a: sequence alignment details (Naulia, T. (2015).)

Naulia, T. (2015) developed DNA QR code scanner that helps in identification of species present in the meat products supporting practical application of DNA barcoding.This identifies the origin of species of the meat products with help of direct scanning printed DNA QR code Naulia, T. (2015). This tool uses Smith-Waterman local sequence alignment Naulia, T. (2015). The DNA barcodes of meat species which are common are used as reference barcodes Naulia, T. (2015).

Figure 2b: Alignment sequence of sequences of DNA (JX426135) (Seq. 1) along with JN632605 (Seq. 2). Naulia, T. (2015)

The DNA QR code scanner provides tool for identifying the species origin of meat products by direct scanning printed DNA QR code Naulia, T. (2015). The DNA QR code of an unidentified specimen is compared with the reference DNA barcodes stored in device memory to find the matching species by local sequence alignment of Smith-Waterman Naulia, T. (2015). The reference DNA barcodes comprises with the barcodes of common meat species Naulia, T. (2015).

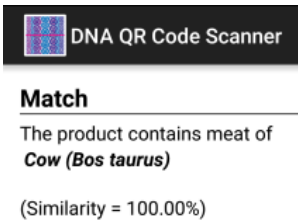

Figure 3: Identification of beef species *Bos taurus* with 100% similarity using DNA QR code scanner (Naulia, T. (2015).

Demerits:

Although DNA barcoding is used worldwide for species identification, it is lacking in certain aspects. For instance, this method cannot distinguish closely linked species, for instance, cow and buffalo (Dawn E. Kane and Rosalee S. Hellberg 2015). Furthermore, the mixture of meat specimens sourced from different species affect the PCR amplification of targeted DNA (Dawn E. Kane and Rosalee S. Hellberg 2015). Species identification using bovine or chicken DNA was unattainable in the case where these were present as 10:1 in the mixture over the DNA of human beings (Dawnay et al. 2007). Besides, DNA barcoding technique may be not highly efficient in taxonomy in the cases where more overlap exists in intraspecific variation (Meyer, C.P.; Paulay, G. 2005) and not able to differentiate breeds. This problem may be tackled with Next Generation Sequencing (NGS).

Moreover, DNA barcoding may suffer from shortage of sufficient reference sequences that are required for sequence comparisons (Eugene H.K. Wong and Robert H. Hanner 2008). We propose that the reported error may be solved by increasing awareness of DNA barcoding among potential researchers and expand horizons of DNA sequence libraries in combination with whole genome sequencing based on requirements and available facilities. Girish et al. (2005) has raised question mark on utility of DNA barcoding by stating that this tool cannot be used for regular meat identification process, is not cost effective and requires more time for its implementation. However, this method is cost effective in the

government research centres since they charge less for processing the biological samples for molecular identification as compared with private laboratories. The time required to complete the process depends of availability and working of primers and other required lab facilities in working mode such as DNA sequencer and PCR reactors.

To add, the meat products originated from breeds are not suitable for identification by DNA barcoding (Andrea Galimberti et al., 2013) since hybridization generates genetic introgression which is generally found in livestock such as cattle in which breeds are produced by hybridization techniques (Kikkawa et al., 2003; Nijman et al., 2003; Verkaar et al., 2003). In turn, this area is open for future research. In contrast, the proposed ideas doesn't fit in the DNA barcoding profile because it used mitochondrial genes which are inherited maternally resulting nullity of effect on identification of breed by COI gene, especially, in the breeds that are produced by mating of their natural parents. We suggest to implement NGS to solve the presented contrast of breed identification, if needed.

Anita Spychaj et al. (2016) obtained amplicons of length <300bp for meat products that indicated the presence of either heated or high pressure treated meat products and Abd El-Azeim A. Ahmed et al., (2016) obtained PCR product of 116 bp belonging to beef specimens collected from marketplaces Giza and Cairo. These report apparently indicate that the environmental factors prevailed during processing of meat products affect the gene length obtained during DNA barcoding process limiting its applicability since such a short fragment doesn't give reliable identification of a species.

The established processes of meat product preparation may include steps like boiling and sterilization as well as salting, curing, smoking, cooking, pre-drying that may affect the quality of DNA due to their degrading effect and addition of fat along with other additives (Dawn E. Kane and Rosalee S. Hellberg 2015).

Identification of many domestic animals such as cattle, sheep, goat, domestic pigs, turkey or chicken is focused by applications of PCR systems (Girish et al., 2005; Stirtzel et al., 2007). La Neve et al. (2008) used PCR in combination with sequencing for differentiating game meat products from domestic animals such as cattle, sheep and goat. Although only PCR products are used by authors for species identification, these are not robust because DNA sequences provide more accurate information by comparing with available global gene data which is not possible with only PCR products.

Whole genome sequence has been used for studying animals (Y. Ge et al. 2017). M. Staats et al. (2016) claimed that standard mini -barcode is not available in order to verify the different meat products. The efficiency of amplification methods is being critically challenged with accelerated complexity as well as diversity of meat products (Yinan Zhang et al. 2020). DNA barcode based on COI genes have limitations as well as shortcomings to identify meat products of live stock (Yinan Zhang et al. 2020), and for such identification, enough and supporting data is not available (Kwong, S. et al. 2012).

Improvements in presented research:

Dawn E. Kane and Rosalee S. Hellberg (2015) proposed that there is need to check the sensitivity of self-designed PCR primers for amplification of meat specimens. In addition, the application of this newly emerged identification tool has reached to identification of diverse food materials such as meat (D'Amato et al., 2013). Govt. of India had launched cow protection act in the year (https://lj.maharashtra.gov.in/Site/Upload/Acts/H%2062-2016.pdf).

NCBI and BOLD:

The important aspect of using molecular investigations of plants and animals is the availability of reference sequences in DNA databases such as NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and BOLD (https://ibol.org/) (Andrea Galimberti et al., 2013). Unfortunately, ambigious data has been detected in public records (Bridges P. D., et al., 2003; Forster P., 2003; Harris, 2003; Nilsson R. H. et al., 2006, Ross and Murugan, 2006; Yao Y.G. et al., 2004). If the reference sequences would not be available in BOLD database or showed more than 1% variation, then the query sequences can be identified using GenBank (Avise 2000). For identification of received pieces of meat specimens, sequences can be queried against GenBank (NCBI) (Benson D. A. et al., 2007) with help of BLAST (Basic Local Alignment Search Tool) algorithms (Altschul S. F., et al., 1997).

Next Generation Sequencing:

Yanyi Pan et al. (2020) collected the real food products (pork ball, beef ball, shrimp ball,fish ball, Chinese sausage, sausage, modulation beefsteak) which were highly processed. 12 species were present in fresh meat samples under study including water buffalo and cattle, domestic pig and chicken, sheep, silver carp, grass carp, tile fish, blue scad, prawn, pomfret. The samples, grouped in 4 groups A, B, C and D were processed further for PCR direct sequencing, cloning sequencing, and Next Generation Sequencing with various compositions (table 2). Group A included 12 various species of food products and group mixture of 12 diverse species which were passed for PCR direct sequencing and cloning sequencing, respectively. For animal species confirmation, group A was set.

With sequencing of PCR products, separate identification of twelve raw meat specimens was performed (Yanyi Pan et al. 2020). Groups B and C were set in order to compare obtained results between clone sequencing, and NGS technology for detection of unique species in the samples that are mixed (Yanyi Pan et al. 2020). In turn, in all groups, meat species are same. In case of group B, twelve raw meat specimens were mixed as equal-weight together, and then clone sequencing was performed. In fact, group C and Group B were same in relation with preparation but Group C was studied with NGS (amplicon sequencing). Group C was same as group B but passed for NGS. Group D containing seven products which were commercial were analyzed through NGS. Yanyi Pan et al. (2020) authenticated animal species origin in seven highly processed real food products with NGS method (table 2).

Table 2: Name along with composition as well as methods of sequencing for every sample belonging to groups A, B, C, and D. (Yanyi Pan et al. 2020)

Sample Group	Sample Composition
Sequencing Method	
Group A (S1-S12)	12 different species treated separately
PCR-direct sequencing	
Group B (B1–B3)a	Sample of the 12 different species mixture
Cloning sequencing	
Group C (C1–C3)a	Same as B1–B3
Next-generation sequencing	
Group D (D1–D7)	7 commercial products

Next-generation sequencing

aB1–B3 and C1–C3 are parallels for the corresponding group.

The DNA barcoding technology can be having applications in the future meat science (JIANG Shuai et al. 2016). Recently, (Yanyi Pan et al. 2020) proposed that cytochrome oxidase I (*COI*) gene fragment can be combined with next-generation sequencing (NGS) for identification animals such as bovine in the meat products that are processed. The Next Generation Sequencing (NGS) can overcome the probability of false negative results in the process of identification of unknown component from the mixture using cloning sequence technology (Yanyi Pan et al. 2020). For identification of species, techniques of molecular fingerprinting in addition to PCR gene chip are useful (N. Z. Ballin et al. 2010; A. K. Lockley and R. G. Bardsley, 2010), which are based on DNA fragments as well as PCR techniques viz. PCR-SSCP, real time PCR, PCR RAPD applied for food authenticity (N. Haider et al. 2012, J. H. Kuo et al. 2017, H. Ozpınar et al. 2013, H. ¨ Ozpınar et al. 2013, C. Sarri et al. 2014).

Unfortunately, it is rarely used for authentication of products that contain processed meat (X. Cheng et al. 2015, F. Bertolini et al. 2015). The efforts taken by scientific community to make the food authentic is very important because it directly affects the public health (N. Z. Ballin et al 2009). Yanyi Pan et al. (2020) stated that the techniques used in order to identify the heavily processed food originated from animals is crucial part of authenticity of food even if there is need of further development. The traditional identification method based on morphology is not useful for the processed as well as cooked products of meat (Yanyi Pan et al. 2020).

With the intention of detection of animal based component in meat products, High-throughput Sequencing was used (Yinan Zhang et al. 2020). Molecular marker (partial CYTB) also works efficiently for detection of ingredients of animals present in meats which are present at less quantity as equal as 1% (Yinan Zhang et al. 2020). As well, this method is effective to identify the food specimens which are unknown from the mixed components of animals indicating its valuable application in future (Yinan Zhang et al. 2020). Additionally, traditional method of using quantitative PCR can be used for identification process (Yinan Zhang et al. 2020).

Future Perspective:

We propose that this DNA QR code scanner can be used for identification of meat species using their whole genome sequence and for meta-genomics investigations as well. We can detect even meat species of human origin as well to restrict cannibalism or human criminal cases (figure 4). We need to develop very accurate identification system of cow based on molecular markers. Currently, we can identify bovines only in general and not a cow specifically.

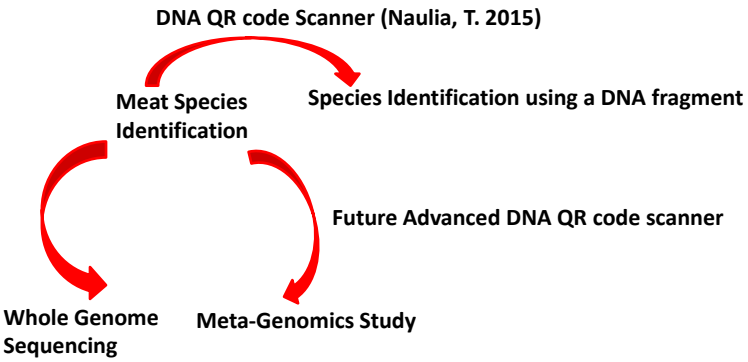

Figure 4: Future applications of DNA QR code scanner.

Conclusion:

The DNA barcoding technology has worked better for authentication of foods based on meat and assisted to restrict food frauds and adulterations. However, future work should focus on meta-genomics approach for authentication of meat and its products since investigation of multiple chromosomes in single attempt is more authentic than single gene or chromosome study. This may help restrict illegal food trades and cheats.

Author contributions:

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