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Department of Toxicology, Forensic
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Cairo University

Use of the Recent Technology in Identification and Detection of Meat Adulteration in Some Animals

Thesis presented by

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ABSTRACT

This study aimed to evaluate the performances precipitation test (a standard protocol applied in Egypt on batches or raw material and finished beef products) for identification of animal species in experimental mixture of fresh minced cattle meat with minced meat of camel, donkey, pig and dog meat with known formulations. In addition, conventional PCR procedure was utilized for detecting different animal species in experimental freshly minced meat samples. Precipitation technique was performed using specific antiserum, which was prepared previously; when the precipitate was formed, it considered a positive result. PCR technique was performed for 35 cycles. The amplified DNA fragments were run on agarose gel stained and visualized using a UV transilluminator. The present result revealed that, precipitation technique was successfully used for identification of individual meat species and detection of adulteration of cattle meat by another one species and low effective in identification of more than three species in freshly minced meat mixture. Agarose gel analysis of PCR product amplified with species specific primers showed that mitochondrial DNA fragments of cattle, camel, donkey, pig and dog meats were 271, 208, 439, 212 and 322 base pair after an amplification of 35 cycles. PCR method could detect animal meat species in freshly minced meat mixtures of two, three species and more than three species. On the other side, PCR could not identify of camel in meat mixture of four species in presence of donkey meat and in meat mixture contain five different species.

Key words: Identification - Meat - Precipitation test - PCR

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INTRODUCTION AND AIM OF THE WORK

Meat species specification needs specialized attention in the management of food quality system. It is a vital field to ensure the food safety to the consumers. The adulteration of inferior quality meat into superior quality meat is a common practice all over the world. The problems of legal or forensic cases particularly related to prevention of meat adulteration in Egypt and related laws existing worldwide can be solved by using meat species-specification techniques. Meats are very susceptible to be adulterated because it is expensive as compared to other food types. The detection and quantification of adulterants by developing consistent analytical methods are very important for the protection of wealth and health of consumers **(Manjula et al., 2009)**.

Minced meat productions remove the morphological characteristics of muscle, making it difficult to identify one type of muscle from another. For this reason meat substitution with unspecified species, usually of lower quality, is the most common form of economic adulteration in the minced meat industry, constituting a fraudulent act that could have economic and health repercussions. Meat species adulteration concerns consumers in terms of economic loss, food allergies, religious observance, and food safety. Species substitutions, such as substitution of horse meat for beef, pork and sheep meat, have been reported in several countries **(Al-Jowder et al., 2002 and Ospina et al., 2012)**.

Minced meat is still facing some unfaithful manufacturing practices and fraud in the form of adulteration with less costly cuts from different animal species. As the physical, chemical and anatomical methods are more suitable for raw meat, minced or comminuted meat requires sophisticated techniques. For some consumer groups, as Muslims, the contamination of food with meat of pig is strictly prohibited **(Sahilah et al., 2011)**. Meat adulteration can occur for a variety of reasons often linked to financial gain which may be achieved to improve the perceived quality of the products, mimic an established brand, and reduce manufacturing costs or for product extension purposes **(Ballin, 2010)**.

In order to prevent fraud of minced meat in the national and international markets, regulatory authorities and food processing companies are increasingly vigilant and require a rapid and specific analytical procedure for authentication **(Ahmed et al., 2007)**. Most of the methods used for species identification of raw meat (include sensory analysis, anatomical differences, histological differentiation of the hair that may exist in the meat, properties of tissue fat, and level of glycogen in muscle tissue, as well as electrophoresis and DNA hybridization) have limitations in its use. This is due to problems in specificity (sensory analysis, glycogen level, histological differentiation, properties of tissue fat, and immunological methods), complexity (electrophoresis and DNA hybridization), high cost (DNA hybridization), and some requirements for baseline data about the differences in protein compositions **(Singh and Sachan, 2011; Ali et al., 2015 and Andrea et al., 2015)**. In the recent past, DNA molecules have been used as target compounds for species identification due to their high stability and unique variability, which allow the differentiation of closely related species. Among DNA based methods, Polymerase Chain Reaction (PCR) is an effective technique that is highly accurate and relatively fast. The conventional PCR method has a satisfactory performance in the qualitative detection of meat species that are undesirable by consumers for health (allergic reactions) reasons, ethnic, or religious values **(Kesmen et al., 2007 and Chandrika et al., 2009)**.

Therefore, the aim of this study was to utilize conventional PCR procedure for detecting different meat species when they are present in the same experimental mixture of fresh minced beef with known formulations. In addition, considerable interest would be directed for the evaluation of the performances of the precipitation test (a standard protocol of testing in Egypt on batches or raw material and finished beef products) for detection of animal species in minced meat.

LITERATURE REVIEW

Meat Adulteration

Meat adulteration is one of the most important problems in Egypt especially with the low income to make meat products of lower prices. The identification of meat species is performed for a variety of reasons as ethnic and economic. Mixing of different meat species followed by its grinding and/or heat processing aids to the difficulties of discrimination of the meat origin, which limits its detection by many analytical techniques. Hence, it is an important task for food control laboratories to be able to carry out species differentiation of raw materials to be used for industrial food preparation and the detection of animal species in food products.

The variety and quality of meat and its delicacy is dependent on the meat type. The variation in the value of meat of various species is also dependant on local choice of the consumers and on nutritional status of the meat. Therefore, to earn more money from the meat business various types of adulterations are very common. As the world population rises, the demand for meat products continues to escalate in almost all regions of the globe, especially in developing countries. Meat is, however, one of the most highly priced food commodities in mostly all countries **(Hargin, 1996 and Delgado, 2003)**, other aspects includes quality and nutritional attributes. Modern consumers are increasingly aware of their health and are demanding more comprehensive information on the origin, composition and safety of the foods they consume **(Verbeke and Ward, 2006)**. Meat has not been widely associated with adulteration since this has most often been marketed as fresh, easily recognizable joints. However, with the escalating prices of commercial meat commodities, the globalization of food trade and the increased processing of meat into value-added products, the incidence of meat adulteration and fraud has become more commonplace **(Flores-Munguia et al., 2000 and Vandendriessche, 2008)**.

Meat adulteration is a legal term meaning that a food product fails to meet federal or state standards. Adulteration is addition of a non-food item to increase the weight/quantity of the food item in raw form or prepared form, which may result in the loss of actual quality of food item. Among meat and its products one of the items used to adulterate are water, dead carcasses, and carcasses of animals other than the animal meant to be consumed. Adulteration can occur for a variety of reasons, often linked to financial gain. Increases in profitability may be achieved by adulteration to improve the perceived quality of products, mimic an established brand, and reduce manufacturing costs or for product extension purposes (**Khadijah et al., 2012**).

Adulteration of high-priced meat with cheaper meat is one of the most common examples of fraudulence prevalent in meat industry without any consideration of economic, religious or health implications (**Grundy et al., 2012**). Typical cases of intentional meat adulteration involve the substitution or addition of animal or plant proteins, which are cheaper proteins, such as soyabean or grain derivatives not declared as such in the ingredient list (**Flores-Munguia et al., 2000**). The identity of the ingredients in processed or composite mixtures is not always readily apparent (**Aida et al., 2005**). Besides substitution of one species with another, fraudulent substitution of tissue with collagen and offal might also be profitable. For simplicity, authentication problems with respect to meat and meat products could be grouped into four major categories: meat origin, meat substitution, meat processing treatment and non-meat ingredient addition (**Ballin, 2010**).

Camel meat is a good source of meat in areas of climate adversely affects other animals. Meat tastes depend on the sex, age, feeding condition and health condition of the animal and has similar taste of beef. In Egypt, camel meat is environmentally adaptable alternative source of meat and mainly consumed by lower income groups. Camel meat is limited in its acceptance because of the

extreme toughness of meat at the economic age due to the extremely heat stable collagen and elastic connective tissues **(Kurtu, 2004)**.

Adulterations of pig meat in food or processed food are possible due to substitution of high quality meat to cheaper materials. The potential uses of pig meats are possible as replacement of beef, chicken and goat meat, due to its cheap price. Besides the tight enforcement from local authority for Halal certification applications, which complied with Halal standards and integrity, scientific evidence against fraud is vital in supporting the Halal authentication **(Nakyinsige et al., 2012)**.

Analytical techniques, which are appropriate and specific, have been developed to deal with adulteration. The most suitable technique for any particular sample is often determined by the nature of the sample itself to identify what makes meat and meat products Halal **(Sahilah et al., 2011)**.

Minced meat is still facing unfaithful manufacturing practices and fraud in the form of adulteration with less costly cuts from the same or different animal species, mechanically recovered meat, offal, blood, eggs, gluten, water or other cheaper proteins of animal or vegetable origin. The food industry is increasingly directing its efforts to produce and commercialize functional foods where the reduction or even elimination of some undesirable and unhealthy components as saturated fat is an important goal **(Ospina et al., 2012)**.

Regional and traditional meat products should be characterized by stable quality and sensory values and this can only be ensured when the same raw material and the same technology are applied. The steadily growing demand for regional and traditional products as well as higher prices consumers are ready to pay for them may give rise to temptation to counterfeit this kind of food article **(Colombo et al., 2002 and Zin, 2005)**

Horse meat is not harmful to health and is eaten in many countries; it is considered a taboo food in many countries, including the UK and Ireland. The

analysis stated that 23 out of 27 samples of beef burgers also contained pig DNA; pork is a taboo food to the Muslim and Jewish communities.

Methods Detecting Meat Adulteration

Meat species specification is an area, which needs specialized food quality management system. It is a vital field to ensure the food safety to the consumers and it conserves the laws related to meat and meat products. The adulteration of inferior quality meat into superior quality meat is a common practice all over the world (**Singh and Sachan, 2011**). In order to protect consumer interests and to combat the continuing problems of food fraud and adulteration scientific expertise and technologies are constantly being developed and advanced to test the authenticity of foods and feeds. In order to prevent food fraud in the national and international markets, regulatory authorities and food processing companies are increasingly vigilant and require a rapid and specific analytical procedure for authentication. Although authenticity testing and analytical techniques have improved immeasurably over years and nowadays a wide variety of techniques and methods are available, each of these techniques is appropriate and specific to deal with a particular adulteration problem. In general, methods for these purposes need to be specific, sensitive, rapid, economic and able to analyze various meat products and to provide quantitative results (**Meza-Márquez et al., 2010**).

The ability to detect less desirable or objectionable species in meat products is important not only for economic, health, religious and ethical reasons, but also to ensure fair trade and compliance with legislation (**Ballin et al., 2009; Nakyinsige et al., 2012 and Spink and Moyer, 2011**). Several techniques for detection of meat species in adulterated meat have been started from simple physical tests to recent sophisticated molecular techniques. Methods used for identification of species of origin of raw meat include sensory analysis, anatomical differences, histological differentiation of the hair that may possibly exist in the meat, properties of tissue fat, and level of glycogen in