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Advanced Studies to Detect Commercial Adulteration in Meat Products at Ismailia Markets

Thesis

Presented by

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About the Thesis

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Abstract

Nowadays, the fraudulent replacement or adulteration of high quality components with their inferior or cheaper alternatives becomes a common practice prevalent in meat industry. Accordingly, a total of 120 commercial beef and chicken meat product samples (20 each of hawawshi, fresh oriental beef sausage, beef luncheon, beef burger, chicken luncheon and chicken burger) were collected from Ismailia city to be subjected to proximate analysis, then compared with the Egyptian standards to determine their acceptability. Additionally, the significant differences of chemical parameters between the examined meat products were determined. Additionally, 60 samples (10 each) of the previously examined samples were analyzed by the conventional Polymerase chain reaction (PCR) technique for the detection of undeclared meat species. The results of hawawshi samples revealed that 80%, 60% and 10% were adulterated with chicken, sheep and equine species, respectively. In addition, 20% considered adulterated as they did not contain beef meat in spite of their selling as beef products. While for sausage samples, there were 20% adulterated due to absence of beef meat, in addition to 80%, 50% and 10% were adulterated with sheep, chicken and equine species, respectively. Additionally for beef luncheon samples, there were 70%, 30%, 20% and 10% adulterated with chicken, sheep, dog and equine species, respectively. While for beef burger samples, the results showed that 100%, 50% and 30% were adulterated with chicken, sheep and equine species, respectively. However for chicken luncheon samples, there were 60%, 20% and10% adulterated with beef, dog and absence of chicken species, respectively. Moreover for chicken burger samples, 40%, 40%, 20% and 10% were adulterated with beef, sheep, absence of chicken and dog species, respectively. from the results, it was obvious that 90%, 90%, 90%, 100%, 70% and 60% of hawawshi, sausage, beef luncheon, beef burger, chicken luncheon and chicken burger samples, respectively were adulterated by undeclared species with a total percentage of 83.3%. In addition, the present study showed the most adulterating species in the examined beef and chicken meat products. Concerning beef meat products, the major adulterating species were chicken (75%), followed by sheep (55%), equine (15%) then dog (5%). Likewise, for chicken meat products, the major adulterating species were beef (50%), followed by sheep (20%) then dog (15%). additionally, the most adulterating species in beef meat products, were chicken (75%), followed by sheep (55%), equine (15%) then dog (5%). Likewise, for chicken meat products, the major adulterating species were beef (50%), followed by sheep (20%) then dog (15%). Additionally, 8samples (2 each) of hawawshi, sausage, beef burger and chicken burger, which previously proved their adulteration with chicken and equine species, were retested with the rapid onsite Meat FlowThrough^{TM} test. In consistency with results, these rapid tests were robust and fast in their ability to detect meat species adulteration within few minutes in the varied meat products, in addition to their highly field portability that all the materials required to conduct them can be readily packaged as a kit.

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Introduction

Adulteration is a serious meat safety and quality issue which becomes the focus of attention for the food industry and consumers in the last few decades **(Ahmed *et al.*, 2016)**. The high price of meat and passiveness of consumer safety warranty further encourage the sellers to substitute components with other replacers in the manufacturing of meat products **(Roostita *et al.*, 2014)**. The meat products adulteration can take many forms such as complete or partial omission or substitution of valuable constituents with undeclared alternatives to increase product bulk or weight or to make the product appears of better value than it is **(Hargin, 1996)**.

Nowadays, due to the high varieties, longer shelf-life, relatively cheaper prices and great changes in life styles, consumption of the different meat products has become very popular **(Lakzadeh *et al.*, 2016)**. Hawawshi, sausages, luncheons and burgers are from the most popular consumed meat products in local markets. They are ready to eat and ready to cook products with very simple preparation, which makes them available for consumers to be used as a quick meal. The main component of these products is meat, usually from beef or chicken with combination of vegetables and spices **(Lukman *et al.*, 2009)**. These fast food products are preferred by lots of people either old or young because of several advantages, although may be false but attractive as that they are delicious, filling, affordable and readily available **(El Shobaki *et al.*, 2014)**. Some of them are industrial and others are handmade which are commonly sold in fast food restaurants, supermarkets or street vendors and no considerable action has been applied to control their safety **(Hajimohammadi *et al.*, 2014)**.

These meat products are generally prepared from ground meat as a raw material, as well as other various non-meat ingredients or additives from

different origins and suppliers which are combined at the formulation stage according to the criteria of composition, sensory characteristics, legal regulations, functionality and also production cost **(Jiménez-Colmenero *et al.*, 2010)**. Therefore, the use of low quality ingredients in their processing yields low quality meat products **(Edris *et al.*, 2012)**. Increases in profitability may be achieved by adulteration to enhance the perceived quality of products, reduce manufacturing costs or for product extension purposes **(Ahmed *et al.*, 2016)**. The detailed information on the chemical and nutritional contents is essential for consumers in choosing meat products **(Erwanto *et al.*, 2012)**.

On another side, the meat used in meat products is exposed to severe morphological changes due to grinding operations which increase the possibility of fraudulent activities by some producers due to the complexity of detection **(Mehdizadeh *et al.*, 2014)**. Among the most common fraudulent practices is the insertion of lower quality animal tissues in meat products based on rapid urbanization and industrialization which has increased the chances of meat species adulteration with meat of inferior and taboo species in meat products **(Mane *et al.*, 2006)**.

The widespread of species adulteration in retail markets may be attributed to the inadequate meat inspection and the lack of suitable and affordable analytical methods. However, the ability to detect less desirable or objectionable species in meat products is important for people whose religious practices limit the types of meat they eat as Muslim communities who are particularly concerned about the meat they eat and the accurate labeling which is critical to detect the species that are considered not permissible to eat **(Farouk, 2013)**. Thus, identifying the species of meat in the finished meat products is the main target to fulfil Halal requirement and Islamic regulations **(Hamzah *et al.*, 2014)**.

Additionally, the trending to halal product items has been expanding somewhat even among the non-Muslim consumers because of their perceived

quality attributes and significantly reduced risk to be a carrier of zoonotic diseases **(Gregory, 2008)**. In other cases, misleading labels may be harmful for individuals who have food allergies and the consumption of some types of meat may create health concerns **(Wang *et al.*, 2004)**. Additionally, adulteration detection is also important to avoid economic loss, ensure fair trade and compliance with legislation **(Spink and Moyer, 2011)**.

Hence, by regard to the above concerns, consumers have the right to be sure that the information declared about meat products is correct, particularly at a time when they are increasingly expressing the desire to make products choices that are consistent with their lifestyles and well-being **(Cawthorn *et al.*, 2013)**. Therefore, the high demands for more transparency in meat industry have led to perform strategies to identify meat species in meat products, and can be routinely monitored by food authorities. In the past, there were insufficient methods for effective detection of these kinds of adulteration include sensory analysis, anatomical differences, histological differentiation of the hair that may possibly exist in the meat, properties of tissue fat, level of glycogen in muscle tissue, as well as electrophoresis and deoxyribonucleic acid (DNA) hybridization **(Brodmann and Moor, 2003)**.

Most of these methods have been reported to have limitations in use due to problems in specificity, complexity, high cost or some requirements for baseline data about the differences in protein compositions. Therefore, there is a need for the development of a more accurate, fast and easy-to-use methods due to the limitations of the existing methods mentioned above **(Matsunaga *et al.*, 1999)**.

Nowadays, the molecular methods have become an everyday tool to resolve a series of problems and questions in the field of species identification, fraud and traceability. Therefore, these methods can be used for the identification of meat products species in order to ensure human safety and

religious issue **(Rezazadeh *et al.*, 2014)**. For this purpose, a range of analytical techniques have recently been developed based on detecting protein or DNA molecules **(Lakzadeh *et al.*, 2016)**.

Currently, the DNA-based methods such as the Polymerase Chain Reaction (PCR) technique have becoming the methods of choice for meat production control which have extensively been applied for the detection of animal species in a wide range of meat products **(Verkaar *et al.*, 2002)**. PCR is an appropriate, sensitive, specific and rapid method for suitable identification of animals' DNA **(Fajardo *et al.*, 2010)**. While among the protein based methods, immunoassay is the most widely used with several companies supplying kits for a range of species **(Chisholm *et al.*, 2005)**.

From another point of view, although the previous methods are the most specific and sensitive methods for meat species identification, they require some expensive laboratory equipments and a certain degree of knowledge and experience **(Macedo-Silva *et al.*, 2000)**. The adoption of these methods by the food control laboratories is limited by factors such as cost and complexity **(Lockley and Bardsley, 2000)**.

Thus, a useful strategy for compliance monitoring can comprise a system of screening then confirmation analysis. A rapid, onsite, low cost field test can be used to identify presumptive positives and only these positive samples will be confirmed using the lab based confirmatory methods. This screening system needed to be broadly reactive to a wide range of species, demonstrate good sensitivity, low cost and easy use in the field. Conceptually, quality control applications by using multiple species specific tests may be incorporated into a rapid visual test format that can be used by state regulators to verify compliance during routine inspections **(Muldoon *et al.*, 2004)**. Recently, commercial kits of these onsite rapid tests are available for the determination of animal species and

they give sensitive and reliable results, but their performances are still not widely used **(Giovannacci *et al.*, 2004)**.

By keeping the above facts in view, the present study aimed to evaluate the nutritional aspects of some commercial meat products and ensure their compliance with the Egyptian Standards as well as detection the extent of meat products adulteration by undeclared species prevailed on the local markets, the most commonly implicated commodities and the most substituted species, by using simple, sensitive and applicable methods for authentication.

Therefore, the objectives of this study were carried out by fulfill the following:

I. Proximate Analysis of Some Commercial Meat Products:

1. Determination of moisture content.
2. Determination of protein content.
3. Determination of fat content.
4. Determination of total ash.
5. Calculation of total carbohydrate content.
6. Calculation of red meat content.

II. Detection of Meat Species Adulteration by Application of Conventional Polymerase Chain Reaction (cPCR) Technique: It was used for the detection of meat species adulteration of some meat products sold in commercial markets.

III. On-Site Detection of Meat Species Adulteration by Application of Raw Meat FlowThrough™ Test: It was used for onsite qualitative determination of meat species in meat products by visual inspection. It was a simple portable test required no additional equipments, and quick as typically took 12 minutes to be performed.

Review of Literature

In recent decades, meat products have become a trade objects and possess high commercial value that lead to the increase of the international meat trade and the consumer demands, but at the same time, attempts to adulterate meat products have become widely prevalent **(Siklenka *et al.*, 2004)**. Adulteration is a legal term meaning noncompliance of a product with the declared information **(Mehdizadeh *et al.*, 2014)**. As the identity of the ingredients in processed or composite mixtures of meat products is not always readily apparent, so the verification that the components are authentic and from sources acceptable to the consumers is required **(Lockley and Bardsley, 2000)**. Meat products adulteration constitutes economic fraud, and raises ethical, religious and food safety concerns. Thus, from the major issues of the meat industry today are the production of high quality products, determination of authenticity and detection of adulteration **(Ayaz *et al.*, 2006)**.

Nutritive Quality of Meat Products:

The first consumer right is getting a meat product of good nutritive quality. Meat products composition assessment is becoming a very important issue in order to avoid unfair competition among producers and allow consumers having products with accurate composition **(Soares *et al.*, 2010)**. The chemical analysis reflects the quality of nutritive values of meat products which are important factors for consumer health and acceptability **(Ahmad *et al.*, 2015)**.

Babji *et al.* (2000) evaluated local and franchise beef and chicken burgers for their proximate compositions. The results revealed that the mean values of local beef burgers were 13.27%, 19.97%, 49.89%, 14.25%, 2.16% and 66.18% for protein, fat, moisture, carbohydrate, ash and meat contents, respectively and

the mean values of franchise beef burgers were 20.03%, 18.23%, 58.31%, 1.31%, 1.48% and 62.08% for protein, fat, moisture, carbohydrate, ash and meat contents, respectively. While, the mean values of local chicken burgers were 14.26%, 18.65%, 55.68%, 7.89%, 1.83% and 65.52% for protein, fat, moisture, carbohydrate, ash and meat contents, respectively, and the mean values of franchise chicken burgers were 20.47%, 6.75%, 67.42%, 3.48%, 1.51% and 71% for protein, fat, moisture, carbohydrate, ash and meat contents, respectively.

Hamed (2001) examined 30 samples each of luncheon and fresh sausage from different supermarkets in Giza and Cairo to determine their nutritive values and the results revealed that the mean values of luncheon samples for moisture, protein, fat and ash contents were 57.91%, 12.61%, 18.68% and 3.56%, respectively. While, the mean values of sausage samples for moisture, protein, fat and ash contents were 56.50%, 13.89%, 18.66% and 3.53%, respectively.

Nouman *et al.* (2001a) reported that the mean values of moisture, protein, fat and ash contents of beef sausage samples were 46.5%, 16.1%, 20% and 4.6%, respectively.

Nouman *et al.* (2001b) analyzed beef luncheon samples and found that the mean values of moisture, protein, fat and ash contents were 52.2%, 13.3%, 17.5% and 3.9%, respectively.

Abd El-Aziz (2002) estimated that the percentages of the moisture, protein and fat contents of beef burger samples were 58%, 13% and 14.55%, respectively. While, the percentages of the moisture, protein and fat contents of beef sausage samples were 52%, 13.3% and 29%, respectively.

Doğu *et al.* (2002) examined 30 sausage samples from five meat plants with high capacity to determine their chemical compositions. The results revealed that the moisture content of the samples ranged from 42.26% to 53.68% with an average 47.58%, the protein contents ranged from 15.24% to 20.13% with an average 17.16%, while the fat content ranged from 23.33% to 32.00% with an average 27.66%.

Mohammed (2002) found that the moisture contents in beef burger and beef sausage samples were 62.7% and 59.9%, respectively. While, the protein contents were 11.6% in each. Moreover, the fat contents were 22.4% and 25.8%, respectively.

Abd El-Aziz (2004) collected 50 samples each of beef burger and beef luncheon from different supermarkets and restaurants in Assiut Governorate for their chemical examination. The results showed that the mean values of fat% in beef burger and beef luncheon were 18% and 19%, respectively.

Ambrosiadis *et al.* (2004) performed chemical analysis on 67 samples of Greek traditional sausages and found that the moisture, protein, fat and ash contents were 49.17%±7.05%, 17.62%±2.67%, 29.74%±8.02% and 2.99%±0.55%, respectively.

Hassan and Yehia (2004) collected 90 random samples of locally manufactured beef burger and sausage from three different processing plants to determine their chemical criteria. The results revealed that the mean values of moisture content of beef burger samples of plants A, B and C were 59.1± 1.0%, 63.8± 1.2% and 66.1± 1.07%, respectively, while for sausage samples were 57.5±1.0%, 62.7±1.3% and 64.6± 1.2%, respectively. Additionally, the mean values of protein content of beef burger samples of plants A, B and C were 14.8± 0.7%, 12.1± 0.9% and 10.3± 0.6%, respectively, while for sausage

samples were 15.3±0.9%, 10.4±0.8% and 9.0± 0.6%, respectively. Moreover, the mean values of fat content of beef burger samples of plants A, B and C were 20.6± 0.7%, 21.3± 1.1% and 22.0± 0.9%, respectively, while for sausage samples were 21.6%, 21.5% and 22.4%, respectively. Additionally, the results showed that 20%, 60% and 86.6% of beef burger samples produced by plants A, B and C were disagreed with chemical profiles stipulated by Egyptian standards, while 6.7%, 33.3% and 73.3% of sausage samples of the three plants were disagreed as a result of low protein.

Kamkar *et al.* (2005) analyzed 68 random samples of sausages with different meat contents ranged from 40% to 90% produced by different factories to determine their nutritive values. The results showed that the mean values of moisture, protein, fat and ash contents in sausages with meat content from 40% to 50% were 54.95± 4.16%, 12.76±2.38%, 20.66± 2.24%, and 2.93± 0.6%, respectively. While, the mean values of moisture, protein, fat and ash contents in sausages with meat content from 51% to 60% were 57.32±3.15%, 12.68±1.88%, 19.62±3.64%, and 2.98±0.12%, respectively. Additionally, the mean values of moisture, protein, fat and ash contents in sausages with meat content from 61% to 90% were 58.52±5.62%, 13.99±1.99%, 17.93±5.71% and 2.85±0.26%, respectively.

Maha and Sohad (2005) collected some luncheon and sausage samples from Giza supermarkets for their chemical examination. The results revealed that the mean values of moisture content were 46.7% and 55.6% for luncheon and sausage samples, respectively.

El-Sayed (2006) examined 100 beef burger samples collected from different factories A, B, C, D in the Egyptian markets for their chemical evaluation and compared the results with the Egyptian Standards. The results

showed that the mean values of moisture% in the examined samples related to factories A, B, C and D were 59.22%, 59.21%, 28.67% and 61.67%, respectively and the percentages of non accepted samples based on moisture % were 12%, 40%, 28% and 60%, respectively. While, the mean values of protein % in the examined samples related to factories A, B, C and D were 15.16%, 14.56%, 14.06% and 12.73%, respectively and the percentages of non accepted samples based on protein % were 40%, 68%, 84% and 100%, respectively. Moreover, the mean values of fat % in the examined samples related to factories A, B, C and D were 18.09%, 17.85%, 19.93% and 22.78%, respectively and the percentages of non accepted samples based on fat % were 12% and 88% related to factories C and D, respectively. Additionally, the mean values of ash % in the examined samples related to factories A, B, C and D were 2.65%, 2.96%, 2.76% and 2.63%, respectively.

El-Tahan *et al.* (2006) determined the chemical properties of some chicken products purchased from local markets in Cairo City and showed that the protein percentage ranged from 15.2% to 15.6% in burger samples, 11.3% to 14% in luncheon samples and 13.5% to 14.96% in sausage samples. While, the fat percentage ranged from 13.61 % to 15.37 % in burger samples, 4.33 % to 5.42% in luncheon samples and 7.18% to 9.81 % in sausage samples.

Fath El-Bab *et al.* (2006) performed chemical analysis to 80 frozen sausage samples collected randomly from different markets in Giza province. The results revealed that the mean values of moisture, protein and fat contents were 61.74±0.23%, 16.42±0.80% and 15.79±0.81%, respectively.

Alina and Ovidiu (2007) examined 14 sausage samples for the determination of the quantity of total proteins in them and the results represented that the average of protein was 14.11 ± 1.4%.

Dharmaveer *et al.* (2007) evaluated sausage samples for their proximate compositions and found that the overall mean of moisture, fat, protein and ash contents were 55.48%, 17.05%, 18.36% and 3.00%, respectively.

Prayson *et al.* (2008) evaluated eight different brands of beef burgers for their moisture and meat contents and found that the moisture content ranged from 37.7% to 62.4% with mean value 49%, while the meat content ranged from 2.1% to 14.8% with mean value 12.1%.

Quasem *et al.* (2009) described the proximate composition of some types of sausages and revealed that the percentage of carbohydrate, fat, protein, ash and moisture contents of the samples were 4.3%, 16.7%, 12.75%, 2.27% and 63.94%, respectively.

Schmid *et al.* (2009) determined the proximate composition of various commercially available sausages and the results showed that the sausages contained a range of 58% to 65% moisture, 11% to 16 % protein, 16% to 23% fat and 0.5% to 1.4% carbohydrate.

Al-Dughaym and Altabari (2010) analyzed the chemical composition of marketed chicken burgers from two different factories to assure their quality in the aspects of nutritive value and found that the mean values of moisture, protein, fat, and ash contents were 66.01%, 16.82%, 8.26%, and 2.05%, respectively from one factory, and 68.60%, 15.07%, 11.13%, and 2.10%, respectively from the other factory.

Ali (2011) investigated the quality parameters of fifty commercial beef burger samples collected from different supermarkets in Giza and Cairo governorates and found that the mean values were 66.12%, 8.80% and 20.45%

for moisture, protein and fat contents, respectively. As regard to the results, the protein content was low while the moisture and fat contents were high.

Ramadhan *et al.* (2011) analyzed ten selected brands of commercial chicken burgers for their proximate compositions and the results showed that the samples contained a range of 46.72% to 69.37% moisture, 11.08% to 18.77% proteins, 9.08% to 20.54% fat, 1.50% to 2.96% ash, 2.56% to 21.27% carbohydrates and 59.34% to 95.91% meat contents.

Al-Bahouh *et al.* (2012) evaluated the quality of 10 different chicken burger brands (2 local and 8 imported) in different co-operatives, fast food restaurants and retail suppliers located in the six governorates in the State of Kuwait. The results revealed that the overall moisture, fat, protein, carbohydrate, ash and meat contents in local chicken burgers were 66.99%, 4.84%, 18.5%, 7.75%, 2.17% and 71.84%, respectively. While, the overall moisture, fat, protein, carbohydrate, ash and meat contents in imported chicken burgers were 62.11%, 10.81%, 14%, 10.83%, 2.23% and 57.66%, respectively.

Edris *et al.* (2012) examined 50 samples of beef meat products and found that the mean values of moisture content of beef burger and beef luncheon were 61.28±0.17% and 58.76± 0.14%, respectively. While, the mean values of protein contents were 15.22±0.18% and 10.03±0.12%, respectively. Additionally, the mean values of fat contents were 19.80±0.19% and 19.25±0.21%, respectively and the mean values of ash content were 3.36±0.07% and 4.29±0.10%, respectively. As regard to results, the misbranded samples of beef burger and beef luncheon according to protein content were 16% and 44%, respectively and according to fat content were 24% and 48%, respectively.

González-Tenorio *et al.* (2012) examined twenty traditional sausage samples for their chemical evaluation and found that the mean values of the studied samples were 42.8±11.4%, 33.4±12.0%, 18.2±3.8%, 2.9±0.8% and 2.7±1.8% for moisture, fat, protein, ash and carbohydrate contents, respectively.

Iordan *et al.* (2012) analyzed different types of sausages to determine their chemical characteristics and found that the moisture content ranged from 33.5% to 46.8%, the fat content ranged from 21.77% to 37.8% and the protein content ranged from 16.23% to 22.8%.

Nada (2012) determined the chemical profile of some beef meat products represented by 25 each of luncheon, burger and sausage collected from supermarkets in El- Menoufia governorate. The results revealed that the averages of moisture content in beef luncheon, beef burger and beef sausage were 58.76±0.14%, 61.28±0.17% and 62.98±0.19%, respectively. Additionally, the averages of protein contents were 10.03±0.12%, 15.22±0.18% and 10.37±0.20%, respectively. Moreover, the averages of fat content were 19.25± 0.21%, 19.80±0.19% and 24.61± 0.26%, respectively. While, the averages of ash content were 4.29± 0.10%, 3.36± 0.07% and 3.08± 0.07%, respectively. In addition, the misbranded samples based on protein were 44%, 16% and 28% in beef luncheon, beef burger and beef sausage, respectively, while the misbranded samples based on fat were 48%, 24% and 36%, respectively.

Ahmed *et al.* (2013) evaluated the chemical compositions of beef sausage samples collected from three locations of Khartoum state and found that the moisture contents of samples from Khartoum, Khartoum north and Omdurman markets were 68%, 62% and 60%, respectively. While, the fat contents were 4.5%, 7.4% and 11.9%, respectively. Moreover, the protein contents were 15%,

17.4% and 19.9%, respectively. In addition, the ash contents were 1.08%, 0.98% and 0.87%, respectively.

Carmen *et al.* (2013) analyzed chicken burger samples for moisture, fat and protein contents in order to assess their nutritive compositions. The results showed that the moisture contents ranged from 66.40% to 67.51%, fat contents ranged from 6.88% to 8.65%, while the protein contents ranged from 19.39% to 21.39%.

El Zahaby (2013) analyzed beef meat product samples represented by 25 each of burger, luncheon and sausage collected from different supermarkets located in EL-Kalyobia Governorate to evaluate their chemical profile and their acceptability to the Egyptian standards. The obtained results showed that the mean values of protein content in burger, luncheon and sausage samples were 10.54%, 10.65% and 10.08%, respectively. Moreover, all the samples were unaccepted based on protein % when compared with the Egyptian standards. While, the mean values of fat contents were 24.46%, 10.91% and 25.13%, respectively and all the samples of beef burger and sausage were unaccepted, while all luncheon samples were accepted based on fat content.

El Shobaki *et al.* (2014) determined the nutritive values of some hawawshi samples purchased from the Egyptian markets and the results showed that the nutrient compositions of hawawshi were 28.8%, 25.2%, 2.9% and 38% for protein, fat, ash and carbohydrate contents, respectively.

Ahmad *et al*. (2015) collected 50 frozen chicken burger samples from commercial markets of Egypt (25 samples) and Saudi Arabian (25 samples) for their chemical evaluation. The results revealed that the mean values of fat, moisture and protein contents in the samples of Egyptian markets were 16.0%, 67.0% and 15%, respectively. While the mean values of fat, moisture and

protein contents in the samples of Saudi markets were 4.6%, 81% and 17%, respectively.

Cunningham *et al*. (2015) analyzed 41 samples of fresh beef sausages purchased from Australia for their chemical evaluation. The results revealed that the fat content was14.9±4.0 %, the protein content was 14.0±1.5%, while the moisture content was 62.5±3.9 %.

Talib (2015) evaluated the chemical values of some beef sausage, beef burger and beef luncheon samples sold in supermarkets in Mansoura city and the obtained results showed that the moisture content for beef burger samples ranged from 56.58% to 59.9%, while for beef luncheon samples was 58.0% and for beef sausage samples ranged from 53.62 to 56.75%.

Alamin (2016) evaluated the chemical composition of different types of sausages and found that the moisture, protein, fat and ash contents of beef sausages were 70.32%, 18.53%, 3.45% and 1.33%, respectively.

Hussain *et al*. (2016) evaluated 30 samples of five different poultry meat products for assessment of their quality parameters. By regarding the results of the chicken burger samples, it was evident that the moisture content had a minimum value of 66.76%, a maximum value of 69.05%, with an average 67.95%, while the protein content ranged from 13.32% to 16.36% with an average 14.97%. Moreover, the fat content ranged from 9.73% to 12.00% with an average 10.64%. Finally, the ash content ranged from 2.13% to 2.37% with an average 2.28%.

Mohamed *et al.* (2016) examined 40 beef luncheon samples produced by eight different meat processing plants (five samples each) from different

production lots and the results revealed that the ash content ranged from 2.9% to 3.7% with mean value of 3.4%.

Oluwaseun (2017) evaluated the proximate composition of some beef burger and sausage samples sourced from united trading company in Lagos. The results revealed that the moisture contents of beef burger and beef sausage samples were 55.21% and 60.14%, respectively. While, the protein contents were 6.38% and 6.91%, respectively. Moreover, the fat contents were 2.23% and 1.93%, respectively. Finally, the ash contents were 1.06% and 1.37%, respectively.

Yagoup *et al.* (2017) determined the chemical composition of Sudanese beef burgers produced in different processing plants designed as A, B and C. The results showed that the moisture contents for burger samples from processing plants A, B and C were 60.68±0.59%, 62.20±0.87% and 61.23±0.90%, respectively. While, the protein contents were 21.81±0.54%, 20.81±0.62% and 21.26±0.86%, respectively. Moreover, the fat contents were 7.85±0.07%, 6.72±0.04% and 7.79±0.26%, respectively. Finally, the ash contents were 2.05±0.03%, 1.89 ±0.06% and 2.37±0.44%, respectively.

Meat Species Adulteration of Meat Products:

One of the main meat quality-related issues is the authentication of animal species in meat products, as meat from highly valuable species may be substituted, partially or totally, by similar but cheaper or banned ones **(Di Pinto *et al.*, 2015)**. Thus, detection of species adulteration has become a challenging area in the meat science **(Myers *et al.*, 2010)**. Meat species specification needs specialized attention in the system of meat quality management. It is a vital field to ensure the meat product safety for the consumers and conserve the laws related to meat and meat products **(Ahmed *et al.*, 2016)**. The possibility of

substitutions of animal species from one to another in meat products leads laboratories to apply reliable and specific methods for identifying these species. Some testing characteristics like becoming fast, accurate, sensitive, selective and user friendly are commonly requested for acceptance of a new analytical method **(Ilhak and Arslan, 2007)**. Most of the successful analytical methods utilized for meat authentication have relied on the detection of species-specific proteins or DNA **(Ballin *et al.*, 2009)**.

1. DNA based techniques:

Recent developments in meat products have remarkably changed the food matrix lowering the reliability of analytical methods based on sensorial, anatomical, morphological and histological differences in detection of adulteration **(Weiss *et al.*, 2010)**. DNA identification methods generally give better resolution and confirmatory identification than the traditional morphological methods and are the most useful tools for determining animal species in commercial meat products. Nowadays, DNA based molecular techniques are preferred because they offer the greatest potential as they are stable and not tissue dependent **(Ahmed *et al.*, 2016)**. In addition to, the identification based on DNA can provide the possibility of distinguishing meat species of closely related animals **(Rezazadeh *et al.*, 2014)**.

- **Mitochondrial Genome:**

PCR techniques based on conserved mitochondrial DNA (mtDNA) primers have been developed for species identification in meats as rapid and relatively inexpensive methods **(Girish *et al.*, 2005)**. The cytochrome b gene, localized on the mitochondrial genome, has been determined as a powerful marker for identifying species with DNA analytical techniques **(Abdulmawjood and Bülte, 2002)**.

Jorde *et al.* (1998) reported that species identification and authentication based on DNA analysis is more sensitive and reliable, since it is independent on the tissues being compared. In particular, due to the high mutation rate of mitochondrial DNA (mtDNA), 10 times greater than nuclear DNA, aimed mutations accumulate very quickly allowing the discrimination of closely related species.

Montiel-Sosa *et al.* (2000) stated that the identification of species using mtDNA for PCR assays offers a series of advantages as that: the mt-DNA genes are present in thousands of copies per cell, the large variability of mt-DNA allows reliable identification of precise species in mixtures, the target compound for species identification is a DNA molecule which has higher stability when compared to protein molecule, in addition to their presence in most biological tissues.

Partis *et al.* (2000) mentioned that the use of mitochondrial DNA for the detection of species in processed meat is based on the high copy number of small, circular mitochondrial DNA in the cells. Additionally, the chances for their survival under different processing conditions are higher, making it ideal for processed meat species identification.

Girish *et al*. (2005) reported that the variable regions of the mitochondrial gene have thousands of copies per cell along with very small size of mitochondria. These peculiarities ensure a sufficiently high quantity of PCR products and increase the probability of achieving a positive result even in the severely fragmented DNA resulted from intense processing conditions or when small amounts of fresh or processed tissues are included.

Ballin *et al*. (2009) stated that the mitochondrial DNA is used now for the purpose of meat species identification as it has a higher thermal stability,

presents in the majority of cells and potentially enables identical information to be obtained from the same animal, regardless to the origin of the tissue.

Ballin (2010) mentioned that DNA exists and is identical in almost all cells, and the unique variability and diversity afforded by the genetic code permits the discrimination of even closely-related species.

Rojas *et al*. (2011) stated that the mitochondrial based DNA analysis has many advantages derived from the fact that there are many mitochondria per cell and many mitochondrial DNA molecules within each mitochondrion, making it a naturally amplified source of genetic variation.

Sumathi *et al.* (2015) reported that species detection based on mtDNA is popular due to its different specificity expressed in the species or genera. There are approximately 104 copies of mtDNA available per cell compared to only one copy of genomic DNA. Thus, it is more efficient to detect species-specific DNA using mtDNA than genomic DNA.

Ahmed *et al*. (2016) mentioned that the mitochondrial genome is easier to be isolated than the nuclear genome, presents in high number of copies, has smaller size and rapid accumulation of mutations and the post PCR analysis is much simpler.

- ## Stability of DNA in Meat Products:

Lockley and Bardsley (2000) mentioned that DNA based methods were widely used in the nutrition and meat area because DNA molecules are strong enough that will be maintained during the meat processing and therefore are good target for identification of different animal species. In addition, DNA molecules are stable and have high thermal stability that makes DNA based techniques suitable for analysis of heat processed products.

Wolf and Lüthy (2001) reported that DNA is a remarkably stable molecule allowing its extraction from all kinds of tissue due to the ubiquity of DNA in every type of cell.

Lenstra and Lees (2003) stated that DNA is relatively stable at high temperatures, meaning that it can be analyzed not only in fresh and frozen meat products, but also in heat treated processed, degraded and mixed commodities.

Mane (2004) mentioned that DNA-based techniques were widely utilized in identification of meat species due to stability at high temperature and highly conserved structure of DNA within all tissues of an individual.

Arslan *et al*. (2006) stated that the heat stability and large copy number of mitochondrial DNA in meat tissue contribute in the protection and survivability of the fragments of DNA that were sufficient enough to be amplified by PCR techniques.

Spychaj and Mozdziak (2009) reported that DNA molecule is highly stable against many factors such as high temperature, pressure and chemical compounds.

De Masi *et al.* (2015) stated that the detection of the specific DNA molecule which is a relatively stable molecule allows analysis of processed and heat treated meat products.

Lakzadeh *et al*. (2016) found that the presence of targeted DNA was successfully identified in the commercial examined meat product samples, and the amplification was not affected by the use of spices and other ingredients in the mixtures.

- **Polymerase Chain Reaction Application in Meat Species Detection:**

A number of Polymerase chain reaction (PCR) based assays have been developed and evaluated for species detection and mislabeling discovery in foodstuff **(Jonker *et al.*, 2008)**. Polymerase Chain Reaction (PCR) is an effective DNA based technique that is highly accurate and relatively fast. The conventional PCR assay is an easily affordable and reliable method for the routine analysis of animal meat products in food industry **(Arslan *et al.*, 2006)**. It has a satisfactory performance in the qualitative detection of meat species **(Ali *et al.*, 2014b)**.

Matsunaga *et al*. (1999) described a polymerase chain reaction (PCR) technique for the identification of multiple meat species includes cattle, pig, chicken, sheep and horse as raw materials for meat products. The PCR primers were designed to give different length fragments from the examined species of meat. The results revealed that the species-specific DNA fragments succeeded to be identified by electrophoresis of PCR products.

Montiel-Sosa *et al.* (2000) improved a reliable and rapid PCR method for detecting a PCR-amplified band from pork and designed a highly species-specific primers for pork mtDNA. The results showed that PCR technique could be useful in detecting both pig meat and fat in meat mixtures, including those dry-cured and heated by cooking. Additionally, the absence of response in PCR-amplified samples or mixtures from bovine, ovine, chicken and human was also demonstrated.

Partis *et al*. (2000) described an investigation for the use of a PCR technique as a routine analytical tool for species testing by generating DNA

fingerprints for 22 animal species and found that cooking the tissues did not affect the DNA extractions or the profiles generated.

Calvo *et al*. (2001) used and evaluated a PCR procedure to detect pork in heated and unheated meat, sausages and patties by isolation of DNA specific porcine element. The results revealed that pork has been identified in both heated and unheated meat products.

Abdulmawjood *et al.* (2003) applied a PCR method for the detection of dog meat with analyzing the cytochrome b gene sequence of its species which could be differentiated with species-specific oligonucleotide primer. The results showed that the use of this oligonucleotide primer allowed a direct identification of dog meat in meat mixtures even after heat treatment.

Rodríguez *et al*. (2004) described a PCR assay for the specific and qualitative detection of pork, beef, sheep and goat in raw and heat-treated meat mixtures and the results showed clear species identification. As regard, this assay could be useful for the accurate identification of these species, avoiding mislabeling or fraudulent species substitution in meat mixtures.

Chisholm *et al*. (2005) successfully tested commercial meat products spiked with horse or donkey meat for demonstrating the applicability of the PCR assays to detect the low levels of horse or donkey meat with designing the primers to the mitochondrial cytochrome b gene.

Farouk *et al.* (2006) applied a polymerase chain reaction (PCR) technique for detecting pig meat or its ingredients in meat products obtained from various local hypermarkets by identifying the sequence of a certain gene found uniquely in pork and using its sequence to design specific primers for the PCR. The results revealed that the PCR technique used for isolation of pig DNA from

various samples was reliable and the specific porcine gene fragment can be amplified using the specific DNA primers.

Ilhak and Arslan (2007) determined the origin of some meat species by applying a polymerase chain reaction (PCR) technique, using species-specific primers by preparing test meat mixtures of 5%, 2.5%, 1%, 0.5%, and 0.1% levels of pork, horse and dog meat to beef, sheep, and goat meat. Then, samples taken from those combinations were analyzed by PCR for species determination. The results indicated that meat species were accurately determined in all combinations by PCR that can be useful for fast, easy, and reliable control of adulterated meat products.

Jain *et al*. (2007) studied the detection of animal species in meat samples including cattle, buffalo, sheep, goat, pig, chicken and horse by applying a PCR assay using cytochrome b gene variability. The results revealed that PCR technique could be used successfully as a routine method, being highly sensitive, reproducible, rapid, simple and not expensive for differentiation of meat species.

Kesmen *et al.* (2007) applied a species-specific PCR assay for the detection of low levels of pork, horse and donkey meat in sausages. As regard to results, application of PCR assay on DNA extracted from sausage samples that previously prepared from binary meat mixtures allowed the detection of each species when spiked in any other species at the 0.1% level. Hence, PCR assay could be used to determine mislabeled or fraudulent species substitution in comminuted meat products.

Martín *et al*. (2007) described a PCR method based on mitochondrial gene for the specific and qualitative detection and identification of dog species in meat and tested the specificity of the primers against 32 non target species

including some mammals and birds species. The results revealed that the PCR assay allowed the detection of raw and heated tissues of dog in meat mixtures even when the concentration of the target species was reduced to 0.1%. Additionally, the performance of the method was not affected by prolonged heat-treatment (up to 133°C for 20 min). Consequently, it could be available to verify the origin of raw materials in products submitted to denaturing technologies.

Abdeen (2008) identified some meat products among them beef burger, sausage and luncheon for the detection of adulteration with donkey, dog, pig and chicken meat. The results reported that 20% of beef sausage samples, 26.7% of beef luncheon samples and 20% of beef burger samples were adulterated, with a total percentage of adulteration 55.9%. Additionally, the most adulterating species in the samples were pork (11.8%), followed by donkey (7.5%), chicken (3.2%) then dog meat (1.1%).

Abdel-Rahman *et al.* (2009) applied a PCR method for identification of dog, donkey and horse meat by extraction of DNA from a very small amount of muscles (0.05 g) to amplify species-specific DNA sequences of these species. The results showed that the using of these species-specific primers allowed a direct and rapid identification and detection of adulteration of dog, donkey and horse meat even after homogenizing.

Ghovvati *et al*. (2009) examined some sausage samples for identification of the presence of fraudulently added meat species (ruminant, poultry and porcine) by utilizing a polymerase chain reaction assay. The results demonstrated that none of the samples was adulterated with porcine residuals, but 40% of samples were contained poultry residuals.

Murugaiah *et al.* (2009) applied an accurate analytical technique for pork, beef and chicken meat identification based on PCR analysis by using cytochrome b gene of mitochondrial DNA for enforcement of labeling regulations. The results revealed that PCR technique is useful and feasible to trace meat adulteration and differentiate species present in mix meat. It can provide a useful laboratory tool for species identification, especially for meat traceability and Halal authentication.

Kesmen *et al*. (2010) described a specific PCR method for identification of horse, donkey and porcine meats in traditional Turkish sausage samples which were prepared from binary meat mixtures obtained by adding different amounts (0.0%, 0.1%, 0.5%, 1.0% and 5.0%) of horse, donkey and porcine meats into either cattle or sheep meats. Specific oligonucleotide primers of horse, donkey and porcine species were designed on the mitochondrial DNA. The results showed that each meat species could be identified at all the levels studied in the range of 0.1% to 5% in all sausage samples. Consequently, the PCR method could be used as a routine control method in food control laboratories for the identification of horse, donkey and porcine meats in the meat products.

Soares *et al.* (2010) used a species-specific polymerase chain reaction (PCR) assay for the simultaneous detection of pork and poultry meat species. The results showed that the applied PCR assay presented a low cost, fast, easy and reliable alternative to estimate the level of poultry meat adulteration by the addition of pork meat.

Abd El Sadek (2011) examined 120 ready to cook meat product samples including beef burger, sausage and hawawshi, randomly collected from Cairo, Giza and Kalubia governorates for detection of their adulteration by illegal substitution with cheap or prohibited meat. The results revealed that 25% and

5% of beef burger samples were adulterated with equine and dog meat, respectively. While, there were 10% and 5% of sausage samples adulterated with equine and dog meat, respectively. Additionally, 15% of hawawshi samples were adulterated with equine meat.

Ahmed *et al.* (2011) analyzed fifty beef meat product samples of minced meat, raw kofta, sausages and beef burger collected from Assiut retail markets by PCR technique for detection of meat adulteration with chicken, pork and donkey species. The results showed that the adulteration rates with chicken were 57%, 63.6%, 66.7 % and 69% for minced meat, raw kofta, sausages and beef burger, respectively. While, the adulteration rates with pork were 35.7%, 45.5%, 41.7% and 23% for minced meat, raw kofta, sausages and beef burger, respectively. Moreover, the adulteration rates with donkey meat were 7%, 18%, 8% and 7.7% in minced meat, raw kofta, sausages and beef burger, respectively.

Hussien (2011) examined 60 samples of industrial beef meat products (20 each of hawawshi, luncheon and oriental sausage) by applying PCR assay for detection of chicken, donkey and porcine meat. The results showed that 65%, 50% and 5% of hawawshi samples were adulterated with chicken, pork and donkey meat, respectively. While, there were 70%, 45% and 10% of sausage samples adulterated with chicken, pork and donkey meat, respectively. Moreover, there were 25% and 20% of luncheon samples contained chicken and donkey meat, respectively. In addition, none of the luncheon samples was contained pork meat. Totally, the results revealed that 85%, 55% and 95% of hawawshi, luncheon and sausage samples respectively were found to contain undeclared species. While, the major adulterating species in all the examined samples were chicken (53.3%) followed by pork (31.6%) and donkey (11.66%).

Sakalar and Abasiyanik (2011) applied a polymerase chain reaction assay to commercial meat products for the identification of adulteration by the most used species in foodstuffs such as, ruminant, poultry and pork materials. The results showed the presence of poultry meat in red meat products analyzed, although, it was not indicated on the label.

Zarringhabaie *et al*. (2011) designed a fast and reliable polymerase chain reaction (PCR) identification system for testing the pure and mixed species origin of meat samples by designing different primers for each species according to the conserved region of mitochondrial cytochrome b (Cytb) gene. The results revealed different specific amplified fragments of pure meat sources for buffalo, goat, cattle and sheep species.

Ciupa *et al.* (2012) suggested that the PCR assay can be used to determine mislabeled or fraudulent species substitution in comminuted meat products by developing a species-specific PCR for the identification of bovine species in 14 samples of meat products which contained bovine meat in different proportions according to their labels. The results showed that, when applying the assay to DNA extracts from different meat product samples, it was possible to detect each meat species to ensure what the label indicated.

Erwanto *et al.* (2012) reported the ability of using PCR- technique for the detection of pork in meat products for the halal authentication by isolation of genomic DNA of pig, bovine, and chicken, then subjected them to PCR amplification targeting the mitochondrial cytochrome b gene. The result revealed the presence of pork in meat products that can be distinguished among bovine, chicken, and pig samples.

Mohammed (2012) analyzed some beef meat products including 25 each of sausages, luncheon and hawawshi by using PCR technique for detection of

meat species adulteration. The results revealed that the adulteration rates with donkey meat were 8%, 0%, and 12%, in sausage, luncheon and hawawshi samples, respectively. While, the adulteration rates with chicken meat were 24%, 20%, and 20%, in sausage, luncheon and hawawshi samples, respectively. However, none of the samples was adulterated with horse meat.

Cawthorn *et al.* (2013) examined various processed meat products including beef burgers and sausages for the presence of undeclared animal species by using PCR technique. The results showed that the percentages of undeclared chicken, sheep and pork species found in the burger samples were 40%, 35% and 30%, respectively. While, the percentages of undeclared chicken, sheep and pork species found in sausage samples were 39%, 47% and 52%, respectively.

Mane *et al.* (2013) applied a Polymerase Chain Reaction assay specific to pork detection for authentication of meat products processed under different manufacturing conditions. The results showed that the level of detection of pork was less than 1% in admixed meat products having five non-targeted meat species viz. beef, buffalo meat, chevon, mutton and chicken. Moreover, by repeating the experiment several times there were the same results. Thus, the PCR assay is a very simple and useful tool for routine assessment of authenticity of meat products.

Özpinar *et al.* (2013) analyzed 73 samples of different meat products sold in stores, meat selling markets and public bazaars located in different districts of İstanbul province for the detection of animal species notified on the label for assessment of adulteration. The results showed that 50% of sausage samples were labeled incorrectly, while, the mostly detected meat species were chicken,

turkey and sheep species. No pig and equine species were detected in all samples.

Dahlan and Sani (2014) investigated the labeling of 18 processed meat products among them chicken luncheon and chicken burger samples using species-specific polymerase chain reaction (PCR) technique and found that all the chicken product samples were adulterated by beef which were not stated in the product label.

Doosti *et al*. (2014) examined 224 variable meat products, among them 68 sausages and 55 hamburgers by using PCR assay for detection of beef, sheep, pork, chicken, donkey and horse meats. Genomic DNA was extracted and PCR was performed for gene amplification of meat species using specific oligonucleotide primers, and the raw meat samples were used as the positive control. The results found that 6 of 68 sausages (8.82%) and 4 of 55 beef burgers (7.27%) were contained Haram (unlawful or prohibited) meat. These results showed that molecular methods such as PCR are potentially reliable techniques for detection of meat type in meat products for Halal authentication.

Eslami *et al*. (2014) examined 110 raw handmade beef burger samples collected from different areas of Yazd city, Iran by using a conventional PCR assay for detection of beef materials. The results showed that 10 (9.09%) samples did not contain any cow meat and were not in agreement with their label, while 100 samples contained cow meat. Hence, as regarding to results, PCR assay is useful for effective control of adulterated consumer products and violations of labeling requirements for meat products.

Hamzah *et al.* (2014) conducted a study to detect the presence of porcine DNA in some meat products among them chicken burger samples in Malaysia markets using conventional polymerase chain reaction (PCR) technique. The

results demonstrated that none of the chicken burger samples was adulterated with porcine residuals. Hence, the PCR amplification yielded excellent results for identification of pork derivatives in meat products and it is a potentially reliable and suitable technique in routine meat analysis for Halal certification.

Irine *et al.* (2014) proved the success of the PCR techniques in amplifying DNA fragments from dog species tested for species authentication in meat products using cytochrome b gene.

Kesmen *et al.* (2014) applied a rapid and highly specific PCR method for the identification and quantification of pork and donkey meats in raw and cooked binary mixtures of donkey with beef and pork with beef. The results found that the PCR assay could be successfully used with a high degree of specificity and sensitivity in the identification of donkey and pork species for adulteration studies.

Mehdizadeh *et al.* (2014) examined 90 raw beef burger samples including 42 handmade and 48 industrial for detection of adulteration by chicken meat using species specific Polymerase Chain Reaction (PCR). The results showed that 94.4% of all the samples including 100% of handmade and 89.6% of industrial samples, were contained undeclared chicken meat and this high rate might be related to mixing beef with cheaper parts of chicken.

Rahman *et al.* (2014) conducted a polymerase chain reaction (PCR) assay for the assessment of dog meat adulteration in meatballs by amplification of canine mitochondrial cytochrome b gene and tested the specificity of the assay against 11 animals species, while the stability was proven under extensively autoclaving conditions that breakdown target DNA. The results showed that the assay could repeatedly detect 0.2% canine meat tissues under complex matrices using 0.04 ng of dog DNA extracted from differentially treated meatballs.

Additionally, examining some ready to eat chicken and beef commercial meatballs from Malaysian supermarkets for detection of canine tissues was applied and the results revealed no commercial samples were found to be positive. Thus, it was obvious that the assay could be used in halal food industry for the authentication of canine derivatives in processed foods.

Rezazadeh *et al.* (2014) applied a polymerase chain reaction (PCR) assay for assessment the detection of bovine, sheep, pig, horse, donkey and chicken species in raw and processed meat products. Specific primers were designed for the identification of each species. The results revealed that by applying PCR technique, expected base pair fragments of all the species were detectable. Thus, this protocol could be used for identification of raw and processed meat products in various animal species.

Roostita *et al*. (2014) identified forty eight beef meatball samples for pork addition to give halal authentication as safety warranty to the consumer by applying molecular approach such as polymerase chain reaction assay. The results found that all the samples were halal beef meatballs as no porcine DNA sequence was amplified from the 48 tested samples, which showed that no pork was added to any of the samples.

Yosef *et al.* (2014) examined some different packaged beef meat products including burger, luncheon and sausages for the detection of meat adulteration with chicken, turkey, goat, sheep, equine and pig species. The results showed that 66.2% of all samples were labeled incorrectly, with adulteration rate of luncheon samples was 72.7%, of sausage samples was 54.5% and of burger samples was 36.4%. Additionally, the detected meat species were chicken, turkey, goat, sheep and pig species, but no samples were adulterated with equine species.

Abuzinadah *et al.* (2015) detected the fraudulent of illegal substitutions of undeclared species in some chicken products as chicken luncheon and chicken burger by using PCR assay. The results found that all tested products were identified as chicken, but additionally, they were identified as turkey meat, suggesting adulteration with inedible parts of turkey in chicken products ingredients.

Ali *et al*. (2015) described a polymerase chain reaction assay for the accurate identification of five meat species forbidden in Islamic foods. Five pairs of species-specific primers were designed to amplify DNA fragments from cat, dog, pig, monkey and rat meats, respectively. The results showed that all PCR products were identified. Additionally, five different halal branded meatballs purchased from various supermarkets in Malaysia were examined and all the commercial samples were found with negative targeted species.

Di Pinto *et al.* (2015) investigated processed-meat products from Italian markets using the mitochondrial cytochrome b gene qualitative PCR identification system in order to verify the substitution or mislabeling of chicken, pork or horse species. The results revealed high species substitution rate among the meat products, represented as 57% mislabeling cases.

He *et al.* (2015) applied a PCR method for identification of multiple meat species (beef, duck, mutton and pork) in processed meat products. The results suggested that PCR represents a simple, efficient test method as a practical alternative for the rapid detection and identification of meat.

Sakalar *et al.* (2015) designed a polymerase chain reaction (PCR) based assay for the detection of porcine and horsemeat in sausages and found that PCR technique is a potentially sensitive, reliable, rapid and accurate assay for the detection of meat species adulterated with porcine and horse meats.

Zahran and Hagag (2015) analyzed 100 samples of commercial beef meat products (50 each of minced meat and sausages) purchased from popular retail markets in Cairo and Giza governorates for detection of meat species adulteration by performing PCR assay. The results showed that 4 (4%), 3 (3%) and 5 (5%) of all the examined samples were adulterated with sheep, goat and donkey meat, respectively, with 12% total rate of adulteration. Therefore, it could be concluded that molecular method such as PCR is a potentially powerful and reliable technique for detection of adulteration with different meat species in meat products.

Ahmed *et al.* (2016) applied a polymerase chain reaction (PCR) technique for identification of different adulterants species (donkey and pig) in experimental mixture of fresh minced beef with known formulations. The results revealed that PCR assay could be a useful tool for detection of animal species in minced meat when adulterated with more than one different meat species.

Bourguiba-Hachemi and Fathallah (2016) tested 105 samples of raw and processed meat products marketed in the Arabian Gulf region by performing a polymerase chain reaction (PCR) technique for detection of the presence of undesirable and undeclared meat as horsemeat and pork. The results showed the presence of horse and pork DNA in 7% and 26% of tested samples, respectively.

Cetin *et al*. (2016) collected 250 fresh processed beef meat products from local markets and restaurants in the districts of Istanbul mostly with low purchasing power for determining their deliberate or accidental adulterations by applying of PCR technique. The results revealed that chicken tissue was found in 62 (24.8%) of the analyzed samples, while horse tissue was found in 2 (0.8%) samples, however, pork tissue was not detected.

Elbialy *et al.* (2016) examined 10 samples of beef luncheon collected from the local markets in Kafrelsheikh Governorate for detection of adulteration with other types of animal species rather than beef by applying a PCR assay. Results showed that 8(80%) of the 10 luncheon samples were adulterated with goat meat, while one (10%) sample was adulterated with equine meat, which were not in compliance with the label on the examined products.

Kane and Hellberg (2016) tested 48 fresh and frozen ground meat products for detecting the presence of undeclared species targeting beef, chicken, lamb, turkey, pork and horse by using a PCR assay. The results revealed that 38 out of 48 meat products were labeled correctly and 10 were found to be mislabeled, with detection of horsemeat in two of them.

Lakzadeh *et al*. (2016) examined 100 samples of sausage and 50 samples of raw burgers collected from local market by using PCR assay for the identification of chicken tissues adulteration. The results showed that the presence of misused chicken meat in sausage and burger samples were 84% and 26%, respectively.

Alikord *et al.* (2017) identified horse, donkey, pig and other ruminants in raw and processed meat products by using the PCR amplification. Oligonucleotid primers were designed for amplification of species-specific mitochondrial DNA sequences of each species and samples were prepared from binary meat mixtures. The results showed that meat species were accurately determined in all combinations by PCR, rendering this technique suitable for use for industrial meat products.

2. Protein Based Techniques:

Hsieh *et al.* (1995) examined 902 raw and cooked meat product samples from over 500 Florida retail markets for their regulatory control by using an Enzyme-Linked Immunosorbent assays to identify beef, pork, horse, poultry and sheep species. The results showed that 22.9% of cooked ground meats and 15.9% of fresh ground meats were substituted with meat from other undeclared species, whereas all intact cuts were accurately labeled. The species detected in ground beef products were sheep, pork and poultry and the major substituting species was sheep compared to pork and poultry. Additionally, beef was found to be the adulterating species in ground poultry meat sold in retail markets. However, horsemeat was not detected in any of the examined samples.

Chen and Hsieh (2000) applied an enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody to a porcine thermal-stable muscle protein for detection of pork in cooked meat products. Validity of the assay was evaluated with laboratory formulated and commercial meat samples. The results revealed that no cross-reactivity was observed with common meat proteins and the detection limit was determined as 0.5% (w/w) pork in heterogonous meat mixtures. Additionally, the accuracy in analyzing market samples was 100% as verified by product labeling and confirmed by a commercial polycolonal antibody test kit.

Flores-Munguia *et al.* (2000) analyzed 40 samples of two processed meat products, uncooked commercial burger and Mexican sausage from local food stores by agar gel immunodiffusion assay (AGID) to identify bovine, porcine, equine and avian species. The results detected undeclared equine species in 9 of the 23 burger meat samples and undeclared equine and porcine species in 5 of the 17 sausage samples and these results showed violations in practices of the regional meat industry.

Macedo-Silva *et al.* (2000) used the dot-ELISA method to identify the meat of different animal species and to detect adulteration of hamburgers by producing antisera to bovine, chicken, swine and horse albumin which could detect the meat extract of the homologous species at concentrations as low as 0.6%. The results showed that the anti-albumin antisera could identify bovine, chicken, swine and horse meat with adequate specificity and sensitivity both in isolation and when added to hamburger. Additionally, examination of some commercial samples of hamburgers showed no adulteration with bovine, chicken, swine or horse meats.

Ayaz *et al.* (2006) analyzed 100 meat product samples for species determination by using the cooked meat species identification Kit (ELISA-TEK, Gainesville, FL) prepared with monoclonal antibody technique. The results indicated that 22.0% of all the samples were not in compliance with the labels and the main adulterants in the meat products labeled as beef were poultry or mixture of beef and poultry.

Joseph *et al.* (2006) tested one hundred marketed beef sausage samples for detection of adulteration with pork using an indirect sandwich ELISA. The samples were produced by two major local manufactures designated as A and B (50 samples from each manufacture), where manufacturer A processed both beef and pork sausages, while manufacturer B processed beef sausages only. The results found that forty-four samples tested positive and all were from manufacturer A, giving 44% rate of adulteration.

Abd El-Aziz (2009) analyzed different beef meat product samples (among them 50 sausages and 50 beef burgers) by using AGID technique for detection of meat species adulteration. The results showed that the rate of adulteration with chicken and pork was 32% and 14% in sausage and 32% and 2% in beef

burger, respectively. While, donkey species was detected only in beef burger at rate of 2%.

Hsieh and Ofori (2014) applied a reliable monoclonal antibody (mAb) based Enzyme-Linked Immunosorbent Assay (ELISA) for horse meat detection by developing two mAbs characterized as horse-selective and employing these mAbs on competitive ELISAs (cELISAs). The results revealed that cELISAs were found to be capable of detecting levels as low as 1% of horse meat in raw, cooked, and autoclaved ground beef or pork, being useful analytical tools for addressing the health, economic, and ethical concerns associated with adulterating meat products with horse meat.

Applications of Onsite Testing in Meat Authentication:

The commercial rapid kits are available efficient tests used for the determination of animal species by visual inspection within short time, giving sensitive, rapid and reliable results **(Giovannacci *et al.*, 2004)**. They have unique applications for fast and on-site screening of large number of samples for detection of adulterating species, and only samples that appeared positive can then be submitted for confirmation by the standard laboratory methods. This analytical approaches could save both the time and costs **(Muldoon *et al.*, 2004)**.

Bonwick and Smith (2004) reported that the commercial ELISA kits could be used as a routine meat screening tests for analysis of large number of samples for their authenticity with short time required for analysis.

Giovannacci *et al.* (2004) evaluated the performances of commercial ELISA kits for identification of pork, beef, sheep and poultry species in forty commercial meat products. The results found that twenty products were in

complete agreement with labels, while the other products showed non-labeled species. Additionally, some species were not detected although they were mentioned on the labels. As regards to results, the ELISA kits led to the identification of one to three animal species for each analyzed product with the detection of low contents of them, even in highly processed foods. Additionally, they had the advantages of being robust, cheap and easy to perform.

Muldoon *et al.* (2004) described a rapid immunochromatographic test strip that had unique applications for screening large numbers of samples for prohibited materials in approximately 10 minutes, and can be performed without sample preparation step and any specialized equipment, in addition to its high sensitivity that gave no false positives and no false negatives at a detection limit of 0.1%.

Asensio *et al.* (2008) provided extensive overview on the applications of the rapid onsite ELISA techniques for meat species discrimination. It was reported that these methods have been widely used because they reduce the use of costly sophisticated equipments and time of analysis and are suitable for routine analysis of a large number of samples. Consequently, applications of these methods could allow consumer protection and confidence, and accurate implementation of the traceability for successful regulatory food controls.

Depamede (2011) produced rapid immunodiagnostic test strips that provide visual detection of the presence of pork components in raw beef and chicken meats and applied them on laboratory prepared adulterated raw meat samples. The samples consisted of pork-in-beef or pork-in-chicken at 1/0; 1/100; 1/1,000; 1/5,000; 1/10,000 (w/w) adulteration levels. In addition, raw beef and chicken meats without pork were included as controls. The results revealed that the analysis was completed in 10 minutes, the detection limit was 1/5,000 (w/w),

although 1/10,000 was also observed. Finally, this immunodiagnostic test can be conveniently applied to detect low levels of pork components in raw beef and chicken meat products with several advantages as it provides a rapid test period (less than 15 minutes) to get results and is ideally suited for onsite testing by untrained personnel without using specialized equipment.

Ulca *et al.* (2013) analyzed prepared comminuted meat products containing different levels of pork from authentic beef, chicken, and turkey in the raw state and after cooking for 20 min at 200 °C by using commercially available kits for animal species identification. The results revealed that the kit could correctly identify the animal species and could reliably detect the addition of pork at a level below 0.1%. Additionally, 42 commercial processed meat products were examined for species adulteration and the results showed that 36 of 42 samples were negative for the presence of pork (< 0.1%), while four samples were found to be containing pork. However, one sausage sample was labeled as containing 5% beef, but beef was not detected, in addition to a meatball sample labelled as 100% beef was found to contain chicken.

Schmutzler *et al.* (2015) applied a handheld industrial fibre optics spectrometer method ready for on-site detection of pork adulteration in the meat and fat part of sausages. The results revealed that analyses with the on-site setup led to successful separation up to the lowest degree of adulteration (10%), and both meat and fat adulteration could be detected.

Dawnay *et al*. (2016) described the development of an assay using a single probe allowing the rapid screening and authentication of meat products. The assay had been designed for the use of field portable system, a molecular detection platform for non-expert users, took approximately 75minutes from sampling to result. The test allowed detection of target species and closely

related species, which might be used as substitutes. The results showed that the assay was quick to design, characterise and capable of yielding results that would be beneficial in authentication of products.

Lee *et al*. (2016) applied an on-site detection method for the rapid and sensitive identification of pork in processed meat products with minimal equipment and no risk of contamination of samples by using a portable real-time fluorometer. Pork-specific primers were designed based on the mitochondrial D-loop regions, and eukaryotic primers were used for the endogenous control in order to prevent false-negative results. Additionally, forty-two commercially processed meat products were successfully verified for labeling compliance using this method within 30 minutes without the need of nucleic acid extraction.

Masiri *et al*. (2017) conducted a highly specific lateral flow immunoassay that can rapidly identify raw and cooked horse meat down to 0.01% and 1.0% contamination, respectively in xenogeneic meat sources in about 35 minutes with no false positive signals observed. Specificity analysis revealed no cross-reactivity with serum albumins or meat derived from chicken, turkey, pig, cow, lamb and goat. The results showed that the assay required considerably less time to perform than either method. Thus, the development of a highly robust and rapid test method capable of detecting trace amounts of horse meat residues should aid food control authorities in their continued efforts to monitor for horse meat adulteration.

Song *et al*. (2017) described an onsite ultra-fast molecular detection method for meat identification based on polymerase chain reaction (PCR) by using the mitochondrial cytochrome b (Cyt b) as a target gene and the amplicon size was designed to be different for beef, lamb, and pork. The detection limit

was as low as 1% of meat adulteration. The results suggested that the developed assay might be useful in the authentication of meats and meat products in rapid on-site applications.

The Significant Importance for Public Health and Economic Point of View:

Meat adulteration is a considerable problem in the meat industry. The threat and risk of meat species adulteration and mislabeling has become a large concern and challenge for the food control authorities and consumers **(Elbialy *et al.*, 2016)**. Detection of meat adulteration is an important issue from aspects of food control and food regulation. Generally, the inspection of the declared composition of food stuff as notified on its label is officially an obligatory task order to protect the public benefits and health against adulteration and infectious diseases caused by zoonoses **(Özpinar *et al.*, 2013)**.

Hargin (1996) reported that individuals with ethical aversions to consuming certain types of meat and those suffering from meat protein allergies need to be certain that the meat products they purchase contain exactly what is pronounced, and nothing else.

Hsieh (2000) stated that meat species adulteration imposes substantial concerns to consumers in terms of economic loss, food allergy, religious observance and food safety.

USDA-FSIS (2001) stated that meat products adulteration that has been reported in several countries involves several issues such as economic fraud, which violates the food-labeling laws as well as a concern for religious taboos that ban the consumption of specific meat species. Additionally, it is related to

food safety to some extent, with regard to individual allergies, contamination with food borne pathogens, and the spread of the fatal transmissible spongiform encephalopathy.

Brown (2003) mentioned that the entrance of infected nervous system tissues of cattle to the human food chain were the most likely cause of human infection with transmissible spongiform encephalopathy. These tissues such as vertebral columns, fragments of spinal cords and paraspinal ganglia might be included during the manufacture of variety of packaged meat products such as hotdogs, sausages, beef patties, luncheon meats or beef stews after subjected them to a process of compression to yield bone fragments (used for gelatin) and forming a paste of "mechanically recovered meat" (MRM).

Hsien-Çhi *et al.* (2004) reported that in some cases, misleading labels may be harmful for individuals who have food allergies and the consumption of these meat products may create health concerns.

Rodríguez *et al.* (2004) mentioned that the identification of the species origin in meat products is relevant to consumers due to the possible economic loss from fraudulent substitutions or adulterations, the medical requirements of individuals who might have specific allergies and the religious reasons.

Ong *et al.* (2007) stated that predisposed individuals with an allergy against special meat and its products do not demand combined meat products having allergen materials, as their health will be endangered in case of consumption of product containing non declared meat proteins which can induce allergic reactions.

Rastogi *et al.* (2007) mentioned that there are many reasons for the importance of disclosure of adulterated meat products such as the health problems, the unfair trade competition and the religious beliefs.

Asensio *et al.* (2008) stated that porcine meat is an undesirable adulterants when undeclared because of the health reasons, as well as the religious reasons, due to the potential introduction of allergens, bacteria, and parasites.

Fajardo *et al.* (2010) reported that some of the species such as cat and dog are potential carrier of several zoonotic diseases such as trichinellosis which is one of the most important food-borne parasitic zoonosis related to dog meat consumption, thus they are not safe and hygienic for human consumption.

Sahilah *et al*. (2011) mentioned that for some consumer groups such as Muslims, the adulteration of meat products with meat of pig and its remnants, dog and cat are forbidden.

Unajak *et al.* (2011) stated that in Islamic regulations, meat species and safety of meat products are very important for religious and health reasons as food containing pig meat is Haram, and horse and donkey sources is Makrooh for Muslims, and they will not eat meat that is Haram and or Makrooh.

Zarringhabaie *et al.* (2011) reported that the increasing demand for transparency in the food industry derives either from socio-religious reasons (such as vegetarianism, preference for organic products, the absence of pork for Jews and Muslims), health concerns or economic reasons. Therefore, there is a strong demand for appropriate detection methods that allow identification of different species in meat products or of the different components in processed meat.

Nakyinsige *et al.* (2012) mentioned that the major authenticity concerns for Muslim consumer in meat products include pork substitution using prohibited ingredients or pork intestine casings. The porcine derivatives used in the meat processing industry include; pork fat (lard), mechanically recovered meats (MRM), porcine gelatin and porcine blood plasma. Pork and its derivative are Haram to be consumed by Muslims.

Ortea *et al.* (2012) stated that meat products authentication is a major concern not only in order to prevent commercial fraud, but also to assess the safety risks arising from the undeclared introducing of any food ingredient that might be harmful to human health, such as potentially allergenic or toxic compounds, or others that might cause problems for the diets of certain consumers, such as vegetarians or religious groups.

Ekanem *et al.* (2013) mentioned that consumption of dog meat might be a major risk factor for exposure to rabies which could be easily have entered the food chain from consumption of dog meat. Additionally, it is expected that all of the dogs involved are usually strayed, apparently unimmunized, posing a threat to those dealing with the products manufacturing.

Premanandh (2013) mentioned that the consumption of horse meat might be very hazardous for human health due to certain antibiotic drug residues from antibiotic use in horses such as phenylbutazone which are highly toxic for humans. The recent controversies surrounding the horsemeat scandal have forced the authorities to enforce stringent regulations on meat adulterations.

Soares *et al.* (2013) reported that the authentication of species in meat products is crucial to protect the consumer from its implications as economic concerns since it leads to unfair competition among producers, religious

concerns since the consumption of certain species is not allowed in some religions, ethical concerns reflecting lifestyles and health concerns.

Yang *et al.* (2013) reported that the most common sources of human infection with toxoplasmosis are ingestion of tissue cysts in raw or undercooked meat and suggested that consumption of horse and donkey meat may represent a potential health risk for human as a source of infection with *Toxoplasma gondii*, which is a single-celled parasite, causes toxoplasmosis. Pregnant women and individuals who have compromised immune systems should be cautious; for them, as the *Toxoplasma* infection could cause serious health problems.

Eslami *et al*. (2014) mentioned that the identification of meat species in different meat products is an appropriate action in molecular epidemiological studies of pathogens transmitted by meat. Therefore, detecting of adulterated meat products is very important as the meat could be a source of enter pathogens.

Mehdizadeh *et al.* (2014) reported that adulteration of meat products may be occurred by addition of slaughtering remnants or the waste products, called trimmings. These waste products have lower nutritional value rather than meat and may be contaminated with food borne pathogens. Therefore, the probable presence of these pathogens with insufficient cooking temperature in final products poses a potential health risk for consumers.

Mohamed *et al.* (2016) stated that some meat processors partially or totally replace high quality and expensive meat by lower quality and cheaper materials such as mechanically recovered poultry meat (MRPM), this may lead to quality loss which resulted from the redistribution of the higher initial microbial load which favors the growth of microorganisms and makes it highly perishable raw material.

Oluwaseun (2017) stated that consuming untreated or undercooked meat from pigs or their residuals if used as adulterants in meat products has potential health risks as they may harbor worms. The pork is the carrier of various helminthes like pin worm, hooks worm or tapeworm. Additionally, trichinosis is a parasite disease caused by eating raw or uncooked infected pork with the larvae of roundworm.

Materials and Methods

I. Proximate Analysis of the Samples:

1. Collection of Samples:

In this study, a total of 120 different commercial beef and chicken meat product samples were randomly collected from street vendors, local and high fast food restaurants, butchers and retail markets located in various regions in Ismailia city during the year 2015. The beef meat product samples represented as 20 each of hawawshi, fresh oriental beef sausage, beef luncheon and beef burger, while the chicken meat product samples represented as 20 each of chicken luncheon and chicken burger. All the samples were wrapped, identified then transported in icebox container to the Central Lab, Faculty of Veterinary Medicine, Suez Canal University for their proximate analysis.

2. Preparation of Samples:

The samples were prepared and examined according to the technique recommended by **(AOAC, 2003)** as follows:

Each sample was grounded by passing through meat chopper with opening ≤ 3mm, and then the chopped material was thoroughly mixed and transferred to suitable container with airtight cover.

3. Determination of Moisture Content (AOAC, 2003):

A. Analytical procedure:

- About 5grams of each prepared sample were weighed in an empty dry covered dish that was previously weighed.
- The sample was spread across the bottom of the dish.

- The dish with the sample was put in a drying hot air oven that was adjusted at 105˚C until two successive constant weights were obtained. The drying oven should not be overloaded.
- After the drying, the samples were removed from the oven and placed in a desiccator for about 30 minutes to be cooled at room temperature, then were accurately weighed.

B. Calculation:

The moisture content was calculated according to the following equation:

$$\text{Moisture \%} = \frac{(W_1 - W_2)}{W} \times 100$$

Where: W = weight (gram) of sample before drying.
W_1 = weight (gram) of the dish with the sample before drying.
W_2 = weight (gram) of the dish with the sample after drying.

4. Determination of Protein Content (AOAC, 2003):

It was done by using the Kjeldahl digestion block **Kjeldatherm** and distillation systems **Vapodest 50s (Gerhardt, Germany)** as follows:

A. Analytical procedure:

Digestion

- About 100 grams of each prepared sample were placed in a 250 ml digestion tube and 2 catalyst tablets **(Kjeltabs CX, catalogue No. 12-0328)** were added followed by carefully adding of 20 ml concentrated nitrogen free sulphuric acid down the side of the flask.
- Digestion tubes were put into the pre-heated block at 400 °C for 30 to 40 minutes, the time was needed till the sample was turned translucent.

- During the entire digestion period, the scrubber **(Turbosog, catalogue No. 12-0057)** should be worked for the suction of the digestion fumes.
- About 1200 ml of 15 % caustic soda (NaOH) were recommended for washing the bottle.
- The samples were left about 30 minutes to be cooled; during this time, the scrubber should continue working.

Distillation

- The Vapodest 50s was started to be used with a blank distillation in order to be warmed up and cleaned.
- All chemicals (boric acid 4%, NaOH 40%, deionized water and standard acid) were checked that they were presented in the needed quantity.
- After the digested sample had cooled down, the water steam distillation was performed according to the following program as showed in **table A**:

Program parameter	VAP 50s
H_2O Addition	~90 ml
NaOH Addition	~80 ml
Distillation Time	240 s
Steam Power	100 %
H_3BO_3 Addition	70 - 80 ml
Suction Sample	30 s
Suction Receiver	30 s
Titration	automatic
Calculation	automatic

Titration

- About 3 - 4 drops of an indicator solution **(Merck)** were added to the receiving solution.
- The titration was run with HCl (0.1 mol/l) until the color changed from green to violet.

- The consumption of the standard acid solution was determined.

Blank Value

- For the determination of the blank value, the analysis (digestion and distillation) was run just using the given chemicals but with 1gram saccharose (free of nitrogen) instead of the sample.
- The consumption of the standard acid solution was determined.

B. Calculation

The nitrogen % (N %) was calculated as follows:

$$\mathbf{N\ \%} = \frac{1.4007 \times C \times (V - V_b)}{E}$$

Where: C = H^+ ion concentration of the standard acid solution: hydrochloric acid C = 0.1 mol/l alternative: sulphuric acid C = 0.05 mol/l.
V = consumption of the standard acid solution (sample) (ml).
V_b = consumption of the standard acid solution (blank sample) (ml).
E = initial sample weight (g).

Then, Protein % = N % × 6. 25

5. Determination of Fat Content (AOAC, 2003):

Extraction of fat was done by using the **Soxtherm Manager (catalogue No. 13-0012, Gerdhart, Königswinter,Germany)**.

A. Analytical procedure:

- About 3 - 5 boiling stones were put into each extraction beaker.
- The beakers were dried in a drying oven for about one hour at 103±2 °C.
- After cooling off to room temperature in the desiccator, each beaker was weighed.
- About 200 grams of each sample were mixed using a glass rod with about 10 grams sodium sulphate, put into an extraction thimble and covered with cotton wool.

- Any remaining fat traces on the glass were taken up with some cotton wool, soaked with extraction agent, and put into the extraction thimble as well.
- The extraction thimble with the sample was dried for 1 hour in the drying oven at 103 °C and after a short cooling off, placed into the thimble holder and put into the extraction beaker.
- The parameters of the program were adjusted as in **table B**:

Program Step	Parameter	Comment
Extraction Temperature	**150 °C**	
Reduction Interval	**4 min**	
Reduction Pulse	**2 s**	
Hot Extraction	**30 min**	**Sample must be completely immersed**
Evaporation A:	**5 x Interval**	**After A the level of the solvent should be at least 10 mm below the thimble**
Rinsing Time	**60 min**	
Evaporation B:	**3 to 4 x Interval**	**After B the extraction beaker should be more or less free of extraction agent**

- After the program was finished, the extraction beakers were dried in the drying oven for 60 minutes at 103± 2 °C, then, put into a desiccator, left to cool down to room temperature and weighed.
- This procedure was repeated until two successive weights were obtained.

B. Calculation:

The fat content (%) of each sample was calculated using the following formula:

$$\text{Fat \%} = \frac{(m_2 - m_1)}{m_0} \times 100$$

Where:

m_1: Mass of the empty extraction beaker with boiling stones in grams.

m_2: Mass of the extraction beaker with fat after drying in grams.

m_0: Weight of sample at the start of the analysis in grams.

6. Determination of Total Ash (ISO, 936/1998):

A. Analytical procedure:

- A porcelain dish was heated for 20 minutes in a muffle furnace set at 550 °C.
- The dish was cooled in a desiccator to room temperature and weighed (m_0) on the analytical balance.
- About 2 grams of each prepared tested sample was transferred to the dish. It was spread out evenly and the dish was weighed (m_1) again.
- The dish with its contents was placed for 1 hour in the drying oven, which was set at 103 °C.
- The dish was removed from the oven, placed on an electric hot-plate or over a gas flame and heated progressively until the substance was carbonized with the evolution of smoke. Carefully, carbonization was continued until smoke evolution ceased. The sample material should neither ignite nor burn with a flame.
- The dish was transfered to a cool muffle furnace and the temperature was raised to 550 ± 25°C.
- After 4 hours, the dish was removed with its contents from the muffle furnace and was allowed to cool in the desiccator to room temperature.
- The ash was inspected, if it was still black, it was treated with a few drops of hydrogen peroxide or water and the procedure was repeated. If the ash had a grey-white appearance, it was weighed on the analytical balance (m_2).

B. Calculation

The ash of each tested sample was calculated using the following equation:

$$wa = \frac{(m_2 - m_0)}{(m_1 - m_0)} \times 100$$

Where: wa is the percentage of ash of the tested sample.
m_0 is the mass of the empty dish, in grams.
m_1 is the mass of the dish with the tested portion, in grams.
m_2 is the mass of the dish with the ash, in grams.

7. Calculation of Total Carbohydrate Content (AOAC, 2003):

The total carbohydrate content of each sample was calculated by difference using the following formula:

Carbohydrate % = 100 – (Protein% + Fat% + Water %+ Ash %).

8. Calculation of Red Meat Content (McLean, 2007):

The calculation of meat content was occurred by the following equation:

$$\text{Fat Free Meat Content \%} = \frac{\text{Total Nitrogen \% - Non Meat Nitrogen\%}}{\text{NF}} \times 100$$

Where:

- Total Nitrogen is the total amount of nitrogen originating from the samples.
- Non Meat Nitrogen = carbohydrate % $\times \frac{\text{CNF}}{100}$

  (CNF = the Carbohydrate Nitrogen Factor = 2)
- NF is the Nitrogen Factor **(AMC, 1993; AMC, 2000)**.

II. Detection of Meat Species Adulteration by Using Conventional PCR Technique (cPCR):

Sixty samples (10 of each type) were chosen from the previously examined samples and transported to Animal Health Research Institute, Giza, Dokki, to be analyzed for detection of meat species adulteration using conventional Polymerase Chain Reaction (PCR) technique.

A. Extraction of DNA (According To QIAamp DNA Mini Kit Instructions, Catalogue No.51304, Qiagen Pvt. Ltd):

QIAamp DNA mini kit was a commercial tissue kit provided silica-membrane-based nucleic acid purification from different types of samples. The procedure did not require mechanical homogenization, so total hands-on preparation time was only 20 minutes. The contents of the kit were as shown in **Table C**.

Quantity	Contents
50	**QIAamp Spin Columns in 2 ml Collection Tubes**
150	**Collection Tubes (2 ml)**
10 ml	**Buffer ATL**
1.25 ml	**QIAGEN protease (Proteinase K)**
12 ml	**Buffer AL**
19 ml	**Buffer AW1 (concentrate)**
13 ml	**Buffer AW2 (concentrate)**
22 ml	**Buffer AE**
1	**Handbook**

DNA extraction was performed using QIAamp DNA Mini Kit as the Manufacturer's protocol described below:

Cell Lysis from Animal Tissue:

- 25 mg of each sample was weighed, cut up into small pieces, grinded and transferred to 1.5 ml microcentrifuge tube.
- 180 µl of Buffer ATL were added to the sample. Then 20 µl of QIAGEN protease were added and mixed by vortexing.
- The tube was incubated at 56 ºC overnight or until tissue had completely lysed.

Protein Precipitation:

- The tube was briefly centrifuged to remove drops from the inside of the lid.

- 200 µl of Buffer AL were added to the sample.
- The solution was well mixed by pulse vortexing for 15 seconds to yield a homogeneous solution then incubated at 70 ºC for 10 minutes. (A white precipitate might be formed on addition of Buffer AL, which would be dissolved during incubation at 70 ºC.)
- The tube was briefly centrifuged to remove drops from the inside of the lid.

DNA Precipitation:

- 200 µl of ethanol 96-100 % **(Applichem)** were added and well mixed by pulse vortexing for 15 seconds.
- After mixing, the tube was centrifuged briefly to remove drops from the inside of the lid.

DNA Hydration:

- The mixture (including the precipitate) was carefully applied to the QIAamp mini Spin Column (2 ml collection tube) without wetting the rim. The cap was closed, and the tube was centrifuged at 8000 rpm for 1 min.
- The QIAamp Spin Column was placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded.
- The QIAamp Spin Column was carefully opened and 500 µl Buffer AW1 were added without wetting the rim. The cap was closed, and the tube was centrifuged at 8000 rpm for 1 min.
- The QIAamp Spin Column was placed in a clean 2 ml collection tube, and the collection tube containing the filtrate was discarded.
- The QIAamp Spin Column was carefully opened and 500 µl Buffer AW2 was added without wetting the rim. The cap was closed, and the tube was centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min.

- The QIAamp Spin Column was placed in a new 2 ml collection tube and the old collection tube containing the filtrate was discarded. The tube was centrifuged at 20,000 x g (14,000 rpm) for 1 min.
- The QIAamp Spin Column was placed in a clean 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate was discarded.
- The QIAamp Spin Column was carefully opened and 200 µl Buffer AE was added. It was incubated at room temperature (15-25˚C) for 1 min, then centrifuged at 6000 x g (8000 rpm) for 1 min.

B. Amplification of the Extracted DNA:

Oligonucleotide Primers:

Six sets of oligonucleotide primers synthesized by **Midland Certified Reagent Company-oilgos (USA)** were incorporated in the present study for detection of beef, pig, equine, dog, sheep and chicken species in the examined samples. These primers were published by **(Tasara *et al.*, 2005)** for pig, **(Abdel-Rahman *et al.*, 2009)** for dog and **(Doosti *et al.*, 2014)** for beef, chicken, sheep and equine. Their specific sequences and amplified products are showed in **Table D**.

Primer name	Oligonucleotide Primers (Primer Sequence5'-3')	Amplicopns (bp)
Beef	**GCCATATACTCTCCTTGGTGACA**	**271 bp**
	TAGGCTTGGGAATAGTACGA	
Pig	**CTACATAAGAATATCACCCAC**	**290 bp**
	ACATTGTGGGATCTTCTAGGT	
Equine	**TTCTGCTCTGGGTGTGCTACTT**	**221 bp**
	CTACTTCAGCCAGATCAGGC	
Dog	**GGAGTATGCTTGATTCTACAG**	**808 bp**
	AGAAGTGGAATGAATGCC	
Sheep	**ATGCTGTGGCTATTGTC**	**274 bp**
	CCTAGGCATTTGCTTAATTTTA	
Chicken	**GGGACACCCTCCCCCTTAATGACA**	**266 bp**
	GGAGGGCTGGAAGAAGGAGTG	

PCR Mastermix:

EmeraldAmp® GT PCR mastermix (Code No. RR310A kit, TakaraBio, Japan) was used according to its manufacture instructions. It was consisted of an optimized buffer, PCR enzyme, dNTP mixture and a density reagent in 2X premix. The reaction was performed by thawing, gently vortexing then briefly centrifuging all solutions. A thin walled PCR tube was placed on ice and the following components were added for each 25 µl reaction as shown in **table E:**

Component	Volume/reaction
Emerald Amp GT PCR mastermix (2x premix)	**12.5 *µl***
PCR grade water	**4.5 *µl***
Forward primer (20 pmol\ *µl*)	**1 *µl***
Reverse primer (20 pmol\ *µl*)	**1 *µl***
Template DNA	**6 *µl***
Total	**25 *µl***

Amplification Protocol:

The cycling conditions of the different primers during DNA amplification was performed according to specific authors and **Emerald Amp GT PCR Master Mix (Takara, Japan)** kit as shown in **Table F**:

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Beef	**94˚C 5 min.**	**94˚C 30 sec.**	**48˚C 30 sec.**	**72˚C 30 sec**	**35**	**72˚C 7 min.**
Pig	**94˚C 5 min.**	**94˚C 30 sec.**	**52˚C 40 sec.**	**72˚C 40 sec.**	**35**	**72˚C 10 min.**
Equine	**94˚C 5 min.**	**94˚C 30 sec.**	**52˚C 30 sec.**	**72˚C 30 sec.**	**35**	**72˚C 7 min.**
Dog	**94˚C 5 min.**	**94˚C 30 sec.**	**48˚C 45 sec.**	**72˚C 45 sec.**	**35**	**72˚C 10 min.**
Sheep	**94˚C 5 min.**	**94˚C 30 sec.**	**48˚C 30 sec.**	**72˚C 30 sec.**	**35**	**72˚C 7 min.**
Chicken	**94˚C 5 min.**	**94˚C 30 sec.**	**60˚C 30 sec.**	**72˚C 30 sec.**	**35**	**72˚C 7 min.**

C. Agarose gel electrophoreses (Sambrook *et al.*, 1989) with modification:

Preparing the Gel:

- Electrophoresis grade agarose (1.5 g) was prepared in 100 ml Tris borate (TBE) buffer in a sterile flask.
- The flask was heated in microwave to dissolve all granules with agitation, and then allowed to cool at 70˚C.
- 0.5µg/ml ethedium bromide **(Sigma, Aldrish, Germany)** was added and mixed thoroughly as a florescent dye to stain gel during examination by UV transillumination.
- The warm agarose was poured directly in gel casting apparatus with desired comb in opposite position to make wells for the samples, and then left at room temperature for polymerization.
- Once the gel had cooled and solidified, the comb was removed.
- Ten µl of DNA molecular weight markers (DNA ladder) were directly loaded into wells of the gel. The ladders used were:

  Gel Pilot 100 bp ladder **(catalouge No. 239035, QIAGEN, USA)**.
  Number of bands: 6
  Size range: 100-600 bp.

  Gene ruler 100 bp DNA ladder **(catalouge No. SM0243, Fermentas)**.
  Number of bands: 10
  Size range: 100-1000 bp.

- Twenty µl of each PCR product samples, negative control and positive control were loaded into the remaining wells of the gel. DNA of raw meats was used as a positive control, while nucleus free water was used as negative control. Positive and negative controls were always present for each batch of extraction.

- The gel was placed into an electrophoresis tank, and electrophoresis buffer was poured into the tank until the surface of the gel was covered. The buffer conducted the electric current.

Separating the Fragments:

The electrical current was turned on; the power supply was 1-5 volts/cm of the tank length. The negatively charged DNA moved through the gel towards the positive side.

Visualizing the Results:

- The run was stopped after about 30 minutes and the gel was removed from the electrophoresis tank and transferred to UV cabinet.
- The gel was photographed by a gel documentation system.
- Then estimation of the size of the DNA in each sample could be applied by matching them against the closest band in the marker.

III. On-Site Detection of Meat Species Adulteration by Raw Meat FlowThrough™ Test (Bio-Check, United Kingdom):

The Raw Meat FlowThrough™ Test (RMFT) is a simple qualitative test offers an ideal testing solution for on-site determination of species by visual inspection with a limit of detection 1%. It is simple and quick to be performed, required no additional equipment and all reagents were pre-dispensed. It is available in the form of packs for five different meat species including cow, horse, pig, poultry and sheep. Two species specific kits were imported from United Kingdom, Bio-Check Company, the poultry specific kits **(Catalouge no. R6058, Blue)** and the horse specific kits **(Catalouge no. R6051, Orange)**. Each pack contained five tests for each species.

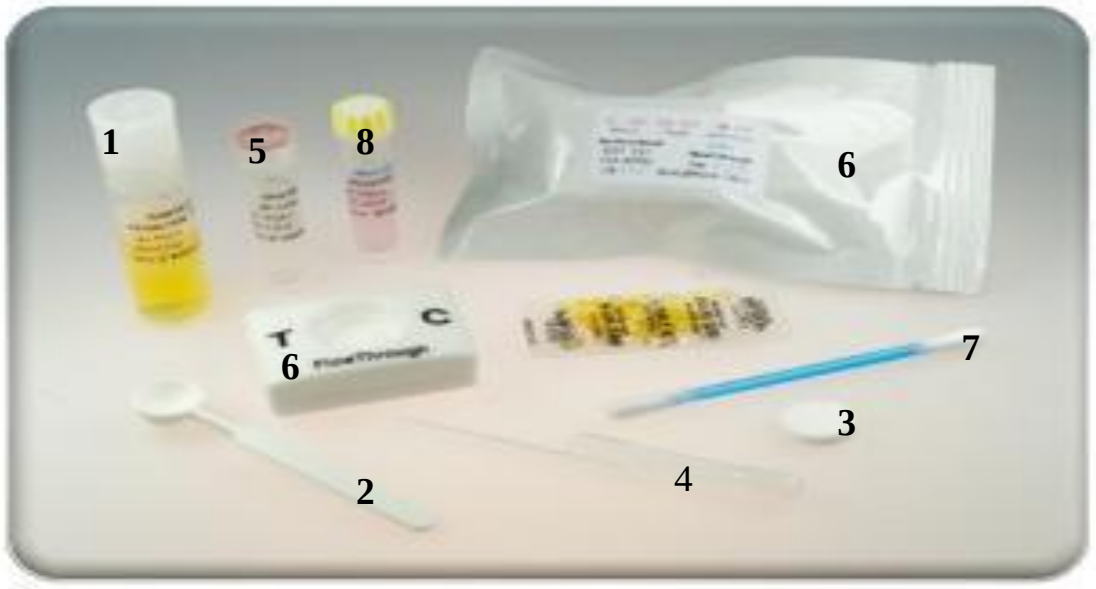
For the poultry specific kits, 4 samples from those previously examined by PCR technique were chosen to be retested, in addition to one sample of raw chicken meat which was used as positive control. The chosen samples were

represented as 1 each of hawawshi, sausage and beef burger samples from those that previously proved their adulteration with chicken species, in addition to one chicken burger sample which considered adulterated as did not contain chicken species.

Likewise, for horse specific kits, 4 samples (1 each of hawawshi, sausage, beef burger and chicken burger) were chosen to be retested from those previously examined by PCR technique and proved their adulteration with equine species. In addition to, one sample of horse meat was used as positive control.

Luncheons meat could not be tested as it is cooked and the FlowThrough™ Test acts on raw meat only. The components of a single test were as shown in **table G:**

No.	Quantity	Contents
1	**1**	**Push-cap tube with yellow extraction solution.**
2	**1**	**Sample scoop, 0.5cc.**
3	**1**	**Separation disc.**
4	**1**	**Self-measuring pipette.**
5	**1**	**Screw-cap tube containing diluent liquid**
6	**1**	**RMFT unit in foil pouch with desiccant.**
7	**1**	**Cotton bud.**
8	**1**	**Pink Colour Reagent in coloured cap tube.**



Species detection was performed as the manufacturer's instructions described below; it typically had taken 12 minutes to be done (included 2 minutes for extraction):

Sample Preparation:

Ground or minced meats required no further preparations.

Extraction:

- The sample scoop was filled with a portion of the sample (0.50g).

- The cap was removed from the yellow extraction solution tube and the sample was added by slowly applying downward pressure on the handle. Then the tube was recapped.
- The extraction tube was shaked vigorously for 1 minute.

- The cap was removed from the yellow extraction solution tube and the separation disc was placed into the tube so as it was level and flush with inside walls.
- The disc was carefully pushed down into extract using scoop handle to separate liquid from meat paste.

Dilution:

- The upper bulb of a clean self measuring pipette was tightly squeezed.

- The pipette tip was inserted into the liquid sample extract above the disc and slow pressure was released on the bulb until the solution overfilled the pipette tube into the lower bulb.

- The cap was removed from the diluent liquid tube and the pipette tip was inserted into it with squeezing the upper bulb to add the extract to the diluent tube.

- Diluent tube was recaped and gently inverted several times to mix.

- RMFT unit was removed from the pouch.

Addition of Diluted Extract:

- The cap from the diluent tube containing extract was removed and the diluted extract was carefully added to the well of the RMFT unit.

- About 5 minutes were waited until diluted extract was completely absorbed in to the RMFT unit.

- Any particulates and liquid on the test area surface and around the rim of the RMFT unit well were gently removed by using both ends of a clean cotton bud.

Addition of Colour Reagent*:*

- The cap was removed from the pink Colour reagent tube and the contents were carefully added to the well of the RMFT unit.
- About 5 minutes were waited until the pink color reagent was completely absorbed into the well of the RMFT unit.

Reading the Result:

- The appearance of a clearly visible, pink Test spot on the left of the test area (T) indicates the presence of meat at about 1% or more in the sample being tested.
- A pink control spot of medium intensity should always appear on the right hand (C) side of the test area; this indicated that the extract was suitable, the test had been performed correctly and all reagents are functional. If a control spot did not appear, the result was invalid and should be repeated.

IV. Statistical analysis

Data obtained was subjected to statistical analysis using a computer program system **(SPSS, version 16)**. Means were compared using least significant difference (LSD).

Results

Table 1: Statistical Analytical Results of Proximate Compositions of Hawawshi Samples (n=20):

	Minimum	Maximum	Mean ± S.E.*
Moisture%	**50.98**	**63.41**	**58.18 ± 0.85**
Protein%	**6.23**	**13.32**	**9.61 ± 0.45**
Fat%	**21.24**	**34.95**	**26.36 ± 1.00**
Ash%	**1.79**	**3.15**	**2.58 ± 0.09**
Carbohydrate%	**0.23**	**6.63**	**3.26 ± 0.39**
Red Meat Content %	**25.09**	**58.26**	**40.32 ± 2.11**

***S.E.**= Standard error*

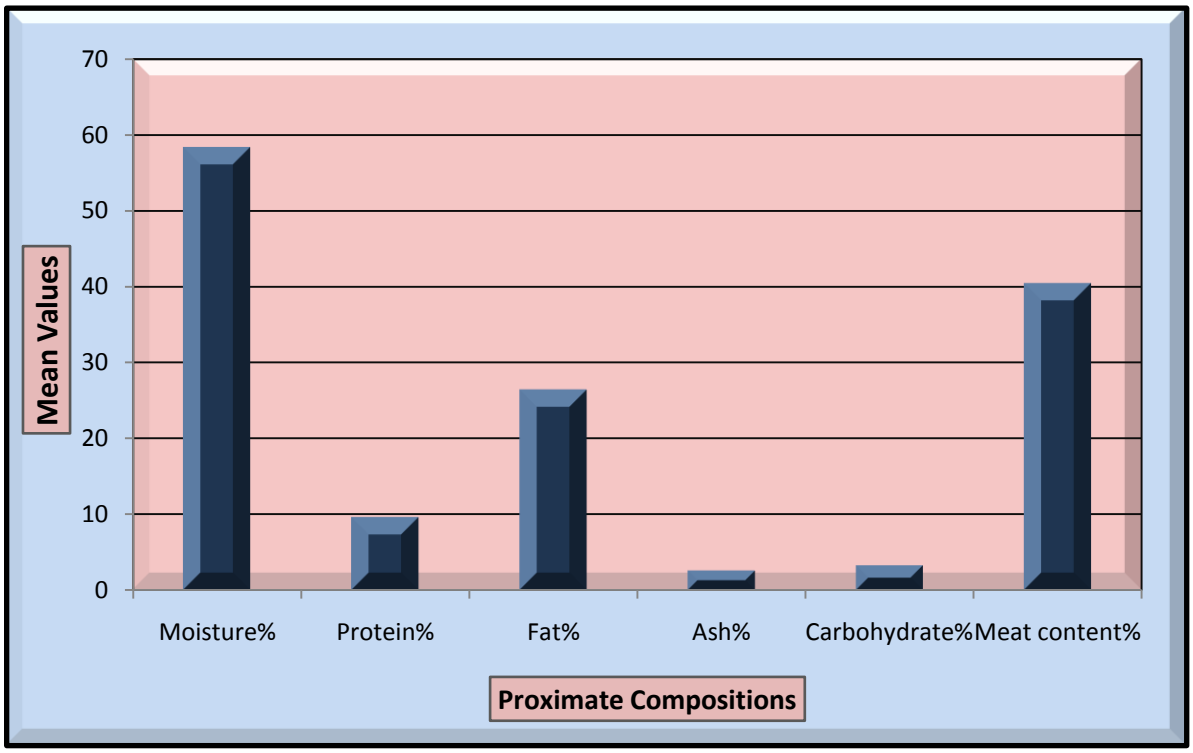


Figure 1: Mean Values of Proximate Compositions of Hawawshi Samples:

Table 2: Statistical Analytical Results of Proximate Compositions of Beef Oriental Sausage Samples (n=20):

	Minimum	Maximum	Mean ± S.E.*
Moisture %	50.14	72.36	60.56 ± 1.58
Protein %	10.14	18.56	15.56 ± 0.55
Fat %	8.24	29.45	19.14 ± 1.67
Ash %	1.50	4.59	3.13 ± 0.21
Carbohydrate %	0.03	6.33	1.58 ± 0.41
Red Meat Content %	41.99	81.32	67.33 ± 2.56

***S.E.**= Standard error*

Table 3: Acceptability of Beef Oriental Sausage Samples According to the Egyptian Standards for their Proximate Compositions (n=20):

	EOS* (1972/2005)	Accepted Samples		Non Accepted Samples	
		No.	%	No.	%
Moisture%	Not more than 60%	10	50	10	50
Protein%	Not less than 15%	14	70	6	30
Fat%	Not more than 30%	20	100	0	0
Ash%	Not more than 5%	20	100	0	0
Carbohydrate%	------	---	----	---	-----
Red Meat Content%	Not less than 60%	15	75	5	25

EOS * = Egyptian Organization for Standardization

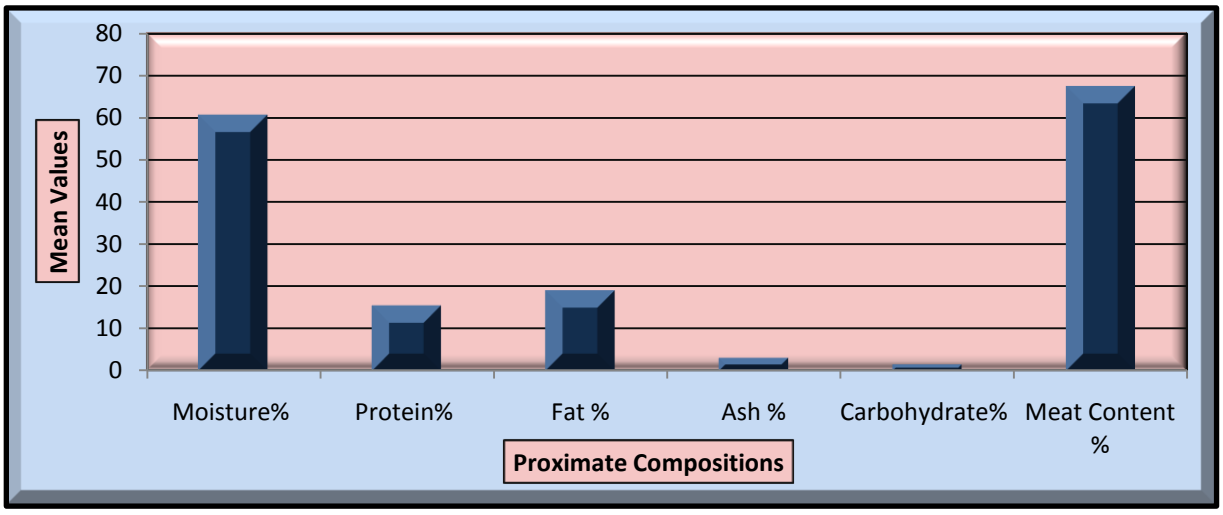


Figure 2: Mean Values of Proximate Compositions of Beef Oriental Sausage Samples:

Table 4: Statistical Analytical Results of Proximate Compositions of Beef Luncheon Samples (n=20):

	Minimum	Maximum	Mean ± S.E.*
Moisture%	56.44	69.11	62.52 ± 0.95
Protein%	3.45	15.71	8.49 ± 0.86
Fat%	3.11	8.56	5.37 ± 0.32
Ash%	2.47	4.75	3.13 ± 0.12
Carbohydrate%	10.00	30.32	20.47 ±1.49
Red Meat Content%	0.33	62.81	26.02 ± 4.45

***S.E.**= Standard error*

Table 5: Acceptability of Beef Luncheon Samples According to the Egyptian Standards for their Proximate Compositions (n=20):

	EOS* (1114/2005)	Accepted Samples		Non Accepted Samples	
		No.	%	No.	%
Moisture%	Not more than 60%	7	35	13	65
Protein%	Not less than 15%	2	10	18	90
Fat%	Not more than 35%	20	100	0	0
Ash%	Not more than 3.5%	15	75	5	25
Carbohydrate%	-------	-----	----	----	----
Red Meat Content%	Not less than 80%	0	0	20	100

EOS * = Egyptian Organization for Standardization

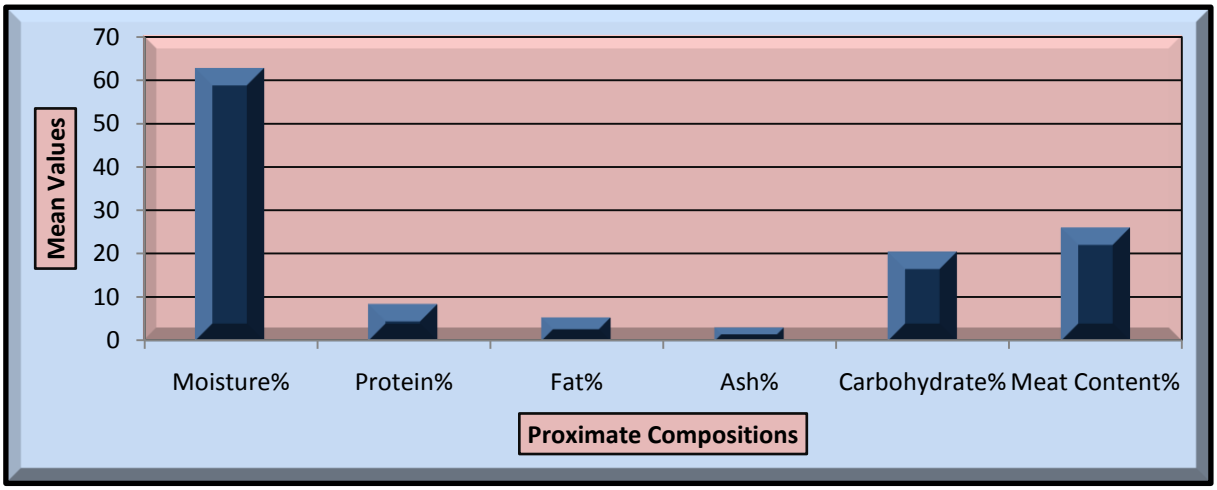


Figure 3: Mean Values of Proximate Compositions of Beef Luncheon Samples:

Table 6: Statistical Analytical Results of Proximate Compositions of Beef Burger Samples (n=20):

	Minimum	Maximum	Mean ± S.E.*
Moisture%	54.95	68.75	61.44 ± 0.79
Protein%	5.96	16.85	11.68 ± 0.74
Fat%	8.95	30.64	16.27 ± 1.50
Ash%	3.04	4.85	3.94 ± 0.12
Carbohydrate%	0.86	12.82	6.65 ± 0.72
Red Meat Content%	23.12	70.60	47.58 ± 3.19

***S.E.**= Standard error*

Table 7: Acceptability of Beef Burger Samples According to the Egyptian Standards for their Proximate Compositions (n=20):

	EOS* (1688/2005)	Accepted Samples		Non Accepted Samples	
		No.	%	No.	%
Moisture%	Not more than 60%	10	50	10	50
Protein%	Not less than 15%	3	15	17	85
Fat%	Not more than 20%	13	65	7	35
Ash%	--------	----	----	----	----
Carbohydrate%	Not more than 10%	15	75	5	25
Red Meat Content%	Not less than 60%	3	15	17	85

EOS * = Egyptian Organization for Standardization

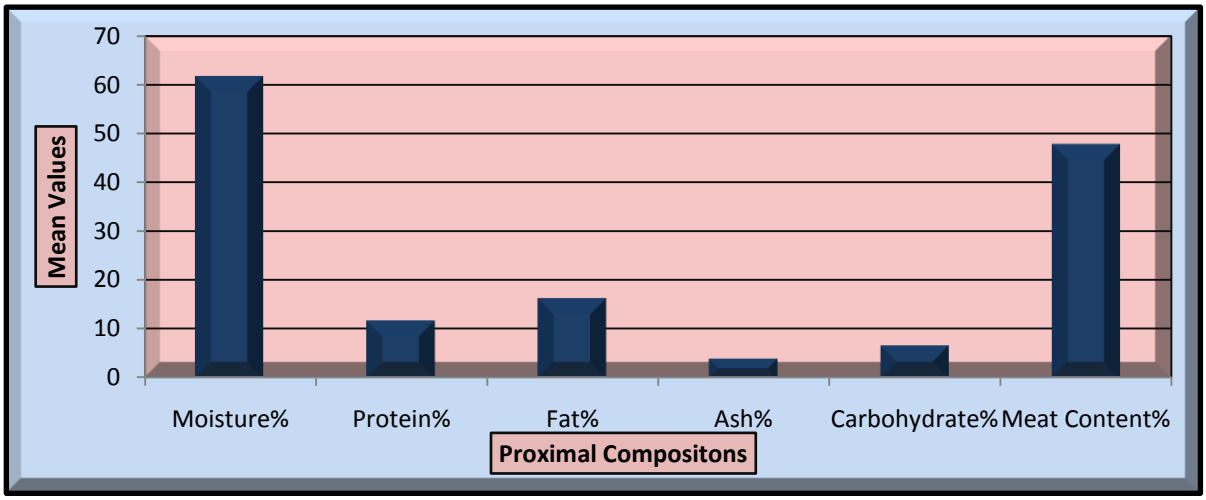


Figure 4: Mean Values of Proximate Compositions of Beef Burger Samples:

Table 8: Statistical Analytical Results of Proximate Compositions of Chicken Luncheon Samples (n=20):

	Minimum	Maximum	Mean ± S.E.*
Moisture%	**63.11**	**71.48**	**67.34 ±0.45**
Protein%	**9.87**	**17.54**	**12.93 ±0.50**
Fat%	**1.89**	**7.84**	**4.13 ±0.38**
Ash%	**2.58**	**3.98**	**3.44 ±0.09**
Carbohydrate%	**7.23**	**15.35**	**12.14 ±0.52**
Red Meat Content%	**36.70**	**75.64**	**52.20 ±2.56**

S.E. = Standard error*

Table 9: Acceptability of Chicken Luncheon Samples According to the Egyptian Standards for their Proximate Compositions (n=20):

	EOS* (1696/2005)	Accepted Samples		Non Accepted Samples	
		No.	%	No.	%
Moisture|%	**Not more than 60%**	**0**	**0**	**20**	**100**
Protein%	**Not less than 12%**	**12**	**60**	**8**	**40**
Fat%	**Not more than 35%**	**20**	**100**	**0**	**0**
Ash%	**Not more than 3.5%**	**10**	**50**	**10**	**50**
Carbohydrate%	**-------**	**---**	**----**	**----**	**----**
Red Meat Content%	**Not less than 80%**	**0**	**0**	**20**	**100**

EOS * = Egyptian Organization for Standardization

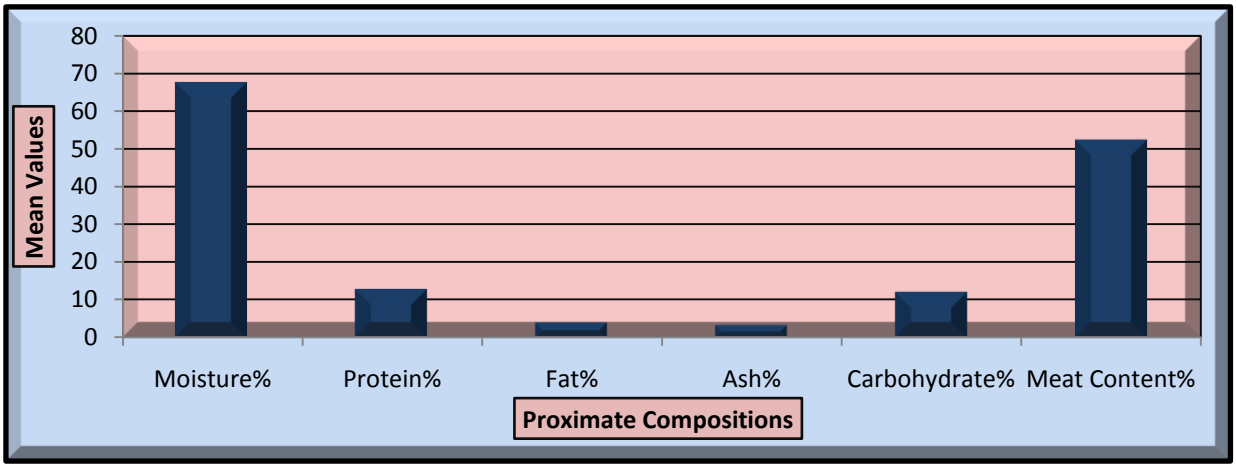


Figure 5: Mean Values of Proximate Compositions of Chicken Luncheon Samples:

Table 10: Statistical Analytical Results of Proximate Compositions of Chicken Burger Samples (n=20):

	Minimum	Maximum	Mean ± S.E.*
Moisture%	**58.74**	**68.45**	**64.26 ± 0.58**
Protein%	**6.48**	**16.82**	**11.72 ± 0.60**
Fat%	**3.54**	**12.45**	**7.70 ± 0.59**
Ash%	**2.24**	**3.98**	**3.21 ± 0.12**
Carbohydrate%	**4.25**	**19.55**	**13.08 ± 0.78**
Red Meat Content%	**18.45**	**74.46**	**46.11 ± 3.08**

S.E. = Standard error*

Table 11: Acceptability of Chicken Burger Samples According to the Egyptian Standards for their Proximate Compositions (n=20):

	EOS* (2910/2005)	Accepted Samples		Non Accepted Samples	
		No.	%	No.	%
Moisture%	**Not more than 70%**	**20**	**100**	**0**	**0**
Protein%	**Not less than 12%**	**8**	**35**	**12**	**65**
Fat%	**Not more than 15%**	**20**	**100**	**0**	**0**
Ash%	**Not more than 2.5%**	**5**	**25**	**15**	**75**
Carbohydrate%	**-------**	**---**	**----**	**----**	**-----**
Red Meat Content%	**Not less than 60%**	**3**	**15**	**17**	**85**

EOS * = Egyptian Organization for Standardization

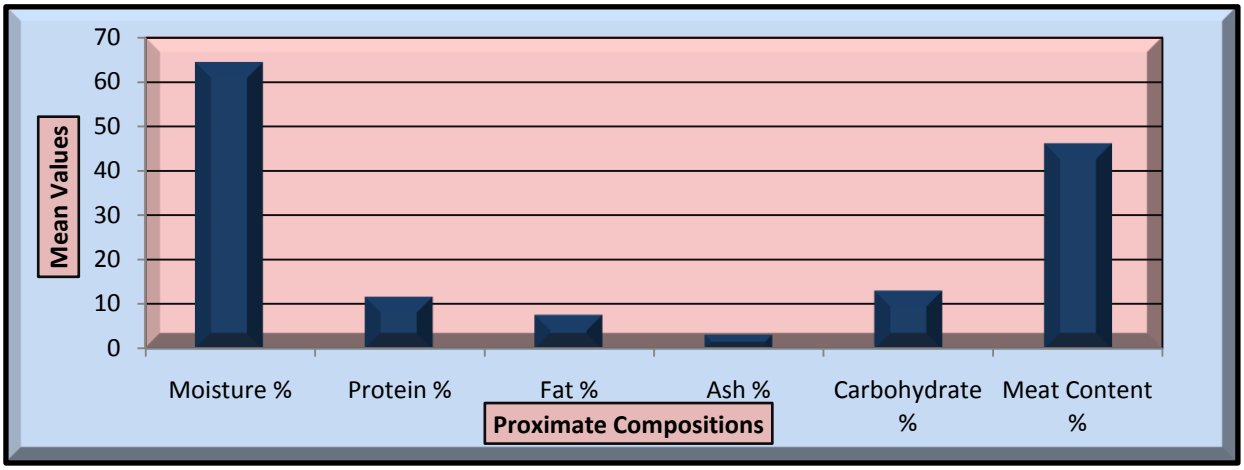


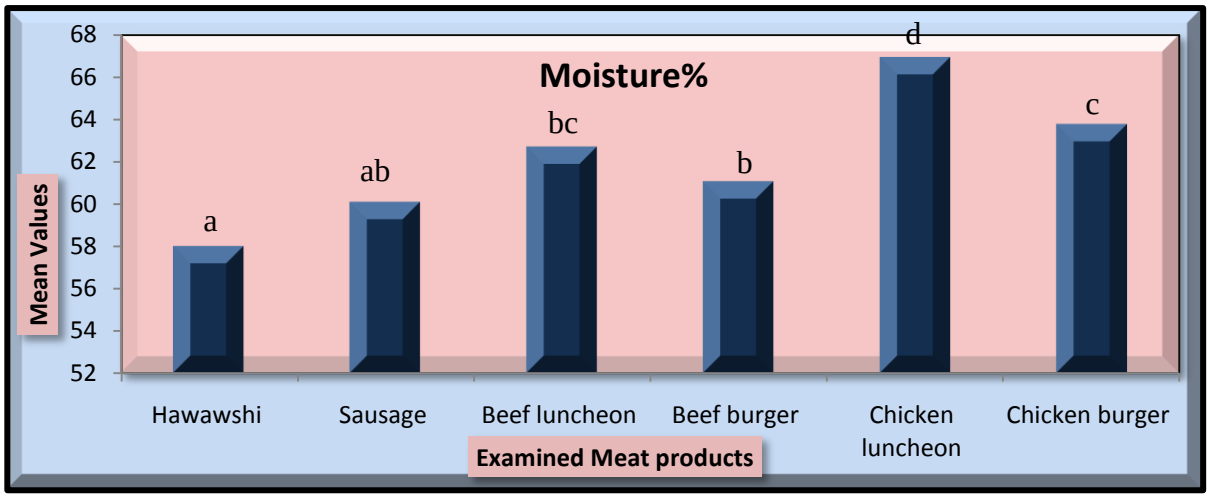
Figure 6: Mean Values of Proximate Compositions of Chicken Burger Samples:

Table 12: The Significant Differences between Mean Values of Chemical Parameters of the Different Examined Meat Products:

	Hawawshi	Sausage	Beef luncheon	Beef burger	Chicken luncheon	Chicken burger
Moisture%	57.99±0.79^{a}	60.06±1.42^{ab}	62.66±0.84^{bc}	61.02±0.81^{b}	66.87±0.44^{d}	63.72±0.57^{c}
Protein%	9.64±0.40^{a}	15.59±0.50^{b}	8.50±0.75^{a}	11.54±0.71^{c}	12.45±0.45^{c}	11.34±0.53^{c}
Fat%	26.54±0.92^{a}	19.54±1.47^{b}	5.25±0.32^{cd}	17.13±1.58^{b}	4.65±0.39^{c}	8.26±0.79^{d}
Ash%	2.65±0.09^{a}	3.24±0.17^{b}	3.07±0.11^{b}	4.01±0.11^{c}	3.40±0.09^{b}	3.17±0.12^{b}
Carbohydrate%	3.18±0.38^{a}	1.56±0.34^{a}	20.49±1.26^{b}	6.29±0.64^{c}	12.63±0.49^{d}	13.49±0.66^{d}
Red Meat content%	40.50±1.92^{a}	67.52±2.31^{b}	26.05±3.84^{c}	47.16±2.99^{ad}	49.67±2.34^{d}	44.14±2.70^{ad}

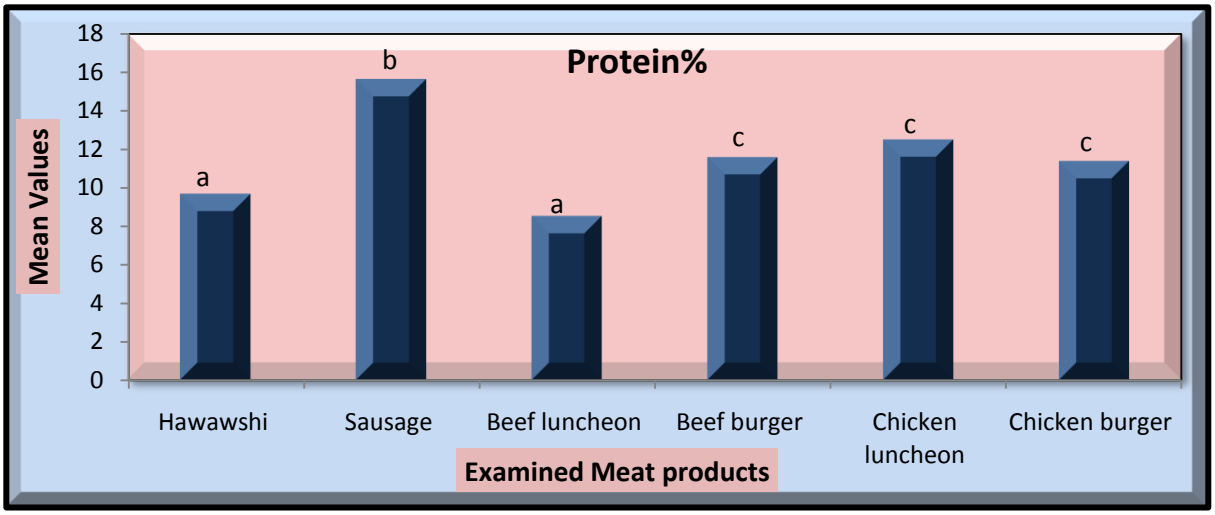
The mean difference is significant at the 0.05 level.

Mean Values with the same letters in each raw are not significant difference.



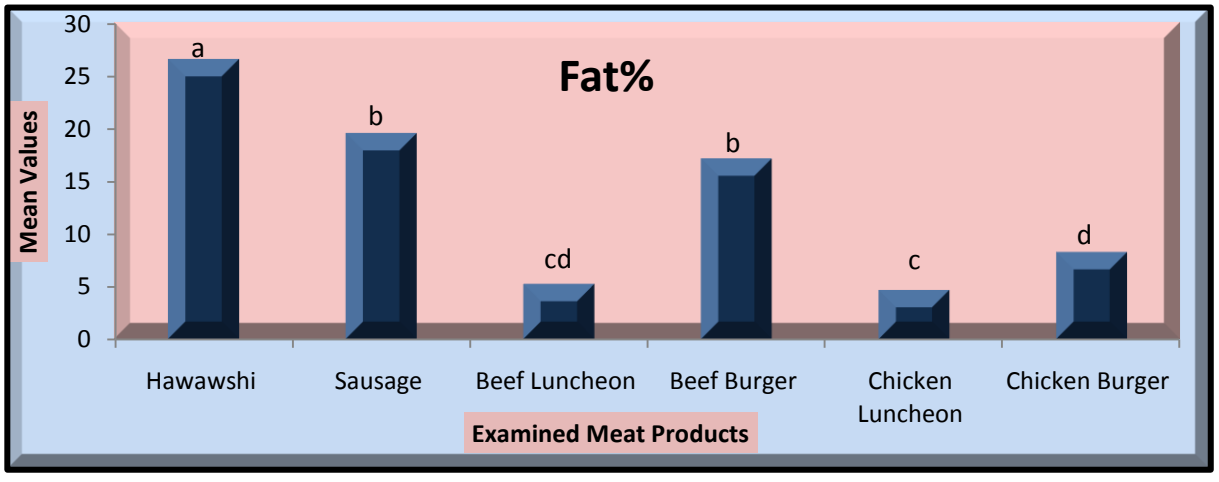
Columns with the same letters are not significant difference.

Figure 7: Mean Values of Moisture Content of the Examined Meat Product Samples:



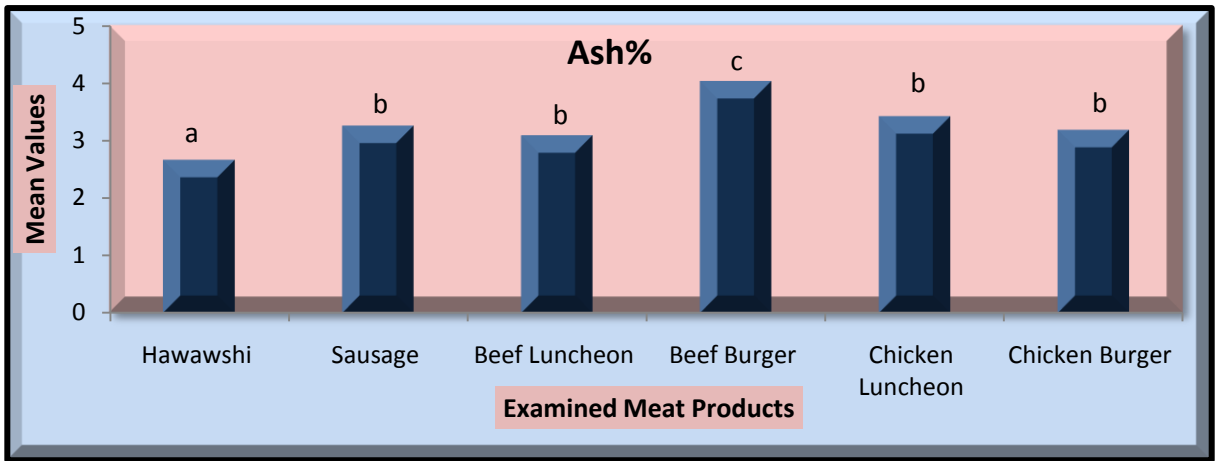
Columns with the same letters are not significant difference.

Figure 8: Mean Values of Protein Content of the Examined Meat Product Samples:



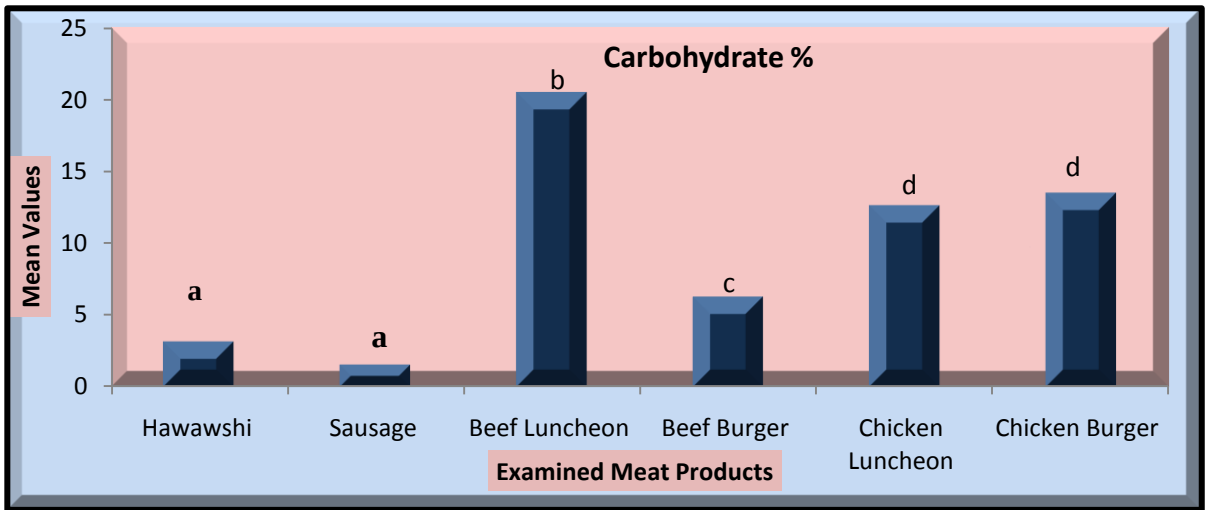
Columns with the same letters are not significant difference.

Figure 9: Mean Value of Fat Content of the Examined Meat Product Samples:



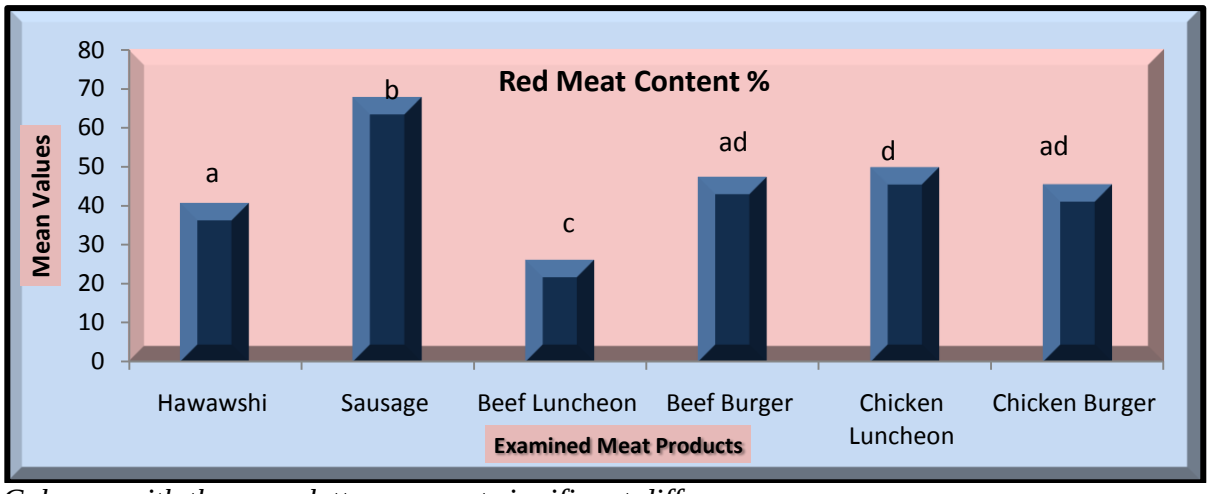
Columns with the same letters are not significant difference.

Figure10: Mean Values of Ash Content of the Examined Meat Product Samples:



Columns with the same letters are not significant difference.

Figure 11: Mean Values Of Carbohydrates Content of the Examined Meat Product Samples:



Columns with the same letters are not significant difference.

Figure12: Mean Values of Red Meat Content of the Examined Meat Product Samples:

Table 13: Detection of Adulteration of Hawawshi Samples with Different Meat Species by Conventional PCR Technique:

Sample No.	Animal Species Detected by PCR					
	Beef	Pig	Equine	Dog	Sheep	Chicken
1	+	-	-	-	-	+
2	+	-	-	-	+	+
3	+	-	-	-	-	-
4	+	-	-	-	+	+
5	+	-	+	-	-	+
6	+	-	-	-	+	+
7	+	-	-	-	+	+
8	+	-	-	-	+	+
9	-	-	-	-	-	+
10	-	-	-	-	+	-
Detected Samples	8	0	1	0	6	8
Percentage of Adulteration	20	0	10	0	60	80

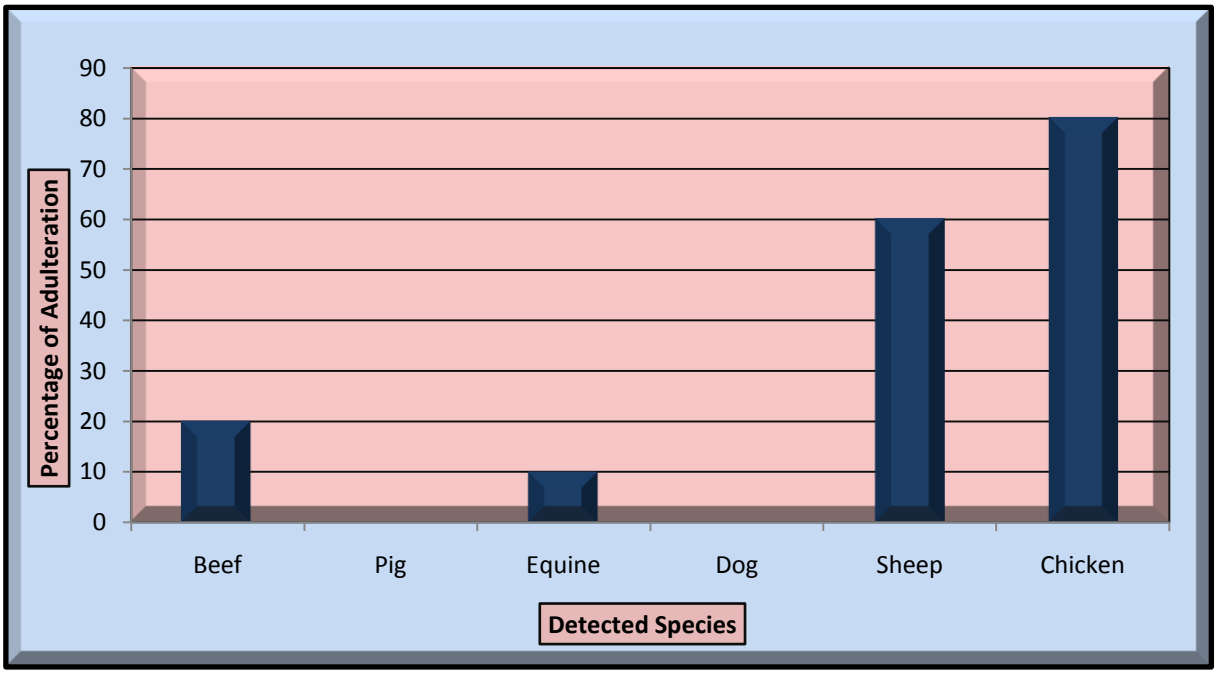


Figure 13: Percentage of Adulteration of Hawawshi Samples by Different Meat Species Detected by Conventional PCR Technique:

Figure 14: Electrophoretic Gel Figures of Adulterated Hawawshi Samples with Different Meat Species Detected by Conventional PCR Technique Where, Lane L: Ladder, Lane P: Positive, Lane N: Negative, Lane 1 to 10: Numbers of Examined Samples:

Figure 14a: Beef Species

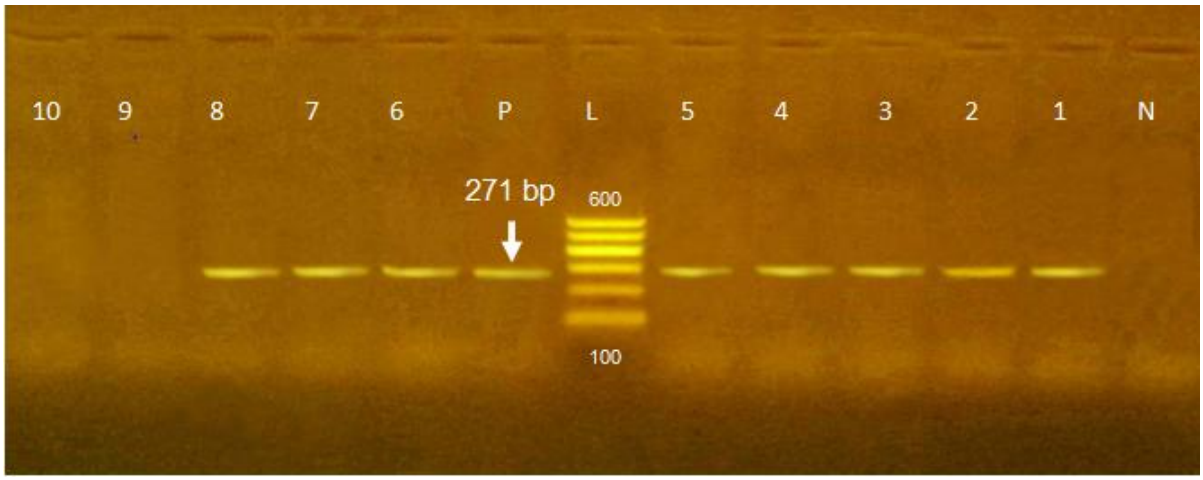


Figure 14b: Pig Species

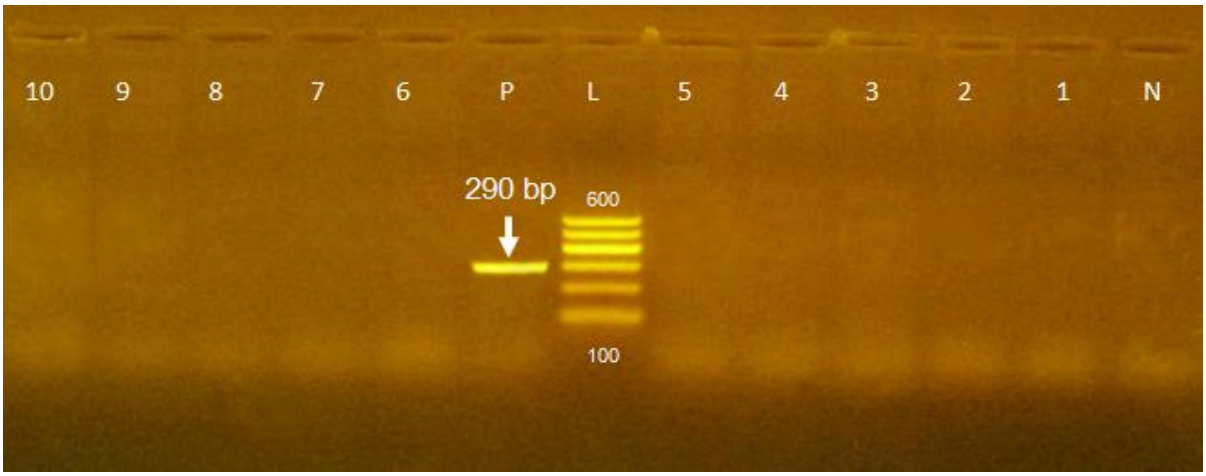


Figure 14c: Equine Species

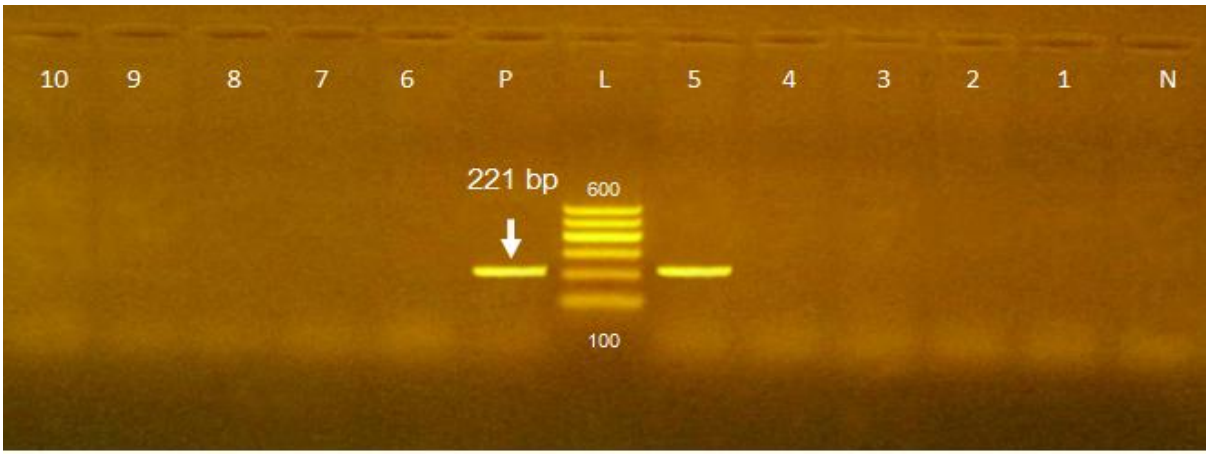


Figure 14d: Dog Species

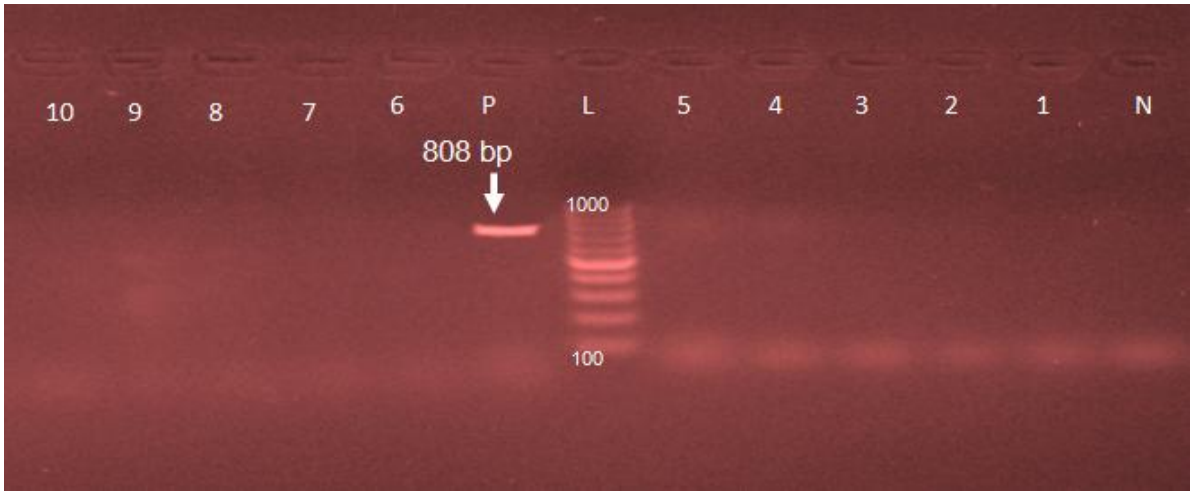


Figure 14e: Sheep Species

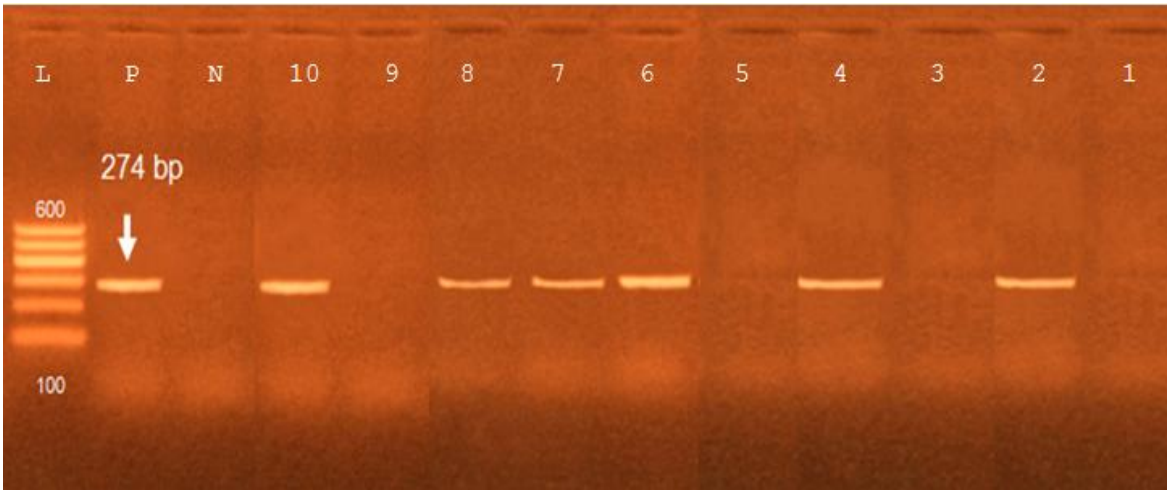


Figure 14f: Chicken Species

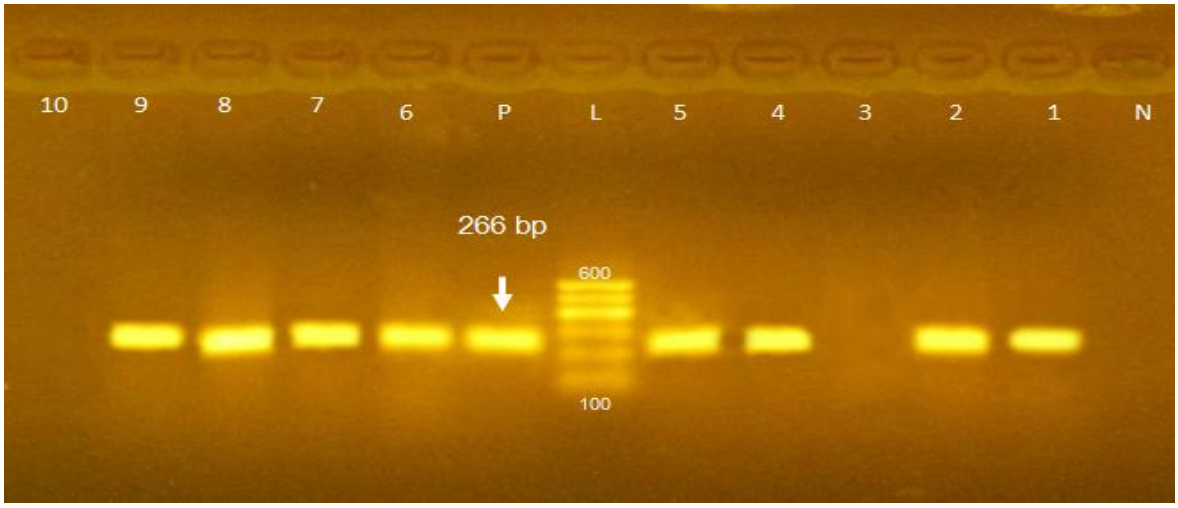


Table 14: Detection of Adulteration of Beef Oriental Sausage Samples with Different Meat Species by Conventional PCR Technique:

Sample No.	Animal Species Detected by PCR					
	Beef	Pig	Equine	Dog	Sheep	Chicken
11	+	-	-	-	+	+
12	+	-	-	-	+	+
13	+	-	-	-	+	-
14	+	-	-	-	+	+
15	+	-	-	-	+	-
16	-	-	-	-	-	-
17	-	-	-	-	+	+
18	+	-	+	-	+	-
19	+	-	-	-	+	+
20	+	-	-	-	-	-
Detected Samples	8	0	1	0	8	5
Percentage of Adulteration	20	0	10	0	80	50

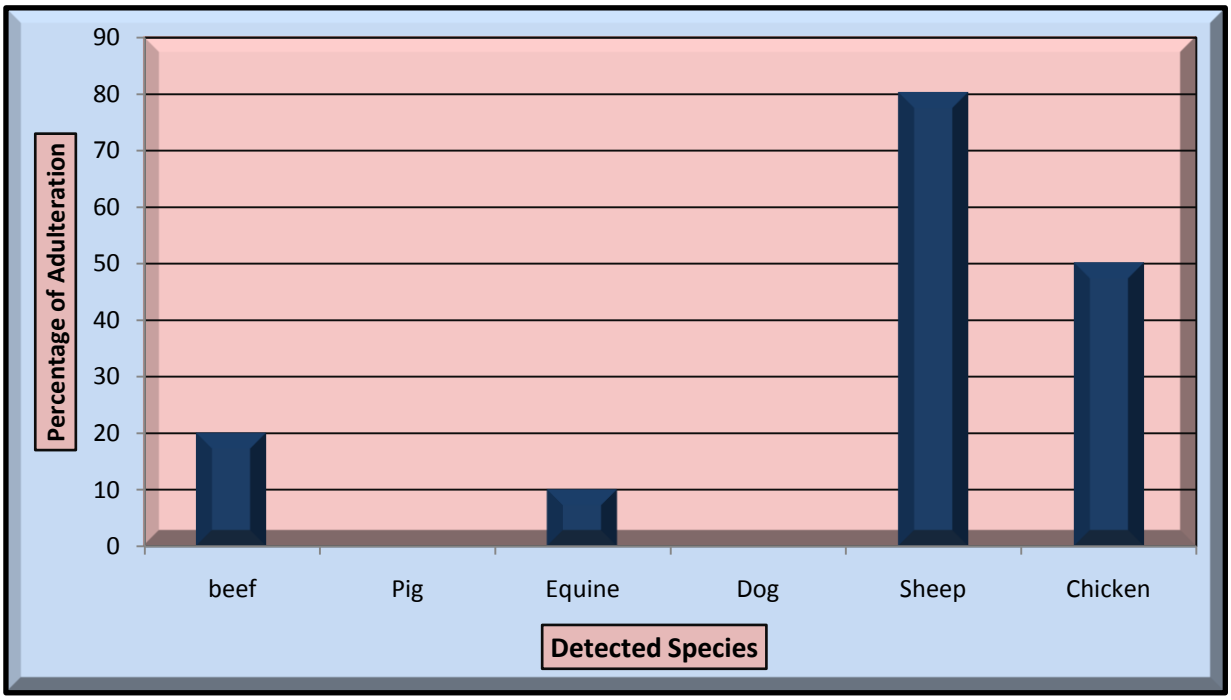


Figure 15: Percentage of Adulteration of Beef Oriental Sausage Samples with Different Meat Species Detected by Conventional PCR Technique:

Figure 16: Electrophoretic Gel Figures of Beef Oriental Sausage Samples of Different Meat Species Detected by Conventional PCR Technique Where, Lane L: Ladder, Lane P: Positive, Lane N: Negative, Lane 11 to 20: Numbers of Examined Samples:

Figure 16a: Beef Species

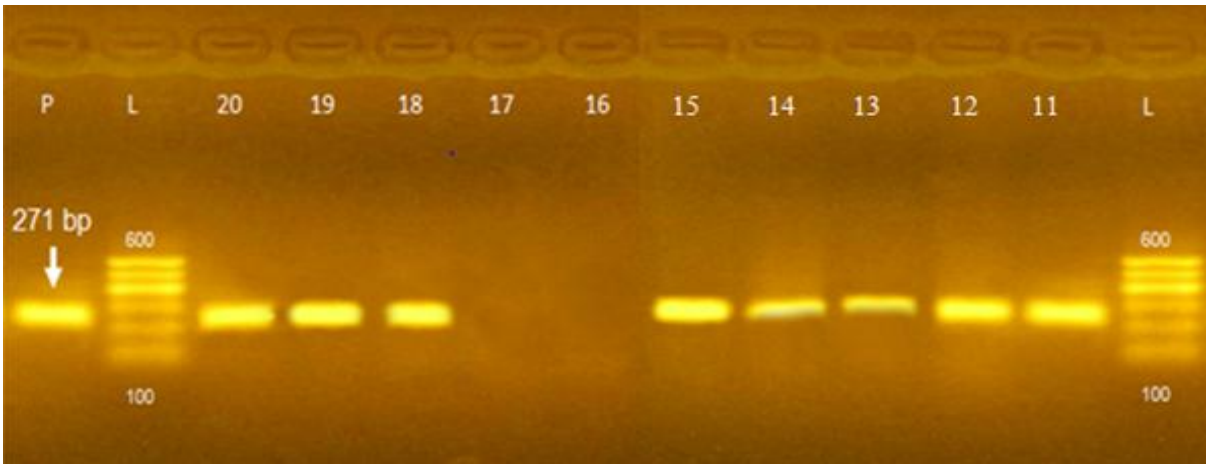


Figure 16b: Pig Species

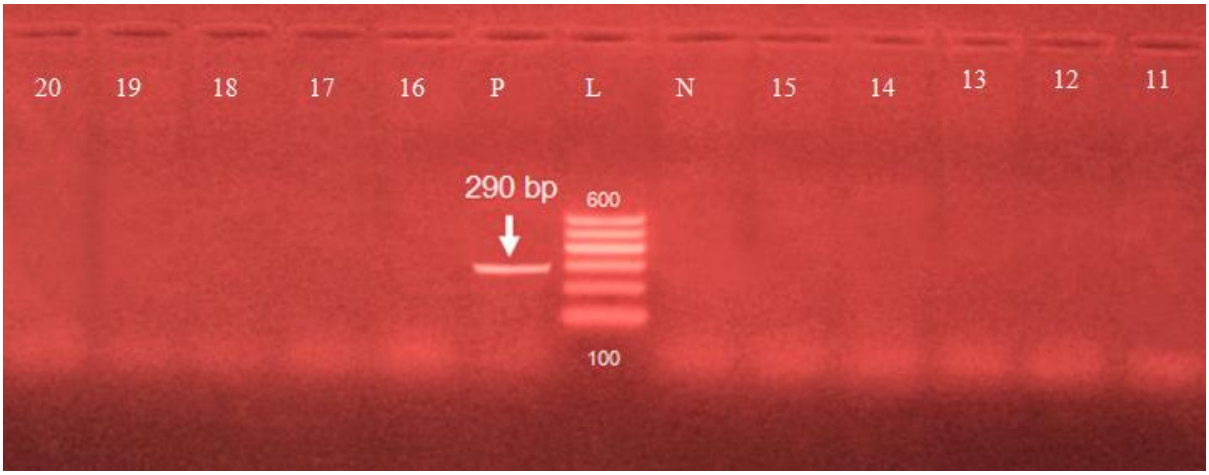


Figure 16c: Equine Species

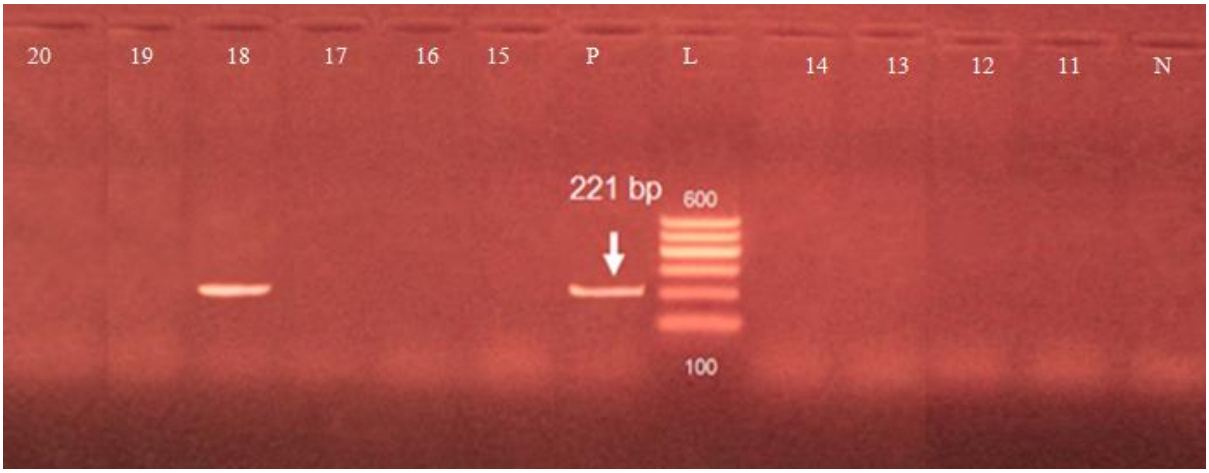


Figure 16d: Dog Species

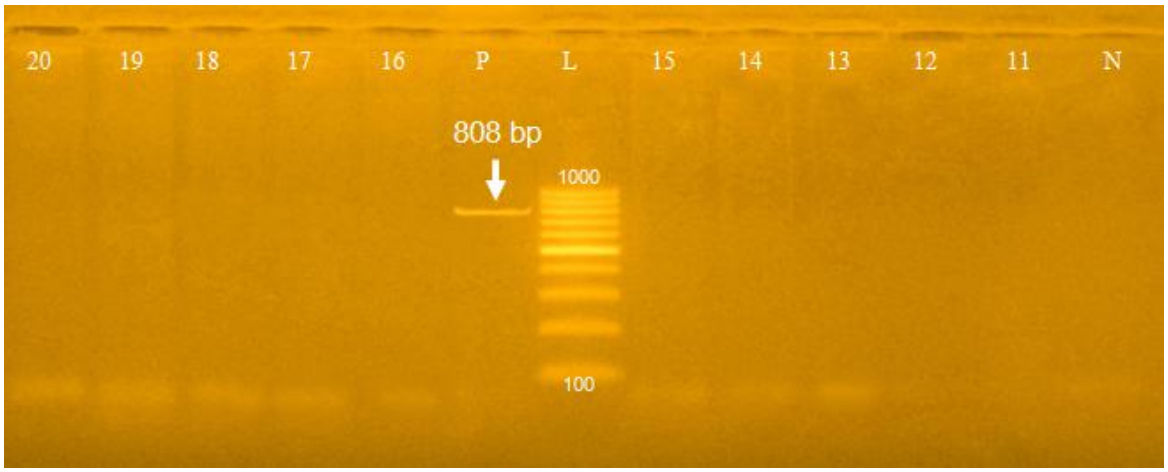


Figure 16e: Sheep Species

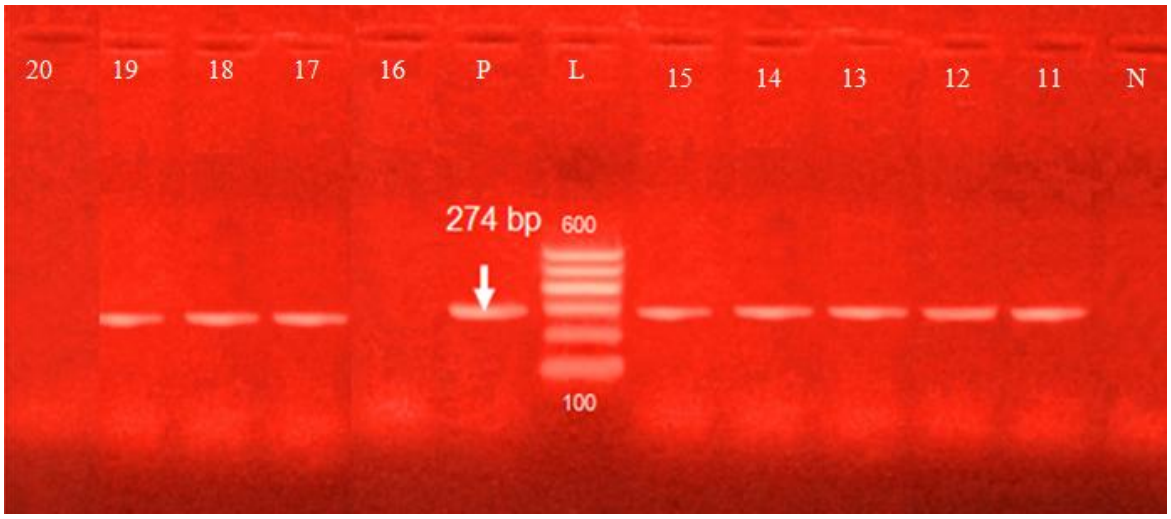


Figure 16f: Chicken Species

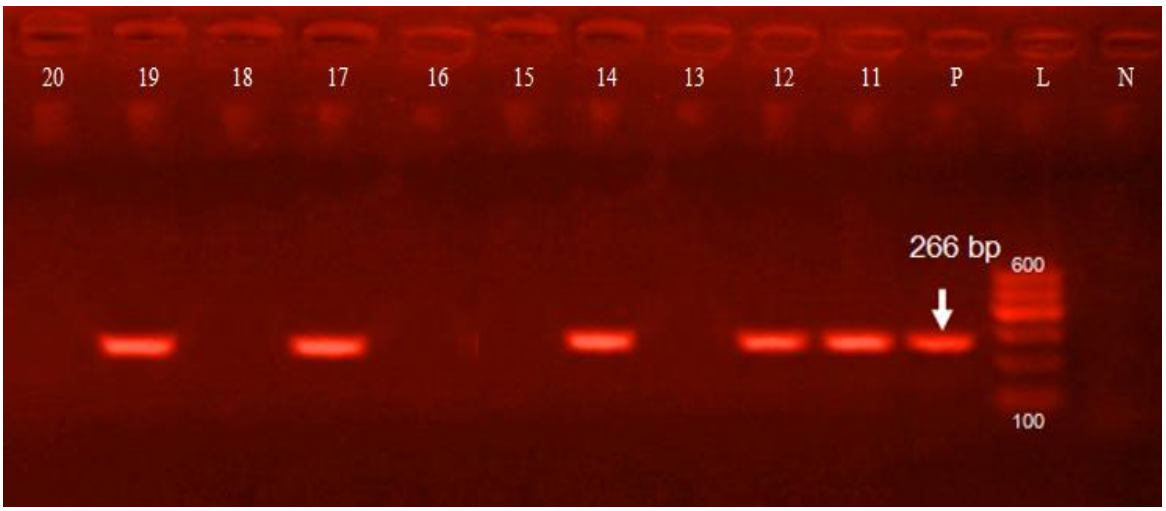


Table 15: Detection of Adulteration of Beef Luncheon Samples with Different Meat Species by Conventional PCR Technique:

Sample No.	Animal Species Detected by PCR					
	Beef	Pig	Equine	Dog	Sheep	Chicken
21	+	-	-	-	-	-
22	+	-	-	+	-	+
23	+	-	-	+	-	-
24	+	-	-	-	+	+
25	+	-	-	-	+	-
26	+	-	-	-	-	+
27	+	-	-	-	-	+
28	+	-	-	-	-	+
29	+	-	+	-	+	+
30	+	-	-	-	-	+
Detected Samples	10	0	1	2	3	7
Percentage of Adulteration	0	0	10	20	30	70

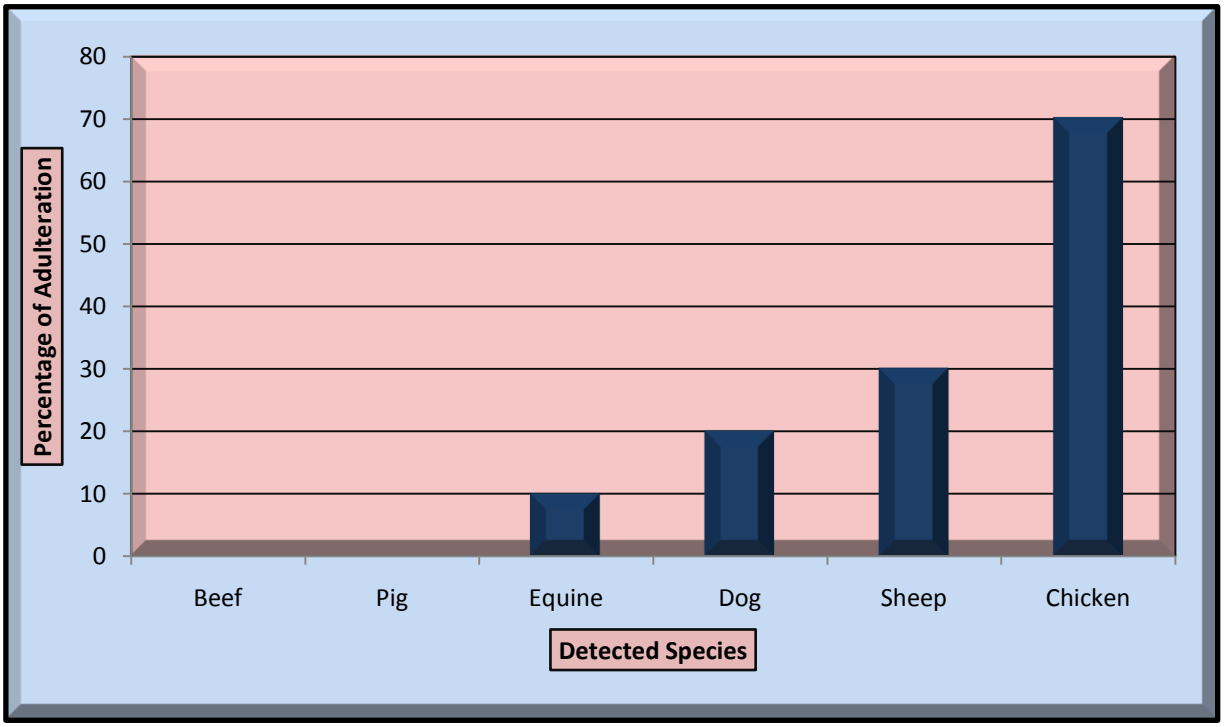


Figure 17: Percentage of Adulteration of Beef Luncheon Samples with Different Meat Species Detected by Conventional PCR Technique:

Figure 18: Electrophoretic Gel Figures of Beef Luncheon Samples of Different Meat Species Detected by Conventional PCR Technique Where, Lane L: Ladder, Lane P: Positive, Lane N: Negative, Lane 21 to 30: Numbers of Examined Samples:

Figure 18a: Beef Species

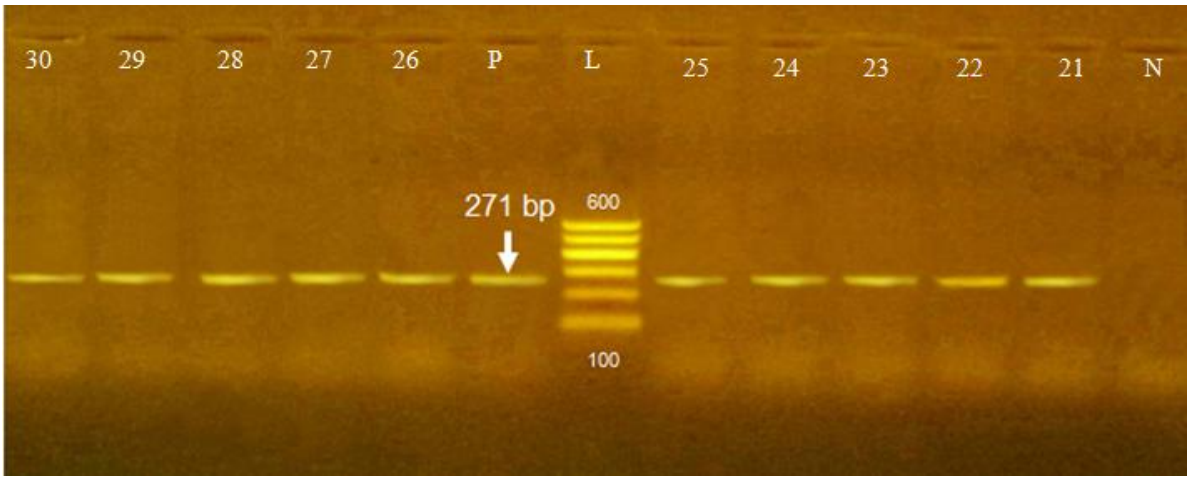


Figure 18b: Pig Species

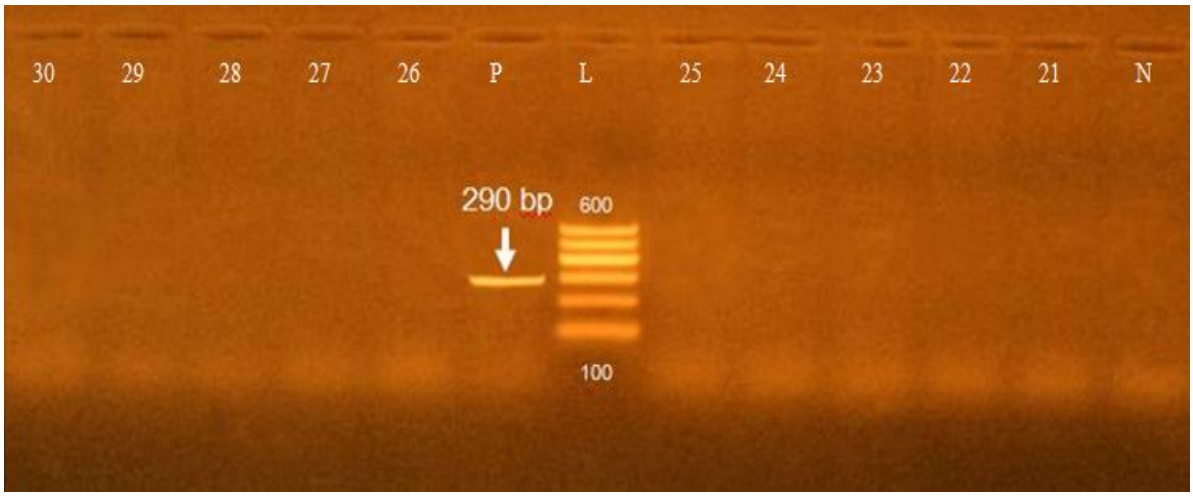


Figure 18c: Equine Species

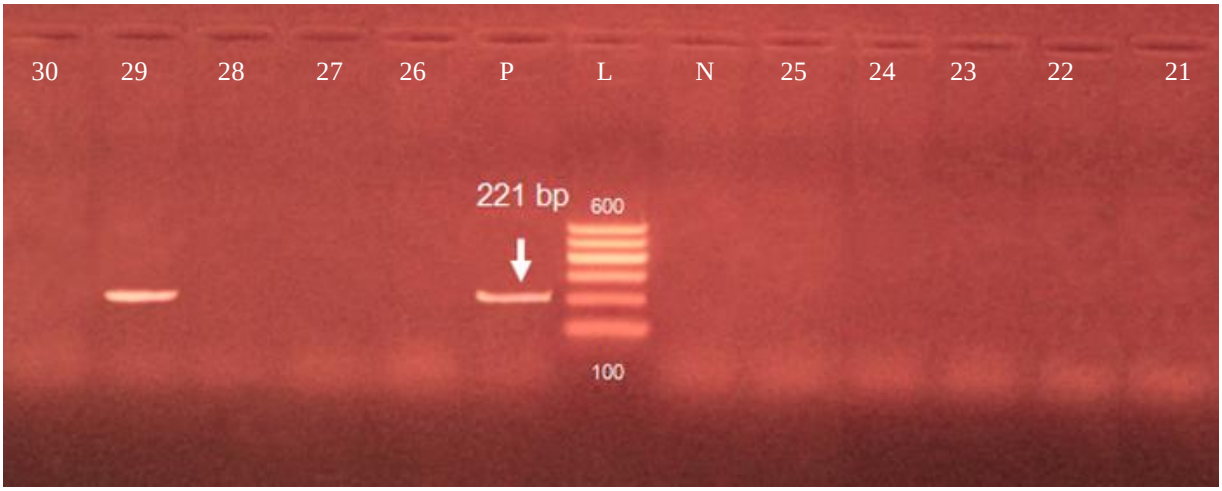


Figure 18d: Dog Species

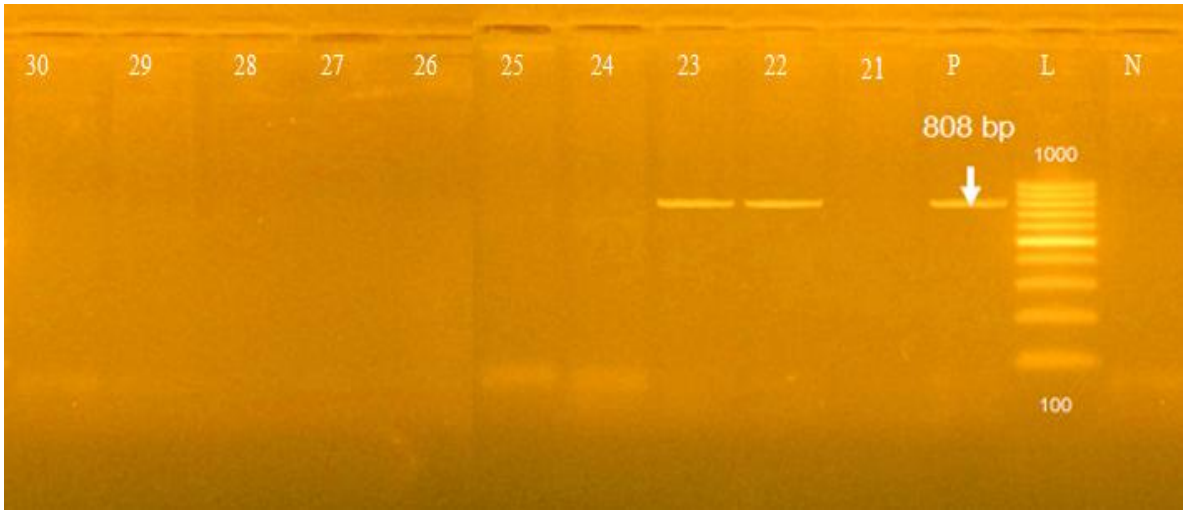


Figure 18e: Sheep Species

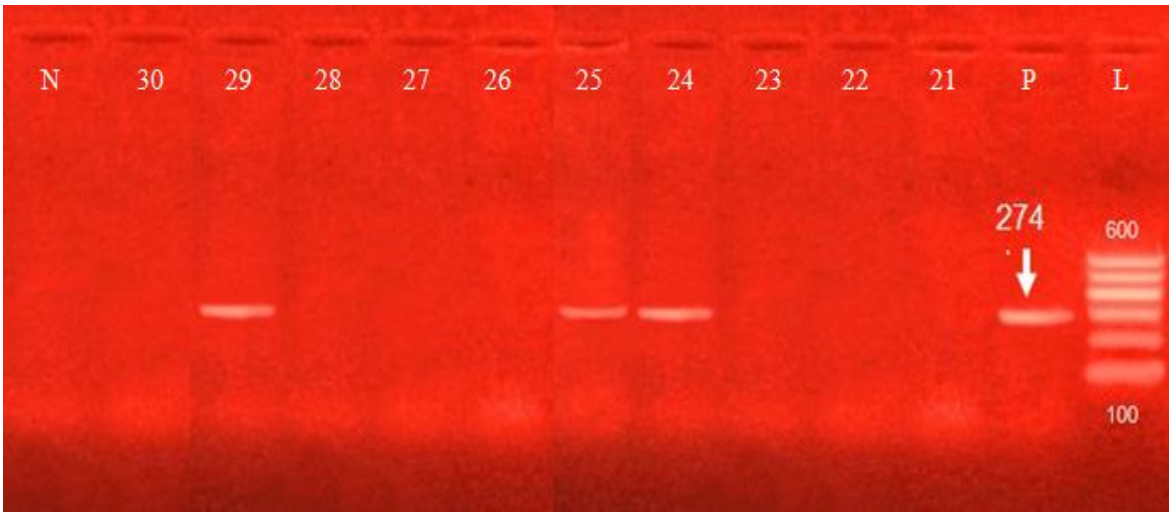


Figure 18f: Chicken Species

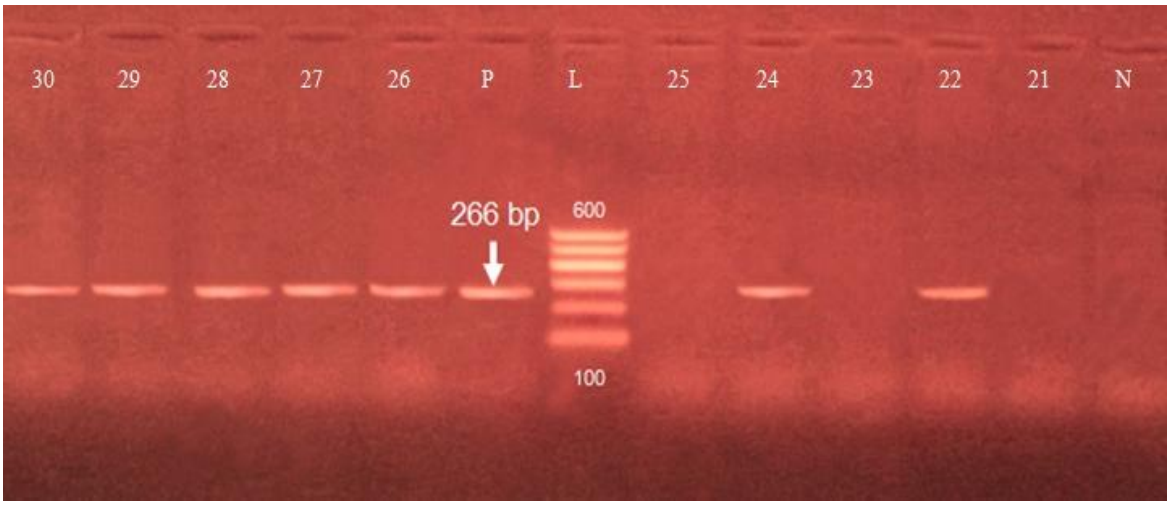


Table 16: Detection of Adulteration of Beef Burger Samples with Different Meat Species by Conventional PCR Technique:

Sample No.	Animal Species Detected by PCR					
	Beef	Pig	Equine	Dog	Sheep	Chicken
31	+	-	-	-	-	+
32	+	-	-	-	-	+
33	+	-	-	-	-	+
34	+	-	-	-	-	+
35	+	-	-	-	+	+
36	+	-	+	-	+	+
37	+	-	+	-	+	+
38	+	-	+	-	+	+
39	+	-	-	-	-	+
40	+	-	-	-	+	+
Detected Samples	10	0	3	0	5	10
Percentage of Adulteration	0	0	30	0	50	100

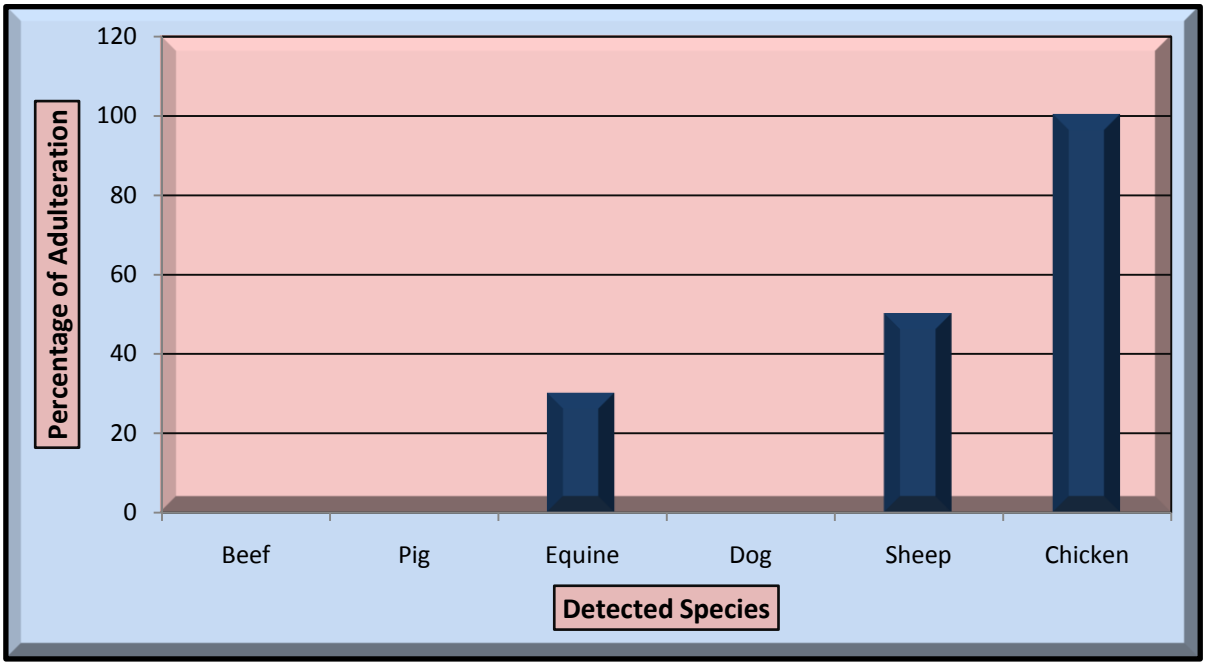


Figure 19: Percentage of Adulteration of Beef Burger Samples with Different Meat Species Detected by Conventional PCR Technique:

Figure 20: Electrophoretic Gel Figures of Beef Burger Samples of Different Meat Species Detected by Conventional PCR Technique Where, Lane L: Ladder, Lane P: Positive, Lane N: Negative, Lane 31 to 40: Numbers of Examined Samples:

Figure 20a: Beef Species

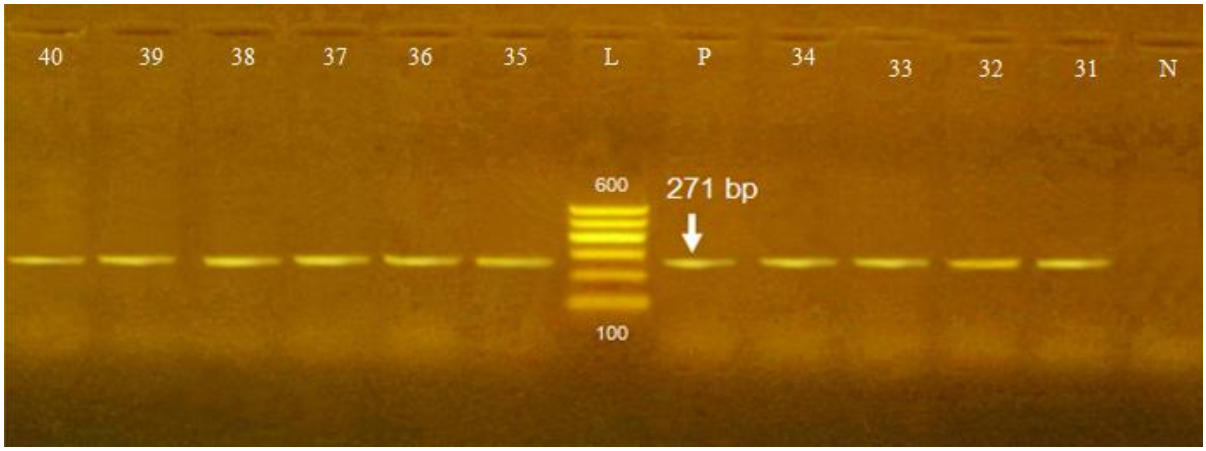


Figure 20b: Pig Species

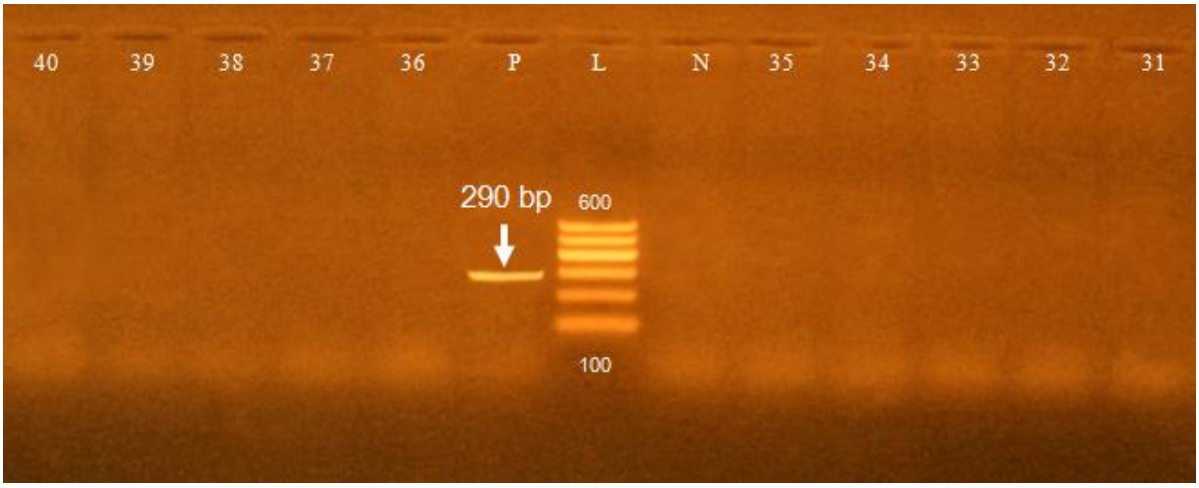


Figure 20c: Equine Species

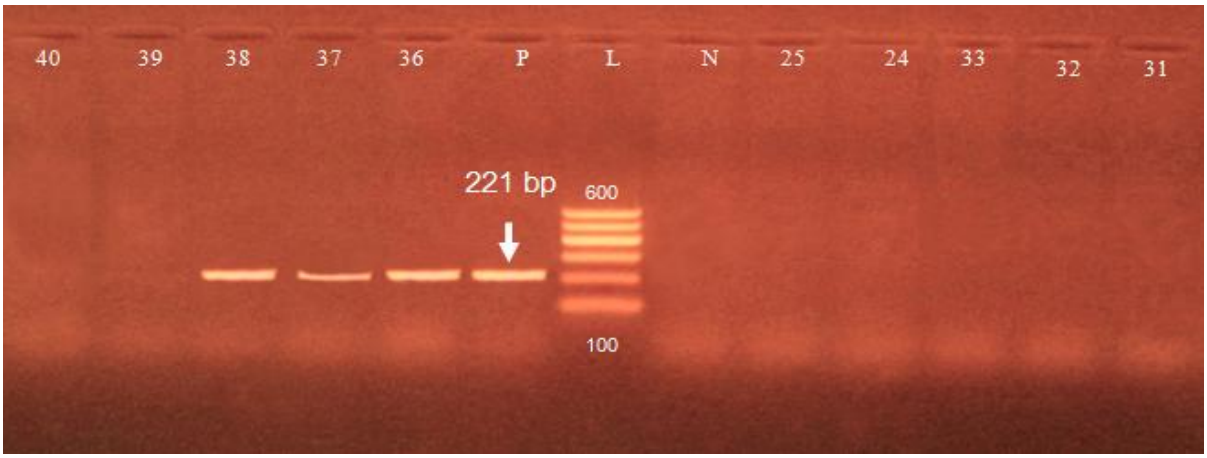


Figure 20d: Dog Species

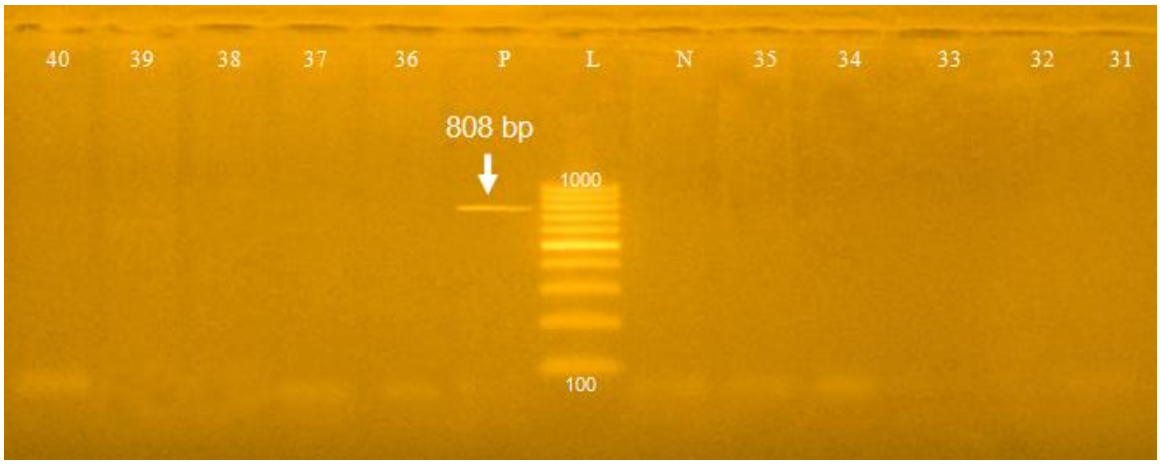


Figure 20e: Sheep Species

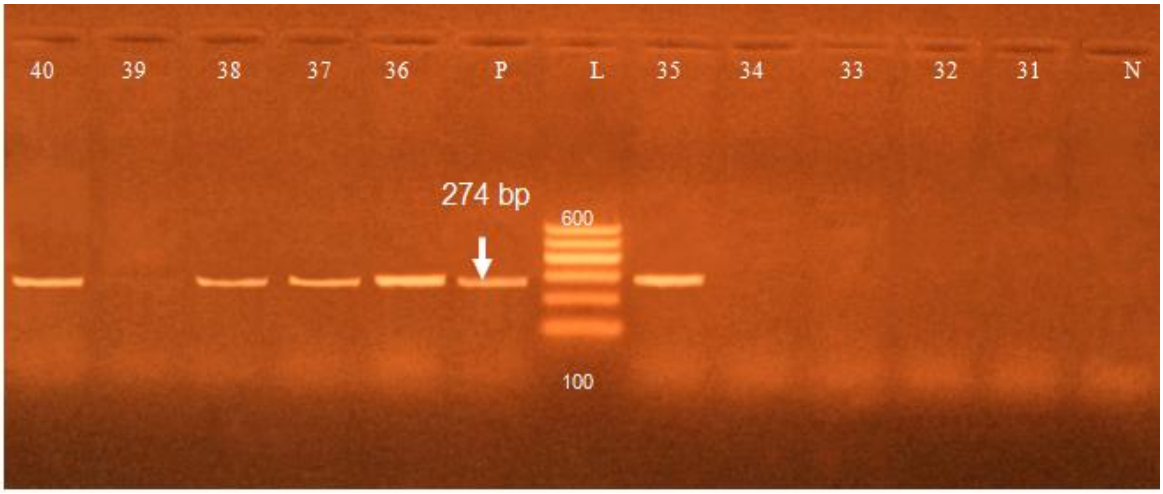


Figure 20f: Chicken Species

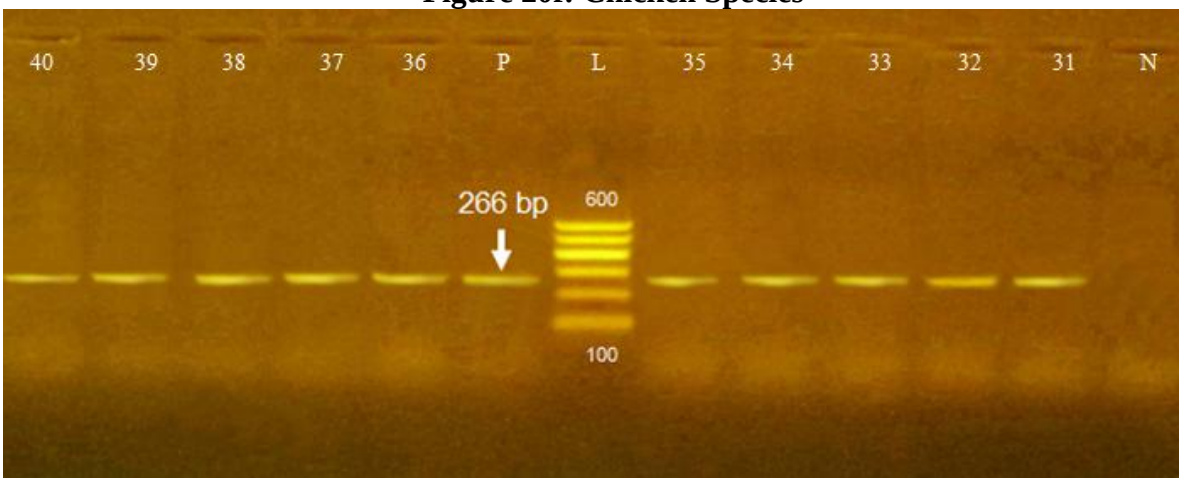


Table 17: Detection of Adulteration of Chicken Luncheon Samples with Different Meat Species by Conventional PCR Technique:

Sample No.	Animal Species Detected by PCR					
	Beef	Pig	Equine	Dog	Sheep	Chicken
41	+	-	-	-	-	+
42	+	-	-	-	-	+
43	+	-	-	+	-	+
44	-	-	-	-	-	-
45	+	-	-	+	-	+
46	+	-	-	-	-	+
47	+	-	-	-	-	+
48	-	-	-	-	-	+
49	-	-	-	-	-	+
50	-	-	-	-	-	+
Detected Samples	6	0	0	2	0	9
Percentage of Adulteration	60	0	0	20	0	10

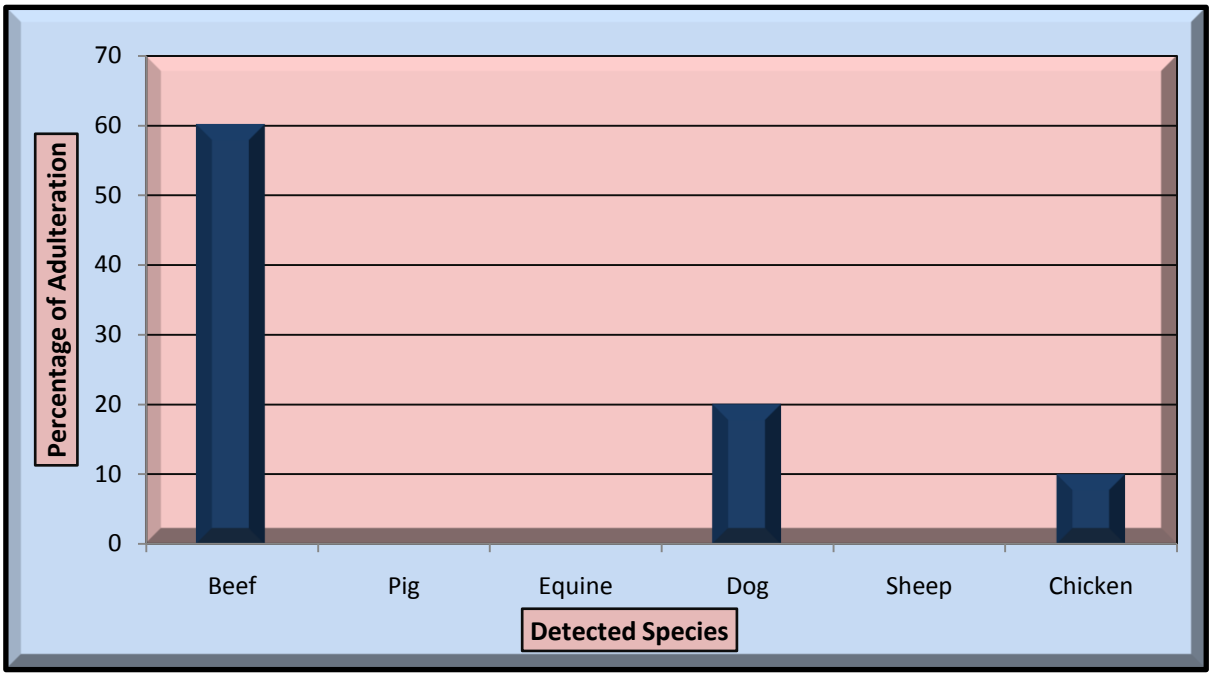


Figure 21: Percentage of Adulteration of Chicken Luncheon Samples by Different Meat Species Detected by Conventional PCR Technique:

Figure 22: Electrophoretic Gel Figures of Chicken Luncheon Samples with Different Meat Species Detected by Conventional PCR Technique Where, Lane L: Ladder, Lane P: Positive, Lane N: Negative, Lane 41 to 50: Numbers of Examined Samples:

Figure 22a: Beef Species

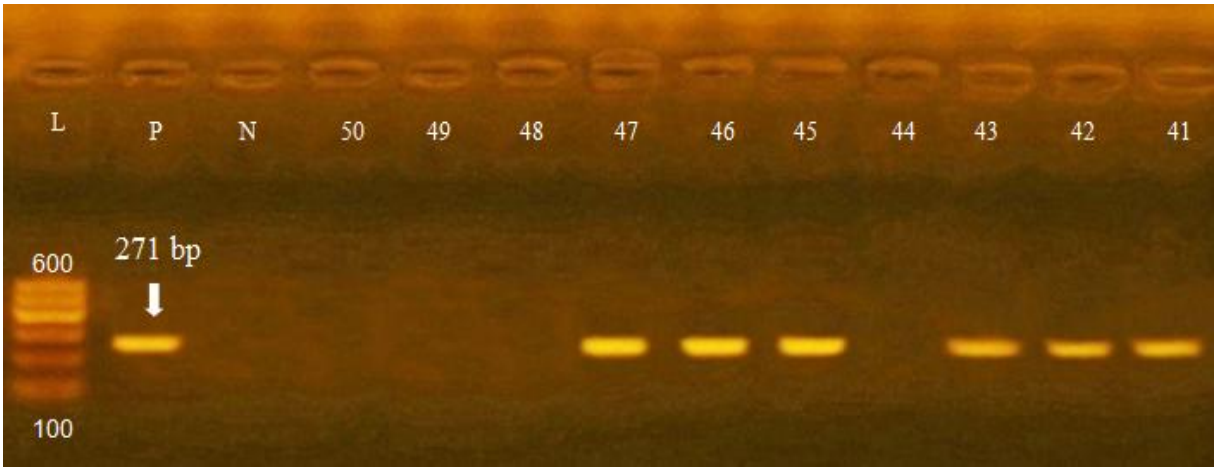


Figure 22b: Pig Species

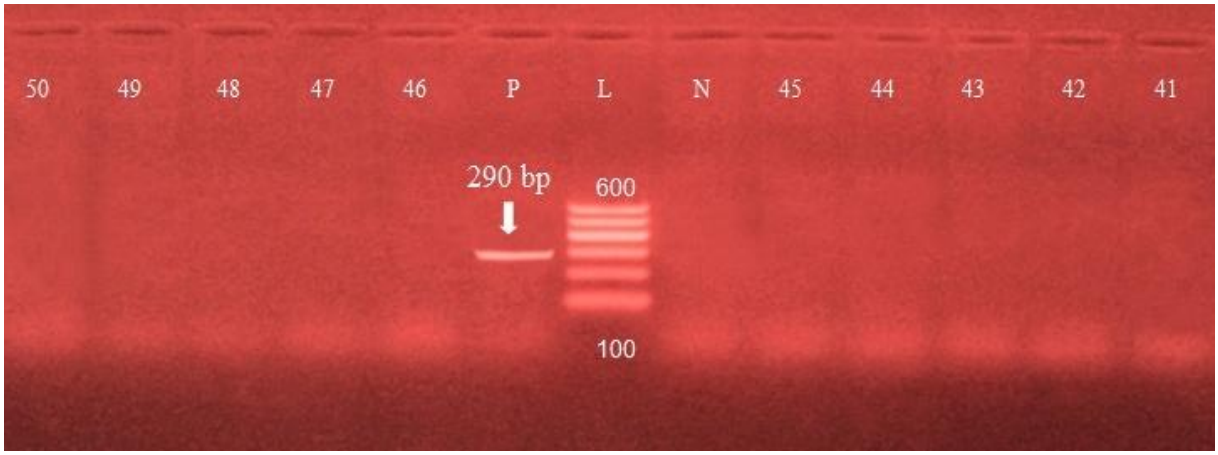


Figure 22c: Equine Species

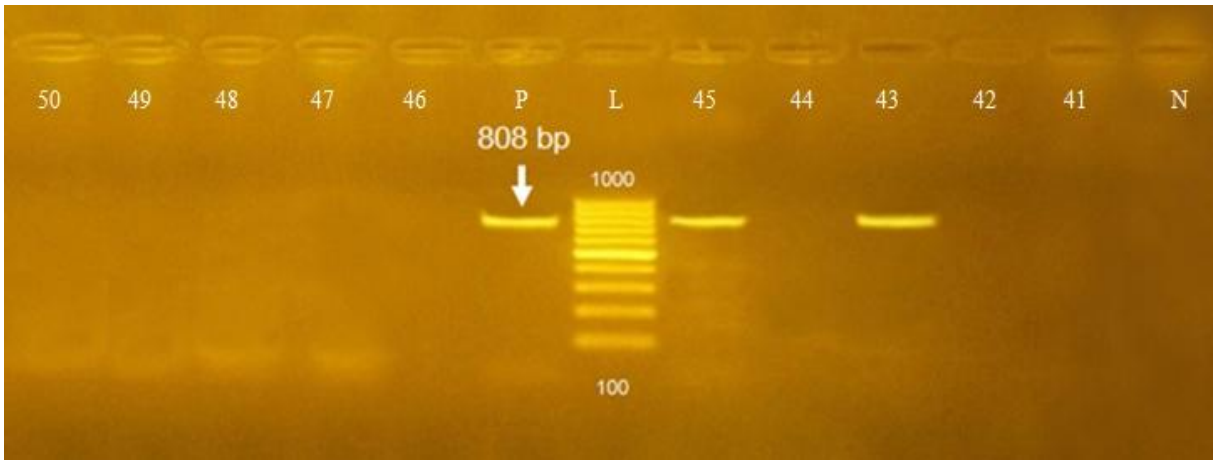


Figure 22d: Dog Species

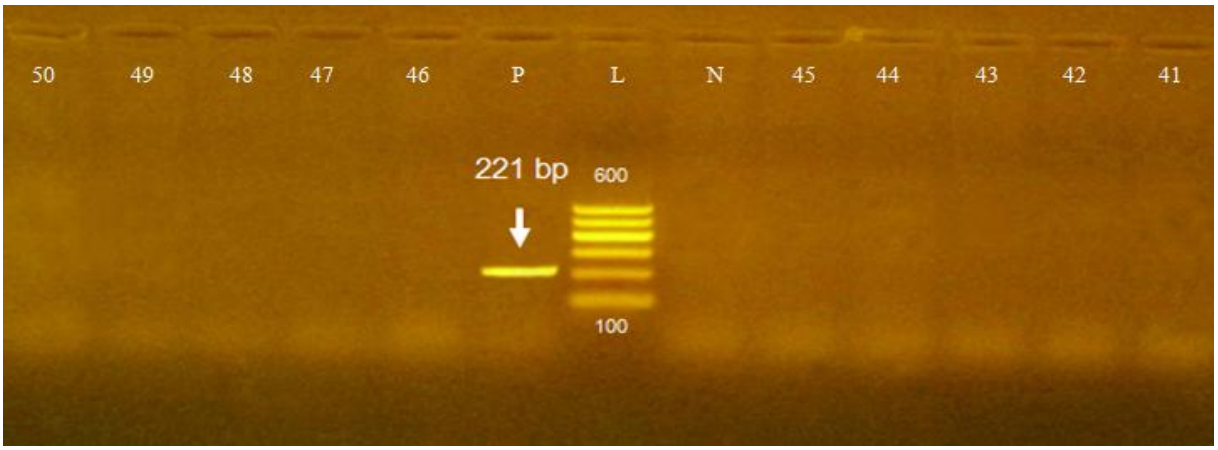


Figure 22e: Sheep Species

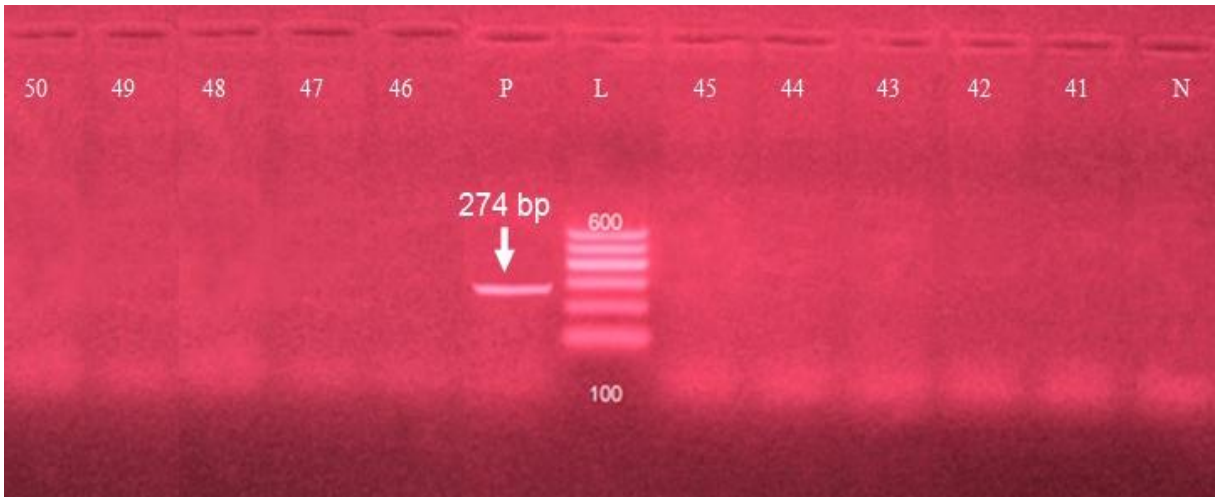


Figure 22f: Chicken Species

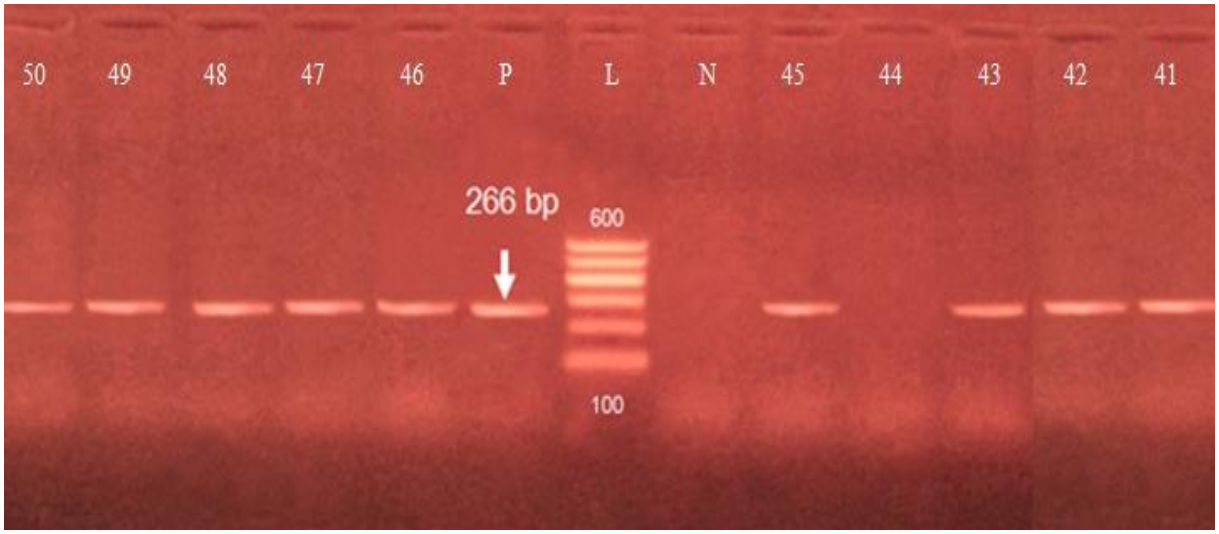


Table 18: Detection of Adulteration of Chicken Burger Samples with Different Meat Species by Conventional PCR Technique:

Sample No.	Animal Species Detected by PCR					
	Beef	Pig	Equine	Dog	Sheep	Chicken
51	+	-	-	+	+	-
52	+	-	-	-	+	+
53	-	-	-	-	-	+
54	+	-	-	-	-	+
55	+	-	-	-	+	+
56	-	-	-	-	+	+
57	-	-	-	-	-	+
58	-	-	-	-	-	+
59	-	-	-	-	-	+
60	-	-	-	-	-	-
Detected Samples	4	0	0	1	4	8
Percentage of Adulteration	40	0	0	10	40	20

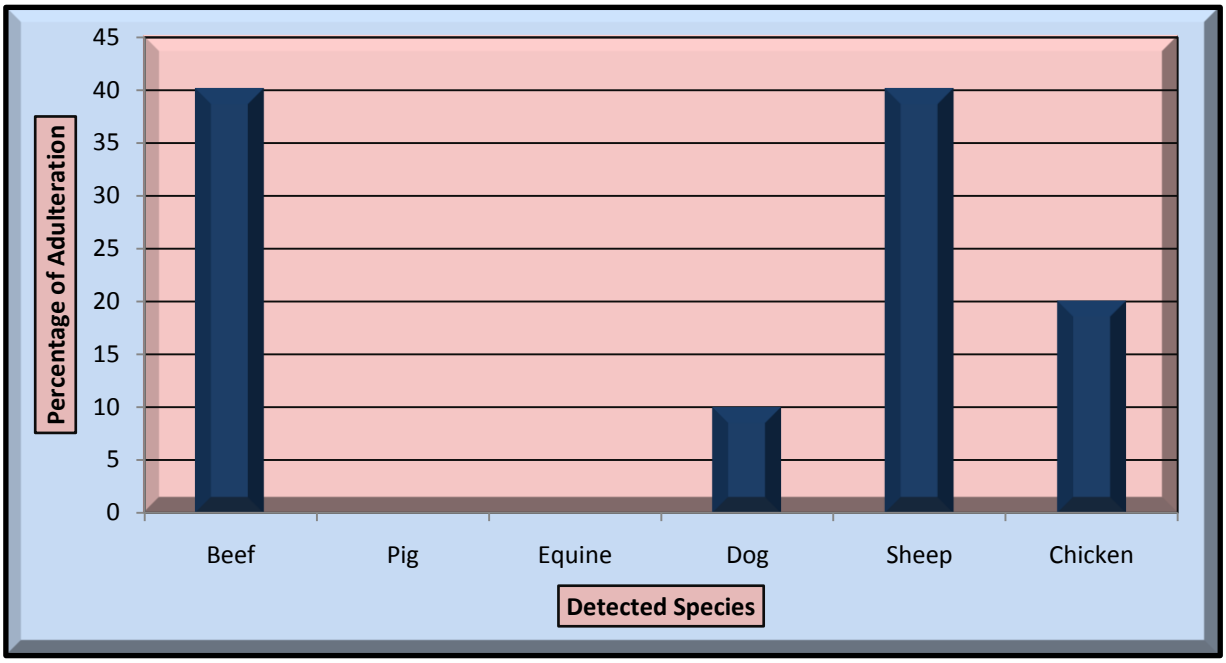


Figure 23: Percentage of Adulteration of Chicken Burger Samples with Different Meat Species Detected by Conventional PCR Technique:

Figure 24: Electrophoretic Gel Figures of Chicken Burger Samples of Different Meat Species Detected by Conventional PCR Technique Where, Lane L: Ladder, Lane P: Positive, Lane N: Negative, Lane 51 to 60: Numbers of Examined Samples:

Figure 24a: Beef Species

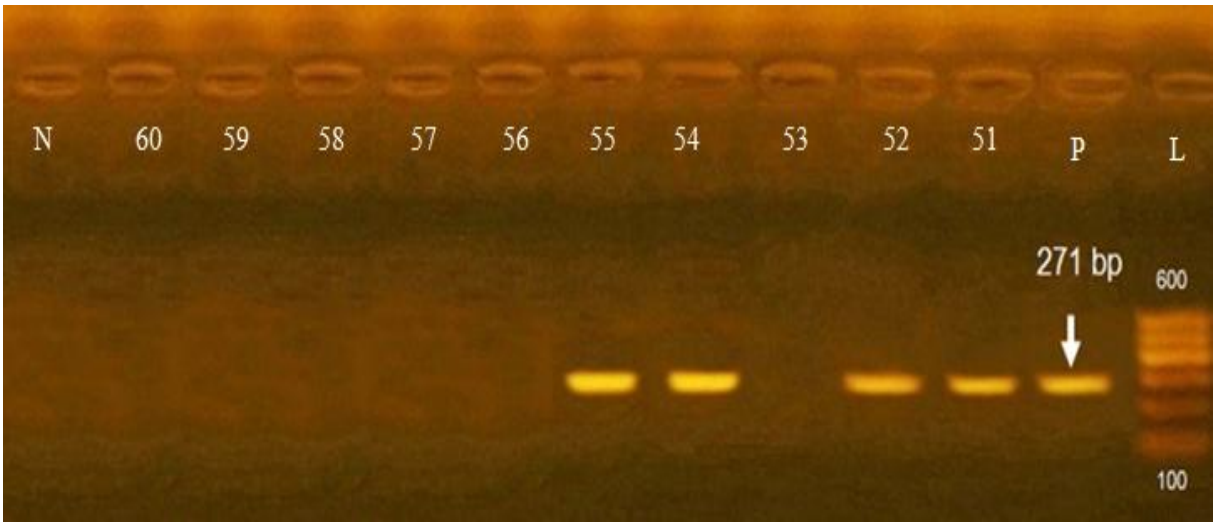


Figure 24b: Pig Species

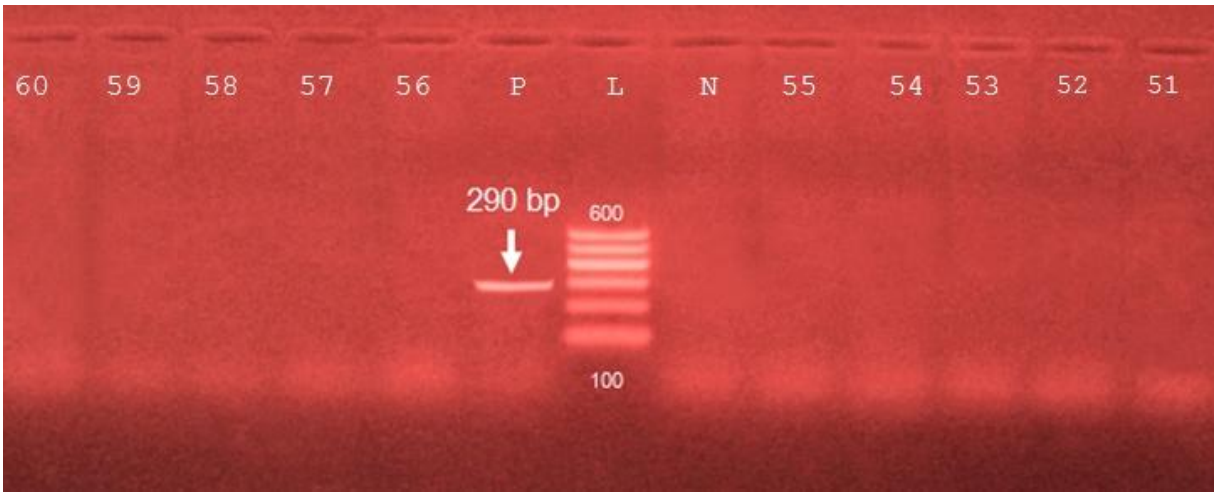


Figure 24c: Equine Species

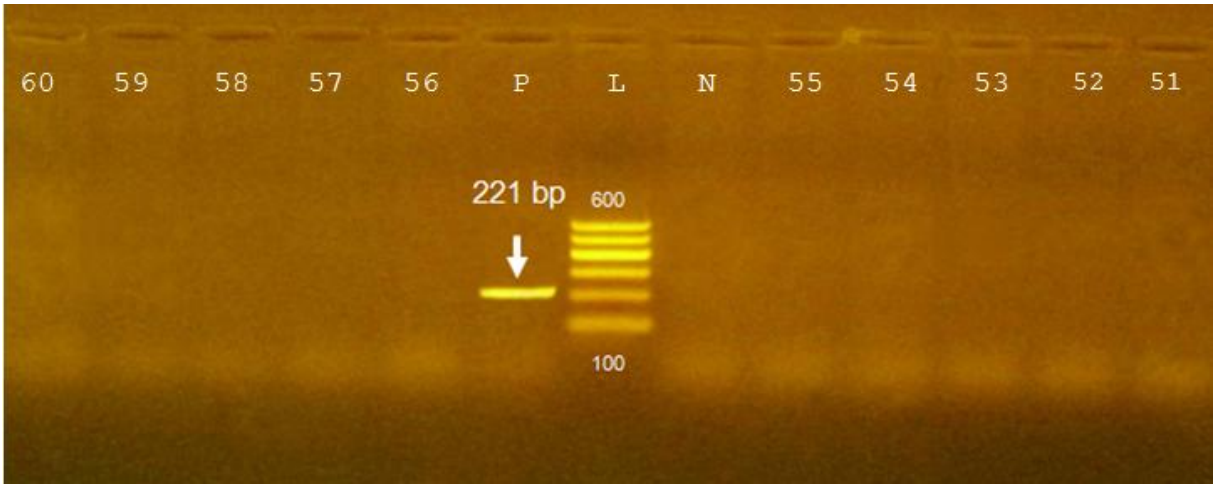


Figure 24d: Dog Species

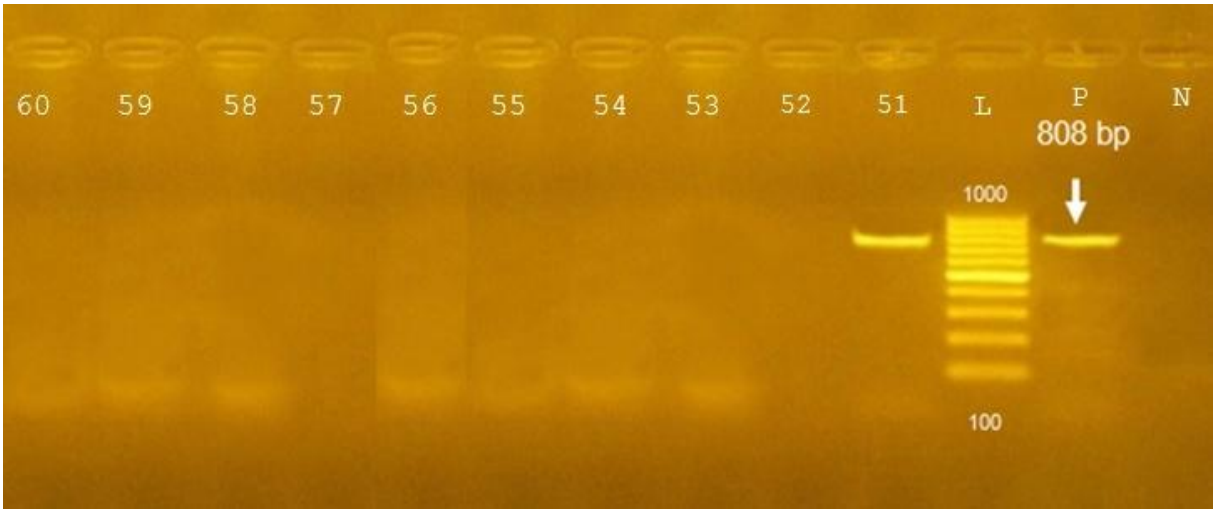


Figure 24e: Sheep Species

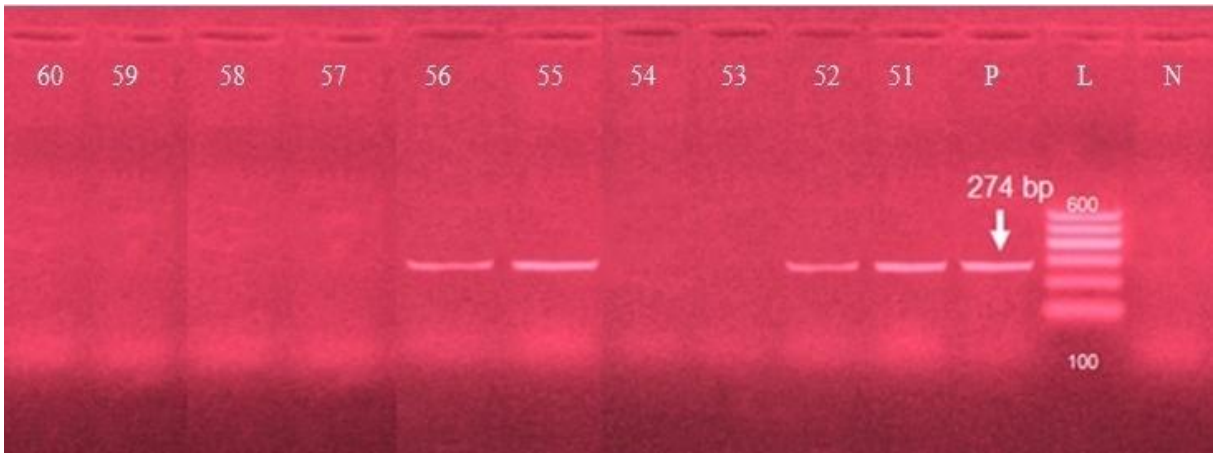


Figure 24f: Chicken Species

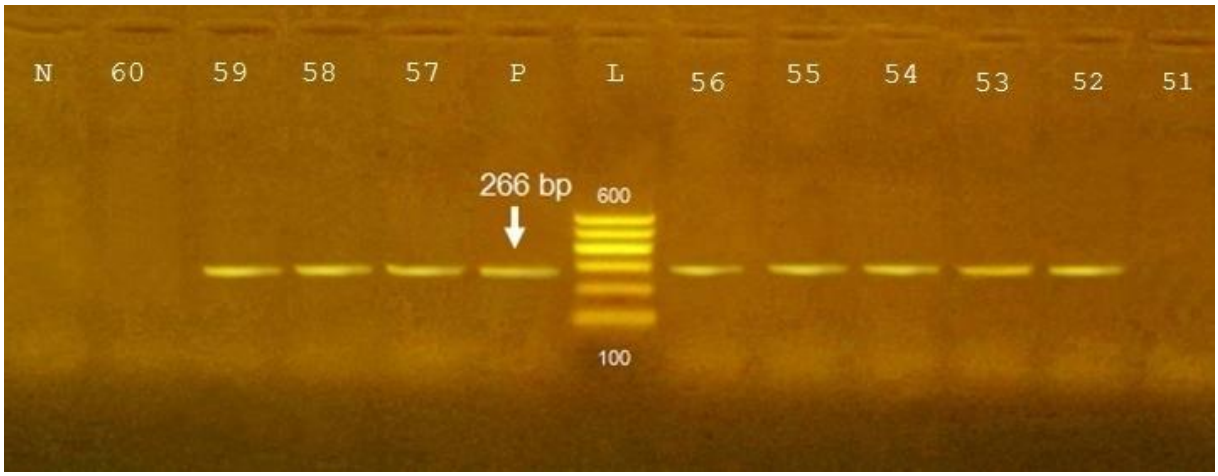


Table 19: General Adulteration Detected in Different Meat Product Samples by Conventional PCR Technique:

	Examined Meat Products						Total
	Hawawshi	Sausage	Beef Luncheon	Beef Burger	Chicken Luncheon	Chicken Burger	
No. of Examined Samples	10	10	10	10	10	10	60
No. of Adulterated Samples	9	9	9	10	7	6	50
Percentage of Total Adulteration	90%	90%	90%	100%	70%	60%	83.3%

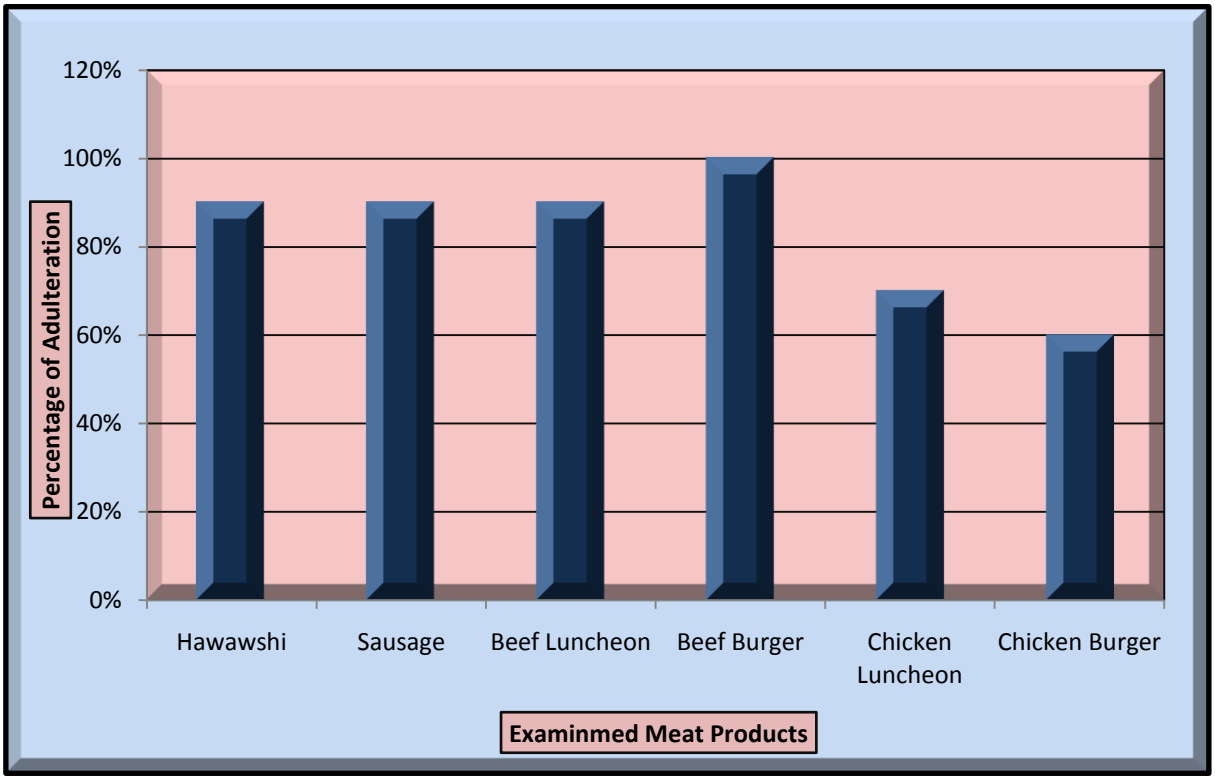


Figure 25: Percentage of General Adulterations Detected in Different Meat Product Samples by Conventional PCR Technique:

Table 20: The Most Adulterating Species Detected in the Examined Beef Meat Products by PCR Technique:

Adulterating species	Examined Beef Meat Products				Total	
	Hawawshi	Sausage	Beef Luncheon	Beef Burger	No.	%
Chicken	8	5	7	10	30	75
Sheep	6	8	3	5	22	55
Equine	1	1	1	3	6	15
Dog	0	0	2	0	2	5
Pig	0	0	0	0	0	0

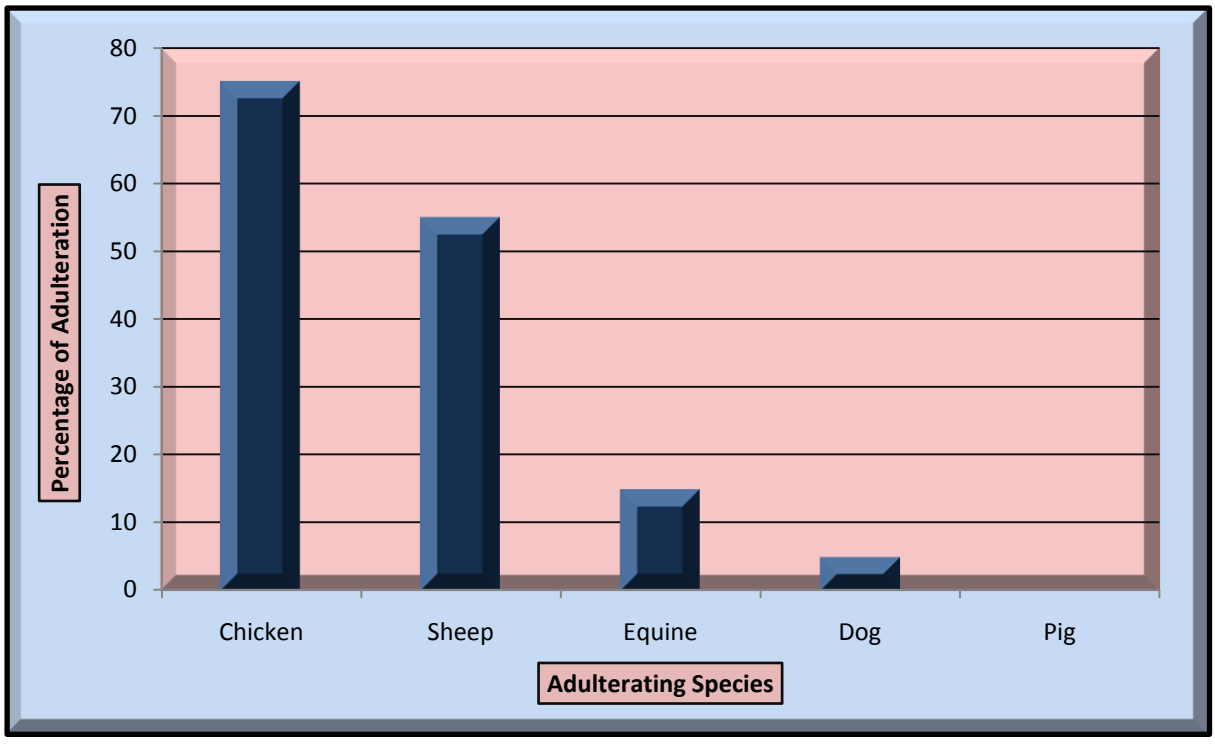


Figure 26: Percentage of the Most Adulterating Species in the Examined Beef Meat Products:

Table 21: The Most Adulterating Species Detected in the Examined Chicken Meat Products by PCR Technique:

Adulterating Species	Examined Chicken Meat Products		Total	
	Chicken Luncheon	Chicken Burger	No.	%
Beef	6	4	10	50
Sheep	0	4	4	20
Dog	2	1	3	15
Equine	0	0	0	0
Pig	0	0	0	0

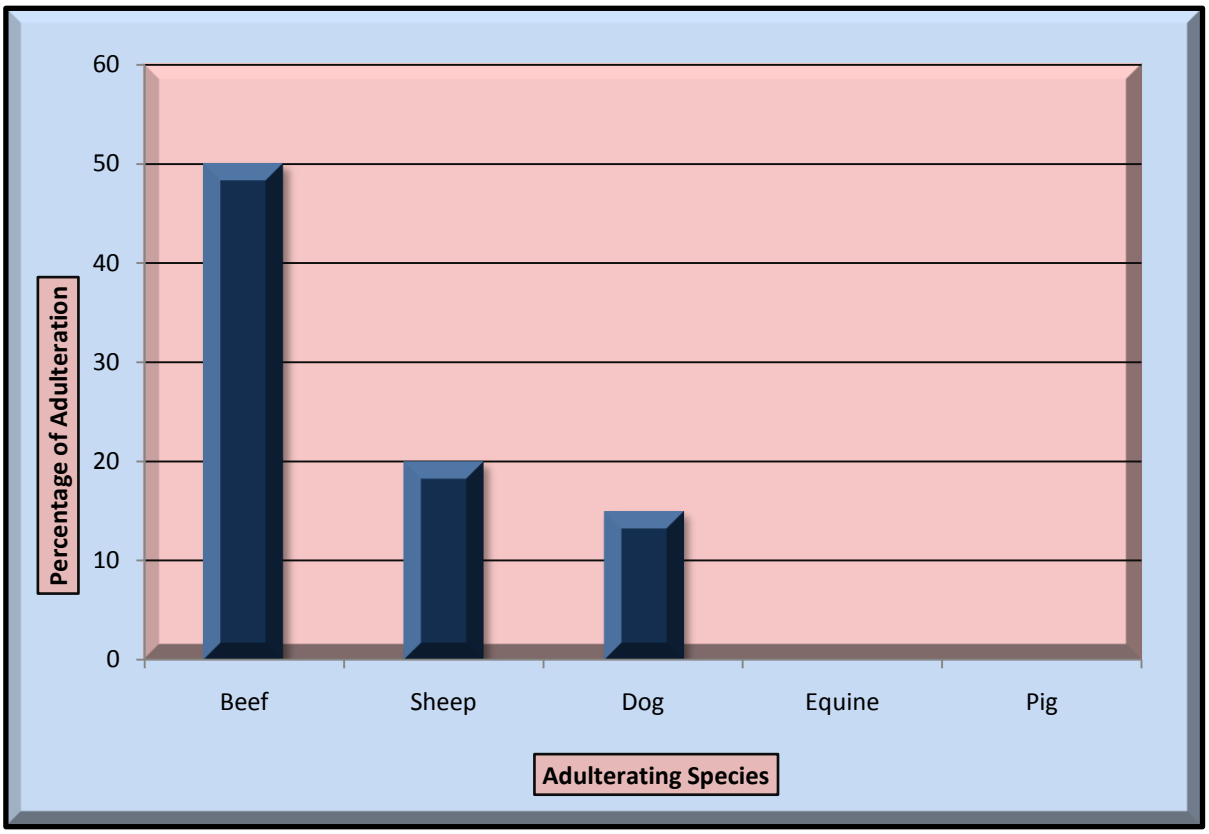


Figure 27: Percentage of the Most Adulterating Species in the Examined Chicken Meat Products:

Table 22: Application of Poultry Specific Kits of Raw Meat FlowThrough™ Test for Detection of Meat Product Samples Adulteration with Chicken Species, Where C1: Raw Chicken Meat Used as Positive Control:

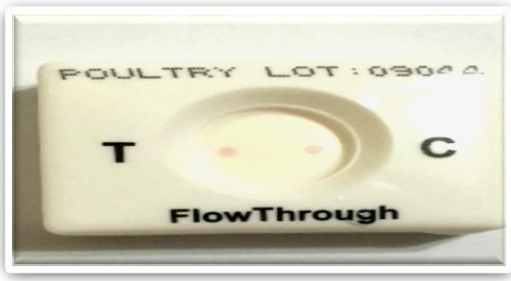
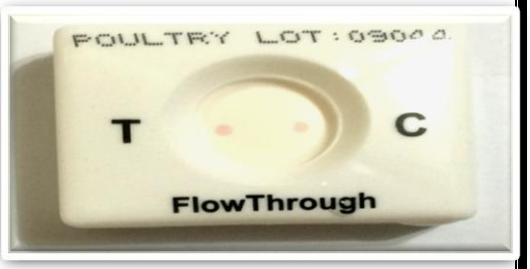
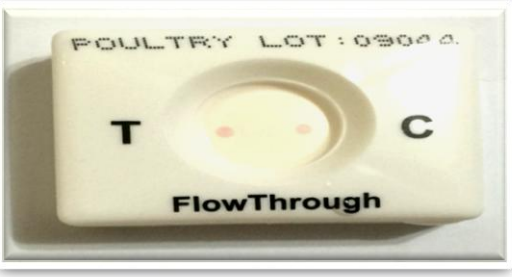
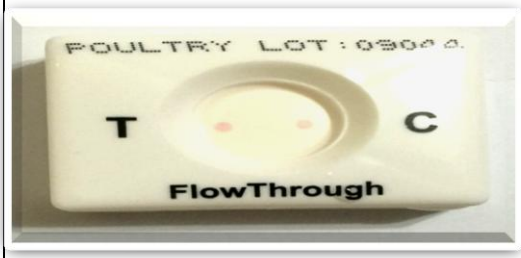
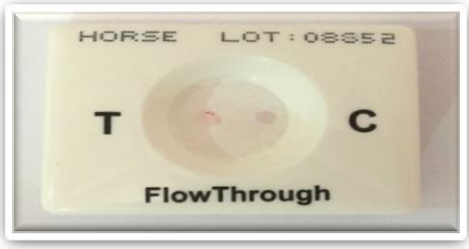
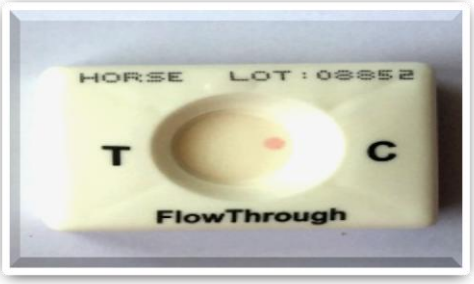
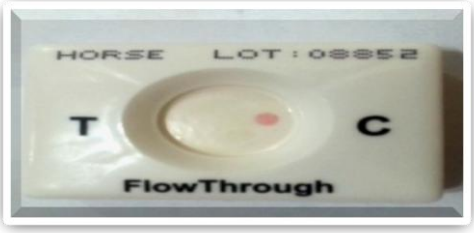
Sample ID Code	Description of The Sample	Test Spot	Control Spot	Detected / Not Detected	
C1	Raw chicken meat	✓	✓	Detected	
1	Hawawshi	✓	✓	Detected	
2	Beef Oriental Sausage	✓	✓	Detected	
3	Beef Burger	✓	✓	Detected	
4	Chicken Burger	----	✓	Not Detected	

Table 23: Application of Horse Specific Kits of Raw Meat FlowThrough™ Test for Detection of Meat Product Samples Adulteration with Equine Species, Where C2: Raw Horse Meat Used as Positive Control:

Sample ID Code	Description of The Sample	Test Spot	Control Spot	Detected / Not Detected	
C2	Horse Meat	✓	✓	Detected	
5	Hawawshi	----	✓	Not Detected	
6	Beef Oriental Sausage	----	✓	Not Detected	
7	Beef Burger	----	✓	Not Detected	
8	Chicken Burger	---	✓	Not Detected	

Discussion

With the continuous escalation of commercial meat products prices, the globalization of meat products trade and the increase of processing the meat into value-added various products, the incidence of adulteration and fraud has become more commonplace **(Vandendriessche, 2008**). Thus, the determination of meat products quality and detection of meat species are very important issues for consumers, vendors and government agencies **(Sakalar *et al.*, 2015)**, which help to safeguard public health, lifestyle of consumers, food choice, religious faith and fair-trade economy **(Karabasanavar *et al.*, 2014)**.

Nutritive Evaluation of the Examined Samples:

Meat products are formed of meat as a main ingredient, mixed with other components such as fat, water, salt and curing ingredients or spices which have been processed and transformed into various products for several objectives such as preserving the products for long periods, using of the total carcass, increasing of palatability and variety or finally enhancing convenience **(Cobos and Díaz, 2015)**. Although the production of high quality meat products is an important objective of the meat industry today, but adulteration of these comminuted meat products has been also a wide spread problem in retail markets **(Al-Bahouh *et al.*, 2012)**. The consumers always want to buy high quality products; however, fraudulent or unintentional mislabeling still exists and may not be detected, resulting in poor quality products **(Calvo *et al.*, 2001)**. Adulterated product is defined as "the product which is incompatible with what is declared by the seller" **(Montowska and Pospiech, 2010)**. Thus, the need for determining the constituents of the meat products is constantly increased **(Eslami *et al.*, 2014)**.

Currently, the Egyptian Organization for Standardization and Quality set standard limits of chemical components for meat products at Egyptian markets. As regard, the proximate analysis was applied in the current study to reflect the nutritive values of some meat products and ensure their compatibility with the Egyptian standards, which is an important factor for consumer health and acceptability.

1. Hawawshi:

Hawawshi is a famous traditional Egyptian meat product consisted of minced meat mixed with spices, chopped onions, garlic, pepper, parsley and sometimes chillies. The ingredients are placed between two circular layers of dough or in a whole loaf of baladi bread, and then baked in an oven. It is served in some restaurants, usually as a take-away **(Abdel Hafeez *et al.*, 2016)**.

The statistical analytical results of the proximate composition of hawawshi samples were showed in **table 1** and **figure 1**. The moisture content had a minimum value of 50.98% and a maximum value of 63.41% with a mean value of 58.18 ± 0.85%. While, the protein content ranged from 6.23% to 13.32%, with a mean value of 9.61±0.45%. In addition, the fat content ranged from 21.24% to 34.95%, with a mean value of 26.36±1.00%. Additionally, the ash content had a minimum value of 1.79% and a maximum value of 3.15% with a mean value of 2.58±0.09%. While, the carbohydrate content ranged from 0.23% to 6.63%, with a mean value of 3.26±0.39%. Finally, the red meat content ranged from 25.09% to 58.26%, with a mean value of 40.32±2.11%.

The obtained results were nearly similar to those obtained by **El Shobaki *et al*. (2014)** of ash % which was 2.9%, and higher than their results of fat% which was 25.2%. However, the results were lower than their findings of protein and carbohydrate contents that were 28.8% and 38%, respectively. Whereas there

are no specific standards for hawawshi as a meat product, so the results were not compared with the standards.

The obtained results illustrated that most of the hawawshi samples were relatively low in protein and in meat contents. Shortage of protein content in some meat products might be attributed to the use of improper meat cuts, the use of meat trimmings in preparation, or the substitution with non meat components as meat proteins are relatively more expensive than non meat components **(Lawrie, 1998)**. Currently, the substitution of some ingredients with other non-meat ingredients might be practiced among processed meat industries for economic purposes as the replacement of ingredients from animal origin with that of plants **(Egbert and Payne, 2009)**.

2. Fresh Oriental Beef Sausage:

Sausages are processed meat products that contained a mixture of minced or comminuted meat and fatty tissues combined with numerous non-meat ingredients and additives (salt, herbs, spices, etc.) that stuffed into casings, commonly natural casings from intestine to be formed into discrete units. The fresh sausages are sold without any heat treatment that are generally stored and commercialized chilled or frozen **(Feiner, 2006)**.

The legal requirements of sausage were established by the Egyptian Organization for Standardization and Quality No. 1972 **(EOS, 2005a)**, where moisture, protein, fat, ash and red meat contents are 60%, 15%, 30%, 5% and 60%, respectively.

It is evident from the results showed in **table 2** and **figure 2** that the moisture content ranged from 50.14% to 72.36%, with an average 60.56±1.58%. While, the protein content ranged from 10.14% to 18.56%, with an average

15.56±0.55%. In addition, the fat content ranged from 8.24% to 29.45%, with an average 19.14±1.67%. While, the ash content had minimum value of 1.50% and maximum value of 4.59% with an average 3.13±0.21%. Additionally, the carbohydrate content ranged from 0.03% to 6.33%, with an average 1.58±0.41%. Finally, the red meat content ranged from 41.99% to 81.32%, with an average 67.33±2.56%.

Likewise, the current results nearly agreed with the results of **Mohammed (2002)** for moisture content which was 59.9%, agreed with the results of **Hassan and Yehia (2004)** and **Ahmed *et al.* (2013)** for protein contents which were 15.3% and 15%, and agreed with the results of **Kamkar *et al.* (2005)** and **Schmid *et al.* (2009)** for fat contents which were 19.62% and 19.5%, respectively. In addition, **Hamed (2001)**, **Dharmaveer *et al.* (2007)** and **Nada (2012)** reported nearly similar results for ash content which were 3.53%, 3.00% and 3.08%, respectively and **Schmid *et al.* (2009)** reported nearly similar results for carbohydrate content which ranged from 0.5% to 1.4%.

While, lower results for moisture contents were obtained by studies conducted by **Hamed (2001)**, **Nouman *et al.*(2001a)**, **Abd El-Aziz (2002)**, **Doğu *et al.* (2002)**, **Ambrosiadis *et al.* (2004)**, **Hassan and Yehia (2004)**, **Kamkar *et al.* (2005)**, **Maha and Sohad (2005)**, **Dharmaveer *et al,* (2007)**, **Iordan *et al.* (2012)**, **González-Tenorio *et al.* (2012)** and **Talib (2015)** which were 56.50%, 46.5%, 52%, 47.58%, 49.17%, 57.5%, 57.32%, 55.6%, 55.48%, 40.15%, 42.8% and 55.18%, respectively. Similarly, other researchers reported lower results of protein contents as **Hamed (2001)**, **Abd El- Aziz (2002)**, **Mohammed (2002)**, **Kamkar *et al.* (2005)**, **Alina and Ovidiu (2007)**, **Quasem *et al.* (2009)**, **Schmid *et al.* (2009)**, **Nada (2012)**, **El Zahaby (2013)** and **Cunningham *et al*. (2015)** which were 13.89%, 13.3%, 11.6%, 12.68%, 14.11%, 12.75%,13.5%, 10.37%, 10.08% and 14.0%, respectively. Additionally,

lower results of fat contents were achieved by **Hamed (2001)**, **Fath El-Bab *et al.* (2006)**, **Dharmaveer *et al.* (2007)**, **Quasem *et al.* (2009)**, **Ahmed *et al.* (2013)**, **Cunningham *et al*. (2015)** and **Alamin (2016)** which were 18.66%, 15.79%, 17.05%, 16.7%, 4.5%, 14.9% and 3.45%, respectively. In addition to, lower results of ash contents were found by **Ambrosiadis *et al.* (2004)**, **Kamkar *et al*. (2005)**, **Quasem *et al*. (2009)**, **González-Tenorio *et al.* (2012)**, **Ahmed *et al.* (2013)** and **Alamin (2016)** which were 2.99%, 2.98%, 2.27%, 2.9%, 1.08% and 1.33%, respectively.

Otherwise, higher findings of moisture contents were obtained by **Fath El-Bab *et al.* (2006)**, **Quasem *et al*. (2009)**, **Schmid *et al.* (2009)**, **Nada (2012)**, **Ahmed *et al.* (2013)**, **Cunningham *et al.* (2015)** and **Alamin (2016)** which were 61.74, 63.94%, 61.5%, 62.98%, 68%, 62.5% and 70.32%, respectively. While, higher results for protein contents were reported by **Nouman *et al.* (2001a)**, **Ambrosiadis *et al.* (2004)**, **Doğu *et al.* (2002)**, **Fath El-Bab *et al.* (2006)**, **Dharmaveer *et al*. (2007)**, **Iordan *et al*. (2012)**, **González-Tenorio *et al.* (2012)**, **Talib (2015)** and **Alamin (2016)** which were 16.1%, 17.62%, 17.16%, 16.42%, 18.36%, 19.51%, 18.2%, 27.07% and 18.53%, respectively. Additionally, higher results of fat contents were found by **Nouman *et al.(*2001a)**, **Abd El- Aziz (2002)**, **Doğu *et al*. (2002)**, **Mohammed (2002)**, **Ambrosiadis *et al.* (2004)**, **Hassan and Yehia (2004)**, **Iordan *et al*. (2012)**, **Nada (2012)**, **González-Tenorio *et al.* (2012)** and **El Zahaby (2013)** which were 20%, 29%, 27.66%, 25.8%, 29.74%, 21.6%, 29.7%, 24.61%, 33.4% and 25.13%, respectively. Currently, higher results for ash content was found by **Nouman *et al.* (2001a)** which was 4.6%. Finally, higher results of carbohydrate content were reported by **Quasem *et al.* (2009)** and **González-Tenorio *et al*. (2012)** which were 4.3% and 2.7%, respectively.

By comparing the results with the Egyptian Organization for Standardization and Quality No. 1972 **(EOS, 2005a)**, there were 50%, 30% and 25% of the samples unaccepted based on their moisture, protein and meat contents, respectively, while all the samples were accepted based on their fat and ash contents as showed in **table 3**.

Similarly, **Hassan and Yehia (2004)** recorded that 6.7%, 33.3% and 73.3% of sausage samples of plants A, B and C were disagreed with the Egyptian standards because of low protein. While, **Nada (2012)** found the unaccepted samples were 28% and 36% according to the protein and fat contents, respectively. In addition, **El Zahaby (2013)** revealed that all the samples (100%) were unaccepted based on protein and fat contents when compared with the Egyptian standards.

The low protein content could be due to adding too much filling of non proteinaceous materials in the formulation of the product like the wheat flakes, root beet as a coloring and filling material which is consequently reflected on the final protein and meat content of the product **(Fath El-Bab *et al.*, 2006)**.

3. Beef Luncheon:

Luncheon is one of the most common emulsion type cooked meat products in Egypt. Its basic raw material is beef in chopped or comminuted form with additional ingredients as spices, soya protein, starch, nitrite, salt, ascorbate, and phosphate **(Mohd Abdullah, 2007)**. The legal requirements of this meat product were established by the Egyptian Organization for Standardization and Quality No. 1114 **(EOS, 2005b)**, where moisture, protein, fat, ash and red meat contents are 60%, 15%, 35%, 3.5% and 80%, respectively.

Regarding the results recorded in **table 4** and **figure 3**, it was evident that the moisture content had a minimum value of 56.44% and a maximum value of 69.11%, with an average 62.52 ± 0.95%. While, the protein content ranged from 3.45% to 15.71% with an average 8.49±0.86%. In addition, the fat content ranged from 3.11% to 8.56% with an average 5.37±0.32%. However, the ash content ranged from 2.47% to 4.75% with an average 3.13±0.12%. Additionally, the carbohydrate content ranged from 10.00% to 30.32% with an average 20.47±1.49%. Finally, the red meat content ranged from 0.33% to 62.81% with an average 26.02±4.45%.

The current results of ash contents agreed with **Hamed (2001)**, **Nouman *et al*. (2001b)** and **Mohamed *et al.* (2016)** whose results were 3.56%, 3.9% and 3.4%, respectively. While, lower results of moisture content were obtained by **Hamed (2001)**, **Nouman *et al.* (2001b)**, **Maha and Sohad (2005)**, **Edris *et al.* (2012)** and **Talib (2015)** which were 57.91%, 52.2%, 46.7%, 58.76% and 58.0%, respectively.

However, higher finding of protein contents were obtained by **Hamed (2001)**, **Nouman *et al.* (2001b)**, **Edris *et al.* (2012)** and **El Zahaby (2013)** which were 12.61%, 13.3%, 10.03% and 10.65%, respectively. Additionally, higher results of fat contents were recorded by **Hamed (2001)**, **Nouman *et al.* (2001b)**, **Abd El-Aziz (2004)**, **Edris *et al.* (2012)** and **El Zahaby (2013)** which were 18.68%, 17.5%, 19%, 19.25% and 10.91%, respectively. Also, **Edris *et al.* (2012)** had found higher results of ash content which were 4.29%. It was obvious from the results that most of the beef luncheon samples were relatively low in protein, meat content and fat contents, while high in carbohydrate contents.

The obtained results were compared with the Egyptian Organization for Standardization and Quality Control No. 1114 **(EOS, 2005b)** that recorded in **table 5**. The results revealed that 100%, 90%, 65% and 25% of the examined samples were unaccepted based on their meat content, protein, moisture and ash contents, respectively. Similarly, **El Zahaby (2013)** revealed that all the samples were unaccepted based on protein contents when compared with the Egyptian standards. While, **Edris *et al.* (2012)** found that the misbranded samples were 44% and 48% according to protein and fat contents, respectively.

The low protein and meat contents achieved by the previous results might be attributed to the illegally replacement of high quality and expensive beef meat partially or totally by lower quality and cheaper materials such as mechanically recovered meat (MRM) to reduce products cost. The mechanically recovered meat is the product of the removal of any remnant flesh from bone after manual deboning. It was developed for the poultry industry as a result of increasing the poultry production **(Mohamed *et al.*, 2016)**. It is pasty in nature, with good binding capacity and good technological properties which encouraged some meat processors to substitute meat partially or totally by it in most meat and poultry products **(Serdaroğlu *et al.*, 2005)**. However, it is generally considered to be of poor quality due to the partial removal of connective tissue during processing such as collagen, which has poor amino acids composition, that lead to the production of poor quality products **(Henckel *et al.*, 2004)**. Additionally, this may lead to quality loss which resulted from the redistribution of the higher initial microbial load which favors the growth of microorganisms and makes it highly perishable raw material **(Mohamed *et al.*, 2016)**.

From another side, the high carbohydrate contents and low fat content might be attributed to the substitution of low cost fat replacers. The most currently used fat replacers instead of meat ingredients are gums, inulin,

maltodextrins, oat flour, starches which are used as carbohydrate-based fat replacers in meat products, whereas olestra, other lipid (fat/oil) analogs are used as fat-based replacers. These finds might be a result of adulteration and trials to reduce the cost, which made the products non-compatible with the standards **(Tokusoglu and Ünal, 2003)**. Additionally, the soy protein, which plays a significant role in the modification of the functional characteristics of meat product, can also be used to replace part of the animal fat **(Mahmoud and Badr, 2011)**. From another side, low fat could also be achieved as more water might be added in meat batter which consequently increased the moisture content **(Colmenero, 1996)**.

4. Beef burger:

The term "burgers" was taken originally from the word "hamburger" which presumably is a product that originated from Hamburg City in Germany. Burgers mostly consist of meat as a raw material in addition to plant-based proteins (e.g. soya bean protein isolate) and starch (e.g. corn starch) which are used as fillers, stabilizers and to improve the texture of the product **(Al-Bahouh *et al.*, 2012)**.

The legal requirements of beef burger are established by Egyptian Organization for Standardization and Quality Control No. 1688 **(EOS, 2005c)**, where moisture, protein, fat, carbohydrate and red meat contents are 60%, 15%, 20%, 10% and 60%, respectively.

From the results recorded in **table 6** and **figure 4,** which showed the statistical analytical results of proximate composition of beef burger samples, it is obvious that the moisture content ranged from 54.95% to 68.75% with an average 61.44±0.79%. In addition, the protein content ranged from 5.96% to 16.85% with an average 11.68±0.74%. While, the fat content ranged from

8.95% to 30.64% with an average 16.27±1.50%. Moreover, the ash content ranged from 3.04% to 4.85% with an average 3.94±0.12%. In addition to, the carbohydrate content ranged from 0.86% to 12.82% with an average 6.65±0.72%. Finally, the red meat content ranged from 23.12% to 70.60% with an average 47.58±3.19%.

The current results of moisture contents nearly agreed with **El-Sayed (2006)**, **Edris *et al.* (2012)** and **Yagoup *et al.* (2017)** which were 61.67%, 61.28% and 61.23%, respectively. In addition to their agreement of the protein content with **Mohammed (2002)** which was 11.6% and their agreement of the ash content with **Edris *et al*. (2012)** which was 3.36%.

While, lower results of moisture contents were obtained by **Babji *et al.* (2000)**, **Abd El- Aziz (2002)**, **Hassan and Yehia (2004)**, **Prayson *et al.* (2008)** and **Talib (2015)** which were 49.89%, 58%, 59.1%, 49% and 58.24%, respectively. In addition, **Ali (2011)** and **El Zahaby (2013)** found lower results of protein contents, which were 8.80% and 10.54%, respectively. Moreover, **Abd El- Aziz (2002)** and **Yagoup *et al.* (2017)** reported lower results of fat, which were 14.55% and 7.79%, respectively. While, lower results of ash contents were recorded by **Babji *et al.* (2000)**, **El-Sayed (2006)** and **Yagoup *et al.* (2017)** which were 2.16%, 2.63% and 2.37%, respectively. Additionally, **Prayson *et al.* (2008)** found lower results of meat content which was 12.1%.

However, higher findings of moisture contents were obtained by **Mohammed (2002)** and **Ali (2011)** which were 62.7% and 66.12%, respectively. In addition to, **Babji *et al*. (2000)**, **Abd El- Aziz (2002)**, **Hassan and Yehia (2004)**, **El-Sayed (2006)**, **Edris *et al.* (2012)** and **Yagoup *et al.* (2017)** recorded higher results of protein contents which were 13.27%, 13%, 14.8%, 12.73%,15.22% and 21.26, respectively. Moreover, **Babji *et al.* (2000)**,

Mohammed (2002), **Abd El-Aziz (2004)**, **Hassan and Yehia (2004)**, **El-Sayed (2006)**, **Ali (2011)**, **Edris *et al.* (2012)** and **El Zahaby (2013)** revealed higher results of fat contents which were 19.97%, 22.4%, 18%, 20.6%, 22.78%, 20.45%, 19.80% and 24.46%, respectively. Additionally, higher results of carbohydrate and meat contents were found by **Babji *et al.* (2000)** which were 14.25% and 66.18%, respectively.

The acceptability of the examined samples according to the Egyptian Organization for Standardization and Quality Control No. 1688 **(EOS, 2005c)** for their proximate compositions was achieved in **table 7**. The results revealed that 85%, 85%, 50%, 35% and 25% of the samples were unaccepted based on their protein, red meat content, moisture, fat and carbohydrate contents, respectively.

Based on the previous results, most of the burgers were low in protein and meat contents. Likewise, **Hassan and Yehia (2004)** found that 20%, 60% and 86.6% of samples produced by different plants A, B and C were disagreed with chemical profiles stipulated by Egyptian standards because of low protein content. Similarly, **El-Sayed (2006)** found the non accepted samples according to moisture, protein and fat contents were 60%, 100% and 88%, respectively. Additionally, **Edris *et al*. (2012)** reported the misbranded samples according to protein and fat contents were 16% and 24%, respectively. Moreover, **El Zahaby (2013)** revealed that all the samples were unaccepted based on protein and fat contents when compared with the Egyptian standards.

The low protein content in the samples might be due to the replacement of protein sources by other cheap ingredients or partially substituted with non-meat protein sources. Binders and fillers like rusk, bread crumbs and plant-derived proteins are commonly incorporated in the burgers as a substitute of animal

protein to reduce the cost **(Ballin, 2010)**. Non meat protein sources such as egg, whey protein, and soy protein, are able to improve the flavor and texture of burgers by increasing the fat and moisture binding ability **(Kassem and Emara, 2010)**. Definitely, these ingredients minimize the production cost and also enhance sensory quality but have failed to fulfill the prescribed limit for proteins **(Turhan *et al.*, 2007)**.

5. Chicken Luncheon:

The production and consumption of poultry products have been increased globally. The increase in chicken meat products popularity has been noted by the fact that it can be processed into ready to eat meals **(Barbut, 2002)**. The processed products subjected to reformulation of the poultry meat with addition of different additives, which virtually had a great influence on the quality of the final product **(Radhakrishnan and Kumar, 2006)**. The legal requirements of chicken luncheon were established by Egyptian Organization for Standardization and Quality No. 1696 **(EOS, 2005d)**, where moisture, protein, fat, ash and red meat contents are 60%, 12%, 35%, 3.5% and 80%, respectively.

Table 8 and **figure 5** showed the statistical analytical results of proximate compositions of the examined chicken luncheon samples. The results indicated that the moisture content had a minimum value of 63.11% and a maximum value of 71.48% with an average of 67.34±0.45%. While, the protein content had a minimum value of 9.87% and a maximum value of 17.54% with an average of 12.93±0.50%. Additionally, the fat content ranged from 1.89% to 7.84% with an average 4.13±0.38%. Moreover, the ash content ranged from 2.58% to 3.98% with an average 3.44±0.09%. While, the carbohydrate content ranged from 7.23% to 15.35% with an average 12.14±0.52%. Finally, the red meat content ranged from 36.70% to 75.64% with an average 52.20 ±2.56%.

The current results agreed with **El Tahan *et al.* (2006)** who showed that the protein percentage ranged from 11.3% to 14% and the fat percentage ranged from 4.33 % to 5.42%. By comparing the results with the Egyptian Organization for Standardization and Quality Control No. 1696 **(EOS, 2005d)**, 100%, 100%, 50%, and 40% of the examined samples were unaccepted based on their meat content, moisture, ash and protein contents that were revealed in **table 9**.

Although the fat contents were expected to be within 35%, but the present study pointed out that all the chicken luncheons had fat contents less than 10%. This result might be due to the substitution of fat replacers instead of natural fat to avoid the rancid effect of oxidized fat which are used to reduce formulations costs as well as retain the desirable sensory characteristics of juiciness and mouth feel of the product that might be affected when the fat contents are reduced **(Ibrahim *et al.*, 2011)**. Additionally, more water might be added in the formulations of meat products to increase the volume of the product since these ingredients can absorb water and bind well with the meat. As a result, they are used to increase the size and weight of the final products without any regard to the nutritional value which gave indication of fraudulence **(González-Tenorio *et al.*, 2012)**.

Additionally, the increase in carbohydrate content might be attributed to the increase in starch content as extender to substitute the raw meat in manufacturing meat products by the inclusion of high amounts of binders and fillers such as rusk, cereals, breadcrumbs and soy protein, in addition to hydrocolloids (gums, starches, dextrins). The main reason behind this might be the manufacture plans to reduce the cost and increase the marginal profit **(Lukman *et al.*, 2009)**.

From another side, by assuming that the common salt is the principal source of ash, the increase in ash content was an indicative of higher amounts of common salt being used **(González-Tenorio *et al.*, 2012)**. Additionally, the high ash content might be due to the addition of high amount of mechanically recovered meat (MRM) in the formulation of luncheon, which could be correlated with high bone content in the components **(Field, 2000)**.

6. Chicken burger:

Processed chicken-based products such as burgers have been distributed through wholesalers and restaurants and widely consumed by the people. Furthermore, local industries have grown up to accomplish the demands from these products **(Guerrero-Legarreta and Hui, 2010)**. The legal requirements of chicken burgers were established by Egyptian Organization for Standardization and Quality Control No. 2910 **(EOS, 2005e)**, where moisture, protein, fat, ash and red meat contents are 70%, 12%, 15%, 2.5% and 60%, respectively.

It is evident from the results showed in **table 10** and **figure 6** that the moisture content ranged from 58.74% to 68.45%, with an average 64.26±0.58%. In addition, the protein content ranged from 6.48% to 16.82%, with an average 11.72±0.60%. While, the fat content ranged from 3.54% to 12.45%, with an average 7.70±0.59%. Moreover, the ash content ranged from 2.24% to 3.98%, with an average 3.21±0.12%. Additionally, the carbohydrate content ranged from 4.25% to 19.55%, with an average 13.08±0.78%. Finally, the red meat content ranged from 18.45% to 74.46%, with an average 46.11±3.08%.

The current results of fat content nearly agreed with those reported by **Carmen *et al.* (2013)** which was 7.76%. While, lower findings for moisture contents were reported by **Ramadhan *et al.* (2011)** which was 58.04%, and lower findings for fat contents recorded by **Babji *et al.* (2000)** and **Al-Bahouh**

***et al.* (2012)** which were 6.75% and 4.84%, respectively. Additionally, **Babji *et al.* (2000)**, **Al-Dughaym and Altabari (2010)**, **Ramadhan *et al*. (2011)**, **Al-Bahouh *et al.* (2012)** and **Hussain *et al*. (2016)** reported lower results of ash contents which were 1.51%, 2.05%, 2.23%, 2.17% and 2.28%, respectively. Also lower findings of carbohydrate contents were recorded by **Babji *et al*. (2000)**, **Ramadhan *et al*. (2011)** and **Al-Bahouh *et al.* (2012)** which were 3.48%, 11.91% and 7.75%, respectively.

However, higher findings of moisture contents were obtained by **Babji *et al*. (2000)**, **Al-Dughaym and Altabari (2010)**, **Al-Bahouh *et al.* (2012)**, **Carmen *et al.* (2013)**, **Ahmad *et al.* (2015)** and **Hussain *et al.* (2016)** which were 67.42%, 66.01%, 66.99%, 66.95%, 67.0% and 67.95%, respectively. Additionally, higher results of protein contents were reported by **Babji *et al.* (2000)**, **El Tahan *et al.* (2006)**, **Al-Dughaym and Altabari (2010)**, **Ramadhan *et al.* (2011)**, **Al-Bahouh *et al.* (2012)**, **Carmen *et al.* (2013)**, **Ahmad *et al.* (2015)** and **Hussain *et al*. (2016)** which were 20.47%, 15.4%, 16.82%, 14.92%, 18.5%, 20.39%, 15% and 14.97%, respectively. Moreover, higher findings of fat contents were found by **El Tahan *et al.* (2006)**, **Al-Dughaym and Altabari (2010)**, **Ramadhan *et al.* (2011)**, **Ahmad *et al.* (2015)** and **Hussain *et al.* (2016)** which were 14.49%, 8.26%, 14.81%, 16.0% and 10.64%, respectively. Finally, **Babji *et al*. (2000)**, **Ramadhan *et al*. (2011)** and **Al-Bahouh *et al.* (2012)** reported higher results of meat contents which were 71%, 77.62% and 71.84%, respectively.

Table 11 showed the acceptability of the examined chicken burger samples according to the Egyptian Organization for Standardization and Quality Control No. 2910 **(EOS, 2005e)** for their proximate composition. The results revealed that 85%, 75% and 65% of the examined samples were unaccepted based on their meat content, ash and protein content, respectively.

With regard to the results, the low level of protein might be due to the utilization of trimmings and cuts of lower price for the formulation of chicken burgers **(Babji and Yusof, 1995)**. Moreover, the addition of fat replacers in poultry burgers caused significant changes in chemical composition of final product in terms of fat reduction **(Carmen *et al.*, 2013)**. While, the increase in the ash content could be achieved by the addition of spices for seasoning, high fiber carbohydrate, starches, cereals, soya-protein and salt. In addition to incorporation of mechanically deboned chicken meat also might be another factor contributed in higher ash content **(Babji *et al.*, 2000)**.

Additionally, the present study showed the significant differences between nutritive parameters of the different examined meat products in **table 12**. As regard to **moisture contents (figure 7)**, the results showed no significant differences ($P \leq 0.05$) between hawawshi and sausage, no significant differences between sausage, beef luncheon and beef burger, and no significant differences between beef luncheon and chicken burger. On the other hand, there were significant differences between chicken luncheon and all the other products. The variability observed between the examined samples could be mainly attributed to large variations among samples for fat used in their manufacture process **(González-Tenorio *et al.*, 2012)**. Additionally, this variation might be influenced by the variable amount of lean meat added, the use of sodium chloride or the addition of water that was added to facilitate the chopping of meat and the mixing of the ingredients. Added water aids in dissolving sodium chloride and curing salts to give better distribution in the mass. Texture and tenderness of the finished products are markedly affected by the added water content **(Edris *et al.*, 2012)**.

While according to **protein contents (figure 8)**, there were no significant differences ($P \leq 0.05$) between hawawshi and beef luncheon and no significant differences between beef burger, chicken luncheon and chicken burger.

However, there were significant differences between sausage and all the other products. The protein represents the most important class of functional ingredients because it possess a range of dynamic functional properties such as structure formation, color texture, thickening, emulsification, foaming, elasticity and provides essential amino acids so it fulfill functional and nutritional requirements **(Dogan *et al.*, 2005)**.

However, by concerning **fat contents (figure 9)**, there were significant difference ($P \leq 0.05$) between hawawshi and all the other products, while there were no significant differences between sausage and beef burger, between beef luncheon and chicken luncheon and between beef luncheon and chicken burger. The function of fat is mainly influencing the sensory quality of products, particularly their flavor **(Suman and Sharma, 2003)**. The variations in the fat contents of meat products might be attributed to the differences in meat cuts and fatty portions used or due to using of improper formulation of such products or the addition of fat replacers, which are the main cause of low fat in the final product **(Mousa *et al.*, 1993)**. Fat as a major component is used for its sensory and physiological benefits that contribute to the flavor, taste and aroma/odor of the final products **(Moghazy, 1999)**.

In addition, regarding to **ash contents (figure 10)**, there were significant difference ($P \leq 0.05$) between hawawshi and all the other products, and between beef burger and all the other products, while there were no significant differences between sausage, beef luncheon, chicken luncheon and chicken burger. Ash is the sum of the total minerals presented in a product such as sodium, phosphorus and iron, that can be contributed by the meat as raw material, salt and spices added **(Fernández-López *et al.*, 2006)**. The ash content in meat products not only depends on muscle minerals but also on the curing salt added **(Kirk and Sawyer, 1991)**. The ash content is influenced by type of meat

used, spices as well as binders and fillers used **(Edris *et al.*, 2012)**. The importance of the high ash content comes from its ability to increase the size and weight of the meat products by the activation of proteins to increase hydration and water-binding capacity **(Desmond, 2006)**. The differences of ash content might also be due to the decrease of moisture content which was associated with storage and handling proceedings with extension in storage period **(Xiong *et al.*, 1999)**.

Moreover, according to the **carbohydrate contents (figure 11)**, the mean values showed no significant difference ($P \leq 0.05$) between hawawshi and sausage, and between chicken luncheon and chicken burger. While, there were significant differences ($P \leq 0.05$) between beef luncheon and all the other products, and between beef burger and all other products. Carbohydrates in meat products are mainly from the use of starches as ingredients. Starches, such as maize, tapioca, rice, potato, and wheat, have been used in processed meat products as meat filler and water binder **(Joly and Anderstein, 2009)**.

While, concerning **red meat contents (figure 12)**, there were no significant differences ($P \leq 0.05$) between hawawshi, beef burger and chicken burger, and no significant differences ($P \leq 0.05$) between beef burger, chicken luncheon and chicken burger. However, there were significant differences ($P \leq 0.05$) between sausage and all the other products, and between beef luncheon and all the other products. It was evident that there were differences in compositional quality of various meat products. These might be due to differences in the type of ingredients used, different formulations and different processing techniques. The quality of these products significantly could be affected by processing, raw materials and ingredient factors either from nutritional value or from overall acceptability by consumers. The composition of each meat product was greatly varied from one product to another as it

contained different kinds of tissues and sometimes a mixture of meat of various organs **(Lawrie, 1998)**. The meat products had a wide varieties of chemical composition which affect the nutritive values and reflect the quality of additives **(Ahmad *et al.*, 2015)**.

Totally, the nutritive evaluation of meat products is very important because consumers ' knowledge of them has increased and they like using nutritional ready-to-use meat products **(Lakzadeh *et al.*, 2016)**. Additionally, from another view, the identification of species in meat products is becoming a very important issue concerning the assessment of their composition, which is necessary to provide consumers accurate information about the products they purchase **(He *et al.*, 2015)**. Hence, it is critical to develop precise and reliable methods intended to detect animal species in meat products which may be substituted or mixed with other undeclared species **(Mehdizadeh *et al.*, 2014)**.

Detection of Species Adulteration:

Nowadays, meat products are still facing some unfaithful manufacturing practices and fraud in the form of adulteration with different animal species **(Ahmed *et al.*, 2016)**. The common fraudulent practice found in the meat products line is the use of a less costly or banned type of species in substitution of more expensive or authenticated ones. Many meat products may contain several species in different proportions mixed together and undetectable by the naked eye or by eating **(Zarringhabaie *et al.*, 2011)**.

The adulteration by substitution of undeclared species has always been a concern for various reasons such as public health, religious factors, wholesomeness and unhealthy competition in meat market **(Ahmed *et al.*, 2016)**. Additionally, it should be cleared to enable consumers, particularly those

suffering from a food allergy or intolerance, to make informed choices **(Di Pinto *et al.*, 2015)**.

Since meat adulteration is of great importance from both economic and health point of view, the demands for the development of accurate, rapid and inexpensive analysis methods are increasing **(Mehdizadeh *et al.*, 2014)**. However, mixing of different species followed by grinding or heat-processing aids to the difficulties of discrimination of meat origin and limits the detect ability of many analytical techniques **(Ballin *et al.*, 2009)**.

Progress in the area of authentication of traded meat products requires the use of molecular tools to ensure proper species identification, thus enhancing the application of effective food control regulations and consumer protection **(Marín *et al.*, 2013)**. In recent years, molecular authentication methodologies have been developed, and these methods have been successfully applied for species authentication in meat products **(Stamoulis *et al.*, 2010)**. Hence, in order to identify the meat and meat products coming from different animal species, various methods based on analysis of species-specific components such as protein and DNA have been developed **(Kesmen *et al.*, 2010)**.

Application of Conventional PCR Technique to the Examined Meat Products:

In recent years, many techniques based on DNA analysis were adopted for meeting the needs of the meat industry and carrying out investigations aiming to species identification. The polymerase chain reaction (PCR) method used in this study deserves special attention because it is characterized by high sensitivity and specificity as well as relatively short period of time necessary to perform the analysis **(Yosef *et al.*, 2014)**. These properties make it ideal for identification of species origin of processed meat and meat products **(Mane *et al.*, 2012)**. It was

proved to be one of the most powerful tools for assessing species identity, food traceability, safety and fraud **(Di Pinto *et al.*, 2015)**. Its technique can briefly be described as the amplification of one or more specific fragments of the DNA by an oligonucleotide primer that binds to the fragments **(Kesmen *et al.*, 2010)**.

In the current study, conventional PCR assay using specific primers for detection of beef, pig, equine, dog, sheep and chicken species was applied on some commercial processed meat products. It was noticed that DNA was successfully extracted from all examined samples of meat products. All resulted PCR products were clearly visible as single bands of expected sizes on agarose gel (271 bp beef, 290 bp pig, 221 bp equine, 808 bp dog, 274 bp sheep and 266 bp chicken). All positive and negative controls gave the expected results. Additionally, the amplification was not affected by additives or processing as well as the presence of DNA from the other species did not affect the detection of targeted DNA, similar observation was concluded by **(Kesmen *et al.*, 2007)**.

1. <u>Hawawshi :</u>

The results presented in **table 13** and illustrated in **figure 13** showed the detection of adulteration of hawawshi with different species, while **figure 14** showed the clear DNA bands of the detected species on the electrphoretic gel photos. The results revealed that 2 (20%) out of 10 samples were detected with no beef species. Furthermore, 8(80%), 6(60%) and 1(10%) out of 10 samples were contained chicken, sheep and equine species, respectively. None of the examined samples was proved to be adulterated with pig or dog species.

Generally, these results were relatively lower than **Abd El Sadek (2011)** who found that 15% of samples were adulterated with equine, and **Hussien (2011)** who reported that 50% of the samples were adulterated with pork.

Moreover, **Mohamed *et al.* (2012)** reported that the adulteration rate with donkey meat was 12%.

On the other hand, the results were higher than **Hussien (2011)** who found the adulteration rate with chicken and donkey were 65% and 5%, respectively. Additionally, **Mohamed *et al.* (2012)** reported that the adulteration rate with chicken meat was 20%.

In consistence with the previous results, it was noticed that the majority of the hawawshi samples were adulterated by multiple undeclared species (mainly three species) which were likely indications of intentional adulteration. Chicken and sheep were the most used species for adulteration followed by equine. In addition, there were samples considered to be adulterated due to the absence of beef in spite of their sold as beef products. These results might be attributed to the fact that deliberate substitution with undeclared species is more difficult to be detected in such products by visual observation than it was in fresh and intact meat **(Ayaz *et al.*, 2006)**. Processing techniques often lead to changes in the appearance, color, texture and even flavor of meat products, meaning that the origins of constituents can be easily concealed in the meat mixture **(Flores-Munguia *et al.*, 2000)**.

A further reason for these results could be due to the accidental cross contamination during processing by the improper handling and the use of shared equipments **(Owusu-Apenten, 2002)**. In meat processing, it may be inevitable that one species of meat mixed with another during meat operations, such as cutting and grinding via knives, grinders, choppers, and cutting boards **(Zarringhabaie *et al.*, 2011)**.

The presence of chicken species might be regarded to the using of the huge amounts of skin, frames, legs and necks resulted from the massive increase in

poultry production and manufacturing, which are related to the change in consumers eating attitude from consumption of whole chicken to cuts and fillets **(Mohamed *et al.*, 2016)**. Additionally, chicken waste products, called trimmings such as fat, connective tissues, blood vessels, cartilages and even pieces of bone as well as dead carcasses might be mixed with meat and used as adulterants **(Mehdizadeh *et al.*, 2014)**. Moreover, another potential source of undeclared chicken species in the samples evaluated could be with mechanically recovered meat (MRM), which is currently most often produced from chicken carcasses. This paste-like substance, typically obtained by forcing carcass remains through sieves under high pressure to separate edible meat from bones. It might be included as a cheap protein source in comminuted meat products **(Surowiec *et al.*, 2011)**.

While regarding to the presence of sheep species, it might be attributable to the addition of the unmarketable trimmings and wastes as well as the dead carcasses in the meat mixtures for the purposes of increasing their bulk. Otherwise, the improper cleaning of the grinder between each change of meat species prior to grinding might be a reason for sheep species detection **(Ayaz *et al.*, 2006)**.

Another great concern from a regulatory, health and ethical standpoint was the detection of undeclared equine species in even one sample sold as beef. The presence of equine meat regardless to its amount is unacceptable by the consumers as it is disgusting and repulsive for eating. Additionally, foods containing donkey and horse sources are considered to be Haram for Muslims to consume. The repeated blending of prohibited species items such as pork, horse, dog, cat, and rat meats with various products have put the Muslim consumers in red alert in determining the halal status of the marketed products **(Ali *et al.*, 2014b)**. From another view, the equine is not a species commercially processed

for human consumption, as it is not considered a conventional species in the domestic meat supply chain in Egypt, hence, the source of this equine meat might have been processed under non-sanitary conditions, which could pose potential risks to human health. Therefore, it could be a further case of intentional substitution for economic gain **(Cawthorn *et al.*, 2013)**. Additionally, consumption of horse and donkey meat may represent a potential health risk for human as a source of infection with *Toxoplasma gondii*, the causative agent of toxoplasmosis, which causes serious health problems to pregnant women, and individuals who have compromised immune systems **(Yang *et al.*, 2013)**.

Additionally, the absence of pig species in the samples might be attributable to the decision of the Egyptian Government announced in 2009, to slaughter all pigs (about 300,000) in the country to overcome the problem of carrying the virus of swine flu **(Maamoun**, **2009)**. This decision might lead to decreasing the availability of pig meat or its remnants of slaughter to be used as adulterants of meat products. The certainty of no pig species in the examined product was a good and important result based on Muslims consumer safety and warranty as it is an important issue especially for Halal authentication **(Hamzah *et al.*, 2014)**. From another point, consuming undercooked pork within meat products has potential health risks as the pork is the carrier of various helminthes like pin worm, hooks worm, tapeworm (its larval stage is Taenia solium which infects the human nervous system, causing neurocysticercosis), or roundworm which is the cause of trichinosis **(Oluwaseun, 2017)**.

Totally, the above instances of mislabeling represent cases of meat products fraud, which might be a result of factors such as poor traceability, accidental cross contamination resulting from improper handling, inadequate cleaning of equipments between species, or intentional fraud carried out for reasons such as economic gain **(Cawthorn *et al.*, 2013)**. Fraudulent

substitutions of expensive meat with cheaper one or addition of undeclared species in meat products may cause concerns for consumer protection and other economic reasons. Religious strictures, perceived or real health concerns and cultural likes and dislikes are the main drivers of species identification for consumer protection **(Ahmed *et al.*, 2011)**.

2. Fresh Oriental Beef Sausage:

Table 14 and **figure 15** pointed out the detection of adulteration in sausage with different species, while **figure 16** illustrated the different specific amplified PCR products on the electrphoretic gel photos. The results revealed that 2(20%) out of 10 samples did not contain any beef DNA. Additionally, sheep, chicken and equine species were detected in 8(80%), 5(50%) and 1(10%) out of 10 samples, respectively. However, no porcine or dog DNA sequence was amplified from the examined samples.

Generally, these results were relatively lower than **Joseph *et al.* (2006)**, **Abd El-Aziz (2009)**, **Ahmed *et al*. (2011)**, **Hussien (2011)** and **Cawthorn *et al*. (2013)** who found the adulteration rates with pork were 44%, 14%, 41.7%, 45% and 52%, respectively. In addition to **Flores-Munguia *et al.* (2000)** who found undeclared equine species in 5(29%) of the 17 samples. Moreover, **Abd El Sadek (2011)** revealed that 5% of samples were adulterated with dog meat. While, **Ahmed *et al*. (2011)** and **Hussien (2011)** showed that the adulteration rates with chicken were 66.7 % and 70%, respectively.

Otherwise, the results were agreed with **Ghovvati *et al.* (2009)** and **Özpinar *et al*. (2013)** who found that none of the samples was contained pork. Additionally, **Abd El Sadek (2011)** and **Hussien (2011)** found that 10% of samples were adulterated with donkey. While, **Ulca *et al.* (2013)** revealed one sample was labeled as containing beef, but beef DNA was not detected.

On the other hand, the results were higher than **Abd El-Aziz (2009) Ghovvati *et al*. (2009)**, **Mohamed *et al.* (2012)** and **Cawthorn *et al.* (2013)** who reported that the rates of adulteration with chicken were 32%, 40%, 24% and 39%, respectively. Additionally, **Ahmed *et al*. (2011)** and **Mohamed *et al.* (2012)** showed that the adulteration rate with donkey was 8%. Also, the results were more than those reported by **Cawthorn *et al.* (2013)** that sheep species was identified as undeclared species in 47% of the samples, While, **Yosef *et al*. (2014)** detected no equine species in any of the samples.

From the showed results, sheep and chicken species adulterated most of sausage samples and some contained equine species, while others were adulterated as did not contain any beef in spite of their selling as beef products. Sausages as a processed-meat products are susceptible targets for fraudulent due to the economic profit resulted from selling cheaper meats as partial or total replacements for high-value ones **(Soares *et al.*, 2013)**. The morphological characteristics of muscle are removed after grinding and mixing making the origin of meat species is easy to be concealed which makes it difficult to identify one type of muscle from another **(Liu *et al.*, 2006)**. It was previously reported by different researchers that there were frequently substitution of animal fats from one species with those from other species in meat products **(Ballin, 2010)**.

From another standpoint, the presence of different animal species in these products could be due to processing of meat from different species in the same meat plants, so it might be caused by the unintentional and incidental commingling of trace amounts of one type of meat products with another during processing and handling **(Di Pinto *et al.*, 2015)**. Whether deliberate or unintentional, moreover, the effects of meat product misdescription are similar, and include consumer deception, potential health risks and inability of

individuals to choose products on the basis of their religious and ethical beliefs **(Cawthorn *et al.*, 2013)**.

The primary reason for adulteration with chicken species might be their lower price if compared with beef so there could be an economic justification for the addition of chicken to beef products. The popularity of poultry meat also increases the chance of mixing mechanically deboned poultry tissue with ground or comminuted products **(Hsieh *et al.*, 1996)**. Additionally, the fat trimmings or slaughtering residues from poultry or sheep could be added to sausage to extend bulk **(Joseph *et al.*, 2006)**. The assurance of absence of pork in sausage was sensitive issue especially with Muslims. Pork is prohibited to consume, because it is not appropriate with halal clause and the consumption of halal foods was compulsory for Muslims **(Rohman *et al.*, 2011)**.

3. Beef Luncheon:

Detection of adulteration of beef luncheon by different species of meat was revealed in **table 15** and **figure 17**. While, the clear DNA bands of different adulterating species on the electrphoretic gel photos were showed in **figure 18**. The results pointed out that all the examined samples contained beef meat and free from pork meat. Additionally, there were 7(70%), 3(30%), 2(20%) and 1(10%) out of 10 samples proved to be adulterated with chicken, sheep, dog and equine meat, respectively.

These results were lower than **Hussien (2011)** who found that 20% of the samples were adulterated with donkey, and agreed with **Elbialy *et al.* (2016)** who reported that one (10%) sample was adulterated with equine meat. Additionally, the results agreed with **Hussien (2011)** who found that none of the samples were adulterated with pork.

On the other hand, the previous results were higher than **Hussien (2011)** and **Mohamed *et al.* (2012)** who reported that the adulteration rates with chicken were 25% and 20%, respectively. Additionally, **Mohamed *et al.* (2012)** and **Yosef *et al.* (2014)** reported that none of the samples contained equine species.

One possible reason of high adulteration rate occurred in beef luncheon is deliberate adulteration with inexpensive species for economic gain, because it is more difficult to detect adulterant in cooked meat than in fresh meat. After grinding, heating or curing processes which may change the characters of meat, the origin of meat is easily concealed in a meat mixture **(Ayaz *et al.*, 2006)**. Another reason is accidental contamination resulting from improper handling or processing. However, in meat plants processing poultry and ruminant species together, contamination of meat products with another meat species may be occured during meat operation **(Zarringhabaie *et al.*, 2011)**. Adulteration of meat species is important for people whose religious practices limit the types of meat they eat, and for people who have allergies to certain types of meat proteins **(Hsieh *et al.*, 1997)**.

From the obtained results, chicken and sheep species were the most used ones for adulteration. While, dog and equine species were also found, this showed that adulteration was intentional as these species were not included in the food chain in Egypt. The mixing of dog origin materials in products is a serious issue in many religions including Islam **(Khattak *et al.*, 2011)**. The Islamic regulations prohibit Muslims from eating ingredients derived from animals having canine teeth or fangs such as dog, cat, monkey and rat **(Ali *et al.*, 2014a)**. These animals could be obtained without any offered prices and hence there is a significant chance of mixing them in halal foods **(Rahman *et al.*, 2014)**. Dogs are potential carrier of several zoonotic diseases such as

trichinellosis which is one of the most important food-borne parasitic zoonosis related to dog meat consumption **(Fajardo *et al.*, 2010)**. Additionally, the consumption of dog meat might be a major risk factor for exposure to rabies, which could be easily have entered the food chain from consumption of dog meat. Moreover, it is expected that all of the dogs involved are usually strayed, apparently unimmunized, posing a threat to those dealing with the products manufacturing **(Ekanem *et al.*, 2013)**.

4. <u>Beef burger:</u>

The data achieved in **table 16** and **figure 19** showed the adulteration of beef burger by different species of meat detected by PCR technique. While, **figure 20** illustrated the different specific amplified PCR products on the electrphoretic gel photos of the examined samples. The results revealed that all the samples contained beef meat, but also all of them were adulterated with chicken species (100%), while, none of the samples was adulterated with pig or dog meat. Furthermore, the results showed that 5(50%) and 3(30%) out of 10 beef burger samples were adulterated with sheep and equine species, respectively.

These results were lower than **Flores-Munguia *et al.* (2000)** who detected undeclared equine species in 9(39%) of the 23 samples. While, **Abd El Sadek (2011)** revealed that 5% of samples were adulterated with dog meat. Additionally, **Abd El-Aziz (2009)**, **Ahmed *et al.* (2011)** and **Cawthorn *et al.* (2013)** found that the adulteration rates with pork were 2%, 23% and 30%,respectively. While, **Eslami *et al.* (2014)** showed that 9.09% of samples did not contain any cow meat which were not in agreement with their labels.

On the other hand, these results were higher than **Abd El-Aziz (2009)**, **Ahmed *et al.* (2011)**, **Abd El Sadek (2011)** and **Yosef *et al.* (2014)** who

revealed that 2%, 7.7%, 25% and 0% of samples were adulterated with equine species, respectively. In addition to, **Abd El-Aziz (2009)**, **Ahmed *et al.* (2011)**, **Cawthorn *et al*. (2013)**, **Mehdizadeh *et al*. (2014)** and **Lakzadeh *et al*. (2016)** who showed that the rates of adulteration with chicken were 32%, 69%, 40%, 94.4% and 26%, respectively. while, **Cawthorn *et al*. (2013)** detected undeclared sheep species in 35% of the samples, respectively.

From the current results, it was obvious that chicken species adulterated all the samples and this high rate might be related to mixing beef with cheaper parts of chicken as the meat products manufacturers or meat processing factories might add different types of meats to species-specific meat product to add bulk or make up the volume of the product. This encourages most of meat processors to use mechanically recovered poultry meat (MRPM) as an inexpensive material to substitute meat in poultry and meat products, with high percentage reached to total meat replacement without any care to its health hazards to consumer or product quality **(Mohamed *et al.*, 2016)**. Another possible reason for mislabeling of meat products include improper cleaning of the grinding equipments in between meat species. But the relatively lower cost of chicken remnants than beef and the inability to distinguish by organoleptic means if an undeclared meat species has been mixed in might indicate the possibility of intentional adulteration for economic reasons **(Spink and Moyer, 2011)**.

Additionally, the presence of equine species in the samples was an unacceptable moral issue as well as represents a potential health risk for human as consumption of horse and donkey meat may be a source of zoonotic diseases **(Yang *et al.*,2013)**.

5. Chicken Luncheon:

Detection of adulteration of chicken luncheon samples with different species was revealed in **table 17** and **figure 21**, while the clear DNA bands of different species on the electrphoretic gel photos of the examined samples were showed in **figure 22**. The results showed that all the examined samples contained chicken species except 1(10%) sample, which did not contained chicken as well as any of the examined species. Additionally, beef and dog species were detected in 6(60%) and 2(20%) out of 10 samples, respectively. However, none of the samples was adulterated with pork, equine or sheep species.

The current results agreed with **Hsieh *et al.* (1995)** who reported that beef was found to be the adulterating species in ground poultry meat sold in retail markets. Additionally, **Dahlan and Sani (2014)** found that chicken luncheon samples were adulterated by beef which was not stated on the labels.

The deliberate substitution of chicken meat with expensive beef or sheep meat is generally not anticipated, one possible explanation of these results that the problem might be centered in the meat grinding operations, market managers readily admitted that they did not routinely clean grinders when changing from ground beef to another meat. They processed several types of meat products, including different meat species, so cross-contamination is bound to occur. Additionally, another reason for the appearance of beef DNA in samples probably might be due to the addition of non-fat dry milk powder in order to increase overall yield, to improve taste and to improve binding qualities **(Di Pinto *et al.*, 2015)**.

From another point of view, the addition of beef or sheep to chicken products was thought to be done by utilizing the unmarketable trimmings left

from expensive meats as well as the dead carcasses in order to extend the bulk of the products. The inedible parts of a meat animal are skin, glands, reproductive organs excluding testicular parts, urinary organs excluding kidney, ears, nails, horns, esophagus, genitals and offals. Some studies have shown that inedible parts of carcasses may be infected with some pathogens such as Brucella melitensis, Brucella abortus, Hepatitis E and coliforms which are potentially risk of zoonosis **(Fatma and Mahdey, 2010)**. Additionally, introducing infected nervous system tissues of cattle to the human food chain by adding them during processing of different meat products were the most likely cause of human infection with transmissible spongiform encephalopathy **(Brown, 2003)**. The regulations to protect the public health against adulteration and zoonoses strictly prohibit the inedible and lower quality meat either to be directly launched or to be processed in the food chain **(Bowling *et al.*, 2007)**.

6. Chicken Burger:

Table 18 and **figure 23** showed the adulteration of chicken burger by different species detected by PCR technique. While, **figure 24** showed the electrphoretic gel photos of the chicken burger samples. The results revealed that 2(20%) out of 10 samples did not contain any chicken species, one sample of them was detected to be adulterated with beef, sheep and dog species together, while the other sample did not contain any one of the examined species. Additionally, the results revealed that 4(40%), 4(40%) and 1(10%) out of 10 samples were adulterated with beef, sheep and dog species, respectively. Finally, none of the samples was adulterated with equine or pig meat.

Likewise, these results were the same as those reported by **Hamzah *et al.* (2014)** who demonstrated that none of the samples were adulterated with porcine residuals, and also similar to **Dahlan and Sani (2014)** who reported a

chicken burger sample contained beef meat species that were not stated in the product label.

From a point of view to the results, beef and sheep are more expensive than chicken, so there is no apparent economic reason for the addition of beef or sheep to chicken products. Possible reasons for their undisclosed presence might be due to cross contamination or the addition of nonconforming meat products to be reworked into non-identical formulations **(Ayaz *et al.*, 2006)**. Additionally, a probable reason for such occurrences could be attributed to actions of 'converting waste to profit', whereby unmarketable, lower-valued beef or sheep trimmings in addition to dead carcasses are incorporated into processed meat mixtures for the purposes of increasing their bulk **(Hsieh *et al.*, 1995)**. Moreover, there are many zoonotic infectious diseases can be transmitted from materials of animal origin such as cyst hydatid, toxoplasma, leptospirosis and brucellosis **(Jackwood and Sommer-Wagner, 2010)**.

Additionally, the absence of pig species in the samples was very important issue for some consumer groups, such as Muslims, as the presence of pig meat or any of its remnants, dog and cat in food are forbidden **(Sahilah *et al.*, 2011)**.

Furthermore, the current study pointed out the general adulteration of different examined meat products detected by PCR technique, which were summarized in **Table 19**. The results revealed that 9 (90%) of hawawshi samples, 9 (90%) of sausage samples, 9 (90%) of beef luncheon samples, 10 (100%) of beef burger samples, 7 (70%) of chicken luncheon samples and 6 (60%) of chicken burger samples were adulterated with undeclared species **(Figure 25)**. These results indicated that 50 out of 60 different raw and cooked meat product samples with a total percentage of 83.3% were contained undeclared species.

These results were higher than that reported by **Ayaz *et al.* (2006)** who found that 22.0% of all the samples were not in compliance with the labels. While, **Abdeen (2008)** reported that 20% of beef sausage samples, 26.7% of beef luncheon samples and 20% of beef burger samples were adulterated, with a total percentage of adulteration 55.9%. Additionally, **Hussien (2011)** revealed that 55% of luncheon samples, 85% of hawawshi samples and 95% of sausage samples were found to contain undeclared species and reported a total percentage of adulteration 78.3%. Moreover, **Özpinar *et al.* (2013)** reported that the adulteration was detected mostly in 50% of sausage samples. While, **Doosti *et al.* (2014)** found that 8.82% of beef sausage samples and 7.27% of beef burger samples were contained undeclared meat.

Additionally, **Yosef *et al.* (2014)** showed that the adulteration rates detected in beef luncheon, beef sausage and beef burger samples were 72.7%, 54.5% and 36.4%, respectively, with 66.2% of all samples were labeled incorrectly. While, **Di Pinto *et al.* (2015)** found 57% mislabeling cases among the examined meat products. Moreover, **Zahran and Hagag (2015)** showed that 4%, 3% and 5% of all the examined samples were adulterated with sheep, goat and donkey meat, respectively, with 12% total rate of adulteration. Additionally, **Bourguiba-Hachemi and Fathallah (2016)** showed the presence of horse and pork DNA in 7% and 26% of tested samples, respectively. Finally, **Cetin *et al.* (2016)** revealed that chicken and horse adulteration rate of the analyzed samples were24.8% and 0.8%, respectively.

In addition, the present study showed the mostly adulterating species detected in the examined meat products. **Table 20** and **figure 26** revealed that the major adulterating species of beef meat products were chicken (75%), followed by sheep (55%), equine (15%) then dog (5%). While, for chicken meat products **(Table 21** and **figure 27)**, the major adulterating species were beef

(50%), followed by sheep (20%) then dog (15%). These results were disagreed with **Abdeen (2008)** who found that the mostly detected adulterating species in burger, sausage and luncheon samples were pork 11.8%, followed by donkey 7.5%, chicken 3.2% then dog meat 1.1%. While, **Hussien (2011)** found the most adulterating species in beef luncheon, hawawshi and sausage samples were chicken 53.3%, followed by pork 31.6% then donkey 11.66%.

Totally, the above mentioned findings present an interesting example of how short comings or lack of clarity in local regulations can easily be capitalized on by some meat producers for financial gain, and adulteration of meat products with various undeclared species in different meat products becomes a widespread problem found in our markets. Thus, rapid and useful tests are recommended for exercising more rigorous controls over industrial meat products, for the benefit of target consumers. Most of the approaches applied to detect this type of fraud required wide variety of laboratory-based tools and technologies, as well as the results could take time to be returned to the investigation authorities. Thus, having a presumptive test for rapid identification could lead to savings in terms of cost and time and allow the sample prioritization if confirmatory testing in a laboratory is required later **(Dawnay *et al.*, 2016)**.

Application of On-Site Raw Meat FlowThrough™ Test to the Examined Meat Products (Bio-Check,UK):

There is an ever-increasing need for rapid methods and instrumentation in the field of meat industry in order to deal with several issues including; the monitoring of processes at all stages, showing due diligence in the control of meat quality and achieving rapid results for detecting meat authenticity and/or adulteration. Developments in analytical techniques have led to the emergence

of a wide range of onsite rapid methods to complement the traditional methods. Faster results, higher productivity, lower costs and increased sensitivity are the needed properties for them **(Van Amerongen *et al.*, 2007)**.

Raw Meat FlowThrough™ speciation test used in this study is innovative products designed for deployment and use in meat processor premises as well as in smaller meat analysis laboratories. It was simple and easy test as it required no additional equipments to be performed. It was quick and rapid as typically took 12 minutes. Its principle based on enzyme-linked immunosorbent assay (ELISA) by utilizing highly purified antibodies to detect species-specific animal serum protein (albumin), which is found at high levels in raw meat. Additionally, it was sensitive as had been validated for the detection of adulteration of raw meat products at about the 1% level. Its detection limit (1%) was verified against Laboratory of the Government Chemist (LGC) Reference Materials **(Bio-Check, United Kingdom)**. In addition, it was highly specific as no known false positives.

With respect to the presented results, **table 22** showed the application of poultry specific kits of Raw Meat FlowThrough^{TM} Test for detection of meat product samples adulteration with chicken species. The results revealed that the kits succeeded in fast onsite detection of poultry species in the chosen hawawshi, sausage and beef burger samples, as well as their success in detecting that the chicken burger sample did not contain any poultry species. These results agreed with those of the PCR technique by 100%. Additionally, the control gave positive result.

In consistency with results, these rapid tests were robust and fast in their ability to detect meat species adulteration within few minutes in the varied meat products, in addition to their highly field portability that all the materials

required to conduct them can be readily packaged as a kit. Similarly, **Giovannacci *et al.* (2004)** evaluated the performances of commercial ELISA kits for identification of pork, beef, sheep and poultry species in forty commercial meat products. The results found that twenty products were in complete agreement with labels, while the other products showed non-labeled species. Additionally, some species were not detected although they were mentioned on the labels. Moreover, **Ulca *et al.* (2013)** showed that 36 of 42 samples were negative for the presence of pork (< 0.1%), while four samples were found to be containing pork. However, one sausage sample was labeled as containing 5% beef, but beef was not detected, in addition to a meatball sample labeled as 100% beef was found to contain chicken.

Moreover, the results presented in **table 23** showed the application of horse specific kits of Raw Meat FlowThrough^{TM} Test for detection of meat product samples adulteration with equine species. The results revealed that the retested hawawshi, sausage, beef burger and chicken burger samples gave negative results which showed that they did not contain horse species, although their adulteration with equine species were previously proved by PCR technique, however, the control had given positive results. Therefore, it was suggested that these retested samples might be adulterated with equine species other than horse, as donkey or mule species, as well as the test had not the ability to detect all the equine species but worked only on horse.

Totally, in the light of the current work, it was proposed that the commercial processed meat products can be tested on-site for adulteration detection and only those which tested positive by the kit will need to be taken back to the laboratory for confirmatory analysis **(Muldoon *et al.*, 2004)**. This test can contribute in the performance of precise quality controls of meat products in a fast routine manner. Furthermore, increased precision of the

methods used will allow increased trust in the safety of the products in international trade and will provide reliable evidence for the probable adulteration cases **(Kesmen *et al.*, 2010)**. Additionally, any unintentional low-level presence of trace amounts of one type of meat products with another during processing and handling may be regulated and controlled by the authorities with frequent monitoring procedures at all levels starting from primary production and processors all the way to the end of the supply chain **(Premanandh 2013)**.

Conclusions and Recommendations

In the light of the previous achieved results, it could be concluded that the examined meat products showed a wide varieties of proximate compositions with some of them were not compatible with the Egyptian Standards. The raw material might affect the quality and nutritive value of these products significantly as well as the ingredients added. From another side, it could be concluded that most of the examined meat products were adulterated by more than one meat species (mainly three species) that might be attributed to either intentional adulteration by mixing of lower-cost or banned species into higher cost products or unintentional mixing of meat species due to cross-contamination during processing.

Additionally, it was obvious that the PCR technique is a highly powerful, sensitive, specific and applicable tool for species identification in processed meat products even though adulteration was unintentional and at a very low level. It can be used as a routine control method in food control laboratories for the verification and control of adulterated commercial meat products and can provide superior levels of precision to authentication monitoring and law enforcement.

Likewise, the raw meat FlowThrough™ kits were easy-to-use, robust, onsite tests efficiently detected low levels (<1%) of meat species adulteration in raw meat products in under 15 minutes. Their application can enable meat inspectors as well as regulatory agencies to more efficiently manage and control the adulteration of meat products by rapid effective decision-making and thereby control the spread of the problem and improve its eventual eradication, as well as save laboratories time and money and give them peace of mind to the integrity of the raw materials and products. However, there is a need for increasing the range of the detected animal species in order to include most of the animals that may be used for adulteration.

Against this backdrop, this study may contribute to the performance of precise quality controls of meat products in a routine manner, production of quality and safe products in the meat industry, as well as protection of public health. Additionally, transparency can be enhanced on the local market, consequently the public confidence in the meat supply chain will certainly be promoted and regained, and the demand for processed meats may be maintained or even increased.

Recommendations

Proper actions should be taken to limit such frauds with taking the following recommendations in consideration:

- High quality, pure and non-adulterated raw meat and additives should be used in meat products manufacturing.
- Meat processing plants should process a single species, or should process their products in a separated production line.
- Activating the role of the health surveillance from the responsible bodies in health directorate and logistics directorate and the application of the Egyptian standard specifications during manufacturing processes based on the research results.
- Calls for the production of Egyptian standards specific for hawawshi as it is one of the most common famous traditional Egyptian meat product.
- Calls for the insertion of the permissible limits of carbohydrate contents of each meat product within its Egyptian Standards.
- The governmental food control authorities must continuously regulate meat products using effective methods and upgrade their systems to identify meat products sources and monitor quality to ensure that proper processing has taken place and labeling information reflects actual contents.

- Developing the methods of identifying species of meat in the Egyptian standards of meat products in order to cover the gaps, which are exploited by the offenders.
- Food laboratories need to have available, fast and accurate methods as PCR techniques to ascertain the species used in the manufacture of meat products.
- Calls for using commercial field-test kits for the on-site large-scale screening of meat products as a presumptive test for rapid identification of meat species with saving time, cost and efforts.
- Continuous screening of commercial meat products for adulteration in local markets and restaurants should be applied under veterinary authority.
- Governmental agencies and food industry leaders must be coordinated to improve the regulations, monitoring and analysis of meat for detection of its adulteration.
- Quickly issue in law on food safety that makes one body (food safety authority) is the mechanism of food safety in all its forms, making it easier with the provisions of oversight and control, and prevent conflicts between the parties concerned to control health and meat products.
- Media (newspapers, radio, T.V. and social media) can play an important role in controlling the problem of meat adulteration in Egypt.
- Education of consumers is one of the keystones of the effective control of meat adulteration.
- Consumers should purchase their requirement of meat products from known trusted shops, butchers and restaurants.
- A continuous monitoring scheme by the General Organization of Veterinary Services along with improved detection methodologies and stringent sanctions on defaulters may help to minimize authentication problems in future.

Summary

Recently, the fraudulent substitution or adulteration of high quality components with their inferior or cheaper counterparts is a common practice prevalent in meat industry all over the world including Egypt. Thus, nutritive evaluation as well as species identification of animal tissues in meat products is an important issue in protecting the consumer from illegal or undesirable adulteration, for economic, religious and health reasons.

Accordingly, a total of 120 commercial beef and chicken meat product samples were collected from street vendors, fast food restaurants, butchers and retail markets in Ismailia city. The beef meat product samples were represented as 20 each of hawawshi, fresh oriental beef sausage, beef luncheon and beef burger, while the chicken meat product samples represented as 20 each of chicken luncheon and chicken burger. All the samples were subjected to proximate analysis, and then compared with the Egyptian standards to determine their acceptability. After that, 60 samples (10 each) of the previously examined samples were analyzed by the conventional Polymerase chain reaction (PCR) technique for the detection of undeclared meat species. Additionally, eight samples (2 each) of hawawshi, sausage, beef burger and chicken burger, which previously proved their adulteration with chicken and equine species, were retested with the rapid onsite Meat FlowThrough^{TM} test. The obtained results can be summarized as follows:

- **<u>Nutritive Evaluation of the Examined Samples:</u>**

1) **Hawawshi:** The results revealed that the mean values were 58.18%, 9.61%, 26.36%, 2.58%, 3.26%, and 40.32% for moisture, protein, fat, ash, carbohydrates and meat contents, respectively. Whereas there are no specific standards for hawawshi as a meat product, so the results were not compared with the standards.

2) **Oriental Sausage:** The results showed that the mean values were 60.56%, 15.56%, 19.14%, 3.13%, 1.58%, and 67.33% for moisture, protein, fat, ash, carbohydrate and meat contents, respectively. By comparing the results with the Egyptian Standards, there were 50%, 30% and 25% of the samples unaccepted based on their moisture, protein and meat contents, respectively.

3) **Beef luncheon:** The results revealed that the mean values were 62.52%, 8.49%, 5.37%, 3.13%, 20.47% and 26.02% for moisture, protein, fat, ash, carbohydrate and meat contents, respectively. By comparing the results with the Egyptian Standards, there were 100%, 90%, 65% and 25% of the samples unaccepted based on their meat content, protein, moisture and ash contents, respectively.

4) **Beef burger:** The results pointed that the mean values were 61.44%, 11.68%, 16.27%, 3.94%, 6.65% and 47.58% for moisture, protein, fat, ash, carbohydrate and meat contents, respectively. By comparing the results with the Egyptian Standards, there were 85%, 85%, 50%, 35% and 25% of the samples unaccepted based on their protein, meat content, moisture, fat and carbohydrate contents, respectively.

5) **Chicken luncheon:** The results revealed that the mean values were 67.34%, 12.93%, 4.13%, 3.44%, 12.14%, and 52.20% for moisture, protein, fat, ash, carbohydrate and meat contents, respectively. By comparing the results with the Egyptian Standards, there were 100%, 100%, 50% and 40% of the samples unaccepted based on their meat content, moisture, ash and protein contents, respectively.

6) **Chicken burger:** The results showed that the mean values were 64.26%, 11.72%, 7.70%, 3.21%, 13.08%, and 46.11% for moisture, protein, fat, ash, carbohydrate and meat contents, respectively. By comparing the results with the Egyptian Standards, there were 85%, 72% and 65% of the samples unaccepted based on their meat content, ash and protein contents, respectively.

In addition, the present study showed the significant differences of chemical parameters between the examined meat products. As regard to **the moisture contents**, the results showed no significant differences ($P \leq 0.05$) between hawawshi and sausage, no significant differences between sausage, beef luncheon and beef burger, and no significant differences between beef luncheon and chicken burger. On the other hand, there were significant differences between chicken luncheon and all the other products.

While for **protein content,** there were no significant differences ($P \leq 0.05$) between hawawshi and beef luncheon and no significant differences between beef burger, chicken luncheon and chicken burger. However, there were significant differences between sausage and all the other products.

However, concerning **fat contents,** there were significant difference ($P \leq 0.05$) between hawawshi and all the other products, while there were no significant differences between sausage and beef burger, between beef luncheon and chicken luncheon and between beef luncheon and chicken burger.

While, according to the **ash content**, there were significant difference ($P \leq 0.05$) between hawawshi and all the other products, and between beef burger and all the other products, while there were no significant differences between sausage, beef luncheon, chicken luncheon and chicken burger.

Moreover, with respect to **the carbohydrate contents**, there were no significant difference ($P \leq 0.05$) between hawawshi and sausage, and between chicken luncheon and chicken burger. While there were significant differences ($P \leq 0.05$) between beef luncheon and all the other products, and between beef burger and all other products.

While, regarding to **meat contents**, there were no significant differences ($P \leq 0.05$) between hawawshi, beef burger and chicken burger, and chicken luncheon. However, there were significant difference ($P \leq 0.05$) between sausage and all the other products, and between beef luncheon and all the other products.

Detection of Species Adulteration by Using Conventional PCR Technique:

1) **Hawawshi:** The results revealed that 20% of samples considered adulterated as they did not contain beef meat in spite of their selling as beef products. Additionally, there were 80%, 60% and 10% of samples adulterated with chicken, sheep and equine species, respectively. However, none of the samples was adulterated with pork or dog species.

1) **Oriental sausage:** The results revealed that 20% of samples were adulterated due to absence of beef meat. Additionally, sheep, chicken and equine species were detected in 80%, 50% and 10% of samples, respectively. However, no porcine or dog species were found.

2) **Beef luncheon:** The results pointed out that all samples contained beef meat and free from pork species. Additionally, 70%, 30%, 20% and 10% of samples were adulterated with chicken, sheep, dog and equine species, respectively.

3) **Beef burger:** The results showed that all the samples contained beef meat but also all of them were adulterated with chicken species (100%). While none of the samples were adulterated with pig or dog species. Furthermore, the results showed that 50% and 30% of samples were adulterated with sheep and equine species, respectively.

4) **Chicken luncheon:** The results showed that all samples contained chicken meat except 10% of samples. Additionally, beef and dog species were detected in 60% and 20% of samples, respectively. However, none of the samples was adulterated with pork, equine or sheep species.

5) **Chicken burger:** The results revealed that 20% of samples contained no chicken meat. Moreover, 40%, 40% and 10% of samples were adulterated with beef, sheep and dog species, respectively. While none of the samples were contained equine or pig species.

Additionally, the current study showed the general adulteration of different examined meat products detected by PCR technique. It was obvious that 90%, 90%, 90%, 100%, 70% and 60% of hawawshi, sausage, beef luncheon, beef burger, chicken luncheon and chicken burger samples, respectively were adulterated by undeclared species with a total percentage of 83.3%.

In addition, the present study showed the most adulterating species in the examined beef and chicken meat products. Concerning beef meat products, the major adulterating species were chicken (75%), followed by sheep (55%), equine (15%) then dog (5%). Likewise, for chicken meat products, the major adulterating species were beef (50%), followed by sheep (20%) then dog (15%).

- **On-Site Detection of Meat Species by FlowThrough™ Test:**

In consistency with results, these rapid tests were robust and fast in their ability to detect meat species adulteration within few minutes in the varied meat products, in addition to their highly field portability that all the materials required to conduct them can be readily packaged as a kit.

Poultry specific kits: The results revealed that the kits succeeded in fast onsite detection of poultry species in the chosen hawawshi, sausage and beef burger samples, as well as their success in detecting that the chicken burger sample did not contain any poultry species. These results agreed with those of the PCR technique by 100%. Additionally, the control gave positive result.

Horse specific kits: The results revealed that the retested hawawshi, sausage, beef burger and chicken burger samples did not contain horse species, although their adulteration with equine species were previously proved by PCR technique, however, the control had given positive results. Therefore, it was suggested that these retested samples might be adulterated with equine species other than horse, as donkey or mule species, as well as the test had not the ability to detect all the equine species but worked only on horse.

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الملخص العربي

في الاونة الأخيرة، أصبح الغش التجاري في اللحوم ومنتجاتها من أكثر المشاكل شيوعا علي مستوى العالم. ولذلك فكان الهدف من هذة الدراسة هو التعرف علي القيمة الغذائية لبعض منتجات اللحوم المتداولة بالأسواق والكشف عن الغش بانواع لحوم غير معلن عنها باستخدام تقنيات حديثة يمكن استخدامها بصورة دورية لحماية الصحة العامة للمستهلكيين ضد مخاطر الغش التجاري.

فتم في هذه الدراسة تجميع عدد 120 عينة عشوائية من منتجات اللحوم و الدواجن المتداولة بأسواق مدينة الاسماعيلية بواقع عشرون عينة ل كل من الحواوشي، االسجق الشرقي، اللانشون البقري، البيجرالبقري، لانشون الدجاج وبرجر الدجاج ليتم الفحص الكيميائي لهم وتقدير مدي المطابقة بالمواصفات القياسية المصرية. ثم تم اختيار عدد 60 عينة عشوائية من العينات التي سبق فحصها بواقع10 لكل منهم وذلك للكشف عن وجود الغش بلحوم الخنزير، الخيول، الكلاب، الاغنام أو الدجاج عن طريق استخدام تقنية تفاعل البلمرة المتسلسل ثم اختيار بعض من العينات الأيجابية للغش لفحصها باختبار حديث وسريع يسمي (FlowThrough^{TM}) للكشف عن الغش التجاري في خلال بضع دقائق . وقد أوضحت النتائج ما يلي:

أولا: الفحص الكيميائي لعينات منتجات اللحوم:

١) الحواوشي :

أظهرت النتائج أن نسبة متوسط الرطوبة، البروتين، الدهون، الرماد، الكربوهيدرات و اللحم الاحمر لمنتج الحواوشي كانت 58.18٪، 9.61٪، 26.36٪، 2.58٪، 3.26٪، و 40.32٪ ، على التوالي. ولم يتم مطابقة العينات بالمواصفات القياسية نظرا لعدم وجود مواصفقياسية مصرية لمنتج الحواوشي.

٢) السجق الشرقي:

أوضحت النتائج أن نسبة متوسط ا لرطوبة، البروتين، الدهون ، الرماد ، الكربوهيدرات و اللحم الاحمر لمنتج السجق الشرقي كانت 60.56٪، 15.56٪، 19.14٪، 3.13٪، 1.58٪، و 67.33٪، على التوالي. ومطابقة النتائج بمعايير المواصفلت القياسية المصرية للسجق الشرقي رقم 1972 لعام 2005 أظهرت أن 25٪، 30٪ و 50٪ من العينات كانت غير متطابقة مع المواصفة القياسية على أساس نسبة اللحم الاحمر، البروتين والرطوبة، على التوالي، في حين ان جميع العينات كانت م تطابقة بناء على محتوى الدهون والرماد.

٣) اللانشون البقري:

أثبتت النتائج أن نسبة متوسط الرطوبة، البروتين، الدهون، الرماد، الكربوهيدرات و اللحم الاحمر لمنتج اللانشون البقري كانت 62.52٪، 8.49٪، 5.37٪، 3.13٪، 20.47٪ و 26.02٪، على التوالي. ومقارنة النتائج بمعايير المواصفات القياسية المصرية رقم 1114 لعام 2005 اوضحت أن 100٪، 90٪، 65٪ و 25٪ من العينات كانت غير متطابقة بناء على نسبة اللحم الاحمر، البروتين، الرطوبة والرماد، على التوالي.

٤) البرجر البقري:

كشفت النتائج أن نسبة متوسط الرطوبة، البروتين، الدهون، الرماد، الكربوهيدرات و اللحم الاحمر لمنتج البرجر البقري كانت 61.44٪، 11.68٪، 16.27٪، 3.94٪، 6.65٪ و 47.58٪، على التوالي. ومقارنة النتائج بمعايير المواصفات القياسية المصرية رقم 1688 لعام 2005 كشفت أن 85٪، 85٪، 50٪، 35٪ و 25٪ من العينات غير متطابقة بناء على نسبة البروتين، اللحم الاحمر ، الرطوبة، الدهون والكربوهيدرات، على التوالي.

٥) لانشون الدجاج:

أظهرت النتائج أن نسبة متوسط ا لرطوبة، البروتين، الدهون ، الرماد ، الكربوهيدرات و اللحم الاحمر لمنتج لانشون الدجاج كانت 67.34٪، 12.93٪، 4.13٪، 3.44٪، 12.14٪، 52.20٪، على التوالي. ومن خلال مقارنة النتائج بمعايير المواصفات القياسية المصرية رقم 1696 لعام 2005 كانت 100٪، 100٪، 50٪، و 40٪ من العينات غير متطابقة بناء على نسبة اللحم الاحمر، الرطوبة ، الرماد والبروتين، على التوالي.

٦) برجرالدجاج:

أوضحت النتائج أن نسبة متوسط ا لرطوبة، البروتين، الدهون ، الرماد ، الكربوهيدرات و اللحم الاحمر لمنتج برجر الدجاج كانت 64.26٪، 11.72٪، 7.70٪، 3.21٪، 13.08٪، 46.11٪، على التوالي. وبمقارنة النتائج بمعايير المواصفات القياسية المصرية لبرجر الدجاج رقم 2910 لعام 2005 كشفت أن 85٪، 72٪ و 65٪ من العينات كانت غير م تطابقة بناء على نسبة اللحم الاحمر، الرماد و البروتين، على التوالي.

ثم تم تحديد الفروق المعنوية بين المنتجات التي تم فحصها، فأظهرت النتائج ما يلي:

من حيث نسبة الرطوبة: عدم وجود فروق معنوية بين منتجات الحواوشي والسجق الشرقي، أو بين السجق الشرقي، اللانشون البقري والبرجر البقري ، وايضا عدم وجود فروق بين البرجر البقري وبرجر الدجاج. ولكن كانت هناك فروق معنوية كبيرة بين لانشون الدجاج وجميع المنتجات الأخرى.

من حيث نسبة البروتين: لم تكن هناك فروق معنوية بين الحواوشي و اللانشون البقري أوبين البرجر البقري ولانشون الدجاج وبرجر الدجاج. ولكن كانت هناك فروق معنوية بين السجق الشرقي وجميع المنتجات الأخرى.

من حيث نسبة الدهون: أظهرت النتائج فروق معنوية بين الحواوشي وجميع المنتجات الأخرى، بينما عدم وجود فروق معنوية بين السجق الشرقي والبرجر البقري أوبين اللانشون البقري وبرجر الدجاج.

من حيث نسبة الرماد: أظهرت فروق معنوية بين الحواوشي وجميع المنتجات الأخرى، وبين البرجر البقري وجميع المنتجات الأخرى، في حين لم تكن هناك فروق معنوية بين السجق الشرقي، اللانشون البقري، لانشون الدجاج وبرجر الدجاج.

من حيث نسبة الكربوهيدرات: أظهرت النتائج عدم وجود فروق معنوية بين الحواوشي والسجق الشرقي، وبين لانشون الدجاج وبرجر الدجاج. في حين كانت هناك فروق معنوية بين اللانشون البقري وجميع المنتجات الأخرى، وبين البرجر البقري وجميع المنتجات الأخرى.

من حيث نسبة اللحم الكلي: أظهرت النتائج عدم وجود فروق معنوية بين الحواوشي، البرجر البقري، برجر الدجاج ولانشون الدجاج. ولكن كانت هناك فروق معنوية بين السجق الشرقي وجميع المنتجات الأخرى، وبين اللانشون البقري وجميع المنتجات الأخرى.

ثانيا: الكشف عن غش اللحوم في المنتجات باستخدام تقنية تفاعل البلمرة المتسلسل:

١) الحواوشي:

كشفت النتائج أن أكثر معدلات الغش في الحواوشي كانت بالدجاج يليها ا لأغنام ثم الخيول بنسبة 80٪ ، 60٪ و 10٪، علي التوالي. بالإضافة إلى أن 20٪ من العينات تم الغش بعدم وجود اللحم البقري بها. وأثبتت النتائج عدم وجود حالات غش بلحم الخنزير أو الكلاب. ثم أوضحت أن اجمالي نسبة الغش كانت 90٪ من العينات.

٢) **السجق الشرقي:**

أظهرت النتائج أن 20٪ من العينات لا تحتوي علي لحم بقري. بالإضافة إلى ذلك، كشف ت النتائج أن أعلي نسبة غش كانت بالأغنام وهي 80٪، يليها الدجاج بنسبة 50٪ ثم الخيول بنسبة10٪ من العيرات. ولكن لم يتم العثور على لحم الخنزير أو كلاب في العينات التي تم فحصها. وأوضحت النتائج أن اجمالي نسبة الغش كانت 90٪ من العينات.

٣) **اللانشون البقري:**

أشارت النتائج إلى أن جميع العينات التي تم فحصها تحتوي علي لحم بقري، وان جميعها خالية من لحم الخنزير. بالإضافة إلى ذلك، أثبتت النتائج ان نسبة الغش بالدجاج ،الأغنام، الكلاب والخيول كانت 70٪، 30٪، 20٪ و 10٪ من العينات، على التوالي. وأوضحت النتائج أن اجمالي نسبة الغش كانت 90٪.

٤) **البيجرالبقري:**

أظهرت النتائج أن جميع العينات تحتوي على اللحم البقري ولكن جميعها أيضا تم الغش بها بالدجاج بنسبة 100٪. في حين ان جميع العينات خالية من لحم الخنزير أو الكلاب. بالإضافة إلى ذلك، أظهرت النتائج أن 50٪ و 30٪ من العيرات تم غشها بالأغنام والخيول ،على التوالي. وأوضحت النتائج أن اجمالي نسبة الغش كانت 100٪ من العينات.

٥) **لانشون الدجاج:**

أوضحت النتائج أن 10٪ من العينات لا تحتوي على لحم الدجاج ولا تتضمن أيا من الأنواع التي تم فحصها. بينما كشفت النتائج ان نسبة الغش بالبقر كانت 60٪ من العيرات بينما نسبة الغش بلحم الكلاب كانت 20٪. في حين ان لم تثبت النتائج وجود حالات غش بلحم الخنزير أو الخيول أو الأغنام. وأوضحت النتائج أن اجمالي نسبة الغش كانت 70٪ من العينات.

٦) **برجر الدجاج:**

كشفت النتائج أن 20٪ من العينات لا تحتوي على لحم الدجاج بينما وجد ال بقري، الاغنام والكلاب في40٪، 40٪ و 10٪ من العينات، على التوالي. في حين ان النتائج اثبتت عدم وجود حالات غش بلحم الخنزير أو الخيول. وأوضحت النتائج أن اجمالي نسبة الغش كانت 60٪ من العينات.

ثم أوضحت الدراسة أيضا أن اكثر أنواع الفصائل المتواجدة في غش منتجات اللحوم البقري التي تم فحصها كانت الدجاج (75٪)، تليها الأغنام (55٪)، الخيول (15٪) ثم الكلاب (5٪). بينما في حالة منتجات لحوم الدجاج اكثر أنواع الغش كانت بالبقر (50٪)، تليها الأغنام (20٪) ثم الكلاب (15٪).

ثالثا: الكشف الموضعي السريع عن غش أنواع اللحوم عن طريق تطبيق اختبار (FlowThrough^{TM}):

١) **كواشف لحم الدجاج:** أظهرت النتائج أن هذه المجموعات نجحت في الكشف السريع عن وجود الدجاج في عينات الحواوشي والسجق الشرقي والبرجرالبقري ، وكذلك نجاحها في الكشف أن عينة برجر الدجاج لا تحتوي على أي نوع من أنواع الدواجن. وقد اتفقت هذه النتائج مع نتائج تقنية تفاعل البلمرة المتسلسل بنسبة 100٪. بالإضافة إلى ذلك، أعطت العينة القياسية نتيجة إيجابية.

٢) **كواشف لحم الحصان:** أظهرت النتائج أن عينات الحواوشي ،السجق الشرقي ،البرجرالبقري و برجر الدجاج لم تحتوي على لحم الحصان، في حين ان العينة القياسية للاختبار أعطت نتيجة إيجابية، بالاضافة الي أن تفاعل البلمرة المتسلسل قد أعطي سابقا نتائج ايجابية مع لحم الخيول. وبذلك فهذا يوضح أن هذه العينات كانت مغشوشة بأنواع خيول أخري غير الحصان، مثل الحمير أو البغال، فضلا عن أن هذا الاختبار له القدرة علي الكشف عن لحوم الحصان فقط وليس له القدرة على الكشف عن باقي أنواع الخيول.

أوضحت النتائج أن هذه الاختبارات الموضعية قوية وسريعة في قدرتها على الكشف عن غش أنواع اللحوم في منتجات اللحوم المتنوعة في غضون دقائق قليلة ، بالإضافة إلى سهولة نقلها واستعمالها حيث أن جميع المواد اللازمة لإجراءها متوفرة، وهذا يوضح امكانية استخدام هذه الكواشف في الكشف الدوري المبدئي عن وجود الغش في المنتجات الي أن يتم التأكد من العينات الايجابية في معامل الفحص وهذا يؤدي الي توفير الوقت، الجهد والمال.

السيرة الذاتية

الإسم: رحاب السيد مسعد جعفر

تاريخ الميلاد: 1983/1/29م في الإسماعيلية.

الإقامة: محافظة الإسماعيلية

المؤهلات:

- الشهادة الإبتدائية عام 1994م من مدرسة السلام الخاصة للغات بالإسماعيلية.
- الشهادة الإعدادية عام 1997م من مدرسة السلام الخاصة للغات بالإسماعيلية.
- الشهادة الثانوية عام 2000م من مدرسة السلام الخاصة للغات بالإسماعيلية.
- بكالوريوس فى العلوم الطبية البيطرية من كلية الطب البيطرى بجامعة قناة السويس بالإسماعيلية عام 2005م.
- درجة الماجستير فى الرقابة الصحية على الأغذية تخصص الرقابة الصحية على اللحوم بكلية الطب البيطرى بالإسماعيلية عام 2012م.
- تسجيل لدرجة الدكتوراه فى الرقابة الصحية على الأغذية تخصص الرقابة الصحية على اللحوم بكلية الطب البيطرى بالإسماعيلية عام 2012م

جهة العمل: باحث مساعد في معهد بحوث صحة الحيوان، معمل الإسماعيلية.

لجنة الإشراف

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قسم الرقابة الصحية على الأغذية

دراسات متقدمة للكشف على الغش التجارى لمنتجات اللحوم في أسواق الاسماعيلية

رسالة مقدمة من

ط.ب./ رحاب السيد مسعد جعفر

بكالوريوس العلوم الطبية البيطرية كلية الطب البيطرى - جامعة قناة السويس (2005)
ماجستير العلوم الطبية البيطرية كلية الطب البيطرى - جامعة قناة السويس (2012)

وذلك للحصول على
درجة دكتوراة الفلسفة فى العلوم الطبية البيطرية

تخصص
الرقابة الصحية على اللحوم و الأسماك و منتجاتهما و المخلفات الحيوانية

كلية الطب البيطرى
جامعة قناة السويس
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