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Review article

Ensuring Meat Authenticity: Real-Time PCR and Thiamine as Tools for Detecting Adulteration

Amany Abd El-Halim [1*], Nabil Hafez [1], Khalil Khalil[1], Samah Abd El- Tawab[1], Rehab Mahmoud[2]

[1] Department of Food Science and Technology, Faculty. of Agriculture, Fayoum University, Fayoum, Egypt; aaa45@fayoum.edu.eg, kik06@fayoum.edu.eg, saa06@fayoum.edu.eg
[2] Chemistry Department, Faculty of Science, Beni-Suef University, Beni-Suef 62511, Egypt. rehabkhaled@science.bsu.edu.eg).

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CORRESPONDENCE
Amany Abd El-Halim
Email: aaa45@fayoum.edu.eg

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Abstract:

Meat adulteration is a common problem that can lead to moral loss, religious infractions, and major public health hazards. Technologies for rapid detection are essential for monitoring this problem. This article explores the economic issue of food adulteration, particularly in meat products, and the detection methods for beef and pork adulteration, focusing on thiamine content determination and real-time RTPCR methods. Therefore, combining thiamine quantification with (Real-Time PCR) analysis provides a robust and complementary approach to meat authenticity testing. These methods not only enhance the reliability of food labeling but also support compliance with religious and health standards. It is recommended that these techniques be further developed and routinely implemented as part of comprehensive food safety and meat inspection systems. In parallel, (Real-Time PCR) offers high sensitivity and specificity in detecting species-specific DNA, even in thermally processed meat products. This makes it a powerful tool for identifying undeclared meat components and verifying label claims.

Keywords: canned meat, beef, pork, thiamine, Real Time PCR.

Introduction

Important nutrients with a high biological value that are necessary for human nutrition can be found in meat and meat products. High-quality protein and other essential nutrients can be found in meat. Protein, iron, zinc, and B vitamins are all abundant in meat products (Lawrie 1971 and Thatcher 1987).

According to B1, B2, B6, and B12 reports, an adult can get roughly 10% of their daily energy needs and a significant amount of vital nutrients from just 28 g of meat (Esteve et al. 2002).

Research has demonstrated that a high dietary balance of vitamins, minerals, and other useful substances (fats, proteins, and carbohydrates) protects against both major and minor diseases caused by nutrient deficiencies. Amino acids and proteins are good for body building and human development. Therefore, in order to achieve the necessary health benefits, this food item (meat) must be included in a healthy diet as a significant proportion. However, the prevalence of saturated fats, which can cause coronary heart disease and elevated cholesterol if consumed in excess, raises concerns about meat consumption due to its fat content and fatty acid profile (Ahmad et al., 2018).

Since meat is susceptible to chemical and biological contamination and is thought to be a favorable environment for the growth of microorganisms, it has long been recognized as being extremely perishable. Bacteria are the most significant microorganisms. While some bacteria are not contagious by themselves, when they grow in food, they release toxins that are harmful to

humans when consumed. Poor hygiene during processing can make contamination worse (Ali et al., 2018; Kolalou et al., 2004).

Due to its unique biological and chemical composition, meat undergoes progressive degradation from the time of slaughter until consumption. Generally, microbial activity either naturally present in the meat or introduced during processing plays a key role in this deterioration, especially under aerobic conditions. This activity leads to undesirable changes that compromise the quality and safety of the meat (Nychas et al., 2008).

The susceptibility of meat to spoilage is largely attributed to chemical and enzymatic reactions. The breakdown of fats, proteins, and carbohydrates results in the formation of unpleasant odors, flavors, and slime, ultimately making the meat unfit for human consumption. Therefore, monitoring meat spoilage is essential to extend shelf life and preserve nutritional quality, texture, and flavor (Dave & Ghaly, 2011).

Raw meat and its derivatives can act as vehicles for multiple hazards that pose significant risks to human health. These hazards may be chemical, physical, or biological in nature of particular concern are biological hazards, which may arise from microorganisms and viruses present in soil or within the animals themselves (Sofos, 2014).

Common pathogenic bacteria associated with beef consumption include Salmonella spp., Bacillus cereus, Campylobacter spp., Clostridium perfringens, Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Yersinia enterocolitica, and Vibrio parahaemolyticus (Biswas et al., 2011).

Meat preservation has become increasingly vital to facilitate long-distance transport while maintaining meat's texture, color, and nutritional value (Nychas et al., 2008). The objectives of food preservation include extending shelf life, minimizing spoilage and financial losses, controlling foodborne pathogens and intoxications, and ensuring microbiological safety (Pal, 2014).

Several advanced food preservation techniques are utilized within the food industry, often requiring specialized equipment. Among these methods are irradiation and vacuum packaging. Irradiation involves exposing food to ionizing radiation to eliminate pathogenic microorganisms, thereby extending shelf life. Vacuum packaging, on the other hand, works by removing oxygen from the packaging environment, effectively inhibiting the growth of aerobic bacteria responsible for food spoilage (Chellaiah et al., 2020).

Chilling is one of the most widely adopted methods for short-term preservation of meat. By lowering the storage temperature below the optimal range for microbial growth, chilling effectively slows down or inhibits spoilage (Cassens, 1994). An advanced form of this technique, known as super-chilling, involves partial freezing of the water content within the meat. In this process, the product's surface temperature is reduced by approximately 1–2°C, causing a fraction of the water to freeze. Over time, the temperature stabilizes, and the internal distribution of retained ice contributes to maintaining product quality during storage and transportation (Zhou & Liu, 2010; Magnussen et al., 2008).

Freezing is considered one of the most effective techniques for preserving the original quality of fresh meat. Meat generally consists of 50–75% water by weight, and freezing converts a substantial portion of this water into ice (Heinz & Hautzinger, 2009). One of the major advantages of freezing is its ability to retain most of the meat's nutritional content. However, some nutrient loss may occur due to drip loss during thawing. To prevent quality degradation such as freezer burn—an abnormal condition caused by surface dehydration and the concentration of meat pigments it is crucial to use appropriate packaging materials that provide adequate protection (Pal & Devrani, 2018).

Canning is a widely used method of food preservation that involves thermal processing of food followed by sealing it in airtight containers. This technique can extend the shelf life of food products from one to five years, and under specific conditions, even longer. Canned products are consumed globally and represent a significant portion of the food supply in both developed and developing countries (Mol, 2011; Henchion et al., 2014).

Canned meat possesses high nutritional value, being a rich source of protein, vitamin B12, and essential minerals such as sodium and zinc. However, it often contains elevated levels of saturated fats,

cholesterol, and certain food additives like preservatives, which may raise health concerns if consumed excessively (USDA, 2004).

One of the advantages of canning is its ability to preserve the original taste and flavor of the food, which contributes to its widespread application in the food industry. Although canned foods are generally sterilized during processing, failures in sterilization can lead to microbial contamination and spoilage (Desrosier, 2004).

Microbiological evaluation of canned meat products is crucial to detect the potential presence of bacteria introduced during handling or processing, and to assess the overall hygienic conditions associated with production. While the aerobic plate count (APC) does not directly indicate food safety, it serves as a valuable tool for assessing the sanitary conditions of food manufacturing and packaging processes. Elevated microbial counts are often linked to poor hygiene practices during preparation, making food a reliable indicator of the cleanliness of its environment (Ehiri et al., 2001).

Undergone one or more processing techniques such as comminution, curing, dehydration, cooking, or fermentation, among others (Simonin et al., 2012).

Meat adulteration has emerged as a global concern, raising issues related to food authenticity and consumer trust. One of the most common fraudulent practices in the meat industry is the substitution of high-value meats with less expensive, undeclared alternatives (Aida et al., 2005; Abdel-Rahman et al., 2009). This becomes particularly problematic when it involves the inclusion of meat from species prohibited by certain cultural or religious beliefs, such as pork in Muslim and Jewish communities, or dog meat in Islamic and Buddhist traditions (Nakyinsige et al., 2012; Soares et al., 2010).

Complete traceability and transparency across the meat supply chain are not always guaranteed by labeling alone. One study, for example, found that undeclared species were present in 22% of the meat products examined (Ayaz et al., 2006). Due to their accessibility and affordability, chicken and turkey meat are commonly found in products that are labeled as ground beef or pork, as well as in processed meats like cold cuts and sausages, without being declared (Djurdjevic et al., 2005; Ghovvati et al., 2009).

Such recurrent frauds contribute to a decline in consumer confidence, as well as in the credibility of food safety authorities and public health institutions. Therefore, the implementation of a proactive meat safety management system is essential at every stage of the supply chain to detect and address fraud in a timely manner (Houghton et al., 2006; Yeung et al., 2010). This necessitates the development and application of reliable detection methods, which manufacturers can use to ensure product authenticity, and which regulatory authorities can utilize for validation, assessment, and certification purposes (Damez & Clerjon, 2013).

2. REVIEW OF LITERATURE

2.1. Quality Characteristics of Meat:

2.1.1. Chemical Composition and Nutritional Value of Meat.

Eneji (2001) conducted a comparative analysis of the chemical composition of beef and pork before and after canning. The results indicated that the protein content in canned beef ranged from 20.54% to 23.92%, while its fat content varied between 3.0% and 4.5%. In contrast, raw beef contained protein levels ranging from 20.25% to 20.85% and fat content between 1.0% and 1.5%. Regarding pork, canned samples had protein contents ranging from 19.12% to 21.68%, while raw pork contained between 19.19% and 19.83%. The fat content in canned pork was between 2.5% and 5.0%, whereas in raw pork it ranged from 5.0% to 5.5%. Ash content in canned beef and pork was reported between 1.13% to 1.53% and 1.20% to 1.62%, respectively, while in raw samples it ranged from 1.14% to 1.68% in beef and 1.01% to 1.74% in pork.

Generally, the chemical composition of meat after exsanguination can be estimated at approximately 75% water, 19% protein, 3.5% fat, and 2.5% soluble non-protein substances (Lawrie & Ledward, 2006).

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A chemical analysis of meat samples of Lebanese origin, labeled with the "Hana" mark, revealed the highest levels of dry matter and fat, recorded at 44.35% and 19.475%, respectively, in comparison to other meat samples. In contrast, Jordanian meat bearing the "Almaraaia" mark showed the highest percentages of ash (5.025%) and protein (14.925%). Meanwhile, the highest carbohydrate content (5.425%) was observed in meat labeled "Creat cow" from Brazilian origin. Additionally, qualitative assessments of total volatile nitrogen revealed the highest value (13.100 mg/100 g) in Brazilian meat. Furthermore, Brazilian meat with the "Extra" mark exhibited the highest thiobarbituric acid value, indicating a greater extent of lipid oxidation (Al-Khauzai et al., 2010).

Raw meat, luncheon, and burgers were among the local meat products gathered from markets in Zagazig City, Sharkia Governorate, Egypt, and their physical and chemical quality attributes were assessed by Ragab et al. (2019). Americana's beef burgers had the highest protein level (16.90%) among the samples under analysis, while Faragello's beef burgers had the lowest protein content (14.7%). Additionally, the Faragello beef and chicken luncheon samples had the highest total protein content in luncheon products, at 16.0% and 15.05%, respectively.

In another study, Praharasti et al. (2019) investigated the impact of storage time and temperature on the physical and chemical properties of canned beef. The samples were stored at three different temperatures: 35°C, 45°C, and 55°C over a period of six weeks. The study revealed significant changes in the composition of the samples due to varying storage conditions. Moisture content ranged between 38% and 42%, while pH values were recorded between 5.15 and 5.38. The thiobarbituric acid (TBA) values, which indicate the level of lipid oxidation, ranged from 0.12 to 0.27 mg of(malonaldehyde per kilogram) of sample.

2.1.2. Thiamine content in raw and canned meat.

Canned pork products, specifically spiced luncheon meat, were found to retain a considerable amount of vitamin B1 (thiamine) following the entire canning procedure. However, significant losses of the vitamin occurred during the retorting phase used for cooking and commercial sterilization. These reductions accounted for approximately 55.3%, 55.6%, and 41.9% of the initial thiamine content in three different sample batches (Reedman & Buckby, 1943).

Noble (1965) investigated the retention of thiamine and riboflavin in various meat types and cuts subjected to braising. His findings revealed that thiamine retention in beef was about 40% in round roasts and steaks, 30% in flank steaks, and 24% in chuck and short ribs. Riboflavin retention was higher73% in chuck, flank steaks, and round roasts, and 62% in short ribs and round steaks. For veal, steaks retained 48% of thiamine compared to 38% in chops, while riboflavin retention stood at 74%. In pork, thiamine retention varied by cut: 57% in general cuts, 44% in tenderloin, and as low as 26% in chops and spareribs. Riboflavin retention in pork cuts was 83%, 64%, and 72%, respectively. Additionally, the cooking liquid from pork contained between 1–13% of thiamine, while cooking liquids from other meats held 15–30% of either thiamine or riboflavin. Meat weight loss during cooking was reported between 35% and 45% of the raw weight.

Because thiamine is the most heat-labile of the B vitamins and has a high thermal sensitivity, its degradation in food is a major nutritional concern. Its substantial loss during heat processing affects nutrition, but because of its sensitivity, it can also be used as a good gauge of how intense a thermal treatment is. Thus, the effectiveness of heat-based preservation techniques can be evaluated using kinetic data pertaining to thiamine degradation (Stumbo, 1973; Mulley et al., 1975).

Furthermore, the formation of volatile compounds associated with flavor is facilitated by the breakdown of thiamine. Heat, UV light, and Maillard-type reactions are some of the processes that can produce these compounds (Buttery et al., 1984; Hartman et al., 1984; van Dort et al., 1984).

The effects of roasting temperatures and times, which ranged from 100°C to 160°C and 15 minutes to 1 hour, on nutrient losses in meat samples were examined by Awonorln et al. (1991). It was found that thiamine degradation in beef ranged from 15% to 25% (0.006–0.011 mg/100 g), while in pork it ranged

from 20% to 40% (0.116–0.232 mg/100 g). Under the same conditions, the riboflavin content of beef dropped by 10% to 25% (0.019–0.047 mg/100 g), while the corresponding reduction in pork varied from 15% to 40% (0.023–0.061 mg/100 g).

Szymandera-Buszka (2003) reported that the inclusion of iodized table salt or a mixture of treated salts containing NaCl and $NaNO_2$ in ground pork led to an increased loss of thiamine during pasteurization. In a separate study, the same author examined the impact of pork sterilization in the presence of oxidized fats and selected antioxidants specifically casein hydrolysate and rosemary extract on both the quantity and quality of thiamine. The results indicated that thermal sterilization induced the highest losses in both forms of thiamine. When fresh pork was used, the total thiamine loss reached 58%, while the presence of oxidized fats raised this figure to 63%. The extent of thiamine loss was influenced by its binding form. Notably, the application of rosemary extract during heat processing with fresh fats reduced the loss of associated thiamine by 5%, whereas casein hydrolysate reduced it by 4.5%. In the context of oxidized fats, both antioxidants exhibited a similar protective effect, with casein hydrolysate demonstrating slightly greater efficacy.

The effect of heat treatments on the amount of B vitamins in meat products was examined by Riccio et al. (2006). For the simultaneous measurement of vitamins B1, B6, and B12 in homogenized boiled ham and different fortified burgers, they created a quick and accurate technique. High resolution, high sensitivity, and low detection limits were guaranteed by the extraction process in conjunction with HPLC. The findings showed that both mild (70–90°C) and severe (120°C) cooking processes decreased the amount of B vitamins. The residual concentrations after cooking were adequate to meet the recommended daily allowance when meat was fortified with 25 μg/g of B vitamins, suggesting that fortifying meat products with B vitamins is a beneficial practice.

According to Wyness et al. (2011), meat is a substantial source of pantothenic
acid, biotin, thiamine, riboflavin, nicotinic acid, vitamin B6, and vitamin B12.

According to Szymandera-Buszka et al. (2014), thiamine loss in standard systems of minced chicken following pasteurization was found to be between 61 and 71%, whereas sterilization caused a loss of 57 to 67% of thiamine.

The heat-treated meat sample's overall thiamine loss during storage increased by roughly 23% due to the oxidation of fats. The rate of fat oxidation was found to be strongly correlated with thiamine loss. Samples with low oxidation lard showed the least amount of total thiamine loss. Thiamine losses were decreased when antioxidants (casein hydroxyzine or rosemary extract) were added to meat samples (Szymandera-Buszka et al. 2014).

According to a study by Thomas et al. (2015), thiamine is crucial for the production of three important odorants in cooked ham: 2-methyl-3-furanthiol, 2-methyl-3-methyldithiofuran, and bis (2-methyl-3-furyl) disulphide. Analysis showed that when the dose of thiamine is increased, the production of these three aroma compounds increases in a closely correlated manner under the same cooking conditions. It was possible to correlate the amounts of thiamine added in model cooked hams with the amounts of 2-methyl-3-furanthiol generated during the cooking process by using a particular 2-methyl-3-furanthiol extraction–quantification method. Thiamine's function as a precursor to the aroma of cooked ham was brought to light by sensory analysis.

Both traditional heating and microwave heating cause the vitamins in meat to be lost during treatment, particularly vitamin B1. Filtration was the primary method used to detect vitamin B1 loss. According to Pathare et al. (2016), these losses were approximately 15–40% when boiling, 40–50% when frying, 30–60% when roasting, and 50–70% when canning.

According to research, pyrophosphate ester is the form of thiamine that is most exposed to heat, while protein-bound thiamine is the least heat-sensitive. Suparno et al. (2017) demonstrated that heating fish to 121°C for 60 minutes completely destroyed thiamine pyrophosphate, with the ester first partially changing into a free form and then decomposing.

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All meat is a good source of thiamine, but fish has the highest concentrations of the amino acid when compared to other meats other than pork (Ahmad et al., 2018).

2.1.3. Physical Properties of Meat.

In 68 and 108 beef and pork muscle samples, respectively, the impact of pH on the boiling test which is used to assess potential aberrant odors in carcasses was examined. The sensory scores derived from the boiling test were significantly impacted by the pH. The effect on meat odor was especially noticeable. For samples with a pH of less than 6.2, the odor scores in beef stay constant, but at higher pH values, they begin to rise quickly. The rise in pork scores seemed to be linear. The judges characterized the smell of the meat at a high pH as unusual and ammonia-like. The results of the boiling test at meat inspection should be interpreted very carefully when dealing with high pH meat (Korkeala et al., 1988).

One of the most desired characteristics of meat is its tenderness, which is primarily influenced by endogenous enzyme activity during the transformation of muscle into meat and the subsequent storage of the meat. Lactic acid is produced by anaerobic glycolysis in muscle up to 24 hours after slaughter, lowering pH. When there is a sufficient initial concentration of glycogen, the pH drops to roughly 5•5, which stops glycolysis primarily because phosphofructokinase is inhibited. The final pH may reach 7.0 if glycogen is exhausted before slaughter; meat with this pH will bind more water and be more tender than meat with a pH of 5.5. Actin and myosin filaments that overlap combine as a result of a drop in ATP levels during rigor mortis; the more tender the tissue, the less overlap there is (Dransfield and Etherington 1981).

The impact of a high concentration of salt (NaCl) and curing salt was examined in relation to color changes and warmed-over flavor (WOF) in the processing of charqui meats. The thiobarbituric acid reactive substances (TBARS) method was used to measure the WOF of both uncured charqui meat (CH) and cured charqui, which is referred to as "jerked beef" in Brazil (JB). Significant WOF occurred in CH, and sodium nitrite inhibited 40–45% ($p<0.05$) of JB samples after 30 days of storage. The CIELAB system's evaluation of color parameters also showed changes.

According to the a*/b* ratio, the brown color of the CH samples indicated the formation of met myo globin (Fe3+), whereas the deep red color of the JB samples indicated the formation of nitrosyl myoglob in (Fe2+).The presence of denatured met myoglobin (Fe3+) in CH and the formation of nitrosyl myochr omogen (Fe2+) in JB samples were indicated by the a*/b* ratio under cooking.The color of charqui mea t was affected by the iron state, and it appears that nitrite was able to chelate iron ions, preventing the de velopment of WOF (Youssef et al., 2003).

Meat product water holding capacity (WHC) is crucial for product yield, economy, and food quality. Factors like genotype and animal diet affect WHC during growth and development. Stressors like fasting and stunning techniques affect WHC during pre-slaughter. Post-slaughter, methods like cooking and cooling, heating and cooling rate, cooking temperature, and endpoint temperature also affect WHC. 614 winter and summer-killed animals were used in studies.

After 48 hours of chilling, the meat's pH and color were assessed in M. longissimus thoracic.Meat p H was found to be significantly impacted by cattle category, slaughter season, and their interactions.The young bull (A) (6.1) and bull (B) (6.07) groups showed high pH values primarily during the summer. In the winter months, both of the aforementioned groups showed somewhat lower values of 5.94 and 5.65, respectively. While meat from cows had pH values above 5.8, regardless of the season of slaughter, meat from heifers was characterized by proper pH in both of the seasons under analysis. All of the cattle categories that were analyzed showed statistically significant differences in color lightness L* ($P < 0.0001$) (Węglarz 2010).

The American Meat Science Association (2012) states that Figure (A) shows a schematic of the interconversions of myoglobin redox forms in fresh meat. Time, temperature, pH, and mitochondrial competition for oxygen all affect myoglobin oxygenation, also known as blooming (reaction 1 in figure A). More precisely, oxygen penetration beneath the meat's surface is determined by the competition

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between myoglobin and mitochondria for oxygen, which has a major impact on the intensity of surface color (American Meat Science Association 2012).

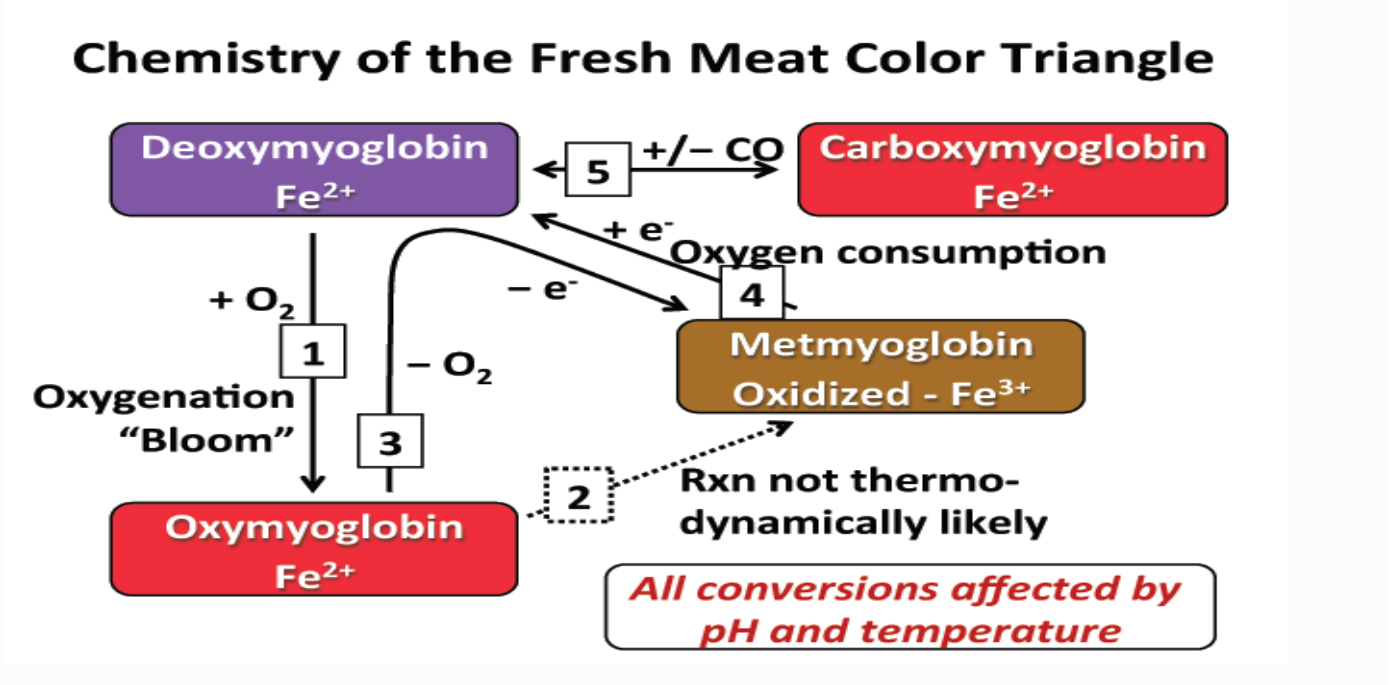

Figure (A): shows a schematic of the myoglobin redox form interconversions in fresh meat.Drs. D. P. Cornforth of Utah State University and M. C. Hunt of Kansas State University provided this informati on.

Puolanne and Peltonen. (2013) investigated the (WH) in meat in the pH-NaCl (ionic strength) combinations that prevail in dry sausages during fermentation and drying. WH in raw beef homogenates, with 230% added water, was determined by centrifugation at pH values of 5.47-4.60, and ionic strengths (μ) 0.50-1.50. The minimum WH in relation to pH was at pH 4.8, but at higher pH values, the WH optimum was at 1.0-1.5μ; at lower pH-values (<5.0) the optimum was more pronounced at 1.0μ.

Meat and meat products' water-holding capacity (WHC) affects their visual appeal, weight loss, and cooking yield in addition to their sensory qualities after consumption. WHC is defined, and the effects of protein and muscle structure on WHC in both raw and cooked muscle are discussed. The following factors are described: age, electrical stimulation, vacuum packing, freezing and thawing, post-mortem pH drop, PSE (Pale, Soft and Exudative) and DFD (Dark, Firm and Dry). Along with the effects of high pressure processing, salting both before and after rigor, ionic strength, and phosphates, the structural changes brought about by cooking and processing are also discussed (Warner 2017).

In a typical Chinese abattoir, Zhang et al. (2018) examined the pH/temperature decline of beef carcasses and the color development as pH decreased during the onset of rigor. The pH/temperature decline for carcasses that exceeded pH 6.0 was modeled using a natural cubic spline model. Along with the same number of normal pHu and intermediate pHu carcasses (5.40–5.79; 5.80–6.10, respectively), six of the 97 carcasses that showed a high (≥6.10) ultimate pH (pHu) (dark cutting) in the M. longissimus lumborum (LL) were sampled in order to assess color development within 24 hours after death. 66.7% of the modeled carcasses were found to be outside the optimal pH/temperature window, with a temperature that was 6.0 degrees below ideal. This suggests that the pH decline needs to be accelerated.

2.1.4. Sensory Properties of Meat.

Along with the Strecker aldehydes, ammonia, and hydrogen sulfide, the dicarbonyl compounds, furfurals, and furanones can affect the aroma of cooked meat on their own. They are also crucial go-betweens for other significant meat flavoring ingredients. The two most reactive of these, H2S and NH3, can interact with the byproducts of lipid degradation and influence the relative production of heterocyclic compounds that result from the Maillard reaction (Mottram 1994).

Depending on the animal's age and diet, aroma or flavor (aroma + taste) can be further differentiated within species, and these variations may affect consumer acceptability [2–5]. Therefore, knowing where the aroma compounds come from is very important. Nevertheless, it is widely acknowledged that the aroma of meat is primarily developed upon heating treatment, where the precursors include thiamine (vitamin B1), glycogen, glycoproteins, nucleotides, nucleosides, free sugars/phosphate, amino acids, peptides, amines, organic acids, and lipids. Some odor-active compounds, such as 4-ethyloctanoic acid (mutton-smell) in sheep, are present in the raw meat and are not significantly impacted by cooking (Rota and Schieberle 2005).

Precursor concentrations fluctuate during the post-mortem phase, mostly due to hydrolytic activity. These precursors take part in reactions that create intermediates when meat is heated. These intermediates can then react with other degradation products to create a complex mixture of volatiles, including those that give meat its aroma (Imafidon and Spanier 1994).

The most crucial factor in identifying an animal species is its aroma, which is followed by its texture; in contrast, the flavors of beef, pork, lamb, and chicken are nearly identical (Matsuishi et al., 2004).

For instance, goats, like other wild animals, have a strong gamy scent, but the meat of chicken, pork, rabbits, turkeys, veal, and lambs has very little or no gamy scent (Rødbotten et al., 2004).

Acceptability is influenced by the aromas released during the cooking of meat as well as the presentation and aromas of the food on the plate. The primary determinants of the product's sensory quality once it reaches the mouth are taste, texture (tenderness, juiciness, fibrousness, greasiness, etc.), and aroma (Resconi et al., 2013).

Controlling the quality of meat, particularly its sensory qualities, is still a major concern for any farm animal production. This is the case for ruminant meat, in particular beef, but also for poultry and pork, although, for them, controlling the technological quality (process ability) is at least as important (Lebret and Picard 2015).

Many years of research, especially in Europe, have improved our understanding of how different production factors affect the quality of the meat and the characteristics of the muscle. In addition, it draws attention to the intricate determinism of the biological traits of meat and muscles, which are frequently influenced by a variety of genetic, animal husbandry, and slaughter systems-related factors (Lebret et al., 2015).

2.1.5. Microbiological status of raw meat and canned meat products.

In 35 samples of canned beef that were gathered from the local market in the Basra Governorate, Alhafeth (2008) determined the total count of E. coli, Staphylococcus aureus, enterococci, psychotropic bacteria, anaerobic bacteria, mold, and yeast. Iraq and reported that the average numbers were 15.62 x 107, 2.54 x 103, 8.22 x 105, 22.027 x 105, 11.22 x 105, 1.742 x 103, 2.82 104 CFU /g; respectively. There was no growth of aerobic bacteria in the test samples.

AL-Khauzai and AL-Grabi (2010) discovered the overall quantity of anaerobic bacteria ranged from 0 to 7 x 101 CFU/g of meat and the average number of Clostridium bacteria was 0 to5 x 101 CFU /g of meat according to international standards.

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Hamasalim (2012) discovered that canned meat products gathered from Sulaimani Markets containe d no aerobic bacteria and that the amounts of anaerobic bacteria were within the permitted limits.

The microbiological characteristics of meat and meat products sold in various local markets in Tripoli, Libya, are reviewed by Altajori and Elshrek (2014). Between 2005 and 2009, studies were carried out independently and at various points in time. In the Tripoli region and its suburbs, samples were gathered primarily from eateries, lodging facilities, homes, and other local marketplaces. The findings showed that samples of beef burgers were heavily contaminated with a number of harmful bacteria, including E. coli (74.5%), E. coli O157:H7 (27.1%), S. Aeromonas (18.6%) and Aureus (28.8%). Studies have shown that meat products marketed in Tripoli are of low quality and unsafe due to the high percentage of pathogenic bacteria present. Fresh sausage samples were contaminated with E.coli O157:H7 (39.3%) and salmonella (2.1%), while chicken burgers had E. coli and Salmonella contamination (10.9%) and 4.68%, respectively. Camel meat samples were found to contain Aeromonas (71%), A. hydrophila, and A. sobria (65% and 35%). The high contamination levels suggest that personal hygiene, sanitation, and manufacturing practices are not being adequately addressed.

Five canned meat samples beef, chicken, fish, hot dogs, and mixed beef and chicken were gathered from local markets in Baghdad, Iraq, and incubated for 30 to 35 days at 40 to 45°C. These samples were analyzed microbiologically by Abdulhay and Salloom (2015). The samples of beef, chicken, fish, hot dogs, and beef and chicken had total bacterial counts of 1x104, 4x101, 1x10, 1x103, and 1x104 CFU/g, respectively. In contrast, the canned beef, chicken, and hot dog samples had coliform counts of 1 x101, 1 x101, and 3 x102 CFU/g, respectively. There was no coliform in the other samples.

Shajahan and John (2016) determined the total viable number, the total number of coliforms, the total number of Streptococcus, the total pseudo number, the total number of bacilli, the total number of staphylococci, the total salmonella and Shigella and the total number of Klebsiella in canned meat sample which were found to be 11.4 x 103, 7.8 x 102, 3.1 x 102, 1.4 x 102, 4.3 x 102, 0.98 x 102, 1.2 x 102 and 0.81 x 102 CFU /g ; respectively.

Raw beef that was randomly selected from Khartoum State markets was examined for microbiological characteristics. The range of the total bacterial count was 4.83–7.88 log10 cfu/g. Notably, every sample tested tested negative for Salmonella (Basheer et al. 2018).

Ali et al. (2018) conducted a bacteriological analysis on 100 randomly selected canned meat samples from various Egyptian supermarkets and retail establishments. The quantity of aerobic bacteria varied from 3x103 to 15x104 CFU /g while the number of anaerobic bacteria ranged between 3x10 to 11x102 CFU /g.

The total bacterial count (TBC), total colon (TCC), total staphylococcus (TSC), and total salmonella were found in various fresh meat samples from Benghazi markets by Mansour et al. (2019). The range of TBC was between 4.41to 6.53 log10 CFU / g, while TSC ranged between 4.3 - 6.2 log10 CFU /g. This microbiological deterioration of the samples may be due to the lack of scientific methods for dealing with meat in butchers and slaughterhouses.

The bacteriological condition of canned meat sold in Beni-Suef City, Egypt, was assessed by Abdel-Atty. et al. (2020). Anaerobic plate counts, Staphylococcus aureus counts, Enterococci counts, total Clostridium counts, and Clostridium perfringens isolation were performed on 150 canned meat samples, 25 of which were each of canned beef, corned beef, canned chicken sausage, canned luncheon, canned luncheon, and canned sausage. The greatest frequency of Clostridia were recorded in corned beef and canned sausage (60% each), while their lowest ones wherein canned beef and canned poultry sausage (28% each). 20%, 24%, 16%, 12%, 24% and 24% of canned beef, corned beef, canned chicken sausage, canned chicken luncheon, canned luncheon and canned sausage, respectivelyexceeded the acceptable S. aureus count limits set by the Egyptian Organization for Standardization and Quality. While canned beef, corned beef, canned chicken sausage, and canned luncheon had detection levels of 12, 28, 4, and 12%, respectively, enterococci were not found in canned chicken luncheon or canned sausage. It was not possible to isolate Clostridium perfringens from every sample that was analyzed.

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2.2. Preservation and Processing of Meat.

2.2.1Chilling:

There are two steps involved in low-temperature meat preservation: chilling the carcass and maintaining the low temperature during display. Lamb preserved only by refrigeration has a limited shelf-life, so it is usually combined with packaging to extend its lifespan either for storage or display (Vieira and Fernández 2014 and Berruga., et al 2005).

Four factors temperature, relative humidity, air velocity, and time can be used to define a cooling rate (McGeehin et al. 2002). Variations in any factor during chilling could reduce the time needed for accomplishing European Union (EU) requirements; however, it has been described that severe variations could have negative effects on meat quality (Muela., et al. 2010). Low-temperature meat preservation consists of two steps: chilling the carcass and keeping the temperature low while it is on display. Lamb that has only been refrigerated has a short shelf life, so packaging is typically used to prolong its storage or display life (Vieira and Fernández 2014 and Berruga et al 2005 .(

A cooling rate can be defined by four factors: temperature, relative humidity, air velocity, and time (McGeehin et al. 2002). Changes in any aspect of chilling could shorten the time required to meet EU regulations, but it has been noted that significant changes could degrade the quality of the meat (Muela et al. 2010).

It is commonly known that the meat industry benefits greatly from both super chilling and ultra-rapid chilling, particularly when shipping fresh meat to far-off markets. By cutting down on the time required to chill carcasses and the initial microbial load, rapid chilling rates increase the shelf life of meat while maintaining its sensory qualities. Furthermore, it would be easy to implement this process in already-existing slaughterhouses, and decreased evaporative losses could offset the increase in plant capital costs (Pja 2016).

The species of origin, initial microbial load, packaging, temperature, and humidity levels during storage all affect how long meat can be kept in the refrigerator. Poultry and pork have a relatively high microbial load at first. Meat handling should be done with extreme caution regardless of the species to prevent additional microbial contamination. Meat spoils over time due to the growth of psychrophilic organisms that are favored by a refrigerated environment (Pal and Devrani 2018).

In contrast to higher temperatures, chilling can slow the growth and metabolic processes of harmful bacteria, viruses, and poisons in food. Due to the rate of cold penetration, some parasites, including all stages of Trichinella spiralis and Taenia cysts in beef, may be totally eradicated by keeping contaminated food at 18ºC for 20 to 30 days. In order to preserve a wide range of food products, including meat, chilling is typically done at temperatures between 0°C and +8°C (Chellaiah et al., 2019).

2.2.2. Freezing:

Food loses heat through convection across its surface and conduction within it during the freezing process, making it an unstable phenomenon of heat transfer (Rahman et al., 2008). Three separate stages make up the temperature reduction process: the pre-cooling or cooling stage, during which the material undergoes a phase change from its initial temperature to its freezing point temperature (Tf), which is frequently associated with crystallization from water; the hardening stage, during which the product reaches the final set temperature (for instance, 18 ° C). It is important to note that the release of the heat of fusion following the initial Super cooling causes an abrupt rise in temperature in typical cooling curves at the start of the process. The process of ice crystallization starts at this point. The system intends to release its latent heat more quickly than the heat extracted from it once the crystal embryos surpass the critical radius of nucleation. For the initial freezing temperature (Tf), the temperature then rises instantly (Rahman 1999; Rahman and Driscoll 1994).

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Rahman et al. (2009) found that the amount of Enterobacteriaceae had decreased over the course of 18 months of frozen pork storage, resulting in a very small total amount of these bacteria at the end of the storage period. According to the findings, the average bacterial count in loin, ham, and belly ribs was 0.36 log10 CFU/g, 0.69 log10 CFU/g, and 1.15 log10 CFU/g, respectively. The quantity of Enterobacteriaceae in belly rib samples decreased by 3 log10 CFU/g after 18 months .

Compared to other preservation techniques, freezing is a crucial way to increase the shelf life of meat and meat products. Long-term storage at low temperatures results in little loss of quality (Soyer et al., 2010).

Muela et al. (2010) assessed how freezing rate affected the quality of frozen meat. Large ice crystals form during slow freezing, which can physically harm muscle tissue and give it a distorted appearance when frozen. In contrast, many tiny ice crystals form evenly throughout the meat tissue during fast freezing. Nearly 98% of water freezes at -20°C, and full crystal formation happens at 65°C. The freezing rate increases as the freezing temperature decreases (Rosmini et al., 2004). A thorough analysis of the effects of freezing and thawing on the physical quality parameters of meat was conducted by Leygonie et al. (2012). When ice crystals form during freezing, the meat's ultrastructure is harmed and its solutes are concentrated, which changes the biochemical reactions that take place at the cellular level and affects the meat's physical quality parameters. Moisture loss, protein denaturation, lipid and protein oxidation, color, pH, shear force, and microbial spoilage were the quality parameters that were assessed. The methods used to lessen the effects of freezing and thawing were also examined.

The study by Jo., et al. (2014) examined the effects of freezing and thawing techniques on meat samples. They found that rapid freezing techniques like CF, IQF, and NF minimized quality deterioration. NCT was found to be better than RT in thawing methods, and the meat quality was influenced by the thawing temperature. The results suggest CF and NCT are the best combination for beef processing.

The study by Al-Sabagh et al. (2016) examined the impact of freezing temperature and storage duration on the amino acid profile and fatty acid pattern of imported beef meat, local beef meat, chicken breast, and Nile tilapia fish. Results showed that freezing reduced the total amount of essential and nonessential amino acids, but no significant differences were found between frozen imported beef meat and local beef meat. The study also found a significant increase in total volatile nitrogen value after frozen storage for imported beef meat.

It may be concluded that in order to preserve and increase the shelf-
life of frozen beef and meat products, freezing time needs to be optimized.
The characteristics of beef quality that are related to nutrient contents like protein, carbs, fat, minerals, a nd fibers are impacted by changes in freezing rate and beef density (Iskandar and Munawar 2019).
According to Hussein et al. (2020), freezing meat inhibits the growth and reproduction of bacteria that c ause damage and spoiling, but it does not stop some changes in the meat's chemical composition.

More recently, Hou et al. (2020) assessed how the freezing process and storage duration affected the alterations in pork longissimus thoracis (LT) quality. The findings demonstrated that pork under immersion solution freezing (ISF) had a better microstructure than pork under air blast freezing (AF), primarily as a result of a higher freezing rate. Compared to AF, the ISF group experienced a significantly higher shear force at day 1 and a significantly lower thawing loss at days 1, 31, and 91 ($P < 0.05$). The color, cooking loss, and sulfhydryl groups did not differ significantly between the two treatments ($P > 0.05$). Storage time had a significant impact on all quality indicators ($P < 0.05$). It was determined that ISF could enhance water-holding capacity and preserve better microstructure and inhibit lipid oxidation during pork LT frozen storage.

2.2.3. Canning:

In order to render low-acid foods "commercially sterile," canning usually entails heating them to 121°C in order to eradicate all mesophilic microorganisms and Clostridium botulinum spores. To do this,

the procedure is used for a duration sufficient to reduce the number of this pathogen's spores by 12 log10. Depending on the makeup of the food, this typically means heating for at least two minutes. Low-acid foods can be stored at room temperature with great stability thanks to this method. However, many food products' quality is impacted by the temperature and time used during canning (Murano 2014).

The most effective way to preserve meat is to can it. The time-temperature relationship required to bring the microbial counts of the most harmful or dominant microorganisms down to a safe level is used to determine the process severity. Prior knowledge of the meat's physicochemical properties, anticipated shelf life, and the target microorganisms' resistance to heat is necessary for process calculations. In canned meats, two heat transfer mechanisms occur: conduction occurs in solid products like sausages, while convection is the predominant mechanism in fluids like soups. Gelling causes luncheon meat and pate, among other gelled products, to transition from convection to conduction (Devine and Decman 2014).

Along with chilling and freezing, the canning process was first used approximately 200 years ago and has long been a primary method of food preservation. When Nicholas Appert found that heating food in a glass container that was tightly sealed prevented food from spoiling, the history of food canning in France began in the late eighteenth century. Later, Bryan Dorkin and John Hall installed the first commercial cannery in England, improving on a technique that Peter Durand had developed for sealing food in tin containers. A few years later, L. Pasteur proved that food deterioration was caused by microorganisms, which provided a plausible explanation for the efficacy of canning (Vergara-Balderas 2016).

Heat, sodium chloride, and sodium nitrite are typically used to prevent C. botulinum from growing in canned meat products. Other preservatives, such as lactates or acetates, are occasionally used; these are frequently sodium compounds. However, because sodium chloride, particularly sodium ion, has detrimental health effects, consumers and health authorities have been asking for less NaCl in meat products over the past ten years (Hansen et al., 2016).

Canning preserves food by heating and sealing it with a vacuum, preventing aerobic microorganisms from growing. Acidic foods, like fruits and tomatoes, have a low pH, limiting pathogen growth. Low-acid foods, like meat, fish, and vegetables, require higher temperatures. Pressure canners or retorts achieve higher temperatures, destroying heat-resistant microorganisms like Clostridium botulinum spores. Acid foods with pH less than 4.5 can be processed safely at 212°F (100°C), similar to boiling water.

Research was done to examine the effects of heat processing on the properties of shelf-stable canned salted beef with tomato gravy. The traditional method of standardizing the product involved using salted beef and tomato gravy to create shelf-stable canned meat. In order to interpret the effects of thermal processing and salt as a preservative, it was then subjected to the optimal preservative concentration and thermal treatments at various temperature time combinations, namely, 110°C, 115°C, and 121°C for 20, 30, and 40 minutes, respectively. For sensory analysis, samples were assessed both at the beginning and at intervals of 0, 15, 30, 45, and 60 days. To illustrate the increase in shelf life stability brought about by the use of curd as an emulsifier (i.e., the effect of preservative and thermal processing), microbiological, chemical, and sensory studies were carried out at 15-day intervals up to 60 days. Thermal processing of shelf-stable canned salted beef with tomato gravy at 121°C for 40 minutes was found to have significantly higher acceptability, while 110°C for 40 minutes was found to have significantly higher adequate protein, fat, and moisture content (Harkal and Manvar 2018).

Microbiological analyses of canned meat were carried out twice in a recent study by Draszanowska et al. (2020): on day 0 (24 hours after storage) and on the final day of storage at 5°C. Microbial counts were measured at <1 log CFU/g to 1 log CFU/g on day 0 and at <1 log CFU/g level on the final day of storage, indicating very low contamination with aerobic mesophilic bacteria. While coliforms and staphylococci were not present in 10 g samples, the numbers of sulfite-reducing Clostridium bacteria were measured at less than 1 log CFU/g. According to the aforementioned findings, the microbiological safety of canned meat during the storage period was ensured by heat processing during canning in jars.

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2.3. Detection of Animal Species in Meat Products.

The application of food labeling laws and consumer protection depend on the detection of species adulteration in meat products. Consumers and food control authorities are increasingly concerned about the dangers of food adulteration and mislabeling.

Every Muslim has an obligation to eat halal food. Pork may contaminate processed meat products. In order to produce halal, safe, and standard meat products, one of the main challenges in meat technology is the identification of meat species in processed meat products. The detection of pork DNA in meat products is extremely noteworthy for halal certification because Muslim consumers are demanding protection from meat products. Because heat treatments negatively affect the targeted proteins and DNA, compromising immuno- and PCR-based detection methods, the analytical techniques for the detection and quantification of species in processed meat products are still in their infancy. In the meantime, the polymerase chain reaction (PCR) technique is used as the identification method due to its high accuracy in detecting porcine DNA in processed meat products and confirming that the product is halal.The identification of meat species in a variety of processed meat products has been the subject of extensive research studies.

Alaraidh (2008) examined thirtythree samples of processed meat products in the Kingdom of Saudi Arabia using the PCR and DNA extraction techniques to identify a particular porcine fragment in the samples and labeled them as halal for the detection of pork.

Meat mixtures comprising species of beef, pork, horses, lamb, chicken, and turkey were identified to a level of 0.05% in a study conducted by Jonker et al. (2008). Levels as low as 0.01% of these types were found by varying the number of cycles.

Mafra et al. (2009) used real-time PCR technology to detect pork in processed meat products that were offered for sale in Portuguese commercial retail establishments. A high correlation coefficient and PCR efficiency ranging from 0.1% to 25% were found and measured for pork. Blind samples were used to successfully validate the methodology, which was then applied to the quantitative evaluation of pork in a range of processed poultry meat product.

Using particular prefixes of swine mitochondrial DNA that were extracted from controlled meat samples, the real-time green PC SYRR I PCR test was created to address the halal certification for processed meat products (Farrokhi et al., 2011).

The ability of real-time PCR (qPCR) detectors based on Minor Groove Binding (MGB) probes to identify and measure pork DNA in binary mixtures of beef and pork meat that have been cooked and sterilized was tested (López-Andreo et al., 2012).For the direct quantification of meat proportions in a binary mixture, qPCR results were calibrated using a single-point matrix standard technique. Additionally, an experiment was conducted to test the relationship between heat treatment and the degree of DNA degradation. DNA ruptured to about 100 basis point fragments after cooking at 65 °C and sterilizing at 126 °C for 10 to 30 minutes, but this still made it possible to detect 5% pork and accurately quantify it in binary mixtures. The findings demonstrated that short qPCR detectors can be used to quantify meat products in processed foods and highlight the need for matrix-adapted standards in the assay.

Soares et al. (2013) obtained a normal titration model from 0.1% to 25% with high linear correlation and PCR efficiency using binary meat mixtures that contained known amounts of pork in poultry meat. The melting curve analysis of the method demonstrated a high specificity, and its successful application to blind meat mixtures verified its effectiveness in detecting pork. The applicability of the method has been fully demonstrated in commercial meat products.

Animal species were identified in certain meat products using the Real Time PCR and DNA microarray techniques. 73 samples of meat and meat products were gathered from various districts in Istanbul province, Turkey, and examined for the presence of animal species listed on the label in a study

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conducted by Özpinar et al. (2013). According to the findings, 39 samples (53.4%) had the wrong label. As a result, it was discovered that the outcomes of the Real Time PCR and DNA Microarray techniques were 100% identical, and both ought to be widely used to identify animal species in meat and meat products.

Pork was detected using the Real Amp checks method in a beef mixture that was thermally treated at 100° C for 15 minutes and contained varying amounts of pork, ranging from 0.01 to 10%. Less than 0.01% of pork was found in meat mixtures using the Real Amp checks, suggesting that this method can be applied to the sensitive and quantitative identification of meat types, including heat-treated meat products (Yang et al., 2014).

To ascertain whether pork was added to 48 samples of beef balls gathered from 21 small business dealers and 21 from the traditional market surrounding the Jatinagur Education Center in Indonesia, (Roostita et al., 2014) employed the PCR technique. Since no tainted beef balls were discovered, the findings showed that all vendors and merchants offered halal beef balls.

Using species-specific primers and a TaqMan probe, Kesmen et al. (2014) employed the real-time PCR assay to detect and measure seagull meat in meat mixtures. In both raw and heat-treated test mixtures, which were made by combining seagull meat with beef and chicken at varying concentrations (0.01–10%), the method was found to be able to detect seagull meat at the level of 0.1%. The researchers concluded by saying that the real-time PCR assay may be a quick and accurate way to regularly identify seagull meat in either raw or cooked meat products.

The pork content of 22 samples of commercially processed meat products, such as lamb, pork, sausage, steak and steak pies, grilled short ribs, and nuggets, was examined by Kim et al. (2016) using the quantitative real-time PCR assay. The findings demonstrated that the real-time PCR technique could identify pork in the various processed meat products with labels stating that pork was used. The examination yielded negative results for every processed meat product that claimed not to contain pork. This study's method demonstrated both sensitivity and specificity in identifying the quantity of pork present in commercially processed meat products.

Ten luncheon (beef) and ten corned beef samples were gathered from the local markets in the Kafr El Sheikh Governorate and tested for mislabeling using the multiplex PCR method in a study conducted by Elbialy et al. (2016). According to the findings, eight out of the ten luncheon samples that were evaluated and all ten samples of corned beef were tainted with goat meat, while one luncheon sample was tainted with horse meat, which was not in accordance with the product's label.

Because of its speed, quality, and sensitivity, Al-Rashedi et al. (2016) found that the cytochrome B gene's specific PCR amplification was helpful in identifying pork minced meat in processed food products.

Among the four different DNA species beef, chicken, goat, and horse specific pork DNA was identified using the Real-time PCR technique. With a concentration of as little as 0.5% of pork DNA in a mixture of processed pork beef products, the results demonstrated that the method could specifically differentiate between pork DNA and the other species (Maryam et al., 2016).

The study compared Real-Time PCR and ELISA-based methods for detecting beef and pork in processed meat products. Real-time PCR detected pork at 0.10% and beef at 0.50% in binary mixtures, while ELISA detected pork at 10.0% and beef at 1.00%. Both methods were successful in identifying species in ground meats, sausage, and deli meats, but pet treats and canned meats proved more challenging The RT-PCR test was recently used by Ghajarbeygi et al. (2018) to identify particular animal species' DNA sequences in samples of meat products. In the samples that were gathered, the distinct DNA sequences of chicken, horse, camel, beef, and turkey were successfully identified. It was determined that RT-PCR, which uses the preserved region of mitochondrial DNA, is an extremely effective technique for monitoring commercial meat products.

According to a more recent report by Chis and Vodnar (2019), real-time PCR analysis has been successfully enhanced and validated for processed meat products, enabling the quantification of meat

levels up to 0.02% for chicken and 0.1% for beef and pork. To make sure the process is accurate, precise, and repeatable, it has been tested on low-meat products like beef bologna, ham, and chicken sausage.

Conclusion

Adulteration of meat is a serious problem with important economic, religious, and health ramifications. Accurate and scientifically proven techniques to identify such fraud are becoming more and more necessary as the consumption of processed and canned meat products rises. Real-Time Polymerase Chain Reaction (Real-Time PCR) and thiamine (vitamin B1) content estimation are two of the best methods for identifying meat adulteration, especially the addition of banned meats like pork. Thiamine determination is a useful chemical indicator because different types of meat have different concentrations of this vitamin, making it possible to distinguish between species like beef and pork. Furthermore, even in meat products that have undergone thermal processing, Real-Time PCR provides excellent sensitivity and specificity in identifying species-specific DNA. Because of this, it is an effective tool for detecting meat ingredients that are not declared and confirming information on labels.

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