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BACTERIOLOGICAL QUALITY AND SHELF LIFE OF SOME MEAT PRODUCTS

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In

(Meat Hygiene)

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1. INTRODUCTION

As a result of inadequate quantities of meat in Egypt and excessive demand of the increased population. Frozen boneless beef is extensively imported from different countries to be sold as such or after being processed.

Technology development in meat processing and handling have given consumers a much greater choice over the food they can buy. So meat hygiene can comprises nearly every aspect of processing from the health of the live animal to the distribution of the final product. It prevent using of harmful ingredients in manufacturing of meat products as well as prevent the sale of contaminated or unwholesome meat.

Meat and meat products are liable to be contaminated with different kinds of microorganisms from different sources, such contaminants may be of public health hazard to consumers or may render the products unmarketable especially in small countries in which the hygienic measures are still under way.

In recent years there has been world-wide renewed interest in meat hygiene. outbreaks of food borne illness associated with bacterial agents are reported every year ***(Vanderline et al., 1998)***. Many of these illness are due to growth of pathogens and / or toxin formation. A list of some important recognized pathogens of meat are *Staphylococcus aureus, Salmonella, Escherichia coli, Shigella, Bacillus, and Yersinia enterocolitica* ***(Archer and Young, 1988 and Johnson, 1994).***

Quality improvement and control is not easy, particularly for very small manufactures who may feel isolated. Many efforts were done to produce a product free from pathogens of public health hazard and with low microbial count in order to improve its keeping quality and keeps its nutritive value to be safe and of high quality.

Chemical methods of food preservation involve direct application of chemical additives, which act as antimicrobial agents, that control the microbial growth in various food system, so extending its shelf-life. ***(Sofo, 1995)***.

It is necessary to produce foods of animal origin using an extended quality definition, not only must the product quality be considered, but quality of production processes must be also included, so that the consumer is ensured that health risks have been excluded and the environmental concerns have been met ***(Branscheid, 1993)***. Therefore, the production, transportation and sale of meat products must be performed with the almost care and preferably be subject to a hazard analysis and critical control point (HACCP) evaluation to prevent the presence of any undue hazard ***(Madden, 1994)***.

The quality of a product may be defined as its measurement against standard regarded as excellent at a particular price which is satisfactory both to the producer and to the consumer.

The common meat products could be classified according to storage and distribution temperatures into three main categories. First category is that include products require cooling (such as slices or thin cuts of bastermi, salami..) and the 2nd is that products

require freezing (frozen meat products as minced meat, beef burger, beef patties ..). The 3rd category were stored and handled at the ordinary room temperature (e.g. closed loaf of basterma, luncheon…..).

In Egypt locally manufactured meat products like luncheon, basterma, sausage etc. are gaining popularity to compensate the shortage in high price fresh meat.

Luncheon is delicious meat products which is available incased form, usually don't undergo further preparation or cooking by the consumers.

Basterma may be defined as a dry salted popular meat product manufactured mainly in middle countries. Basterma is an excellent food article that contains a wide variety of easily digestible nutrients. Besides its high caloric value, it also supplies the consumers with the mineral and animal proteins which of high biological value. On the other hand Oriental sausage is famous frozen product due to delicious, taste and cheaper price.

This study is planned out to fulfill the following:

- Chemical evaluation: determination of pH, Total volatile nitrogen (TVN) and Thiobarbituric acid (TBA) of some meat products like luncheon, basterma and sausage.
- Determination of total aerobic bacterial counts .
- Determination of total coliforms counts .
- Isolation and identification of some potential food-borne pathogens as *Escherichia coli, Salmonellae and Staphylococcus aureus*.

2. REVIEW OF LITERATURE

2.1. Public health importance

June et al., (1953) Mentioned that meat products contain serotypes of *Escherichia coli* may give rise to outbreaks of meat borne gastroenteritis for some consumers.

Lundbeck et al., (1953) stated that an outbreak of human salmonellosis in Sweden was one of the largest outbreaks recorded. It caused 9.000 cases and 90 deaths it was attributed to the consumption of contaminated of insufficient cooked meat.

Freeman (1960) reported that *Escherichia coli, coliforms* and *Enterococci* are indicators for the sanitary quality of food. As the presence of large numbers of these organisms indicates sanitary neglected measures during preparation of meat leading to spoilage, loss of quality or danger to health.

Angelotti et al., (1961) Stated that the presence of staphylococci in raw meat and their known heat resistance suggest that they could be a problem in heat-processed meat products.

WHO (1962) stated that in certain countries which have relatively good hygienic standards and efficient reporting service, more than one half of outbreaks of food-borne diseases were caused by meat and meat products, particularly processed meat. Several important diseases of man were known to be or suspected of being transmitted via meat and meat products as well as other food stuffs, these disease agents in meat products arise either from infection in the animal or through secondary contamination from human being or environments.

Elliot and Micher (1961) observed that under frozen storage *E. coli* died quickly than other member of coliform group.

Bryan (1968) stated that outbreak of salmonellosis had occurred in England and Wales during (1963) and(1964). The vehicle was associated with processed meat products.

Bryan (1975) revealed that 121 outbreaks of salmonellosis in united states between (1963) and (1969) were traced to foods in which red meats and their products constitute (14.9%).

Lee (1977) reported that meat and meat products caused over 70% of successfully investigated outbreaks of human salmonellosis.

Niskanen (1977) reported that the growth and enterotoxin production by *Staph. aureus* were affected by environmental factors such as water activity, pH, sodium chloride conc., temperature and competition with other microbes.

Scotland et al., (1977) determined the ability of certain strains of *E.coli* to produce enterotoxin. They stated that Recent studies showed that the enterotoxigenic strains of *E. coli* have been associated with outbreaks of diarrhea in several countries.

Bergadoll (1979) stated that the symptoms of staphylococcal intoxication are usually appearing within 2 to 4 hours of consumption of contaminated food. Incubation periods are as short as 30 minutes or in excess of 8 hours. The symptoms include nausea, itching and less frequently diarrhea, headache, dizziness and weakness were reported in minority of cases.

Labie (1980) recorded that salmonellae food poisoning come mainly from meat and meat products. The most frequent salmonella serotypes encountered in these outbreaks was *Salmonella typhimurium* 47.8% of identified strains.

Pyathin and Krivoshein (1980) stated that definite *E.coli* serogroups are capable of causing various acute intestinal diseases in humans; certain serotypes are responsible for colientritis in children. They cause diseases in infants of the first months of life and in older infants. While others cause dysentery like disease or cholera like diarrhea. E.coli may also cause peritonitis, meningitis, enteritis, cystitis, pyelitis, pylonephritis, angiocholitis, salpingoopheritis, appendicitis, otitis and purepeeral sepsis.

Bryan (1981) stated that in U.S during 1973-1978 salmonellosis accounted for 40% of the reported cases of food borne disease and 23% of the reported outbreaks of food borne disease. In Canada during 1973-1975 it accounted for 39% of all reported cases of food borne disease and 25% of the reported outbreaks of food borne disease. In addition to workers are unaware that raw meat can carry salmonella, therefore they are unaware that the salmonella can get on their hands, knives, cutting boards or any thing, which may touch raw meat.

Scotland et al., (1981) found that strains of *E.coli*, which belong to enteropathogenic serogroups usually fail to produce heat-labile or heat-stable enterotoxins.

WHO (1983) stated that cases of food poisoning outbreaks due to S.typhimurium were in Denmark (1981) 277 cases, in Belgium (1981) 3 cases; in Ireland (1981) 80 cases; in Norway (from 1975 to 1981) 715 cases; in Poland (1980) 237 cases; in Spain (1981) 37 cases; in Scotland (1982) 84 cases and in England (1982) 22 cases.

Kampelmacher (1983) stated that in the USA, Approximately 20.000 cases of human salmonellosis are registered annually, but due to the fact that there is a great amount of under reporting cases. The real incidence is estimated to be about 2 millions annually.

Archer and Kvenberg (1985) reported that during 1983 human salmonellosis reached approximately 1,147,000 human case in the US and the number of isolates were 38,881 isolate*, E.coli* reached 242,666 case. Other well known food-borne pathogens were *staph. aureus, C. perfringens* and *yersinia enterocolitica* accounted for 33.9, 13.4 and 3.8% respectively of confirmed food-borne disease cases in 1981.

Kristine et al., (1985) reported that enterotoxigenic *E. coli* are a common cause of travelers diarrhea and/or endemic infantile illness and gastrointestinal illness in human in many countries.

Marzouk (1985) investigate the microbial etiology of infantile diarrhea and proved that the isolated pathogen in Alexandria were *E.coli* (54%), *Klebsiella* (13%)*, Salmonella* (1%) *Enterobacter* (1%) and *Rotavirus* (1%).

National Academy of Science (NAS) (1985) stated that improper cooking and handling of raw meats in homes and food service establishment is one of the main reasons for food-borne illness caused by consumption of cooked meats.

McCormick (1986) showed that the laboratory reports for identification of food poisoning microorganisms and salmonellosis in England and Wales increased from 10856 cases in 1980 to 15204 in 1984. He also reported that the number of outbreaks increased from 518 in 1980 to 556 in 1984. the majority (84%) of all outbreaks were due to salmonella infection. Out of the salmonella infections 45% were due to *S.typhimurium*.

Seddik et al., (1988) could isolate forty-four strains of *E.coli* from 60 children suffering from diarrhea at the central hospital of sedfa from these 44 strains, 19 strains were typed as *enteropathogenic E. coli.*

Karmali (1989) stated that although *E.coli* $O_{157}H_7$ was the most serotype commonly isolated from outbreaks, numerous other serotypes were identified from clinical cases Verotoxogenic *E. coli* (VTEC) were associated with a spectrum of disease in human ranging from sub clinical infections to mild diarrhea, haemorrhagic colitis and haemolytic uraemic syndrome (HUS). The epidemiological investigations of outbreaks of human disease implicated under cooked ground beef and unpasterized milk as sources of infection.

Bean and Griffin (1990) examined the etiological agents and food vehicles associated with 7458 outbreaks of food-borne disease reported by the Centers for Disease Control(CDC) between 1973 and 1987.The bacterial pathogens accounted 66% of outbreaks and 87% of cases, *Salmonella* accounted for 28% of outbreaks and 45% of cases, *Staph*. *aureus* was the cause of 13% of outbreaks and 14% of cases, *C. botulinum* caused 8% of outbreaks, *C. perfringens* caused 7% of outbreaks, while *E. coli* caused 1% of outbreaks. When date from 1973-1975 were compared with 1985-1987, 75% increase in the proportion of outbreaks and 13% increase in the proportion of cases were due to *Salmonella* were observed. Also observed that beef is the most frequently food associated with *Salmonella* outbreaks , as it was implicated in 77 outbreaks caused by Salmonella and 51,22 and 2 outbreaks were due to *C. perfringens, Staph. aureus* and *C. botulinum*, respectively. Bacterial pathogens accounted for 90% of deaths, about 88,47,12 and 4 cases of deaths caused by *Salmonella, C. botulinum, C. perfringens* and *E. coli,* respectively.

Cliver (1990) stated that the principal syndromes caused by *enterohaemorrhagic E. coli* have been linked to *E.coli* O_{157} H_7 which are haemorrhagic colitis with bloody stool, Haemolytic Uraemic Syndrome (HUS)leading to renal failure in children and thrombocytopenic purpura syndrome causing brain damage and high mortality rate.

Doyler (1991) stated that *E.coli* O_{157} H_7 was identified as pathogen in 1982 following its association with two food-related outbreaks of an unusual gastrointestinal illness. The organism is now recognized as an important cause of food borne disease with outbreaks reported in the USA, Canada and the United Kingdom. Illness is generally quite sever, and can include three different syndromes, i.e., haemorrhagic colitis, Haemolytic Uraemic Syndrome (HUS) and thrombocytopenic purpura. The organism is typical of most *E. coli*, but dose possess distinguishing characteristics, for example, *E.coli* O_{157} H_7 dose not ferment sorbitol within 24hours, dose not possess beta glucuronidase activity and dose not grow well or at all at 44- 45.5 °C degrees.

Olsvik et al., (1991) stated that the *enterotoxigenic E.coli (ETEC*) strain produce heat labile and heat stable entertoxins. These strains posses adhesion fimbria for intestinal attachment and colonization, some enteropathogenic *E.coli* strains produce cytotoxins, but adhere also to intestinal cells interferring with the electrolyte transport system. Strains possessing invasive properties are *enteroinvasive E. coli (EIEC). Enterohaemorrhagic E.coli (EHEC)* strains also produce cytotoxins (vero-cytotoxins, shiga-like toxins). Food from warm blood animals may be contaminated with *E.coli* but contamination from human sources are more common for food involved in outbreak of disease. In general, strains causing disease in animals possess other colonization factors than those found in human pathogenic strains. *EIEC* are like *shigella* only known to induce disease in man.

WHO (1991) *E. coli* constitutes part of the normal flora of the intestinal tract of human being and most of warm blooded animals, however there are pathogenic strains that cause, distinct syndromes of diarrheal disease and that have been associated with food borne illness.

Eley (1992) recorded that *Staph. aureus* is the second most common cause of food poisoning in the USA and is prevalent in Hungary. In both countries the frequency of outbreaks is though to be linked to dietary habit.

Eley (1992) and Ward et al., (1997) stated that the genes *staphylococcus* comprise three species *Staph. aureus, Staph. epidermidis* and *Staph. saprophyticus* of which *Staph. aureus* is the most concern to food microbiologists. Staphylococcal food poisoning is a syndrome characterized by nausea, vomiting, diarrhea, general malaise and weakness beginning one to six hours (usually 2 to 4 hrs) after ingestion although the illness is seldom fatal and the complications including dehydration, shock and can accompanied with sever attacks. Recovery usually occurs after about 24hours but may take several days.

Jay (1992) stressed on the important bacteria causing food poisoning. These include *Escherichia coli, salmonella, staph., pseudomonas, proteus* and *yersinia enterocolitica*.

Park et al., (1994) stated that staphylococcal food poisoning is caused by the ingestion of enterotoxins produced in food by some strains of *staph aureus*. Growth of enterotoxigenic strains of *Staph. aureus* to a population of at least 5×10^5 cells/gm of food is generally considered necessary for production of sufficient amount of enterotoxin to cause intoxication if the food is consumed.

Feng (1995) stated that *E.coli* O_{157} H_7 was only recognized as a human pathogen a little more than a decade age, yet it becomes a major food borne pathogen. The severity of serotype O_{157}: H_7 infections on the young and the elderly has had a tremendous impact on human health; the implication of acidic foods as vehicles of infection has dispelled the concept that low-pH foods are safe further, the association of non-bovine products with outbreak suggests that other vehicles of transmission may exist for this pathogen.

Roushdy and Sabry (1995) studied 156 cases of diarrheal children and 54 of non-diarrheal children (control) at institute of research for tropical medicine for detection, isolation and identification of suspected causative organism number of bacterial pathogen could be isolated. Enteropathogenic *E. coli (EPEC)*was the most common species 16 (10.25%) and 4(7.4%) followed by *salmonella* 10 (6.4%) and 5 (9.26%) and *yersinia enterocolitica* 2 (1.28%) and negative respectively. The control cases, which proved to contain the diarrheal causative microorganisms, were 14 cases (24.7%) and considered carrier for such organism.

Bettelhein (1996) stated that all mammals are colonized by *E.coli* generally at birth and these organisms become part of their intestinal flora for the rest of their lives. New types are acquired generally by an oral route. some *E. coli* are pathogenic and some may have ability to colonies the human intestine than most others. Recently *enterohaemorrhagic E.coli* have emerged. They can cause a number of intestinal illness in human including bloody diarrhea and HUS. These organisms produce a number of virulence factors particularly the shiga-like toxin (verotoxins). The animal intestine may be the reservoir of these organisms for human infection. Food especially under cooked meat products, have been associated with a number of outbreaks through out the world.

ICMSF(1998) stated that outbreaks of salmonellosis can follow from inadequate cooking, mishandling and recontamination. Raw meats can act as a source of cross-contamination of cooked meats or other foods in the kitchen or in processing plants.

Massoud et al., (1998) stated that staphylococcal enterotoxins exhibit the properties of they have been occurred in food poisoning as well as toxic shock syndrome which is a fulminate bacterial disease. They have evaluated staphylococcal enterotoxins as super antigens in food poisoning by their influence on lymphokine production with special emphasis to interleukin 2 (IL2). They concluded that there was a significant increase in T cells and massive increase in IL2 serum level in staphylococcal food poisoning patients especially among cases with sever toxicity, which may be evidence that staphylococcal enterotoxins could be incriminated as superantigens.

Mead and Griffin (1998) stated that *E. coli* $O_{157}H_7$ is found regularly in the faeces of healthy cattle and is transmitted to humans through contaminated food, water and direct with infected people or animals.

Fawzi (1999) reported that meat and meat products were incriminated in 26 outbreaks (20.9%) of total microbial food poisoning outbreak admitted to Alexandria poison center at Alexandria main university hospital from August 1997 to July 1998. *Staphylococcus aureus* was the most common isolated bacteria (46.8 %) also Salmonellae were involved in 2.7% of total food poisoning outbreaks.

Rajpura et al., (2003) reported that there is an outbreak of infection with *E. coli* O_{157} *phage type 21/28* occurred bet., the 23rd November 2001 and the 7th December,2001 in Eccleston, lancashire. There were 30 confirmed cases (23 with positive faecal isolates and 7 serologically positive). Initial investigations identified the suspected source as a butcher counter in a supermarket in Eccleston. The median age of cases was 60 with a mean of 56 and a range of 2-91 years. Microbiological investigations confirmed the butcher's counter as the source of the outbreak. The epidemiological evidence implicated cooked meats and microbiological evidence confirmed that contamination had occurred between raw and cooked meats. Complete physical separation of raw and cooked meat operations reduces the risk of such outbreaks.

Bang et al., (2008) reported that *Staph. aureus* contamination and enterotoxin production is a potential food safety hazard during the dry step of production of air-dried fresh meat products.

Manning et al., (2008) stated that *E. coli* O_{157} H_7, a toxin-producing food and water borne bacterial pathogen, has been linked to large outbreaks of gastrointestinal illness for more than two decades. *E. coli* O_{157} H_7 causes a wide range of clinical illness that varies by outbreak, although factors that contribute to variation in disease severity are poorly understood. Several recent outbreaks involving O_{157} contamination of fresh produce (e.g., spinach) were associated with more sever disease, as defined by higher Haemolytic Uraemic Syndrome(HUS) and hospitalization frequencies suggesting that increased virulence has evolved.

2.2. Chemical evaluation:

2.2.1. Determination of pH value:

Kauffman et al., (1963) reported that the increased muscle acidity was associated with pale, soft tissues, which yielded higher percentages of expressible juice, dark, dry and firm muscle tissue, exhibiting a relatively high pH. Shrink-less during curing and cooking and was more juicy and tender than pale, soft watery muscle. The cooked muscles possessed pH value about 0.35 unit highest than those of uncooked muscles.

Pearson (1968) stated that 6.5 pH may be considered as indicative for starting spoilage of meat.

Golovkin and Melozova (1970) stated that the pH value was related to protein denaturation by proteolysis.

El-Shazely (1976) reported that the pH of canned luncheon meat imported from Denmark, Yugoslavia and china was 6.45, 6.48 and 6.38, respectively.

El-Khateib(1982) recorded that the mean pH values of fresh, dry and locally manufactured sausage samples were 6.1, 4.71 and 6.15, respectively.

Nelsen and Zeuthen (1983) reported that addition of low pH mixture of sodium pyrophosphate, sodium tripolyphosphate, and sodium poly phosphate had a stabilizing effect upon pH in sausage during storage at 8ºC.

Cuzzoni and Gazzoni (1984) mentioned that the rise of the meat pH values at frozen storage period may be due to breakdown of protein and consequently the increase of ammonia and free amine group produced in meat.

Pikul et al., (1984) mentioned that the putrefaction condition may attributed to the action of certain proteolytic microorganisms which can metabolize meat protein through the action of enzyme and some other which can attack protein but can metabolize peptides or free amino acids, then the degradation product resulting from bacterial metabolism of meat protein in which lead to increase pH value, while souring may be attributed to the condition of anaerobic metabolism of carbohydrate additive to meat product during processing by bacteria, lead to produce various fermentation products primary organic acid, principally lactic acid which lower the pH.

Vural and Öztan (1989a) reported that the pH values of Kavurma (Turkish cooked meat product) were ranged from 6.15 to 6.45 with average 6.26 ± 0.031 .

Smith (1991) mentioned that the pH value as a criteria could not be taken alone as a guide line for freshness in the sausage because the final pH is the summation of the interferring factors coming from the blend of the sausage mass ingredients.

Hoda (1995) found that mean pH value was 5.6 in the examined basterma samples.

Tiryakioğ IU and Yücel (1995) reported that the average pH value of kavurma was 6.39.

Arvanitoyannis et al., (2000) chemical analysis were performed on 48 samples of Cavourmas (a Greek traditional cooked meat products) and found the pH were ranged from 6.1 to 6.7 with a mean value 6.3.

Cetin (2000) found that the average pH value of kavurma in the marketplace in Erzurum was 6.06.

Hala and Hoda (2002) examined the pH value of 10 beef luncheon samples and found the mean value of pH was 5.6±0.05.

Amal (2004) found that the mean pH values the examined luncheon, basterma, fresh and frozen sausage were 5.42, 5.22, 5.17 and 5.41, respectively.

Ambrosiadis et al., (2004) chemical and microbiological analyses were performed on 67 samples of Greek traditional sausage and recorded that the pH values and aerobic plate counts were ranged from 4.67 to 6.09 with a mean value 5.48 ± 0.49 and from 5.14 to 8.96 with a mean value 8.22 ± 0.5 $\log_{10}$ CFU/g, respectively.

Aksu and Kaya (2005) reported the mean pH values of Kavurma stored at 0ºC for 300 days were as follow: no added antioxidants group 0 day: 6.21±0.02, 300 days: 6.32±0.06 and added antioxidants group: 6.26±0.21 and 6.30±0.07, respectively.

Belgin Siriken et al., (2006) determine the pH of 100 Turkish sausage samples collected from shops and markets in the Afyone province, Turkey and found that the pH values ranged from 4.8 to 6.5.

Jonathan et al., (2007) determined the pH of 6 types of traditional dry sausages (A, B, C, D, E and F) from 6 small scale facilities representative of production in the Massif central region in France and found that the pH values were ranged from 5.7 to 6.1, from 5.5 to 6.0, from 5.0 to 5.6, from 5.2 to 6.2, from 5.9 to 6.5 and from 5.4 to 5.9, respectively.

2.2.2. Determination of Total volatile nitrogen value:

Pearson (1976) said that the method of total volatile bases was valid only for fresh or frozen meats and not for bacon and other cured meats. Meat should give a value of less than 20mg percent calculated or a fat free basis and value over 30mg percent was considered to correspond to staleness.

Mousa et al., (1993) found that the average nitrogenous content was 28.16 mg % and 16.1 mg % for the examined samples of basterma and sausage, respectively.

Egyptian Standard (1522/2005) reported that the T.V.N. must be not more than 20 mg % in meat and frozen meat products.

Abd El-Hafiez (2006) chemical evaluation were performed on final basterma product samples and found that pH and TVN value were ranged from 5.8 to 6.0 with a mean value 5.9 ± 0.01 and from 15 to 20 with a mean value 17.46 ± 0.20, respectively.

2.2.3. Determination of Thiobarbituric acid value:

Ockerman (1976) stated that the meat products with TBA > 1mg malonaldehyde / kg could be considered as "rancid".

Gray and Pearson (1987) reported that TBA increased gradually during frozen storage due to formation of malonaldehyde (an end product of oxidative operation of fat) and the lipid oxidation of frozen meat products due to incorporation of air into the meat during processing also mentioned that the reduction of particle size by grinding, chopping, flaking or emulsification results in disruption of cell membranes and incorporation of air into the tissues. Both of these actions increase tissue susceptibility to oxidation and hasten the development of oxidative rancidity.

Pikul et al., (1989) mentioned that the TBA was related to the oxidation in meat products also, some microorganisms can degrade the fat by either lipase hydrolysis enzyme or oxidation by oxidase enzyme which can yield rancidity.

Vural and õztan (1989b) reported that the TBA values of kavurma stored at 0ºC for 180 days were as follow: no added antioxidant group, 0 day: 2.10, 180 days: 2.32mg malonaldehyde/kg, and added antioxidant group: 1.41 and 1.61mg malonaldehyde/kg, respectively.

Monahan et al., (1992) mentioned that oxidative rancidity (warmed over flavor) has been recognized as a problem occurring during the storage of meat. Meat and meat products to under go oxidation depends on several factors including the fatty acid composition and the presence pre-oxidants in muscle. The susceptability of muscle lipids oxidation depend on their degree of unsaturation. The poly unsaturated fatty acid contents of muscle varies between species.

Dinc (1997) reported that TBA values of kavurma increase during storage 0 days: 1.055, 90 days: 1.583mg malonaldehyde/kg.

Papadima et al., (1999) chemical and microbiological analyses were performed on 31 samples of Greek traditional sausage and recorded the rang of TBA value, pH value and aerobic plate count were 0.42-5.33 mg malonaldehyde /kg, 4.74-6.74 and 5.48-9.32 cfu/g respectively.

Arvanitoyannis et al., (2000) chemical analysis were performed on 48 samples of Cavourmas and found the TBA. values were ranged from 0.5mg to 7.3 mg malonaldehyde/kg with a mean value 3.4 mg malonaldehyde/kg.

Gab-Allah and Shalaby (2001) They examined chemically 25 frozen sausage samples were collected from different shops and supermarkets in Alexandria governorate for determination of pH and thiobarbituric acid. The obtained results were ranged from 5.70 to 6.35 with a mean value ±SE 6.10 ± 0.062 and from 0.55 to 0.70 with a mean value 0.66 ± 0.008, respectively.

Abd El-Monem et al., (2002) They examined 10 frozen sausage for determination of thiobarbituric acid and acidity value (pH).which ranged from 0.52 to 1.70 with a mean value 0.68 and from 5.65 to 6.90 with a mean value 5.70.

Aksu and Kaya (2005) reported that the mean TBA values of kavurma stored at 0°C for 300 days were as follow no added antioxidant group, 0 day: 6.74 ± 0.34, 300 days: 18.50 ± 0.77 mg malonaldehyde/kg and added antioxidant group: 7.90 ± 2.30 and 9.79 ± 0.38 mg malonaldehyde/kg, respectively.

2.3. Bacteriological evaluation

2.3.1. Meat quality

Hubber et al., (1958) reported that the effect of cooking on the number of microorganisms in meat was to generally reduce *coliforms*, *staphylococci* and gram-negative bacteria either to a very low level or to completely destruction of them.

Freeman (1960) found that the rate of bacterial count in meat products depends on its initial contamination.

Levine (1961) found that the total bacterial count was the most reliable index for detecting the sanitary condition for proper storage of food products.

Shiffman (1961) stated that the fresh meat and meat products with a bacterial count below 10^5 organisms/gm at 37°C have not been implicated in food poisoning and that microbiological standard for such products should be established at 10^5 per gram.

Sadek (1965) mentioned that the presence of large number of bacteria was an indicative for inadequate sanitary conditions during processing and lead to spoilage of the product and/or public hazard to the consumers.

MeCony and faber (1966) reported that high levels of *staph aureus* growth could be occur in cooked meat without production of detectable amount of enterotoxin A., in the presence of competitive organisms.

Murray (1969) found that more than 5% examined frozen boneless meat samples had aerobic plate count $<10^5$ cells/g.

Weissman and Carpenter (1969) reported that although *salmonellae* were often found in the raw meats used to manufacture processed meat, they were rarely found in the processed product. Also they concluded that *salmonellae* did not survive the heat processing given to these products.

Brown and Twedt (1972) reported that *coliform* organisms were undetectable at 51.1ºC after 12 hours from inoculation.

Dennis et al., (1972) found that the total number of aerobes changed very little during the processing of beef for further cooking. They reported that the mean counts ranged from log 4.06 to 9.94 organisms/gm and the coliform numbers decreased during freezing probably due to loss of viability of vegetative cells. Moreover, the count varied through processing steps.

ICMSF (1974) reported that the microbiological limits for acceptance of boneless frozen meat are aerobic plate count 5×10^5 as acceptable, but 75×10^5 as marginally acceptable, while $> 10^7$ is considered unacceptable.

Bryan (1975) stated that the high bacterial count in the examined samples of meat products may be attributed to contamination of flesh used for manufacture of such products. Moreover, mincing, machines, grinders, equipments, knives and filling or stuffing machines are considered sources of infection and contamination of meat during processing

Powers (1976) stated that the microbiological specification for frozen meat purchased by U.S military and federal agencies are A.P.C 10^6 cells/g and *coliforms* 10^2 cells/g. *E. coli* and *Salmonellae* could not be detected.

Mossel et al., (1978) found that freezing killed a small proportion of all microorganisms present, as more die during frozen storage, but this occurs rather slowly (5% month at -20°C). gram negative bacilli was more susceptible than positive cocci.

Thatcher and Clark (1978) reported that the high bacterial count may be attributed to the unsanitary condition and unsuitable environmental conditions during production, storage and handling of foods.

Johnson et al., (1979) found that A.P.C of examined frozen beef samples ranged from 88×10^3 to 31×10^6 organisms/g.

Robert et al., (1980) stated that *salmonellae* was found in 2 out of 162 examined meat samples.

Ali and Andyne (1981) analyzed bacteriologically 6 lots of ground beef obtained at intervals from local supermarkets and found that the mean value ($\log_{10}$) for aerobic and psychotrophic plate counts were 6.35 and 6.66 organisms/g, respectively.

Goda et al., (1981) examined 318 frozen meat samples imported to Arab Republic of Egypt and they found that the A.P.C ranged from 12×10^4 to 16×10^5 organisms/g with a mean value 1×10^6 organisms/g, while coliform varied from 3×10^2 to 21×10^2 organisms/g with a mean value 15×10^2 organisms/g.

Seddik (1982) examined 335 samples of frozen meat and chicken products for detection of *staphylococcus* organisms. He detected *staph. aureus* in 71.23%, 83.33% and 90% of examined beef, chicken and pork products, respectively.

Amal (1983) examined bacteriologically 10 raw frozen bone-less meat samples and found that the mean counts of *A.P.C, enterobacteriaceae, coliform, faecal coliform*, and *staph. aureus* per gram were $69.78\times10^4 \pm 25.27\times10^4$, $92.52\times10^3 \pm 72.025\times10^3$, $2.735\times10^2 \pm 1.327\times10^2$, 60.3 ± 64.386 and $22.42\times10^{2`} \pm 8.166 \times 10^2$ organisms/g, respectively.