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Thermal and Microbial Quality Assessment of Emulsion Based Meat Sausage

Belal Abdullah Mahmoud Tarda

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By:
Belal Abdullah Mahmoud Tarda

B.Sc: Food Science and Technology. Al-Quds University-Palestine

Supervisor: Dr. Ibrahim Afaneh

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Prepared By: Belal Abdullah Mahmoud Tarda
Registration No.: 20714325

Supervisor: Dr. Ibrahim Afaneh

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The name and signatures of the examining committee members are as follows:

1-Head of Committee: Dr. Ibrahim Afaneh Signature:……..

2- Internal Examiner: Dr. Sulaiman Allousi Signature:……..

3- External Examiner: Dr. David Vagnoni Signature:……..

Jerusalem – Palestine

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Dedication

This thesis is dedicated to my supervisor, my parents, my lovely wife, my colleagues in Al-Quds University, and staff of Salwa Food Company.

Belal Abdullah Mahmoud Tarda

Declaration:

I certify that this thesis submitted for the degree of master is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed:

Belal Abdullah Mahmoud Tarda

2012

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Belal M. Abdullah

Abstract:

This work is aiming to determine the influence of storage time and temperature of major ingredients of meat sausage (Mortadella) which are mechanically deboned meat and chicken skin as approved by Palestinian standards for meat sausages on microbial growth namely; *Total Plate Count, Total coliforms,* and *Salmonella,* and on pH values. Study also the influence of thermal treatment (Pasteurization) conditions of cooking oven like; oven temperature T (out), core temperature, and holding temperature on microbial growth namely; *T.P.C, Total Coliform, Clostridium Perfringens, Staphylococcus aureus, Salmonella,* and *Listeria Monocytogenes*. In addition to study the influence of storage time on each of the microbial growth namely; *T.P.C, Total Coliform, Clostridium Perfringens, Staphylococcus aureus, Salmonella,* and *Listeria Monocytogenes,* chemical composition of final refrigerated meat sausage (Mortadella) namely; moisture analysis, crude fat, ash determination, protein content, peroxide value, residual Nitrites, and pH values, and finally quality assessment and sensory evaluation of processed meat sausage (Mortadella).

The research was based on four categories; frozen minced mechanically deboned meat and frozen chicken skin samples taken under aseptic conditions, and then stored under different temperatures through 15 days, final product stored at temperature (0-4°C) after cooking with reject to different factors like oven temperature, core temperature, and holding time, the work of analytically test periodically done monthly for samples through storage at temperature to determine changes on chemical composition as approved by Palestinian standards for meat sausages, and finally through storage we applied sensory panel test for samples by standard procedure during 4 months.

Results showed that the *Total Plate Count* and *Total coliforms* growth in MDM and chicken skin increased with increasing the period of storage time under sequential temperatures, also sharply increase in *Total Plate Count* growth in MDM at high storage temperature, also showed that the best conditions for storage of MDM and chicken skin was under temperature -17°C. In addition to presence of *Salmonella* under all investigated times and temperatures. Results showed that pH values of MDM at all storage temperatures was decreased gradually for all times investigated but for chicken skin results showed that pH values at storage temperatures was gradually decreased as linear almost for all times investigated. The best conditions for storage MDM and chicken skin was under temperature -17°C.

The analysis of MDM in this study indicated that chemical composition of MDM from Palestinian sources gave suitable characteristics which influence on several functional properties. This study found that there was no vegetative microbial growth for any specific bacteria; except for *T.P.C* through cooking of processed meat sausage (pasteurization) under all investigated conditions, and found that temperature was related directly with time of oven temperatures, core temperature, and holding time in increasing the *T.P.C* population.

Also this study found that microorganism's population of final processed meat sausage was in agreement with Palestinian standard and that there was no vegetative microbial growth for any specific bacteria, except for an exponential increase of *T.P.C* growth during storage time. The analysis of final processed meat sausage in this

study indicated that chemical composition was in agreement with Palestinian standards. There were no clear and significant changes to moisture, fat, ash, and protein during storage time. On the contrary of changes and degradation of peroxide value, nitrites, and pH with time. Results showed that, sweetness, saltiness, smell (odor), and color characteristics of sample were changed clearly, while other characteristics such as; sourness, bitterness, softening, coarse (rough), hard particles, and slicing with time were much more stable.

تقييم جودة مستحاب لحوم السجق بناء على المعاملات الحرارية والجرثومية

إعداد: بلال عبد الله محمود طرده

إشراف: الدكتور إبراهيم عفانة

الملخص:

الهدف من هذه الرسالة هو دراسة تأثير درجة الحرارة وفترة التخزين للمكونات الأساسية لمنتج السوسج (المرتديلا) ألا وهي اللحوم المنزوعة العظم ميكانيكيا وجلد الدجاج كما هو معتمد في المواصفات الفلسطينية على النمو الميكروبي وبالأخص العدد الكلي للبكتيريا والكوليفورم والسالمونيلا وأيضا التغير في درجة الحموضة (الرقم الهيدروجيني). دراسة تأثير ظروف المعاملات الحرارية (البسترة) لعملية الطبيخ مثل حرارة الفرن وحرارة المنتج والفترة الزمنية لمرحلة البسترة على النمو الميكروبي لكل من العدد الكلي للبكتيريا, الكوليفورم, كلوستريديوم بيرفرنجيز, ستافلوكوكاس ايرياس, سالمونيلا, الليستيريا مونوسايتوجينيس, والمكونات الكيميائية للمنتج النهائي خلال التبريد مثل الرطوبة, الدهن, الرماد, البروتين, قيمة البيروكسايد, كمية النيتريت المتبقي, والتغير في درجة الحموضة, وفي النهاية مدى التغير على التقييم الحسي وجودة المنتج خلال فترة التخزين لمنتج السوسج (المرتديلا).

تعتمد الدراسة بشكل أساسي على اربعة عناصر ألا وهي : تخزين اللحمة المنزوعة العظم وجلد الدجاج ضمن ظروف صحية ومطابقة على درجات حرارة مختلفة لفترة 15 يوم, وتخزين المنتج النهائي على درجة حرارة (0-4 مئوي) بعد معاملته حراريا تحت ظروف مختلفة مثل حرارة الفرن وحرارة المنتج وفترة البسترة, والفحوصات التحليلية تمت بشكل دوري وشهري للعينات خلال التخزين لدراسة التغييرات الكيميائية والفيزيائية ومطابقتها مع المواصفات الفلسطينية لهذا المنتج, والعنصر الأخير يتمثل في عمل تقييم حسي للمنتج بشكل دوري خلال فترة الصلاحية الا وهي اربعة شهور.

اظهرت النتائج أن نمو العدد الكلي للبكتيريا والكوليفورم في اللحمة المنزوعة العظم وجلد الدجاج يتزايد مع زيادة فترة التخزين تحت درجات حرارة متسلسلة, وأيضا هناك تزايد حاد لنمو للعدد الكلي للبكتيريا في اللحمة المنزوعة العظم على درجات حرارة عالية خلال التخزين, ولوحظ أن افضل درجة حرارة لتخزين كل من اللحمة المنزوعة العظم وجلد الدجاج هي (-17 مئوي), بالأضافة الى وجود بكتيريا السالمونيلا في جميع الظروف التي تم تخزين

اللحمة المنزوعة العظم وجلد الدجاج عليها. أظهرت النتائج ان التغير في الرقم الهيدروجيني (pH) للحمة المنزوعة العظم على درجات حرارة مختلفة كانت تتناقص تدريجيا مع الوقت ولكن بالنسبة لجلد الدجاج كان التناقص خطيا قدر الأمكان وبسكل تدريجي, فأظهرت النتائج أن افضل درجة حرارة للتخزين هي (-17 مئوي).

تبين من خلال التحليل الكيميائي للمكونات الأساسية للحمة المنزوعة العظم من المصادرالفلسطينية بأنها مطابقة ومناسبة بحيث تحدث المواصفات المطلوبة لأنتاج السوسج (المرتديلا), وأظهرت هذه الدراسة أنه لا يوجد اي نمو ميكروبي لأي نوع من البكتيريا في المنتج النهائي بعد الطبيخ تحت جميع الظروف التي تم تحديدها باستثناء العدد الكلي للبكتيريا وكمية هذا التواجد كانت تعتمد بشل أساسي على الحرارة والوقت لكل من فرن الطبيخ والمنتج نفسه وفترة البسترة.

ايضا اظهرت الفحوصات الميكروبية والكيميائية مطابقة المنتج النهائي (المرتديلا) للمواصفات الفلسطينية وذلك بعد وجود اي نوع من البكتيريا الممرضة فيها باستثناء التزايد الأسي للعدد الكلي للبكتيريا خلال فترة التخزين. لم يكن هناك اي تغيير ملحوظ على المكونات الأساسية للمنتج النهائي للسوسج (المرتديلا) مثل الرطوبة, الدهن, الرماد, والبروتين خلال فترة التخزين على العكس من المكونات الاخرى ألا وهي التغير الملحوظ والأختلاف في النسب مع الوقت لكل من قيمة البيروكسايد والنيترايت ودرجة الحموضة (pH). بناءا على التقييم الحسي لوحظ خلال فترة التخزين ان هناك تغيير ملحوظ لكل من درجة الحلاوة, الملوحة, الرائحة, واللون على العكس كان هناك ثباتية في المواصفات الاخرى خلال فترة التخزين.

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Definitions

D-value: Decimal reduction time is the time required at a certain temperature to kill 90% of the organisms being studied. Thus after a colony is reduced by 1 D, only 10% of the original organisms remain.

Peroxide Value: The amount of peroxide oxygen per 1 kilogram of fat or oil, used as a measurement of the extent to which rancidity reactions have occurred during storage.

Abbreviations

ANFOR: Association French for Standardization

AOAC: Association of Analytical Communities

CFR: Code of Federal Regulations

COFRAC: Committee French Accreditation

DFD: Dark, Firm, and Dry

DWB: Dry Weight Basis

FAO: Food and Agriculture Organization

FDA: Food and Drug Administration

FFA: Free Fatty Acid

FSIS: Food Safety and Inspection Service

GI: Gastrointestinal

GMPs: Good Manufacturing Practices

HACCP: Hazard Analysis of Critical Control Point

IQF: Instant Quick Freeze

MDM: Mechanically Deboned Meat

MSCM: Mechanically Separated Chicken Meat

MSHM: Mechanically Separated Hen Meat

PFIA: Palestinian Food Industries Association

PS: Palestinian Standards

PSE: Pale, Soft, and Exudative

RTE: Ready to Eat

SPP: Sodium Polyphosphate

TBA: Thiobarabituric Acid

USDA: United States Department of Agriculture

WHC: Water Holding Capacity

Al-Quds University

CHAPTER ONE

INTRODUCTION

Chapter One Introduction

1. Introduction:

1.1. Background

The food processing sector has been one of the fastest growing sectors of the Palestinian economy in producing basic products. It has a main role in developing the economy and contributing with more than 24% of the production value, 20.7% of the total added value, and 16.7 % of the total employers in the manufacturing industries and represents14.2 %of the total number of manufacturing establishments during 2008. Food industry development and growth have been obvious in the last decade with prestigious shares of the local stakeholders with total market sales that are approximately $ 520 million per year (PFIA, 2010).

The sector meat production includes; sausages, canned meats, and other meat preparations. The number of factories in this industry is 14 legal factory with 442 workers with an average of around 32workers/unit (PFIA, 2010).

The production capacity of this sector is 2430 tons/month, with a value of 32 million dollars. The added value of production is around 10 million dollars which present 33.6% of total added value (PFIA, 2010).

Meat processing factories are modernly equipped using the latest technologies and most of them are semi- or fully automated (PFIA, 2010).

These factories are taking in consideration hygiene, quality and food safety standards in addition; four factories from this sub-sector are ISO 22000 -certified. Most of these factories are certified with Palestinian quality certificate (PFIA,, 2010).

Meat processing has always been part of FAO's livestock programmes, not only because of the possibility of fabricating nutrient-rich products for human food, but also owing to the fact that meat processing can be a tool for fully utilizing edible carcass parts and for supplying shelf-stable meat products to areas where no cold chain exists (Gunter and Peter, 2007).

Sausages are one of the oldest forms of meat processing and modern sausage technology has its roots deeply embedded in history. Sausages are fresh comminuted meats modified by various processing methods to yield desirable organoleptic and keeping properties (I.V. Savic, 1985).

Sausages are usually defined as comminuted seasoned meats, stuffed into casings; they may be smoked, cured, fermented and heated. They are made from any edible part of the slaughtered, veterinary-inspected animal, and a series of nonmeat ingredients. A sausage formulation is always a compromise between the expected quality of the finished

product, the cost of raw materials and the techniques applied. The production of a wide variety of sausages is possible through the manipulation of different variables such as meat formulation, processing temperature, types of casing and particle size. By altering certain processing treatments, changes occur within the product's texture and flavor, moisture content, percentage of yield and other attributes. The number and variety of sausages are limited only by the manufacturer's imagination and knowledge (I.V. Savic, 1985).

Hort, & Cook (2007) explained that, fat is a critical part of the food matrix for both flavor and texture properties and thereby for overall palatability. Fat acts as a carrier and reservoir of aroma compounds, stimulates the senses during eating and acts as a precursor for certain flavors. Moreover, the amount and composition of fat and its physical state influence the dynamic release of flavor compounds during consumption.

In general, meat is composed of water, fat, protein, minerals and a small proportion of carbohydrate. The most valuable component from the nutritional and processing point of view is protein (Gunter and Peter, 2007).

Gunter and Peter (2007) documented that, the mineral contents of meat include calcium, phosphorus, sodium, potassium, chlorine, and magnesium with the level of each of these minerals above 0.1%, and trace elements such as iron, copper, zinc and many others. Blood, liver, kidney, other red organs and to a lesser extent lean meat, in particular beef are good sources of iron. Iron intake is important to combat anaemia, which particularly in developing countries is still widespread amongst children and pregnant women. Iron in meat has a higher bio-availability, better resorption and metabolism than iron in plant products.

Chicken meat delivers essential vitamins and minerals. The chicken industry is a significant contributor to the economy and, of the land based animal production systems; chicken meat production creates the least environmental burden (The Australian Government's National Food Industry Strategy, 2003).

Jimenez (1996) reported that, complex food matrices including a great variety of components which can interact with flavor compounds affecting their release and perception. Among these components, fat and salt are some of the most interesting since their presence in meat products are in continuously discussion due to health considerations.

Hely and Erminio (2009) evaluated that, sensory characteristics are of a great importance for consumer preferences and satisfaction with foods.

Also Behrends et al. (2005) confirmed that, Flavor and texture, particularly juiciness and tenderness, have a clear relationship to meat palatability.

Heat treatment of processed meat products serves two main purposes, Enhancement of desirable texture, flavor and color, in order to make meat products more palatable and

appetizing for consumption, and reduction of microbial content thus achieving the necessary preservation effects for an extended shelf life (storability) of the products and food safety effects by eliminating potential food poisoning agents, the heating parameters to be applied in meat processing can vary considerably in temperature and time depending on the type of product. Heat treatment methods cause various physical-chemical alterations in meat, which result in the beneficial sensory and hygienic effects on the processed products (Gunter and Peter, 2007).

Heat treatment became the common way of making meat palatable for consumption. The impact of high temperatures induces coagulation and denaturation of meat proteins and structural and chemical changes of fats and carbohydrates, which make meat tastier and also tenderer. In addition, the absorption of nutrients from heat treated meats in the digestive tract of humans is improved (Gunter and Peter, 2007).

Microorganisms are sensitive to heat and are killed at certain temperatures, which may be below 100°C in the case of Pathogenic or above100°C in the case of spore forming microorganisms, so each species of microorganisms reacts differently to heat treatment, due to their different heat resistance, microorganisms are quickly killed when they are exposed to relatively high temperatures. Microorganisms can also be killed at relatively low temperatures, but longer heat treatment periods will be necessary (Gunter and Peter, 2007).

Bio-physically the heat inactivation of microorganisms is relatively complex. The heat destruction of a population of microorganisms does not occur instantly but gradually. Mathematically, it can be expressed by the term “decimal reduction time” (also called D-value. after a defined heat impact period (constant heat) 10% of the original population will survive, after the same impact period again 10% and so on, vegetative microorganisms can all be destroyed at temperatures below 100°C, basically in the temperature range of 60-85°C (depending on the type of microorganisms). Only those microorganisms capable of forming spores (which all belong to the groups of *Bacillus* and *Clostridium*) can survive temperatures of 100°C and above. The heat treatment besides basic food safety aspects affect sensory reasons (Gunter and Peter, 2007).

For processed meat products exact temperature control is indispensable, as a balance between two opposite requirements has to be found, firstly heat treatment temperatures should be raised high enough to accomplish adequate microbial reduction for shelf life extension, secondly heat treatment temperatures should be kept low enough to prevent deterioration of the eating quality, heat treatment of processed meat products will therefore always be a compromise between sensory and hygienic requirements (Gunter and Peter, 2007).

1.2. Literature Review.

In the late 1950s and early 1960s marked changes in the poultry processing industry began. At that time, the poultry industry began marketing more cut-up and further processed poultry meat products. As the popularity of these consumer choices grew along with the increased consumption of poultry meat, more parts such as frames, backs, necks, drumsticks, wings, etc. became available for mechanical separation. In the process of mechanical separation, meat is removed from the skeletal bone tissues by grinding the starting material (frames, necks,…etc.) and passing it through a sieve under high pressure. Most of the bones and cartilaginous materials are removed based on a differing resistance to shear.

The chicken industry has economical interest in the use of hens meat through adequate processes. This literature review evaluates the quality of mechanically deboned meat by microbial growth, chemical characteristics, sensorial properties and functional characteristics, namely; definition of MDM, proximate composition, cholesterol, bones, calcium and iron contents, fatty acid profile, pH, quality of mechanically deboned meat of poultry as raw material for sausages, regarding their chemical composition, microbial growth and functional characteristics.

Further processed poultry will likely continue to grow due to the expansion of fast food outlets, home meal replacements from supermarkets and restaurants, and the continued growth of food service in the health care sector.

1.2.1. Mechanically Deboned Meat (MDM).

The mechanical separation of poultry began in the late 1950s in the U.S.A, but for different reasons. The consumer preference for chicken cuts instead of whole chicken and, later on, the demand for chicken fillets and convenience products, such as nuggets, hamburgers and marinated cuts, required the finding of ways to use backs, necks, and bones left over's from manual deboning processes. These parts make up about 24% of the edible part. From there on, the mechanically deboned (separated) meat of poultry became available and started to be used in the manufacture of several products, such as; sausages, bolognas, salamis and dry soups (Field, 1988 and Froning, 1981).

Yields of mechanically separated poultry range from 55 to 70% (Froning, 1981).The meat-to-bone ratio largely influence the yield from specific parts. In 1994, the USDA indicated that approximately 1 billion pounds of raw poultry material produces about 700 million pounds of mechanically separated poultry (USDA, 1994).This mechanically separated poultry has been formulated into about 400 million pounds of sausages (bologna, salami, and franks) and 300 million pounds of nuggets and patties. Some mechanically separated poultry is combined with other species (e.g., beef and pork) in various sausage products.

In every deboning process, after the removal of the usual meat cuts, there is always an amount of meat which is firmly attached to the bones. Mechanically separated meat is a

product resulting from the mechanical separation of the meats attached to these bones. Normally the mechanical separation is made for bones of irregular shape, more difficult to be manually deboned, such as vertebral column and neck. However, other bones with attached meat, or whole carcasses, can be submitted to mechanical separation.

According to Lyons (2001), the volume of biological material, costs of labor and transportation associated to the slaughtering of laying hens make it one of the main economical and environmental problems of poultry industry. Another area for the use of these poultry is the production of sausages, using mechanically separated hen meat (MSHM) (Mott et al., 1982; Lee et al., 1997; Grunden et al., 1972 and Jantawat and Dawson, 1980). Mechanically-separated chicken meat (MSCM) is made from the deboning and cutting of parts with lower commercial value, such as the back and the neck (Barreto, 1995).

Mechanical separation provides a means of harvesting functional proteins which can be used in the preparation of a variety of further processed meat products. Mechanically separated poultry meat (also reported as mechanically deboned poultry meat prior to 1995) has been widely utilized in further processed poultry meat products such as bologna, salami, frankfurters, turkey rolls, restructured meat products, and soup mixes. This low cost meat source has led to poultry meat products being more cost effective in the market place.

The use of mechanically separated poultry meat is regulated by the U.S. Department of Agriculture Food Safety Inspection Service (Federal Register), while the Food and Drug Administration (FDA) regulates fish and fishery products.

Regulations regarding mechanically separated poultry meat were first established by the Food Safety Inspection Service (FSIS) in 1969. There have been significant changes in the regulations relating to the labeling of mechanically separated poultry meats. Prior to 1996, mechanically separated poultry meat was generically referred to as “mechanically deboned poultry” or “comminuted poultry,” but the label needed only to state “chicken” or “turkey.” As far as labeling was concerned, there was no discernable difference in poultry meat that was processed using a deboning machine or poultry that was hand-deboned.

In response to the lawsuit regarding these labeling inconsistencies, poultry regulations were re-evaluated by FSIS. FSIS suggest that mechanically separated poultry is not different (in consistency and form) from product resulting from mechanical separation of other livestock products. Moreover, the final texture and form of mechanically separated poultry is different from hand-deboned poultry, even if the hand-deboned poultry is further processed through a grinder. As a result of these conclusions, FSIS revised the regulations regarding mechanically separated poultry meat which became effective in November of 1996. Specifically, the poultry products inspection regulations were amended so that mechanically deboned poultry would be required to be labeled as “mechanically separated (kind of poultry)” as opposed to labeling it “ground chicken or turkey” without indication of the mechanical deboning process used. However, this

labeling requirement is dependent on the starting materials. If the starting materials are frames, trim, or parts where most of the meat has been removed, then the labeling should indicate the meat is mechanically separated poultry. However, if the starting materials are parts with the majority of meat still attached or whole birds such as spent fowl, rooster, and mature breeder hen, then the standard of identity on the label can still indicate "ground chicken."

Another change in regulations included the use of mature fowl in baby foods. Many of the original regulations were based on an USDA review of a report on the health and safety of the use of mechanically separated poultry. At that time, there was a concern about the high fluorine (from bone) content in mechanically separated meats from mature fowl. As a result, the past regulations prohibited the use of mechanically separated mature fowl in baby, junior, and toddler foods and limited the amount in other poultry products to 15%.

USDA recently re-evaluated the health report of 1979 (Murphy et al., 1979) and concluded that the fluorine content in mechanically separated poultry was not a health concern. This change of attitude was based on discussions with dentists, medical doctors, and baby food companies, who agreed the chances of developing fluorosis (fluorine toxicity) from overconsumption of poultry products containing mechanically separated mature fowl, were negligible. Therefore, the 1996 regulations removed the prohibition of the use of mechanically separated mature fowl in baby foods.

1.2.2. Microbial Growth of Mechanically Deboned Meat

Fresh cuts or raw materials should not be above 4.4°C (40°F) when received and frozen materials should be below -17.8°C (0°F). Sample temperature checks should be made in the geometric center of boxes or combos. If poultry muscles are received in combos, pull pieces from the center of the container and check the internal temperature. Pieces having temperatures of 7.2°C (45°F) may not have been chilled adequately prior to fabrication or have been temperature abused in transit. In either case, the higher receiving temperature means a potential decrease in shelf-life of the product, accelerated spoilage, or a potential pathogen risk. Real-time recording thermometers may be placed in refrigerated trailers and monitored for temperature deviations during transit (Alan, 2001).

Carcasses are chilled immediately after slaughter to reduce the temperature to <4.4°C (40°F) within 4 and 8 h for broilers and turkeys, respectively (Addis, 1986). A weight loss of 0.5% will typically occur during fabrication of muscles used in further processing. Muscle pieces may be iced in combos or boxed and held in a cooler maintained at -2.2 to 2.8°C (28 to 37°F) for 24 to 36 h. Trimmings held for longer than 36 h should be boxed and frozen quickly to preserve quality. The high relative humidity (~85%) in most coolers assists in preventing carcass shrink and moisture loss.

Muscle pieces, MDM, or MDT destined for frozen storage are boxed in plastic-lined or waxed, cardboard containers for subsequent freezing immediately after fabrication. Generally, the lower the temperature and the more protection from atmospheric oxygen,

the greater the reduction in oxidative rancidity and extension of storage life. At ≤ -10°C (14°F) most microbial growth and enzymatic activity are reduced to almost zero because most of the cellular water molecules are fixed in a crystaline structure, but reactions may continue slowly down to -80°C (-112°F). Most commercial holding freezers range from -17.8 to -28.9°C (0 to -20°F) while air-blast or Instant Quick Freeze (IQF) freezers use high air velocity (2500 ft[762 m]/min at ≤ -28.9°C [-20°F]) to rapidly remove the heat. Powdered carbon dioxide (CO_2) or CO_2 "snow" (-62.2 to -78.3°C [-80 to -109°F]) may be dusted among muscle pieces or MDM/MDT prior to boxing to accelerate the freezing process. However, caution should be used to avoid suffocation due to sublimation of the CO_2 if the boxed product is stored in a closed area such as a refrigerated trailer. If CO_2 snow is used in mixer/blenders, they should be properly vented to avoid the risk of displacing oxygen in the air. In any freezing application, raw or finished products must be packaged to exclude air and protect the surface from excessive drying (freezer burn). Poultry muscle that is frozen and held at -17.8 to -28.9°C (0 to -20°F) should retain its quality for 6 to 10 months (Hedrick et al., 1989). The least desirable temperature for holding frozen meat trimmings is -11.1 to -10°C (12 to 14°F), which is the point of phase transition between intercellular crystalline ice and a combination of ice and water. Frequent cycling of the refrigeration system through this temperature zone causes large ice crystal growth in muscle cells and excessive purge (water loss) when thawed.

Proper thawing prevents excess purge loss and the risk of microbial growth on muscle pieces and in MDP/MDT. Keeping the product in the packaging material during thawing prevents dehydration and drip loss. Raw materials are often thawed or tempered over a 2- to 3-day period in a cooler held at 0 to 2.8°C (32 to 37°F) until the product temperature reaches -3.3 to -2.2°C (26 to 28°F). Boxed product without metal staples or banding can be thawed more quickly by conveying through a microwave tunnel followed by holding approximately 8 h under refrigeration to allow for external-to internal temperature equilibration prior to processing. Refreezing previously frozen product causes loss of proteins, flavor and juiciness, and excessive drip. It also poses some risk for subsequent microbial growth and increases product deterioration and is not recommended (Alan, 2001).

Raw materials should be dated and coded upon receipt, tracked through to final product form, and temperature/processing records should be kept with a designated lot number. Careful monitoring should be performed and raw products rotated through processing on a "first in, first out" basis (Alan, 2001).

Large surface area, release of cellular fluids and heat generated during mechanical deboning enhance bacterial count and growth (Kumar et al., 1986).

As reviewed by European Union (1997) that The quality of mechanically deboned meat (MDM), its good technological characteristics and comparatively low cost make the product a profitable and useful raw material. However, it has to be regarded as a potential health risk. Both red meat and poultry meat can be a source of pathogens like *Salmonella spp., Campylobacter spp*., enterohaemorrhagic *E. coli* like *E. coli 0157:H7, Listeria monocytogenes, Yersinia enterocolitica, Staphylococcus aureus* etc. as well as spoilage

bacteria such as *Pseudomonas* responsible for the development of rancidity. Therefore it is essential that all sanitary and hygienic measures concerning the raw material to be used for the production of MDM are absolutely restricted and controlled. Meat quality can be judged very well by the quality of microbes. Generally it can be said that if the number of bacteria on the surface of meat exceeds 1×10^8 cfu/g, the meat is unfit for human consumption. If the microbe count is 1×10^7 cfu/g the meat is of poor quality. Even that kind of raw material should not be used as raw material for MDM.

By far, the most important factor affecting psychotropic bacterial growth and hence, the shelf-life of fresh poultry is holding temperature. Pooni and Mead (1984) reported that poultry products may be subjected to variations in holding temperature during processing, storage, distribution, and retail sale. Ayres et al. (1950) evaluated the effect of storage temperature on the shelf-life of fresh poultry. The authors reported that the average shelf-life for commercially eviscerated fresh cut-up carcasses was 2–3, 6–8, and 15–18 days, respectively, when held at storage temperatures of 10.6, 4.4, and 0°C. Barnes (1976) demonstrated that turkey carcasses that were stored at -2, 0, 2, and 50°C, developed off-odors in 38, 22.6, 13.9, and 7.2 days, respectively. Daud et al. (1978) reported that broiler carcasses maintained under optimal conditions should have a shelf-life of 7 days when stored at 5°C. The rate of spoilage is twice as fast at 10°C and three times as fast at 15°C, than for carcasses stored at 5°C (Daud et al. 1978). Hence, as storage temperatures were reduced, the shelf-life of carcasses in these studies was extended.

Moreover, Baker et al. (1956) reported that the temperature and time of storage are related to shelf-life because increases in aerobic bacterial counts on ready-to-cook broiler carcasses, stored more than 7 days at 1.7 and 7.2°C, were much greater than increases in bacterial counts for corresponding carcasses stored for shorter periods of time. This study confirmed that carcasses will eventually spoil if held long enough, even if held under appropriate refrigeration and that significantly longer shelf-life is obtained by holding carcasses at temperatures as low as possible.

The temperature at which fresh poultry is held is of great concern to the poultry industry because it is the most important factor that affects the growth of both spoilage and pathogenic bacteria. Olsen (1947) reported that, when considering the relationship of temperature to microbial life, two things must be considered: the holding temperature of the microorganism and the time the microorganism is exposed to that temperature. All living cells respond to variations in temperature in various ways and bacteria, because they are living cells, are no exception. Bacterial metabolism, physical appearance, or morphology may be altered and proliferation may be stimulated or retarded, depending upon the particular combination of temperature and time of exposure. All bacteria are only able to multiply within a defined range of temperatures. Olsen (1947) reported that within this range, there is a minimum growth temperature, below which growth ceases, an optimum growth-temperature, which is the most favorable for rapid growth, and a maximum growth temperature, above which growth ceases. Different species of bacteria may vary not only with regard to the temperature range within which they are able to multiply, but also in their minimum, optimum, and maximum growth temperatures Olsen (1947). The two criteria that are used to determine optimum growth conditions for a

bacterial species are generation time and maximum cell population (Greene and Jezeski 1954).Generation time indicates the speed of cell division; whereas, maximum cell population takes into account cell death as well as cell production.

The minimum, optimum, and maximum growth temperatures for psychrotrophic and mesophilic bacteria are listed in Table 1.1, Olsen (1947) placed bacteria, now considered psychrotrophic, in the psychrophilic category. (Muller, 1903; Zobell and Conn, 1940; and Ingraham, 1958) objected to the term "psychrophiles" because, while many bacteria responsible for spoilage are able to survive and multiply at low temperatures, the temperatures that are optimal for growth are well above freezing. Ayres et al., (1980) reported that the optimum temperature for replication of psychrophilic bacteria is between 5 and 15°C. Much earlier, Muller, (1903) reported that the psychrotrophic bacteria are a group of mesophiles that are able to multiply relatively slowly at a lower temperature range than most other bacteria.

Table.1.1 Minimum, optimum, and maximum growth temperature ranges for psychrophilic and Mesophilic bacteria.

	Maximum	Minimum	Optimum
Psychrophilic	-5 to 0°C	10 to 20 °C	25 to 30 °C
Mesophilic	10 to 25 °C	20 to 40 °C	40 to 45 °C

Source: Adapted from Olsen, (1947).

A more current perspective on bacterial groupings based on growth temperatures is presented in Table 1.2. Many species of bacteria cannot be placed into any single category because their temperature range is very broad (Ayres et al. 1980). Some species of bacteria, such as *Listeria monocytogenes,* are able to grow well at both refrigerator temperatures and warm temperatures. However, these bacteria represent the exception, rather than the rule, when considering separation of bacteria based on their minimum, optimum, and maximum growth temperatures.

Table.1.2 Minimum, optimum, and maximum growth temperature (°C).

	Minimum	Optimum	Maximum
Psychrophiles	≤0	5 to 15	± 20
Low temperature mesophile, psychrotrophic,psychroduric microorganisms	± 10 to +8	20 to 27	32 to 43
Non-fastidious high temperature mesophiles	± 8	35 to 43	43 to 45
Fastidious high temperature mesophiles	20 to 25	37	?

Source: Adapted from Ayres et al. (1980).

Studies conducted on the microbiological effects of storing chicken on ice have been somewhat conflicting. Lockhead and Landerkin (1935) observed that chicken carcasses that are suspended in a refrigerator at -1.1°C do not develop spoilage odors as soon as chickens held at the same temperature surrounded by ice or ice water. In contrast to these

results, Naden and Jackson (1953) reported that there are significant advantages to packing poultry on ice including: (1) fresh quality is maintained longer, (2) drying out is prevented, and (3) the carcasses are more attractive in the display case. Baker et al. (1956) determined that bacterial counts on ready-to-cook poultry, stored on ice for 9 days, were similar to those stored under refrigeration for 5 days at 1.7°C or 4 days at 7.2°C, indicating that storage on ice is more effective to extend shelf-life. However, others have observed that carcasses stored in crushed ice had the same shelf-life as those stored in mechanical refrigerators at -0.6°C (Spencer et al. 1954). It is interesting to note that among four separate studies, all three possible conclusions were reached (i.e., refrigeration is best, ice is best, and no difference). This may be due to the fact that different investigators used different parameters to judge spoilage, such as odor or slime production.

Initial bacterial load immediately after processing has also been shown to affect shelf-life. Brown (1957) demonstrated that an increase in the initial bacterial load results in a concomitant dramatic decrease in shelf-life. This effect is due to the fact that much less time is required for bacterial populations to reach numbers that are high enough to produce spoilage defects when bacteria are high in number initially.

Spencer et al. (1954) identified a number of factors that may affect shelf-life and reported that the scalder water temperature and chlorination of the chiller water were important. Under simulated commercial conditions, carcass halves scalded at 53.3°C had an average shelf-life of 1 day longer than carcass halves scalded at 60°C (both scalded for 40 s). Carcass halves scalded at 53.3°C and cooled for 2 h in ice water containing 10 ppm of residual chlorine had a shelf-life of 15.2 days, as compared to 12.8 days for control halves chilled with non-chlorinated water.

When fresh poultry is placed in a cold environment, conditions for replication of most species of bacteria are no longer optimal. Ayres et al. (1950) reported that, the total number of bacteria on poultry stored at 0°C decreased during the first few days of storage. These authors reported that this decrease was due to the following: (1) the unsuitability of the temperature for reproduction and survival of chromogenic bacteria (pigment producers) and mesophilic bacteria, and (2) insufficient time for psychrotrophic bacteria to begin the exponential phase of growth.

Psychrotrophic bacteria are able to grow at refrigeration temperatures and spoil foods; however, the rate at which these bacteria multiply is greatly reduced. Most species of mesophilic bacteria are unable to multiply at refrigeration temperatures below 5°C. (Russell et al. 1992; Olsen and Jezeski, 1963) reported that generation times for mesophiles and psychrotrophs do not increase proportionally when incubation temperatures are lowered progressively from their optimum growth temperature ranges. As the lower temperature limit for *E. coli* (a mesophile commonly found on broiler carcasses) replication is approached, not only is the doubling time of the bacterium much slower, but there also is a longer lag period before it begins to multiply (Barnes 1976). Barnes (1976) observed that the generation time of *E.coli* at -2, 1, 5, 10, 15, 20, 25, and 30°C was 0, 0, 0, 20, 6, 2.8, 1.4, and 0.6 h, respectively. Elliott and Michener (1965)

reported that when mesophilic bacteria are placed at storage temperatures below 0°C, the generation time may exceed 100 h.

At temperatures that are considered "mild temperature abuse" (around 10°C) the generation times of psychrotrophic bacteria are much shorter than mesophilic populations of bacteria (Firstenberg-Eden and Tricarico 1983). At approximately 18°C, however, the multiplication rate of psychrotrophic and mesophilic populations of bacteria is approximately equal. When storage temperatures exceed 18°C, mesophiles proliferate much more rapidly than psychrotrophic bacteria (Firstenberg-Eden and Tricarico 1983).
Identification of bacteria responsible for spoilage of fresh chicken and other muscle foods dates back to the late 19th century. Forster (1887) reported that most foods are exposed to saprophytic spoilage bacteria that are found in the air, soil, and water. The author mentioned that, when cold storage was to be used as a means of preserving foods, it was important to be able to predict the behavior of these saprophytes over a given range of temperature Forster (1887).

Glage (as reported by Ayres (1960)) was one of the first researchers to isolate spoilage bacteria from the surfaces of meat that had been stored at low temperature and high humidity. This author named these bacteria *Aromobakterien.* Glage observed a total of seven species of spoilage bacteria, one of which predominated. The author reported that these bacteria were oval to rod shaped with rounded ends and that they occurred occasionally in chains. Glage (as reported by Ayres (1960)) revealed that these *Aromobakterien* grew well at 2°C, but very slowly at 37°C, and their optimum growth temperature was 10 to 12°C.

In 1933, Haines (1933) determined that Glage's *Aromobakterien* were similar to isolates that produced slime on meat stored at refrigeration temperatures. Haines (1933) reported that, except for some members of the *Pseudomonas* group and a few *Proteus,* microorganisms found on lean meat stored at 0–4°C mostly belong to the *Achromobacter* group. Others observed that 95% of the bacteria found on fresh beef, immediately after processing that were capable of growth at 1°C, were *Achromobacter* and some *Pseudomonas* and *Micrococcus* (Empey and Vickery 1933). The authors found that during cold storage, populations of *Achromobacter* and *Pseudomonas* were able to increase, while populations of *Micrococcus* significantly decreased.

Various studies by Haines (1937), Empey and Scott (1939) and Lockhead and Landerkin (1935) indicated that species of *Achromobacter* were the predominant spoilage bacteria of fresh meat and poultry. However, Ayres et al., (1950) Kirsch et al., (1952) and Wolin et al. (1957) conducted studies which contradicted these earlier studies. These authors reported that species of *Pseudomonas* were more predominant than *Achromobacter.* These three groups of researchers attributed the discrepancy between their results and those of previous workers to changes in nomenclature used in the sixth edition of Breed et al. (1948) (*Bergey's Manual of Determinative Bacteriology)* from that adopted in the third edition Bergey (1930) that may have been used by Haines (1933), Empey and Scott (1939), and Lockhead and Landerkin (1935).

Brown and Weidemann (1958) reassessed the taxonomy of the 129 psychrotrophic meat spoilage bacteria that had been isolated by Empey and Scott (1939) and the authors concluded that most of these bacterial species were pseudomonads. Empey and Scott (1939) previously classified meat spoilage bacteria as *Pseudomonas* largely on the basis of the production of a water soluble green pigment. Brown and Weidemann (1958) determined that 21 of the strains that were originally classified as pseudomonads on the basis of pigment production, failed to produce any type of pigment. Ayres et al., (1950) using *Bergey's Manual 6th ed.* as their taxonomic guide, reported that bacterial isolates collected from spoiled, slimy carcasses were closely related to these species of *Pseudomonas: ochracea, geniculata, mephitica, putrefaciens, sinuosa, segnis, fragi, multistriata, pellucida, rathonis, desmolytica (um)* or *pictorum.*

These authors revealed that, because of changes in Bergey's Manual between the 3rd (Bergey (1930) and 6th ed., Breed et al. (1948) many bacterial species that were originally reported as belonging to the *Achromobacter* genus should be reclassified as members of the genus *Pseudomonas,* because they move by means of polar flagellation Ayres et al., (1950). Kirsch et al. (1952) in separate studies achieved the same results.

In 1950, Ayres et al. (1950) observed that *P. putrefaciens,* which is a common spoilage bacterium found on meat and poultry, has both lateral and polar flagella. The authors argued that this bacterium should not be placed into the genus *Pseudomonas. P. putrefaciens* is characterized by brownish colonies and is further differentiated from other pseudomonads by its highly proteolytic properties and hydrogen sulfide production.

Later, Halleck et al. (1957) determined that non-pigmented *Achromobacter-Pseudomonas* type bacteria made up approximately 85% of the total bacterial populations on fresh meats during the first two weeks of storage at 1.1 to 3.3°C and during the first week of storage on meat samples held at 4.4 to 6.7°C. These authors reported that *Pseudomonas fluorescens* constituted approximately 80% of the bacterial species on meat toward the end of the storage period; however, at the beginning of the storage period, *P.fluorescens* seldom exceeded 5% of the population on fresh meats (Halleck et al. 1957).

Barnes and Impey (1968) observed that the three genera of bacteria that were most commonly isolated and identified from spoiled chicken were *Pseudomonas, Acinetobacter,* and *P. putrefaciens.* The predominant pseudomonads on spoiled poultry are divided into two related categories: fluorescent or pigmented strains and non-pigmented strains.

Since the time when Barnes and Impey (1968) reported that *P. putrefaciens* was determined to be a primary spoilage bacterium of fresh poultry, this bacterium has been reclassified. *P. putrefaciens* was originally classified as *Alteromonas putrefaciens* (MacDonell and Colwell 1985). It was then changed from *Alteromonas* to *Achromobacter. Achromobacter* was transferred to the genus *Pseudomonas* in the 7th edition of Bergey's Manual Breed et al. (1957). MacDonell and Colwell (1985) placed *P. putrefaciens* into a new genus and named it *Shewanella putrefaciens.* Thornley (1960)

mentioned that *Acinetobacter* was also part of the genus *Achromobacter* until the mid-1960s.

More recently, Russell et al. (1995) conducted a study to identify the bacteria responsible for the production of off-odors on spoiled broiler chicken carcasses, to characterize the odors they produce, and to survey carcasses produced in different areas of the U. S to determine how consistently these spoilage organisms were found. The authors reported that the bacteria isolated from spoiled carcasses that consistently produced off-odors in chicken skin medium, regardless of the geographical location from which the chickens were obtained, were *S. putrefaciens* A, B, and D, and *Pseudomonas* (*fluorescens* A, B, and D, and *P. fragi*). These bacteria produced off-odors which resembled "sulfur," "dishrag," "ammonia," "wet dog," "skunk," "dirty socks," "rancid fish," "unspecified bad odor," or a sweet smell resembling "canned corn." However, odors produced by the spoilage bacteria were varied.

Odors most associated with spoiled poultry, such as "dishraggy" or "sulfurous" odors, were produced by the bacteria that were most consistently isolated, such as *S. putrefaciens* and the pseudomonads Russell et al. (1995).

Bacterial genera that are responsible for off-odors and slime on spoiled chicken are not nearly as prevalent if storage temperature increased. Populations of spoilage bacteria on chickens held at various temperatures, as reported by Barnes and Thornley (1966), are listed in Table 1.3. Immediately after processing, the predominant bacterial species on broiler carcasses are mesophilic, such as micrococci, Gram positive rods, and flavobacteria. However, if carcasses are held at temperature abuse temperatures, such as 10°C, *Acinetobacter, Pseudomonads*, and *Enterobacteriaceae* are able to multiply. For carcasses held at 15°C, *Acinetobacter* and *Enterobacteriaceae*, whose optimum growth temperatures are higher than those of the *Pseudomonads*, predominate (Barnes and Thornley 1966).

Table.1.3 The spoilage flora of eviscerated chickens initially and after storage at 1, 10, and 15°C until spoiled.

	Number of spoiled			
	Initial	1°C	10 °C	15 °C
Total strains	58	40	80	69
Gram positive rods	14	0	4	6
Enterobacteriaceae (lactose pos.)	8	0	3	10
Enterobacteriaceae (lactose neg.)	0	3	12	17
Micrococci	50	0	4	0
Streptococci	0	0	6	8
Flavobacteria	14	0	0	0
Aeromonas	0	0	4	6
Acinetobacter	7	7	26	34
Pigmented *Pseudomonas*	2	51	21	9
Non-pigmented *pseudomonas*	0	20	12	2
Pseudomonas putrefaciens	0	19	4	4
Unidentified	5	0	4	4

Source: Adapted from Barnes and Thornley (1966).

High numbers (10^5 cfu/cm^2) of psychrotrophic spoilage bacteria are required on poultry surfaces before off-flavors, off-odors, and appearance defects are able to be detected organoleptically. Lockhead and Landerkin (1935) were not able to detect off-odors caused by bacteria on uneviscerated broiler carcasses until bacterial concentrations reached 2.5 x 10^6 to 1 x 10^8 cfu/cm^2. Other researchers8 observed that odor and slime were not present until bacteria exceeded 1 x 10^8 cfu/cm^2. Elliott and Michener (1961) were able to detect odor when bacterial concentrations reached 1.6 x10^5 cfu/cm^2. The authors reported that higher numbers of bacteria (3.2 x 10^7 to 1 x 10^9 cfu/cm^2) were required to produce slime.

In another study on sliced beef, Kraft and Ayres (1952) found that off-odor was able to be detected when bacterial concentrations on the surface reached 2 x10^6 cfu/cm^2. The authors reported that incipient spoilage was indicated by the onset of off-odor at 10^6 cfu/cm^2; however, off-odors were more easily recognized when bacterial counts on the surface of meat reached 10^7 cfu/cm^2. More recently, Dainty and Mackey (1992) determined that proteolysis and slime production under aerobic conditions begins when bacterial numbers reach 10^7 to 10^8 cfu/g.

Under refrigeration (<5°C), psychrotrophic bacterial populations are able to multiply on broiler carcasses and produce spoilage defects; however, the mesophilic bacteria that initially predominate on the carcass remain the same or decrease in number (Barnes and Thornley 1966; and Russell et al. 1992). This phenomenon may be explained by examining some of the metabolic changes that occur in these groups of bacteria as they are exposed to refrigerator temperatures.

Culturing psychrotrophic bacteria under refrigeration has been demonstrated to increase their ability to grow at cold temperatures. Hess (1934) found that culturing psychrotrophs

(*P. fluorescens*) at 5°C produced strains that were more active at 0 and -3°C than other strains of *P. fluorescens* that had been incubated at 20°C. Chistyakov and Noskova (1955) were able to successfully adapt a variety of bacterial strains to environmental temperatures as low as -2°C by growing them at 0 to -8°C for 2 years. Ingraham and Bailey (1959) and Wells et al. (1963) suggested that this "process of adaptation" may be the result of cellular reorganization. This "adaptation" is also important for understanding how bacteria react to very low temperatures, such as freezing.

MacFadyen and Rowland (1902) summarized the unique ability of bacteria to survive freezing and thawing by stating the following: "It is difficult to form a conception of living matter under this new condition, which is neither life nor death, or to select a term which will accurately describe it. It is a new and hitherto unobtained state of living matter—a veritable condition of suspended animation."

The effects of freezing on the ability of bacteria to survive and reproduce have been studied as far back as the late 19th century. Burden-Sanderson (1871) reported that all bacteria are not destroyed by freezing. Bacteria isolated from fish were found to be able to multiply at freezing temperatures, such as 0°C (Forster1887). Fischer (1888) isolated 14 different bacterial species that were able to proliferate at 0°C. Another researcher isolated 36 different bacterial species that multiplied at 0°C from sausage and fish intestines (Muller 1903). Microorganisms that are capable of multiplying at 0°C are widely distributed; however, their growth characteristics are described as being similar at 0°C as at higher temperatures; however, the rate of growth is decreased (Muller 1903). Bedford (1933) determined that strains of *Achromobacter* were able to proliferate at temperatures as low as -7.5°C. Others revealed that -10°C is the lowest temperature at which bacteria are able to multiply (Berry and Magoon, 1934).

Berry and Magoon (1934) observed that under specific conditions, moderately cold storage temperatures (-2 to -4°C) may negatively impact bacteria to a greater degree than storage at -20°C. This may be explained by the fact that when cells are frozen rapidly, both intra and extracellular fluid freezes. However, when cells are frozen at a slow rate, an intra- and extracellular osmotic gradient occurs due to freeze concentration. This may result in cellular disruption (Mazur 1984). After freezing various species of bacteria at -190°C for 6 months, MacFadyen and Rowland (1902) found no difference in the vitality of the microorganisms. The normal functions of life cease at temperatures as low as -190°C. The authors hypothesized that intracellular metabolism must also cease as a result of withdrawal of heat and moisture (MacFadyen and Rowland, 1902).

Although bacterial species are in "suspended animation" when frozen, a fraction of the microbial population is killed or sublethally injured during the freezing process (Elliott and Michener, 1960). During frozen storage, the individual cells that survive on meat surfaces can range from 1 to 100%, but averages 50%, depending on the type of food (Elliott and Michener, 1960). Straka and Stokes (1959) observed that some nutrients that are required by bacteria for growth are rendered inaccessible by the freezing process, thereby preventing bacterial multiplication.

Studies have demonstrated that freezing and thawing may enhance the growth rate of surviving bacteria. Hartsell (1951) reported that *E. coli* that are able to survive the freezing and thawing process were able to grow more rapidly than *E. coli* that had not been previously frozen. One reason why the growth of bacteria that have survived freezing may be accelerated is that tissue damage due to freezing may result in nutrient release and increased moisture, such that the tissue becomes a better growth medium (Sair and Cook, 1938).

Because the rate of bacterial growth on meat surfaces is reduced by freezing, it would seem that freezing chicken would be an acceptable means of increasing its shelf-life. However, consumers presently have an aversion to buying frozen poultry. This may be attributed to the fact that in the early to mid-1900s, poultry was often held until it was about to spoil and was then frozen. People who purchased frozen poultry products found that when the product was thawed, it was of inferior quality and spoiled rapidly. Pennington (1910) expressed concern about this when she remarked, "frozen poultry has too frequently been synonymous with carcasses held until they are just about spoiled and then frozen. Hence the consumer gets a low grade product and the reputation of the poultry business suffers." Thus, frozen poultry has not been widely accepted in the U. S.

The effect of freezing on the shelf-life of fresh poultry has been extensively studied. Spencer et al. (1955) reported that carcasses that are frozen and held for two months and then thawed had the same shelf-life as unfrozen controls. Similar observations were made by Spencer et al. (1961) and Newell et al. (1948) who observed no major increases or decreases in shelf-life of chicken carcasses as a result of freezing and thawing. Elliot and Straka (1964) reported that chicken meat, which was frozen for 168 days at -18°C and subsequently thawed, spoiled at the same rate as unfrozen controls.

The processing steps to which birds are subjected are designed to produce wholesome and safe final products. Thus, as birds proceed through processing, there is substantial decrease in overall bacterial load. Removal of feathers, feet, heads, and viscera serve to also remove the bulk of the bacterial load. However, given the nature of modern poultry processing, not all bacteria are eliminated. The remaining bacteria can be transferred among carcasses. The extent to which bacteria are removed from carcasses or transferred among carcasses is a function of the specific processing steps and operational conditions. Although live haul (transportation of live birds from production farms to the processing plant) may not be considered a plant process, cross contamination can be attributed to this step in the process. Transportation coops are often contaminated with *Salmonella* even after washing (Rigby et al., 1980). *Salmonella* from coops can be transferred to birds held in them and to adjacent coops (Wakefield 1999). *Salmonella*-contaminated coops lead to external contamination (feet, feathers, skin) and to cecal and crop carriage (Wakefield 1999; Rigby and Pettit 1980). *Salmonella* originating from live haul equipment can contribute significantly to subsequent cross contamination among carcasses during processing (Wakefield 1999). Factors affecting this cross contamination include close crowding, coprophagy, weather, other stressors, and time the birds are off feed, which is an additive effect of feed withdrawal at the farm, transportation distance, and time birds are kept in the holding yard. Because these factors can affect the spread of *Salmonella*

during the transportation phase, they subsequently affect the level of pathogens entering the processing plant.

The process of scalding the carcass is used prior to feather removal. This process subjects the carcass to immersion in hot water, facilitating the opening of the feather pores so that the feathers may be removed more effectively. There are two types of scalding, hard and soft. A hard scald, in which the carcass is immersed in water that is greater than 55°C, removes the cuticle (or epidermis) of the skin. If the carcass is immersed in water that is ≤55°C the cuticle is not removed and the carcass is considered to be soft scalded. Scalding tends to partially remove dirt, fecal material, and other contaminants found in the feathers. However, these contaminants may be spread to other carcasses through scalder water (Mulder et al., 1978). Most plants use a countercurrent scalder, in which water for the continuous overflow is fed from the cleanest end of the scalder (that ends nearest the picking machines), toward the dirtiest end. This helps to reduce the amount of cross contamination. Tests on scald water have shown that *C. perfringens* and *S. aureus* can be isolated. However, *Salmonella* spp. And *Campylobacter* spp. are usually not isolated (National Advisory Committee on Microbiological Criteria for Foods 1997). In general, scalding has little effect on the microbiological quality and safety of raw poultry products in the retail market (Bailey et al., 1987).

While defeathering of carcasses reduces the overall bacterial load via removal of the feather, the process is of major concern to the poultry industry because the modern mechanical process of feather removal can be a major contributor to cross contamination (Mulder et al., 1978).

This process usually leads to an increase in the number of non-psychotrophic organisms on the individual carcasses (National Advisory Committee on Microbiological Criteria for Foods 1997). Defeathering has also been attributed to an increase particularly in *S. aureus* because the organism becomes embedded in the cracks of the rubber fingers. It has also been attributed to the cross contamination of carcasses by *Salmonella* spp., *Campylobacter* spp., and *E. coli.* This may be due to embedding of these microorganisms in the feather follicles after the feather is removed and before the follicle can reduce in size (National Advisory Committee on Microbiological Criteria for Foods 1997).

Removal of the carcass is another area of special concern in the area of cross contamination. If the intestines of the bird are cut, fecal contamination can occur. This is especially important because of the enteric pathogens that the intestines can harbor. These pathogens can contaminate machinery and workers. Plant personnel that handle carcasses must wash their hands frequently to decrease the possibility of transferring fecal-borne pathogens among carcasses. Continuous flow of chlorinated water over machinery that frequently becomes contaminated with feces and other GI tract contents is used to prevent spread of fecal-borne pathogens via equipment contact (Alan, 2001).

An emerging area of interest concerning cross contamination of commercial broiler carcasses is crop removal. While rupture of the intestinal tract, especially the ceca, has been the major focus of cross-contamination issues, crop removal is also a major

problem. In the plant, crops are more likely than the ceca to rupture by up to 86-fold (Hargis et al., 1995). This problem is further exacerbated by findings that both *Campylobacter* and *Salmonella* can be more readily extracted from the crops of market-age broilers than from the ceca. (Byrd et al., 1998) reported that of 359 birds sampled, 286 (62.4%) harbored *Campylobacter* in the crop, whereas only 9 of 240 (3.8%) of birds sampled harbored *Campylobacter* in the ceca. In a similar study by (Hargis et al., 1995), 286 of 550 (52%) broilers studied were positive for *Salmonella* in the crop, while only 73 of 500 (14.6%) were positive for *Salmonella* in the ceca. These findings show that care must be taken in crop removal during processing to reduce cross contamination of these important pathogens.

Freezing equipment has been a frequent source of this pathogen. *L. monocytogenes* can survive below -1.5°C and thus can persist in freezers (Ryser and Marth 1999). Other environmental sources of *L. monocytogenes* include water, air, personnel, and all product contact surfaces.

Two overall strategies are used by poultry processors to control pathogens in the plant: GMPs and HACCP. The processing plant must provide hygienic environmental and operating conditions (i.e., follow GMPs) such that products are produced in a safe, sanitary, and wholesome manner. As referred to earlier, processing plants must also develop and implement HACCP to control pathogenic bacteria and other food safety hazards. While HACCP must be a separate program, it must also be based on solid GMPs. Therefore, these two pathogen control strategies are interrelated. As part of GMP programs or HACCP, there are specific antimicrobial treatments that can be employed to improve the microbiological safety of poultry meat.

The processing plant should be located, constructed, and maintained in accordance with sound sanitary design and hygienic principles. Because pests can be vectors of food-borne pathogens, premises should minimize pest (i.e., rodents, insects, birds) harborages, such as areas of standing water, trees and shrubbery in close proximity to processing plant, bird nesting sites associated with the building, waste collection sites, etc. For this reason, the processing plant site should be well drained, landscaped with minimal shrubbery, and designed to facilitate waste management.

As overall considerations, facilities should be designed to facilitate product flow and should provide for separation of operations where appropriate. Product should flow from the area of highest microbial load to the area of lowest microbial load (e.g., raw to cooked), and not “back track.” Separation of areas and of employee traffic patterns are an important consideration in preventing microorganisms from moving throughout the plant. Overall layout and design of the plant should also provide for adequate ventilation, lighting, and space for equipment and storage. Without these provisions, maintaining sanitary conditions in the plant will be more difficult.

Walls, doors, ceilings, and floors represent the interior surfaces in the plant, and, therefore, need special attention. The surfaces should be easily cleaned and sanitized,

impervious to water, and minimize niches for collection or entry of microorganisms and pests.

Walls should be solid, sealed for waterproofing, and be free of windows. In terms of the latter, windows are not necessary in well-ventilated and lighted plants. If windows are present, the glass should be unbreakable, the sill should be sloped to prevent collection of debris (bacteria), and windows should not be able to be opened by employees unless required by fire regulations. Doors represent another type of opening in a wall. If doors are present, it is likely that they are there to ensure separation of different areas of the plant; therefore, they should be kept closed during plant operations.

In poultry and other food processing, water has always been a major premises/facility related issue. Water is used extensively in processing and cleaning operations, therefore the plant should have access to a good water source and be able to maintain water quality in the plant. Water system design and plumbing will be important in preventing water contamination in the plant. The water system should be designed to keep potable water protected from wastewater and sewage, and plumbing/maintenance activities should not compromise this protection. In recent years, other water issues, such as availability, adequacy of palatability standards, conservation, reuse, and wastewater treatment, have also emerged. These issues will also impact the plant's water programs.

1.2.3. Microbial Growth of Chicken Skin.

Specific species of bacteria cease to proliferate at a particular temperature as the temperature of their environment is lowered because, as environmental temperature decreases, so does the absorption of nutrients by bacterial cells (Wells et al. 1963). Moreover, as environmental temperature decreases, bacteria begin to increase the content of lipids in their cell membranes.

Graughran (as reported by Wells et al. 1963) observed that as primarily mesophilic species of bacteria are exposed to progressively lower temperatures, the quantity of cellular lipids increases and the degree to which these lipids are saturated increases. As lipids in the cell membrane increase, the absorption of nutrients is inhibited. Eklund (1962) reported that *Brevibacterium linens* contained 7.2% fat when incubated at 25°C, where it grew well; however, at 4°C, it produced 16.7% fat and multiplied poorly. In addition, the author determined that bacterial cells produce more fat at 4°C than at 9.4 or 22°C Eklund (1962). Interestingly, two typical psychrotrophic bacteria species exhibited no such temperature-induced differences when grown at 4°C (Wells et al. 1963).

Research has demonstrated that the amount of lipase produced by psychrotrophic bacteria increases as a result of exposure of the bacteria to cold temperatures. Nashif and Nelson (1953) observed that lipase production by *P. fragi* was high when bacteria were incubated at temperatures between 8 and 15°C; however, production of lipase was almost completely absent at temperatures of 30°C or higher. Other researchers reported that lipase production by *P. fluorescens* was the same when this organism was cultured at 5 or

20°C; however, very little lipase was produced when the bacteria were exposed to 30°C (Alford and Elliott 1960).

Changes in proteolytic activity of bacteria at low temperatures have also been studied. Peterson and Gunderson (1960) reported that production of proteolytic enzymes by *P. fluorescens* was higher when this bacterium was cultured at lower temperatures. Moreover, De Castro et al. (1988) reported that by measuring the production of free amino acids as a result of the production of aminopeptidases and subsequent breakdown of protein, the progression of spoilage on meat surfaces may be evaluated and the quality of the meat may be determined.

Mesophilic species of bacteria decrease utilization of carbohydrates as environmental temperature is reduced, while psychrotrophic species are able to continue to utilize carbohydrates as an energy source. Brown (1957), Ingraham and Bailey (1959), and Sultzer (1961) reported that at reduced incubation temperatures, carbohydrate oxidation rates of psychrotrophic bacteria decrease to a lesser degree than oxidation rates of mesophilic bacteria. Temperature coefficient differences between mesophiles and psychrotrophs have been determined for the following catabolic processes: glucose oxidation, acetate oxidation, and formate oxidation by resting cells (Ingraham and Bailey, 1959). Maintenance of a high rate of carbohydrate metabolism when psychrotrophs are exposed to low temperatures may be one explanation for their ability to maintain their metabolic processes under adverse temperature conditions.

1.2.4. Chemical Composition of Mechanically Deboned Meat.

With the introduction and increasing use of mechanically separated meats in the elaboration of sausages and other industrialized products, many questions arose regarding their nutritional value for human consumption. Studies on the most diversified characteristics of these meats were carried out on contents and quality of proteins, lipids and minerals, bone content, lipid and pigment stability, pathogenic and spoilage bacteria, among others, proved that consumption of MDM does not jeopardize human health and can prevent the wasting of large amounts of minerals, lipids and animal protein. Next, some aspects of the chemical and nutritional composition of MDMs that can influence their use for the elaboration of sausages will be discussed.

When poultry meat is mechanically separated, considerable shearing action causes marked cellular disruption. The extent of the cellular damage is largely affected by the screen size utilized. Schnell et al. (1974) observed that small screen sizes used in the separator will reduce the size of the myofibrils. Breaks were noted at the Z or M bands. Also, the bone marrow is released from broken bones during the separation process, thereby contributing increased lipids and heme components into the separated meat. Lipid and heme fractions further dilute the amount of protein in separated meat.

Proximate composition of various sources of mechanically separated poultry meat is considerable variation in the composition. Factors influencing the composition include bone-to-meat ratio, age of the bird, skin content, cutting methods, deboner settings, and

species. Younger birds generally will have more heme and lipid components from the bone marrow influencing the proximate composition. Skin content may greatly increase the fat content of the resulting separated meat while the collagen from the skin is largely found in the bone residue (Satterlee et al. 1971). However, if cooked carcasses or parts are mechanically separated, the collagen is likely gelatinized thereby increasing the collagen content of the separated meat.

Deboner settings can affect the yields and the proximate composition substantially. If the settings are set for high yields, the fat and ash content in the resultant mechanically separated meat may be largely increased. High settings may also increase the temperature resulting in protein denaturation, which may ultimately affect functionality. Protein quality of mechanically separated poultry meat has received considerable emphasis. Several scientists have observed that the protein quality of mechanically separated poultry is comparable to that found from hand-deboned sources (MacNeil et al., 1978; Babji et al., 1980; Essary and Ritchey et al., 1968; Hsu et al., 1978).

One concern has been the fatty acid and cholesterol content of mechanically separated poultry meat. Moerck and Ball (1973) observed that the bone marrow from chicken broilers contained a higher percentage of phospholipids and cholesterol than that found in other broiler meat. However, the fatty acid composition of chicken bone marrow and mechanically separated poultry meat was quite similar to that from hand-deboned meat sources.

With the advent of mechanical separation of poultry meat, the issue of possible bone content came under close scrutiny. Bone particles from hand-deboned and mechanically separated poultry meat have been characterized (Froning, 1979). Bone particles isolated from hand deboned sources were actually somewhat larger than that obtained from mechanically separated meat. Any bone particles found in mechanically separated poultry meat were indicated to be of a “powdery” form presenting no hazard to the consumer.
Calcium content in terms of bone equivalents is closely monitored today. Several minerals in mechanically separated poultry have been investigated as they may affect health and safety. Murphy et al. (1979) analyzed for several minerals including arsenic, fluoride, cadium, strontium 90, selenium, iron, nickel, copper, lead, and zinc. None of these were indicated to be a health hazard in mechanically separated poultry meat. This may, in part, be due to the exclusion of body parts (e.g., kidneys) from the production flow as has been previously discussed.

High contents of collagen in any meat can negatively influence its technological and nutritional characteristics, since collagen is a protein with inferior functionality and low nutritional value because of its poor balance of amino acids. However, as collagen is strongly attached to the bones, very little of it goes through the ridges of the deboning machines and, consequently, little collagen is incorporated to MDMs (Field, 1988).

A potential aspect of concern by consumers is the cholesterol content, which is higher in MDMs than in manually separated meats as a result of the inclusion of the bone marrow material, which has high cholesterol concentrations.

Ang and Hamm (1982) analyzed cholesterol contents in manually separated chicken meats from the same cuts. Amongst the MDMs, they compared the neck with and without skin and the whole entire back. The results showed that there was lower cholesterol content in the manually deboned meats than in the respective MDMs Table 1.4.

The same authors analyzed cholesterol contents in bone marrow (1992mg/100g) and in the fat of the back (312mg/100g), having indicated that the cholesterol comes both from the marrow and the fat.

Table.1.4 Cholesterol contents of manually and mechanically deboned chicken meats[1].

	Cholesterol (mg/100g sample)	
	Mechanical deboning	Manual deboning
Neck without skin	94	75
Neck with skin	109	94
Back	95	81

[1]Ang and Hamm (1982)

The cholesterol contents in breast and thighs of hens were analyzed by Jantawat and Dawson (1980), who reported that meats from manually deboned hens also presented lower cholesterol contents (43 and 70mg cholesterol /100g sample of breast and thigh) than their respective MDMs (73 and 110mg cholesterol /100g sample of breast and thigh). That is, cholesterol levels in MDMs, although higher than those of manually deboned meats from the same cuts, are not very high in comparison to the contents of bone marrow or fat.

Nonetheless Al-Najdawi and Abdullah (2002) evaluated collagen contents in manually and mechanically deboned meats of whole and skinned hens, and observed higher contents of collagen in the MDMs (3.45 and 3.00% for whole and skinned carcasses, respectively) in comparison to the meats of manually deboned hens (1.60 and 0.85% for whole and skinned carcasses, respectively).

Mechanical deboning of poultry affects the proximate composition of resulting meat. Considerable amounts of lipids present in the raw material are incorporated in the MDMs, diluting protein and increasing the lipid contents of the deboned tissues. These lipids include those present in the bone marrow, the subcutaneous fat, the skin and the abdominal fat, excluding the fat of the viscera removed during the slaughtering process. Moreover, the proximate composition can vary according to the settings and type of machine used for the mechanical separation (Froning, 1981).

Table 1.5 presents the proximate compositions of MDMs from several sources. The lipid content of the MDM is really higher and the protein contents are lower in MDMs in comparison to fillets, where as the protein content is higher, and the lipid content is lower and steadier for MDM of hens than MDM of chicken. It could expect the opposite, since older animals have more fat deposits. However, MDMs of hens are generally extracted

from the whole carcasses, and the higher the meat content in the raw material, the lower the amount of fat in the MDM.

Table.1.5 Proximate composition of manually deboned hen meat and MDMs of hens and chicken (fresh weight basis).

Raw Material	Protein %	Fat%	Moisture%	Ash%	References
Hen MDM	15.5	19.0	63.8	1.3	Hamm and Young (1983)
Hen MDM	15.4	20.4	62.5	1.2	Mott et al. (1982)
Hen MDM	14.2	26.2	60.1	n.a.	Grunden et al. (1972)
Hen MDM	13.9	18.3	65.1	n.a.	Froning (1981)
Chicken back with skin MDM	8.5	30.4	60.0	0.6	Pollonio (1994)
Chicken back without skin MDM	12.4	15.0	70.1	1.1	Pollonio (1994)
Chicken back and neck MDM	9.3	27.2	63.4	n.a.	Grunden et al. (1972)
Chicken back and neck MDM	13.4	14.4	72.2	n.a.	Essary (1979)
Hen breast fillets	23.1	3.4	72.1	1.2	Kondaiah and Panda (1987)
Hen thigh fillets	19.5	8.8	69.9	1.0	Kondaiah and Panda (1987)

Aurelia et al. (2003) compared between different samples of MDMs as shown in Table (1.6). This presents the global chemical composition of MDM. The analytical data indicate significant according to their provenience. Meats originating from the USA are characterized by higher protein content; lower lipid content as well as lower ash content as comparing to the Slovak meat samples. Also Table 1.6 Include chemical composition for MDM from local source.

Table.1.6 Chemical composition of mechanically deboned meat poultry meat.

Sample provenience	Humidity, %	Proteins, %	Lipids, %	Ash, %	pH
USA	70.56	16.34	15.07	0.955	6.61
USA	68.6	16.57	14.33	0.68	6.65
Slovenia	68.46	14.78	15.67	0.95	6.18

Protein and fat were addressed based on the standard of identity of mechanically separated poultry. Mechanically separated poultry cannot contain greater than 25% fat and not less than 14% protein for it to be deemed as mechanically separated poultry **(**PS1-1031**)**. While mechanically separated meat may contain slightly higher amounts of collagen than hand-deboned poultry, protein quality is not greatly affected. Moreover, Froning (1981) reported that protein efficiency ratios of mechanically separated poultry

were comparable to that of casein, a high quality protein. Mechanically separated poultry that is labeled as such must have a minimum protein efficiency ratio of 2.5. Product derived from the newer "advanced recovery meat/bone separating systems" which can be labeled as "meat" (chicken, turkey, beef, etc.) also has particular protein quality standards as defined by a minimum protein efficiency ratio.

Amongst the fatty acids profile of meats, the high levels of unsaturated fatty acids are usually associated to poultry. Unsaturated fatty acids are regarded as beneficial to human health. However, they are more prone to oxidation, causing losses in sensorial quality of meats during the storage.

Several authors evaluated the profile of fatty acids in MDMs with the purpose of comparing it to those found in manually deboned meats, according to the incorporation of the lipids of the bone marrow and the skin of the poultry.

MDMs have been widely used in the emulsified sausages production. Therefore, the emulsification capacity is an important attribute of these meats. The determination of this functional property generally follows the methodology described by Swift et al. (1961), based in the amount of oil that a meat is able to emulsify. The values are generally expressed in ml of oil per 2.5g of meat. Variations in the emulsifying capacity of a MDM may result from its composition, quality and amount of proteins, protein denaturation, freezing and storage. The content of fat in MDM is the main factor that affects its capacity of emulsification.

Froning et al. (1973) studied the influence of the skin content of MDM on several functional properties and observed that as there was an increase in the addition of skin to the carcasses (0; 16.8; 28.9 and 38.9%) before the mechanical separation, there was a proportional increase in the content of fat in MDM (15.3; 24.6; 29.8 and 33.6%), and a reduction in the emulsifying capacity (182, 148, 133, and 127 ml oil/ 2.5g MDM). The increase in the skin level did not cause a significant increase in the collagen content of the MDMs.

Regarding the proteins quality, McMahon and Dawson, quoted by Froning (1981) determined the soluble protein content in manually and mechanically deboned turkey meat. The percentage of these proteins was lower in the MDM than in the manually deboned meat. The emulsifying capacity was higher in the manually deboned meat but, on the other hand, the MDM presented greater water holding capacity.

The most beneficial functionality properties of raw poultry muscle tissues, MDP, and MDT are their ability to retain fluids (water-holding capacity; WHC) and bind (cohesiveness) meat pieces such that the finished product has a whole-muscle texture or that of a solid, homogenous emulsified product matrix. Critical to these properties is the amount of total myofibrillar protein available for binding and WHC, the ratio of moisture to total protein (M:P ratio), and the amount of myofibrillar protein in relation to sarcoplasmic and connective tissue proteins. Lean poultry muscle tissue contains approximately 19 to 23% proteins while MDP or MDT without skin contains 14 to 16%

protein and with skin 11 to 12% protein. Raw materials with approximately 16% protein or higher would be typically classified as good binders for water retention and meat particle binding. However, the type (myofibrillar, sarcoplasmic, connective tissue), physicochemical condition (PSE or DFD), and ratio of the protein types primarily determines their functional properties.

1.2.5. Influence of Temperature and Time of Mechanically Deboned Meat and Chicken Skin on pH Changes.

Numerous research data have shown that freezing and frozen storage do not completely prevent all possible quality changes from occurring; reactions that lead to oxidative changes can proceed at low temperatures, although slowly. It is believed that one of the major environmental factors that results in increased loss of quality for most food is exposure to high temperatures. The higher the storage temperature the greater is the loss of food quality. A knowledge of the rate of food quality deterioration as a function of environmental conditions is necessary to make it possible to predict the extent of high quality food shelf life. Another important aspect of predicting food quality shelf life is determining the criteria for establishing what food quality attribute is to be measured and how much of that characteristic must be lost to establish an end of shelf life as perceived by the consumer. Shelf life here is to be considered as a function of environmental conditions and initial quality level. The amount of change in an initial quality level that can be tolerated before the product is judged to be significantly inferior should establish product shelf life.

Generally, MDMs present higher pH than manually deboned meats, in general as a result of the incorporation of red marrow, in which pH ranges from 6.8to 7.4 (Field, 1988).

According to Beraquet (2000), the pH of manually deboned meat lies between 5.8 and 5.9 for the breast and 6.2 and 6.3 for the thigh, whereas MDMs have values between 6.5 and 7.0. These high pH values favor the water holding capacity. On the other hand it contributed to increase in the bacterial load, as well as speeding up the spoilage process.

Addition of nitrite and erythorbate before storaging the MSML did not affect the final pH of the Mortadella. The elevated pH values of 6.68 for both treatments can be explained by the high content of mechanically separated meat (100% MSML) used in the products. Higher pH values in emulsified meat products improve the emulsification capacity of the myofibrillar proteins and fluid retention (Field, 1988).

This property is related to the weight loss and final quality of the product in which the MDM is used, as a result of the formulation, processing, storage, cooking and freezing. The MDM increases the WHC of the products, for it has a higher pH than the manually deboned meats. Calcium, magnesium, iron and copper decrease the WHC. The presence of the conjunctive tissue, in which the main protein is collagen, makes the WHC decrease when heated at temperatures of 60-65ºC, causing shrinking, deficient skinning, unstable emulsions, gel formations and wrinkling of the external skin of the emulsified products. Freezing decreases the WHC of the MDM, especially when done slowly (Field, 1988).

Two general methods have been accepted to predict food product shelf life. The most common procedure is to expose the food product to some selected single abuse condition and test the food two or three times during some specific period, generally by sensory methods, and then extrapolate the results to normal storage conditions. The other approach is to assume that certain principles of chemical kinetics apply with respect to temperature dependency of the food product deterioration, such as the Arrhenius relationship, and to utilize a more elaborate design, which is more expensive but is likely to provide better data (Labuza, 1985).

As presented by Labuza (1979) the quality loss of most food under storage conforms to the following general equation:

$$dA/dt=k\,(A)^{n}$$

where:
dA/dt is the rate of change in quantity of the quality attribute A with varying time t. A negative sign is used if the deterioration is a loss of A and a positive sign if it is for production of an undesirable end product:

A is the measured amount of the quality attribute at any time t.
t is the time.
k is the rate constant in appropriate units, which depends on the temperature and water activity.
n is a power factor called the order of the reaction which defines whether the rate is dependent on the amount of A present, and it is generally 0, 1, or 2.

Most shelf life data for change in a food quality characteristic, based on some chemical reaction or upon microbial growth, follows a zero-order(n=0) or first order (n=l) pattern. A straight line is obtained by using linear coordinates to plot variation in A versus time, if it is zero-order data, whereas semilogarithmic coordinates are needed to produce a linear plot for first-order data. For second-order data, a linear relationship is obtained by ploting 1/A versus time in linear coordinates. By integrating the above equation within any defined range of A with assumed order of the reaction the following equations are obtained:

Zero-order $A=A_o- k_z\,(t)$
First-order $\text{In } A=\ln A_o- k_1\,(t)$

where A is the attribute value at time t, k_z is the zero-order rate constant in amount/time unit, and k_1 is the first-order rate constant in time^{-1} units (Labuza, 1985).

Labuza (1980) has applied the Arrhenius relationship between temperature and the reaction rate constant to rates of chemical reactions in food systems.
The Arrhenius relation is mathematically represented as:

$$k=k_0 e^{(-E_a/RT)}$$

where k is the rate constant, k_0 is a preexponential constant, E_a is the activation energy in kilojoules per mole, R is the gas constant, and T is the absolute temperature (degrees Kelvin). The Arrhenius principle involves the temperature of the system.

A semi logarithmic plot of the rate constant k (on the log scale) versus 1/T gives a straight line. This type of relationship makes it easier for extrapolation and for obtaining predition of length of shelf life.

It has been observed that for a given amount of food quality deterioration and reaction order, the rate constant is inversely proportional to the time required for reaching some degree of quality loss (Kramer, 1974). A plot of the logarithm of the shelf life (log t_s) versus 1/T is a straight line. Also, if only a small range of temperature is considered it is possible to find that most of the food data yields a linear plot when log ts is plotted versus temperature T. The equation of this line would be:

$$t_s = t_0 e^{-bT}$$

where t_0 is the shelf life at T=0, t_s is the shelf life at T, and b=ln Q_{10}/10 (Labuza, 1985).

By taking the ratio of shelf life between any two temperatures 10 C apart, the Q_{10} of the reaction can be obtained, where:

$$Q_{10} = (t_s \text{ at } T)/ (t_s \text{ at } T+10 \text{ C})$$

In the determination of Q_{10} it is assumed that the equation $t_s=t_0e^{-bT}$ is valid, which has been shown to be true for small ranges of temperature; usually this is not the case for large ranges of temperature (Labuza, 1979).

Some investigators have established time temperature relationships that can be used for predicting the extent of shelf life of refrigerated or frozen muscle food, even though these methods are considered as only rough guidelines (Labuza, 1982; Bailey, 1986). By using these calculations Bailey (1986) estimated the shelf life of frozen pork at -18 C to be about 25 weeks. Similar data were presented by Labuza (1982).

Dawson and Stadelman (1960) reported that the shelf life of chicken breast at 0 C was 9 times that at 20 C. This is a Q_{10} of 3. Bacterial counts on uneviscerated and eviscerated chicken carcasses were used as criteria for shelf life computation (Barnes and Impey, 1975). The counts for both were very similar before storing. They reported that at 4 C the end of shelf life was six days, while at 10 C it was four days, which gives a Q_{10} of two. These two reports exemplify the variability in these indices for computing fresh poultry shelf life.

Sklan et al. (1983b) studied the effect of keeping temperature of 37 C, 4 C and -18 C on lipolysis and oxidative changes in fresh turkey breast and thigh muscle. They reported that lipolysis of phospholipids was observed at 37 C and at 4 C and to a small extent at -18 C, with little change in triacylglyceride levels. At a temperature of 4 C very little or

almost no change occurred in free fatty acids and conjugable oxidation products of turkey breast and thigh meat during the first three to five days. It has been suggested that any chemical change in meat product before a processing operation, greatly affects its stability to a subsequent operation (Jul, 1984; Pikul et al., 1985b). Thus, these observations are important in turkey meat processing if there is a need to hold the product at refrigeration temperatures for 3 to 5 days after packaging and before freezing the product.

Many factors related to the equipment can affect end product quality. For instance, yield is affected by the amount of pressure that is applied when pushing product through the sieve. However, when pressure is increased, the separation process can become slightly less efficient by allowing more bone, sinew, and other non-meat residues in the final product. Processors determine the optimum machine settings to achieve high yield and product quality. Maintenance of the equipment is another factor that affects product quality.

Maintaining sharp edges on cutting surfaces greatly influences the end product texture and consistency. Poor equipment maintenance can cause product to smear and become pasty in texture. Texture can also be altered by changing screen or sieve sizes. Large pore sizes in sieves result in a course textured product.

Product temperature is another factor that can alter end product quality. Most equipment can process meat that is chilled, but not frozen. One Midwest processor has modified the separation equipment so that they are able to process meat that is frozen. This is a tremendous advantage because the product has a superior texture according to Fig.1.1, longer shelf-life, and lower bacterial counts.

Fig.1.1 Example of product from a modified separation process that can be labeled as ground chicken.

The stability of MDM during storage at -12°C was studied by Aurelia et al. (2003), who reported that the stability of MDM is influenced by the oxidation reactions that degraded the lipids and proteins and deteriorate the aroma, texture and color. Also Aurelia et al. (2003) found that the MDM is rich in hemoglobin and myoglobin which in their oxidized form catalyze the oxidation poultry lipids that are rich in phospholipids having a high content of polyunsaturated fatty acids.

Table 1.7 showed that the TBA index represents a reliable indicator for estimating the quantity of peroxidated lipids in the MDM stored under freezing conditions. It measures the concentration of substances generated by lipids decomposing able to react with 2 thiobarbituric acid Aurelia et al. (2003).

Generally, the freezing temperatures (-12 …. -18°C) reduce the rate of proteolytic processes. After five months of storage at -12°C, the accumulation of proteolysis compounds and amino acids deamination and decarboxylation were situated at low level Aurelia et al. (2003).

Table.1.7 The influence of storage period at -12°C upon the titrable acidity, pH, and TBA index of the USA 'MDM.

INDEX	Storage period at -12°C				
	Initial	1 month later	2 month later	3 month later	5 month later
TBA	3.14	4.07	6.73	7.36	11.74
Titrable acidity	0.212	0.216	0.220	0.241	0.249
pH	6.61	6.55	6.55	6.48	6.33

Cholesterol oxidation products (oxysterols) have received considerable attention in recent years because of their biological activities associated with human diseases. The implications of adverse biological effects such as atherogenesis, cytotoxicity, mutagenesis, and carcinogenesis of oxysterols has been reviewed (Hwang, 1991; Kesava et al., 1996) It has been proven that oxysterols associated with lipid oxidation in meat arise from heating (Hwang, 1991; Linseisen J. and Wolfarm G., 1998), storage (Hwang, 1991), various stages of processing, and type of meat product (Higley et al., 1986).

Moreover, cholesterol rapidly undergoes oxidation in the presence of oxygen, light, metal ions, radiation and other compounds, which could generate free radicals (Munch et al., 2000; Kesava et al., 1996; Pie et al., 1990; Yan P. S. and White P. J, 1990)

Mott et al. (1982) compared the MDM of whole hens with the MDM of skinned hens, and found higher concentrations of unsaturated fatty acids in the first. On the other hand, according to Moerck and Ball (1974), the composition of fatty acids in the marrow and in the MDM of chicken was similar to that of the breast, thigh and skin. Jantawat and Dawson (1980) compared mechanically and manually deboned hen meats, and also found very close fatty acids profiles, as shown in Table 1.8.

Table.1.8 Fatty acids profile of MDMs of chicken and hens and in meat of hen breasts.

Fatty acids	MDM hens with skin[1]	MDM hens[2]	Light hen meat[3]		Dark hen meat[3]	
			MDM[4]	Manual[5]	MDM[6]	Manual[7]
……% of the total of fatty acids …..……						
Saturated						
Lauric C12:0	-	-	1.6	1.6	1.2	1.6
Miristic C14:0	1.1	0.9	2.2	2.8	2.5	2.8
Palmitic C16:0	26.3	21.2	23.3	21.4	20.7	17.4
Stearic C18:0	4.4	4.1	7.5	10.4	8.4	11.5
TOTAL SATURATED	31.8	26.2	34.6	36.2	32.8	33.3
Monounsaturated						
Palmitoleic C16:1	7.1	5.4	5.7	6.8	7.4	10.1
Oleic C18:1	41.8	45.5	34.9	32.7	32.7	28.5
Polyunsaturated						
Linoleic C18:2	19.3	22.1	23.2	22.4	25.4	25.5
Linolenic C18:3	Tr	0.8	1.5	1.9	1.7	2.5
Arachidic C20:4	-	-	Tr	Tr	Tr	Tr
TOTAL UNSATURATED	68.2	73.8	65.3	63.8	67.2	66.6

[1]Mott et al. (1982): MDM of spent layers without skin; [2]Mott et al. (1982): MDM of whole carcasses of spent layers; [3]Jantawat and Dawson (1980); [4]MDM gotten from the keel of hen breasts; [5]Fillet manually deboned hen breasts; [6]MDM of hen back and neck; [7]Manually deboned thighs and wings meat.

R.B. Ozkececi et al. (2008) reported that the pH, FFA and peroxide values for mechanically deboned chicken meat were also affected by the prolonged storage time.

1.2.6. Sensory Characteristics of Mechanically Deboned Meat and Chicken Skin.

Browning, graying, or two-toning of the muscle pigments may indicate prolonged storage after fabrication, temperature abuse, or early microbial spoilage of the raw material, while greening, slime formation, putrefaction, souring, musty aromas, or other off-color/aroma characteristics are signs of apparent spoilage. Accidental contamination by approved ingredients, lubricants, chemical compounds, cleaning agents, or holding conditions can also cause discoloration of muscle pieces and trimmings. These can include sanitizing chemicals (chlorine, iodine, or ammonium ions), sulfites (permanent "red" color), microbiological pigments (orange, brown, black, green), and a chalky, dry surface on frozen muscles that indicates freezer burn (freeze dehydration). Excessive purge present in boxes or combos can indicate poor freezing conditions or premature thawing of the product. Quality defects such as pale, soft, and exudative (PSE) tissue or dark, firm, and dry (DFD) muscles can result in reduced product yields and poor product quality (Lawrie, 1991). Likewise, lipid oxidation of the fat may produce rancid or stale

off-odors due to inadequate packaging during storage or especially when precooked products are reheated.

When MDM of whole carcasses of hens is used, these particles are mainly from thighs, which are highly calcified and break into small particles during the grinding in the deboning machine and are incorporated to the MDM (Grunden and Mac Neil, 1973).

Rate of freezing and frozen storage time and temperature are believed to affect turkey fat oxidation resulting in rancid flavor. Very few studies have been conducted to evaluate the effect of freezing methods on the development of rancid off-flavor in turkey meat.

Dawson and Gartner (1983) have reported that extreme mechanical stress and extraction of important amounts of fats and heme components from the bone marrow as well as aeration during the mechanical deboning process produce high oxidative potential of MDM. These factors promote the auto-oxidation of polyunsaturated fatty acids, producing secondary products of fatty acid oxidation such as aldehydes, ketones, hydrocarbons, esters, furans and lactones. These secondary products are responsible for generated rancid flavors (Ladikos and Lougovois 1990).

Stewart et al. (1945) studied the effect of freezing rate on the quality stability of broilers. One group of broilers was held for 18 hr at 1.7 C after slaughter and cut-up before being frozen, and the other group was frozen within two hours of slaughter and cut-up. Freezing time varied from about 10 minutes for birds frozen in a dry ice-alcohol mixture at -68 c to approximately five hours for birds frozen at -20 C. They reported that neither holding the meat products under refrigeration for 18 hr before freezing nor the freezing rates employed affected the aroma, flavor, juiciness, or tenderness as evaluated by a sensory panel. The sensory quality of the broilers decreased with frozen (-23 C) storage time. They reported that microscopic examination showed that both the rate of freezing and time of aging before freezing affected the histological appearance of the muscle fibers.

Muscle from birds frozen faster (-68 C) and within two hours after slaughter had vacuoles within the fibers of breast and thigh muscle. These vacuoles were very numerous in both raw and cooked sections and they were considered an indication of intra-fibrillar freezing, ice crystals having formerly occupied the site of the vacuoles. No intra-fibrillar freezing occurred in muscles frozen at -20 C within two hours of slaughter. They also reported that, in general, intra-fibrillar freezing did not occur in any broiler meat held for 18 hr at 1.7 C before freezing, regardless of the freezing rate used (Stewart et al., 1945).

Klose et al. (1955) studied the effect of frozen storage duration and temperature and storage temperature fluctuation on the quality of turkey meat. The fluctuating temperature condition consisted of a sine wave cycle fluctuating between -23 and -13 C and repeating itself every 24 hr. They reported increased moisture loss with increasing frozen storage temperature of -34, -23, -18, and -13 C. The moisture loss for meat stored under fluctuating frozen temperature was higher than that for the corresponding mean temperature of -18 C. There were no important differences in the turkey meat quality

characteristics due to the effect of constant frozen storage at temperatures of -34, -23, and -18 up to 12 months storage.

Miller and May (1965) studied the effect of freezing temperature (-18, -34 and -68 C) and frozen storage time (0, 1, 3 and 6 mo) and storage temperature (-18, -26 and -34 C) on tenderness of chicken breast meat. They concluded that tenderness of the chicken meat did not change due to freezing temperature (length of time to freeze). Product stored at -34 C was reported as more tender at 1 and 6 mo frozen storage. Product was the most tender at 0 and 1 mo storage at all the temperatures.

Wyche et al. (1972) used freon (-40 C) or blast freezing (-20 C) to freeze cooked chicken breast, thigh, drum and wings. There was no difference in rancidity (TBA values), moisture content, percent fat and- shear value due to freezing rate. These quality characteristics did change with frozen storage of three months. They reported high incidence of visual white desiccated areas "freezer burn" in the liquid Freon frozen parts, an event not observed in blast frozen meat.

Berry and Cunningham (1970) determined TBA values of cooked chicken frozen in a household freezer (-10 C), by blast freezing (-30 C) and liquid nitrogen freezing (50C), and concluded that liquid nitrogen freezing, the faster freezing rate, was the better procedure, blast freezing intermediate and freezing in a household freezer, the least desirable. Klose et al. (1959) suggested a storage temperature of -18 C or lower for frozen cut up chickens. They reported that the storage life of chicken pieces at -7 C ranged from less than a month to 6 months, at -12 C from 3 to 10 months, while at -18 C storage life was at least 6 months. Small flavor losses during low temperature frozen storage of chicken meat were also observed by Jacobson and Koehler (1970).

Uebersax et al. (1977) reported that a faster "CO_2 snow" chilling than conventional blast freezing rendered higher TBA values of mechanically deboned chicken and turkey meat under frozen (-18 C) storage.

Jantawat and Dawson (1977) observed that blast frozen chicken pieces (-18 C for approximately 10 hr) developed lower TBA values than chicken pieces frozen by $C0_2$ tunnel for 6 min (-34 C at entrance and -51 C at exit) after 3 and 6 mo of frozen (-18 C) storage. However, no off-flavor differences were detected between these treatments by a sensory panel.

Based on the above reports it seems quite difficult to draw a definite conclusion regarding the effect of freezing rate on rancidity development in poultry during frozen storage. It has been long accepted and still persists, in some circles engaged in producing, marketing and distributing frozen foods, among consumers and in technical and scientific circles, that a very rapid freezing rate is necessary in order to achieve frozen food of satisfactory quality. However, Jul (1984) after an extensive analysis of the available experimental data, suggests that, apart from the rarely used very slow freezing rate, the rate of freezing has little, or frequently no influence on the quality of the frozen product, as some of the aforementioned experiments suggest. He contends that the resulting high quality of end

products, believed to be due to the "quick freezing" in early experiments, could well have been the result of a superior quality of raw material and a shorter interval between the products being placed in the freezer and the time freezing actually commenced.

Early studies placed great emphasis on the relationship between rapid freezing and ice crystal formation and size in muscle foods. Differences in ice crystal structure (especially crystal size) were deemed to be inversely related to quality of the thawed product without actually analyzing the sensory quality attributes of these muscle foods. Nusbaum et al. (1983), when studying these relationships, suggested that there is no justification for drawing any conclusions between ice crystal microstructure in the frozen state and sensory properties of thawed meat products.

Nusbaum et al. (1983) presented data on freezing rate as it affects some sensory properties of raw hamburger patties. Patties were frozen (unprotected) in liquid carbon dioxide (freezing rate of 5 cm/hr), in an air blast at -30 C (1 cm/hr), in an air blast at -15 C (0.4 cm/hr), and in still air at -10 C (0.3 cm/hr). Their results suggest that there is no sensory improvement in tenderness of the hamburgers by increasing freezing rate from 0.4 cm/hr to 5 cm/hr. Weight loss during freezing and cooking loss decreased with faster freezing rate. There was practically no difference in total preparation loss (weight loss during freezing plus cooking loss) due to variation in freezing rates from 1 cm/hr to 5 cm/hr.

Ice crystals are formed when tissue is frozen. The dissolved substances in the unfrozen tissue fluid become more concentrated as freezing continues and thus, the tissue freezing point decreases apace. During thawing the process is reversed. Does water once removed (by freezing) from its natural position in the cell matrix where it was partly or fully bound to proteins, carbohydrates and so forth, actually completely return to its original position in the system during thawing or will it remain (at least partly) as "free" water, easily lost from the tissue by so called drip loss.

Freezing rate is believed to affect drip loss. Very slow freezing rates of muscle foods have produced increased amounts of drip loss as compared to a faster freezing rate. However, Jul (1984) concluded that in the normal range of freezing rates commercially available today, drip loss is practically independent of rate of freezing. There is also a positive correlation between the amount of water "absorbed" during the chilling operation in poultry processing and the subsequent drip loss during thawing of the frozen poultry. Crigler and Dawson (1968) compared a wide range of freezing rates for chicken breast muscle and reported that drip loss varied quite inconsistently with freezing rate. Carroll et al. (1981) reported that beef semitendinosus muscles frozen at -18 C and stored for up to 46 weeks showed essentially no change in muscle structure attributed to the marrow.

Mechanical separation of poultry meat influences the color of the resultant meat. The process releases heme pigments from the bone marrow into the mechanically separated meat. Froning and Johnson (1973) found that the mechanical separation of poultry meat will increase the heme protein content approximately three times that found in hand-deboned poultry. This increase is primarily due to hemoglobin from the bone marrow.

Hemoglobin is more subject to abnormal color problems since it is more easily oxidized and more susceptible to heat denaturation during processing and storage. Abnormal brown, green, and gray color defects have been reported in further-processed poultry meat products containing mechanically separated poultry. During the separation process the meat is exposed to considerable air, which may accelerate the oxidation of heme pigments.

Composition and processing variables have been shown to affect the color characteristics of mechanically separated poultry meat. Froning et al. (1973) investigated the effect of skin content prior to deboning on the color of mechanically separated poultry meat. Higher skin levels generally increased the lightness and decreased redness of the resultant mechanically separated poultry meat. These color changes were attributed to the dilution of the heme pigments by the additional fat from the skin.

Researchers have attempted to modify color characteristics of mechanically separated poultry meat by centrifugation (Froning and Johnson, 1973; Dhillon and Maurer, 1975). Froning and Johnson (1973) observed that centrifugation increased redness of the mechanically separated poultry meat while Dhillon and Maurer (1975) reported less redness due to centrifugation. The discrepancy may be partially explained by the use of mechanically separated fowl meat in Froning and Johnson's study while Dhillon and Maurer utilized mechanically separated chicken and turkey meat in their study. Cryogenics have been investigated by some researchers as a faster method to cool mechanically separated poultry meat (Uebersax et al., 1977; Cunningham and Mugler 1974; and Mast et al. 1979). Carbon dioxide snow produced a darker and redder meat, which became more dark and gray during subsequent storage. Cooling with CO_2 snow apparently increased the oxidation rate of the heme pigments during storage of the mechanically separated poultry meat.

Certain processes and formulations may affect the color of products containing mechanically separated poultry meat. Dhillon and Maurer (1975) found that 50/50 mixtures of mechanically separated poultry (chicken or turkey) and beef produced summer sausages with excellent color scores. Froning et al. (1971) found that the addition of 15% mechanically separated turkey meat to red meat franks decreased redness as compared to that observed from 100% beef franks. Also, franks containing 15% mechanically separated turkey meat had a slightly higher rate of color fading during storage. However, it was felt that this fading would not be noticed by the consumer. Today, mechanically separated poultry meat is utilized routinely in emulsified meat products in combination with other species.

The mechanical separation process produces considerable cellular disruption and releases hemoglobin and lipids from the bone marrow. Also, heat produced from the separation process may accelerate lipid oxidation if not controlled. Therefore, quality assurance programs must be especially rigorous to reduce flavor oxidation problems during processing and storage. Mechanically separated poultry meat produced today is much better than that marketed 20 to 25 years ago. This is largely due to improved equipment

and a better understanding of factors related to improved handling of mechanically separated poultry meat.

Several studies have emphasized the storage stability of mechanically separated poultry meat and approaches to improve its flavor stability. Dimick et al. (1972) reported that minimal lipid oxidation of mechanically separated meat occurred during 6 days of storage at 3°C. Mechanically separated turkey meat was the least stable during storage at 3°C.

Froning et al. (1971) indicated that mechanically separated turkey meat stored at -24°C for 90 days exhibited high 2-thiobarbituric acid (TBA) values and unexceptable flavor scores. On the other hand, Dhillon and Maurer (1975a, b), reported that summer sausages made from mechanically separated poultry stored for 6 months were highly acceptable. Also, Johnson et al. (1974) reported that mechanically separated turkey meat had minimal lipid oxidation up to 10 weeks of storage. Janky and Froning (1975) observed the interaction of lipid and heme components in mechanically separated turkey meat. Heme oxidation decreased as storage temperatures were reduced from 30 to -10°C. There was a strong interaction of heme and lipid oxidation, particularly between 10 to 15°C. This strong interaction likely contributes to the increased lipid oxidation being experienced during frozen storage of mechanically separated turkey meat. Heme pigments are known to be strong catalysts for lipid oxidation in meat products. During mechanical separation, oxygen incorporation, temperature increases, high pressure, and metal contact with the deboner may further contribute to the lipid oxidation problem.

Spoilage is caused by the accumulation of metabolic byproducts or the action of extracellular enzymes produced by psychrotrophic bacteria as they multiply on poultry surfaces at refrigeration temperatures. Some of these byproducts become detectable as off-odors and slime as bacteria utilize nutrients on the surface of meats. The metabolic byproducts from spoilage bacteria vary depending on the energy source available to them. When populations are low, the bacterial cells utilize glucose as their primary source of energy. The byproducts of glucose metabolism are not usually odorous and do not substantially contribute to spoilage defects. However, as bacterial populations increase and glucose availability begins to decrease, these bacteria begin utilizing other substrates, such as protein, which yields much more odorous end products as reported by Pooni and Mead (1984) .

Others as Nychas et al., (1988); Lampropoulou et al. (1996) have also reported that proteolysis of the skin and muscle tissue begins when concentrations of glucose and/or gluconate have been exhausted. Pooni and Mead (1984) determined that initial off-odors do not result from breakdown of the protein in skin and muscle, as previously thought, but from the direct microbial utilization of low molecular weight nitrogenous compounds such as amino acids, which are present in skin and muscle.

Venugopal (1990) reported that bacteria growing on the surface of muscle (meat or fish) secrete a variety of extracellular enzymes that degrade the muscle tissue, causing extensive damage. Tarrant et al. (1973) and Porzio and Pearson (1980), using SDS-PAGE techniques, demonstrated that these enzymes were able to degrade myofibrillar proteins

extensively into heavy meromyosin, light meromyosin, and meromysin. Schmitt and Schmidt-Lorenz (1992) demonstrated that there was an increase of low molecular weight peptides (less than 50,000 Da) and free amino acids on chicken carcasses stored at 4°C.

Recently, Nychas and Tassou (1997) monitored the progression of spoilage as it relates to depletion of substrates in the muscle tissue. The concentration of glucose decreased progressively and more rapidly toward the end of the storage period and occurred to a greater degree for samples held at higher temperatures. Similar observations were made for concentrations of L-lactate. Concentrations of free amino acids increased as proteolysis occurred throughout the storage period. De Castro et al. (1988) demonstrated that measurement of these free amino acids, due to the production of aminopeptidases and subsequent breakdown of protein, may be used to rapidly determine the bacteriological quality of beef.

Spoilage defects have been the subject of researchers for many years. Glage (as reported by Ayres (1960)) found that spoilage bacteria initially produce a gray coating on the surface of meat which later turns yellow in color. As these bacteria multiply, an aromatic odor accompanies their growth. Eventually, meat surfaces become coated with tiny drop-like colonies which increase in size and coalesce to form a slimy coating. Microorganisms appear first in damp pockets on the carcass, such as folds between the foreleg and breast of a carcass, and their dispersion is promoted by condensation which occurs when a cold carcass is exposed to warm, damp air (Ayres, 1960).

Ayres et al. (1950) identified an ester-like odor which was described as a “dirty dishrag” odor that developed on cut-up chickens. In most cases, off-odor preceded slime formation and was considered the initial sign of spoilage. Immediately after off-odors were detected, many small, translucent, moist colonies appeared on the cut surfaces and skin of the carcass. Initially, these bacterial colonies appeared similar to droplets of moisture; however, they eventually became large, white or creamy in color, and often coalesced to form a uniform sticky or slimy layer. In the final stages of spoilage, the meat began to exhibit a pungent ammoniacal odor in addition to the dirty dishrag odor Ayres et al. (1950), which may be attributed to the breakdown of protein and the formation of ammonia or ammonia-like compounds.

Slime production has also been attributed to proteolytic activity of bacteria growing on the surface of meat and poultry. Various authors have reported that degradation of meat by pseudomonads results in the formation of slime (Nychas et al., 1988; Schmitt and Schmidt-Lorenz 1992; Gill and Newton 1978; and Schmitt and Schmidt-Lorenz 1992).

1.2.7. Process Technology for Manufacturing Sausage (Mortadella).

Formed meat products may be produced by sectioning muscle pieces and combining with a ground or emulsified myofibrillar protein binder and chilled brine. Restructured items have a smaller particle size which is reduced by grinding, flaking, dicing, chopping, slicing, or emulsifying. The particles are then mixed with an appropriate binding material and formed into a specific portion size. Sectioned products are primarily intact muscles

and have a more "whole-muscle" texture than restructured items. Because of the similarity of these two types of formed products, they will be discussed simultaneously with limited distinction between the sectioned and formed or restructuring processes. Federal Regulations (United States Department of Agriculture 1999) 381.159 and 381.171, respectively. For example, "turkey ham" specifies a labeling requirement of "chunked and formed" for thigh pieces ≥0.5" while pieces <0.5" are labeled "ground and formed" or "chopped and formed."

Sectioned and formed poultry products are prepared from well-chilled (-2.2 to 1.6°C or 28 to 35°F) whole muscle pieces or chunks that have been defatted and injected with or marinated in a salt brine containing alkaline phosphates. If cured, sodium nitrite and sodium erythorbate are added to the brine. A functional protein is required to coat the meat particle surfaces, form an interwoven network between meat pieces, and then coagulate when heated to form a solid tissue mass with a meat-like texture. Cold-set binders are available to bind meat pieces without the need for heat coagulation of the myofibrillar proteins.

These include the hydrocolloid sodium alginate which is cross-linked with a calcium salt, transglutaminase, and a fibrinogen-thrombin combination. When using enzymatic binders, processing time may need to be reduced, product temperature kept near freezing to slow the enzymatic reaction, and meat surface moisture minimized to enhance the polymerization reaction occurring between the protein molecules.

Formed products offer the advantages of being: (1) boneless; (2) easily portioned into an appropriate size and shape; (3) lower in cook loss and higher in serving yield, having virtually no waste; (4) uniform in composition for better brine or cure distribution; (5) able to utilize whole muscle pieces with otherwise less utility; and (6) easier to heat, slice, and serve (Pearson and Gillett 1996). Obvious limitations are (1) low quality poultry pieces cannot always be improved; (2) formed products require more equipment, manufacturing technology, additional molds or casings, and handling considerations to avoid pathogen contamination; (3) shelf-life may not be as long as whole-muscle, non-marinated products; and (4) further processing requires a high input of labor and capital.

Emulsified poultry products are processed by homogenizing MDM in a bowl chopper with iced water, salt, cure, dextrose, alkaline phosphates, corn syrup solids, modified starch, spices, sodium erythorbate, and other additives to an end point temperature of approximately 10°C (50°F). Further processing may require passing the batter through an emulsion mill to further reduce particle size and obtain a smooth texture. Batter temperatures should not exceed 12.7°C (55°F) to avoid overheating the fat that would result in processing defects (fat caps, fatting-out) during thermal processing. Sausages are then vacuum encased in a cellulose casing (frankfurters) or moisture-proof fibrous casing (bologna) and fully cooked using a multiple-stage cooking cycle. Application of smoke to the product may be in the form of: (1) a liquid drench applied to the casing surface (sausages); (2) atomization of liquid smoke in the smokehouse; (3) incorporation of liquid smoke into the product formulation; or (4) natural smoke generated from hardwood sawdust. A minimum internal temperature of 68.3°C (155°F) is required if the product

contains ≥100 ppm nitrite or 71.1°C (160°F) if <100 ppm of nitrite is present (Alan, 2001).

In addition to the protein and fat contents, some technological aspects, such as pH, water holding capacity and emulsifying capacity, are the main factors affecting the technological quality of MDM. The functionality of myofibrillar proteins determines the quality of the meat emulsion formed, mainly regarding the texture, the yield of the process, and the emulsion stability. However, oxidized lipids, which can be present in the MDMs, can cause protein polymerization and insolubilization, polypeptide chain rupture, amino acid destruction, and formation of products with protein addition. These interactions influence negatively the functional properties of the meat. The higher the instability of the material in regards to lipid oxidation, as in the case of the MDMs, the greater the effects on the functionality. In systems with high water activity or aqueous solution, proteins form crossed links among themselves in the presence of peroxidized lipids, with simultaneous loss of solubility. Reactions between malonaldehyde, a by-product of the lipid oxidation, and free amino groups of proteins, lead to the formation of irreversible covalent links, with a consequent loss of the solubility of proteins (Pollonio, 1994).

1.2.7.1. Curing Treatment:

The primary purpose and benefit of utilizing sodium nitrite for meat curing is its inhibitory property against *Clostridium botulinum* growth and toxin production. *C. botulinum* is an obligately anaerobic, Gram positive, spore-forming rod that is widely distributed in the soil (Hayes 1992) and therefore has the potential to be a contaminant on the feathers, skin, and intestines of poultry. The spores are extremely heat resistant to normal pasteurization or cooking processes for cooked, cured products, excluding those processed by canning. *C. botulinum* is referred to as Type A to G on the basis of the exotoxin produced during vegetative cell growth. Types A and B are more frequently associated with animal foods. The toxins affect the neurological system of the body, paralyzing muscles and eventually can cause death from respiratory failure or cardiac arrest. Antitoxin administered prior to development of severe symptom development increases survival.

As an antimicrobial, nitrite's effectiveness is dependent on factors such as pH, salt concentration, temperature, and level of contamination. While not preventing germination of spores of clostridia, it inhibits the rate of cellular outgrowth. It is also generally inhibitory to other bacteria but this may be due to the combined effect with salt and/or pH, acting together as outgrowth obstacles (Van Laack 1994). The effective compound is thought to be nitrous acid which is rapidly decomposed as the aqueous pH increases. Nitrite's effectiveness generally increases approximately 10-fold as the pH decreases from 7.0 to 6.0 and is most effective in the range of pH 5.5 to 5.0 (Kraft 1986). Nitrite is thought to exert its bacteriostatic effects through several mechanisms such as reaction with iron-sulfur proteins important in energy metabolism and inactivation of catalase and cytochrome by nitric oxide.

Experience with vacuum-packaged, cured poultry products suggests that similar products packaged in modified atmospheres would benefit from the same preservative factors derived from nitrite addition. However, the main weakness for all cured poultry products may prove to be the extensive use of alkaline sodium phosphates to increase the product's functional properties while likely decreasing the bacteriostatic effects of nitrite. These areas will require further research and experience related to safety and shelf-life extension from delayed microbial outgrowth.

The addition of relatively small quantities of sodium nitrite produces the development of the desired color "pickling red" in processed meat products. Without nitrite meat products turn grey in color when heated (Gunter and Peter, 2007). The principle of curing is not dyeing the product, but chemical reaction of the red muscle pigment with nitrite resulting in a stable red color that does not change during heating and storage (Gunter and Peter, 2007). Sodium nitrite has the ability to react with the red meat pigment to form the heat stable red curing color. Only very small amounts of the nitrite are needed for this purpose (Gunter and Peter, 2007).

Nitrite can be safely used in tiny concentrations for food preservation and coloring purposes. Traces of nitrite are not poisonous. In addition to the reddening effect, they have a number of additional beneficial impacts so that the meat industries widely depend on this substance. Levels of 150 mg/kg in the meat product, which is 0.015%, are normally sufficient to reduce the risk of overdosing of nitrite salt; a safe approach is to make nitrite available only in a homogeneous mixture with common salt generally in the proportion 0.5% nitrite and the balance of sodium chloride (99.5%). This mixture is called nitrite curing salt (Gunter and Peter, 2007).

Many attempts have been made to replace nitrite by other substances, which would bring about the same beneficial effects as listed above. Up to now no alternative substance has been found (Gunter and Peter, 2007). Nitrate and nitrite are used for the purpose of curing meat products. In most countries the use of both substances, usually added as potassium or sodium salts, is limited. Either the ingoing or the residual amounts are regulated by laws (Karl-Otto, 2007). The effective substance is nitrite acting primarily as an inhibitor for some microorganisms. Nitrite added to a batter of meat is partially oxidized to nitrate by sequestering oxygen-thus it acts as an antioxidants-apart of nitrite is bound to myoglobin, forming the heat stable NO-myoglobin, a part is bound to protein or other substances in meat (Karl-Otto, 2007). Nitrate may be reduced to nitrite in raw meat products by microorganisms. As oxidation and reduction may occur the concentrations of nitrite plus nitrate in a product has to be controlled and measured especially if the residual amounts are regulated (Karl-Otto, 2007).

This sum of both compounds is important for the human body. Intake of nitrate with food leads to its absorption over the digestive tract into the blood (Karl-Otto, 2007). In the oral cavity nitrate appears again where it is reduced to nitrite. With the saliva the nitrite is mixed with food, having the same effect as nitrite in a batter (inhibiting growth of some pathogenic microorganisms) and swallowed (Karl-Otto, 2007).

Because of its curing activity nitrite is an important ingredient in meat preblending; it functions also as anti-oxidant and in the production of the typical aroma of cured meats. Nitrite also has important antimicrobial effects, avoiding the production of toxins by *Clostridium botulinum* (Hasiak et al., 1984; Kolodziejska et al., 1990). A maximum of 150ppm of nitrite is allowed by the Brazilian food safety legislation in meat products (Brasil, 1998).

Sodium erythorbate is used in meat products to speed up the formation and stabilize the characteristic pink color of nitrite-cured meats. The utilization of antioxidants can minimize functional deteriorations in muscle proteins during storage (Xiong and Decker, 1995). Erythorbate is an antioxidant, so this effect could also be expected. The Brazilian legislation does not limit the use of sodium erythorbate in sausages (Brasil, 1998).

1.2.7.2. Thermal Treatment:

The use of fire to cook or smoke meat and the use of sunlight to dry meat are parts of human survival and cultural practices. The benefits of heat were probably obvious; perhaps consumers were sick less frequently with cooked as compared to raw meat due to destruction of pathogens. In addition, the meat lasted longer before spoilage, and the palatability was changed (although raw meat eaters at first may not have appreciated the new flavor, texture, or color). Cooking can negatively affect meat quality due to potential loss of some nutrients and potential formation of mutagenic compounds (Engler and Bowers 1976; Chiu et al., 1998).Interestingly, marination prior to cooking may decrease mutagen levels of cooked poultry meat (Chiu et al., 1998).

If the product is cooked, internal temperatures that are adequate for killing target pathogens (lethality) are required (Food Safety and Inspection Service Appendix A 1999). After cooking, products must be stabilized by rapid cooling to prevent outgrowth of *C. perfringens* spores (Food Safety and Inspection Service, Appendix B 1999).

Following proper cooking, products will be free of vegetative pathogens; however, recontamination of product can occur if preventive measures are absent or ineffective. Post-cooking contamination is the primary factor leading to *L. monocytogenes* contamination of ready-to-eat poultry products. *L. monocytogenes* can survive well in processing plant environments and is considered an environmental contaminant (Food Safety and Inspection Service, *Listeria Guidelines for Industry* 1999).

Pamela et al. (1974) reported that both storage time and temperature affected percentages of total cooking loss for mechanically deboned turkey meat. Storage time, but not storage temperature affected eating quality. Juiciness was affected by storage time. TBA values of the mechanically deboned turkey increased with time and temperature of storage.

Several developments in the further-processing of poultry contributed to the utilization of “curing” as a means of providing products with a unique flavor, color, and improved safety. As the consumer demanded more cut-up poultry, uses were needed for the fine fibrous, paste-like mechanically deboned meat obtained from residual carcass frames,

necks, and backs. Since preparation of highly comminuted meat through extensive chopping of whole tissue was the starting point in frankfurter manufacture, chicken and turkey frankfurters were developed directly with mechanically deboned meat. In addition, mechanically deboned chicken and turkey eventually were permitted as a meat ingredient in combination with pork and beef for manufacture of typical red meat frankfurters (Froning et al., 1971).

With the improvement of equipment and worker skills for deboning of carcasses; breast, thigh and drums, whole-muscle strips, chunks, and pieces became available to prepare such cured products as poultry hams, pastrami, and cured, smoked breasts. Another category of products made from coarsely ground boneless poultry meat includes various luncheon items such as salamis and fermented summer-style sausages. Today, along with frankfurters made entirely of poultry meat, these other cured products are marketed as intact or portioned chubs or as presliced products and are very popular with consumers.

Meat in early history was primarily preserved with salt that exerted its inhibitory effect on contaminating bacteria while also dehydrating the meat through osmotic effects on muscle cells at the meat surface. Salt was liberally applied as a rub or packed onto meats and the practice was termed "dry curing." This is similar to old style country cured ham manufactured today. Early salt was frequently impure, possessing a brownish-red color, which resulted in reddening of the meat. The contaminant was later found to be sodium nitrate. In the late 1800s, scientists found that nitrate-reducing bacteria yielded the nitrite ion which was the major active compound in curing reactions. Except for approved nitrate use in the manufacturing of dry cured products, most of which are non-poultry except for some turkey jerky, sodium (or potassium) nitrite is the principal permitted curing ingredient today and its use is regulated by the USDA. The initial regulation permitting the use of nitrite as a curing agent was promulgated in 1925 (Cassens 1990) and with subsequent revisions as to concentrations permitted, it remains in effect today and applies to all cured poultry products as well as other meats. Both the sodium or potassium form of nitrite may be used as long as the concentration does not exceed a limit depending on the product (156 ppm of nitrite for sausages), calculated as sodium nitrite on an in-going or ingredient additive basis. Such limitations have been instituted to reduce the potential formation of carcinogenic nitrosamine byproduct compounds. In addition to reducing nitrites in the formulations, nitrosamine formation can be limited by assuring an adequate reducing environment with ascorbates or erythorbates to enhance conversion of the nitrites to nitric oxide.

In addition to nitrite, several other ingredients are used in poultry meat curing. Nearly all cured products have salt (NaCl) added for flavor as well as antimicrobial and protein functionality purposes. Salt is still considered a primary curing ingredient with nitrite. Examples of other ingredients include various reductants such as ascorbic or erythorbic acid (or their respective sodium salt ascorbate or erythorbate); pH modifiers, such as phosphates, citric acid, or glucono-delta-lactone; and flavorings or flavor enhancers such as sugar, corn syrups, honey, hydrolyzed vegetable protein, autolyzed yeast, spices, and seasonings. Today, the manufacture and safety of cured poultry meat products relies on

nitrite addition as the principal preservative agent, but also includes combination with vacuum or modified atmosphere packaging and refrigeration.

The medium used to apply heat to poultry products (air, steam, oil, or water) is one method of categorizing the different methods of cooking. The commercial processor typically utilizes in-line ovens or fryers whenever possible to maximize throughputs of products adaptable to these methods, including parts, whole-muscle, and chopped-and-formed items. Other products require batch processing under different conditions, such as canned items, kettle-cooked whole birds, and smoked or cured products. In general, for commercially processed poultry products, the in-line ovens and fryers are the preferred methods for many of the mass-produced poultry items marketed at retail and to restaurant or food service customers.

Dry-heat cooking methods such as grilling produce drier product with lower yield than moist-heat cooking methods, such as baking; likewise, higher heat rapid methods such as frying or searing can yield moister product by rapidly cooking the surface and sealing in the juice. Marination is a common approach to reduce these cooking losses, thereby increasing yield and quality.

The end point temperature required by law for pasteurization of poultry products labeled as fully cooked are 71°C (USDA standard for commercial cookers) or 74°C (FDA standard for retailer cooking). However, processors generally cook to a slightly higher temperature (75 to 77°C) for a safety margin against process or product variations. Aside from safety, it is important in maintaining yield not to exceed the end point temperature greatly. The rate of product temperature increase (°C per min) during cooking gradually decreases the closer the product and the cooking (oven, fryer, grill, etc.) temperature become Fig.1.2. So, the closer the product temperature gets to the cooking temperature, the longer each degree of temperature increase takes to achieve. This longer time is critical because it is more time for product moisture and yield to be lost, particularly with dry-heat cooking methods. This may also help to explain the juiciness of some rapid cooking methods.

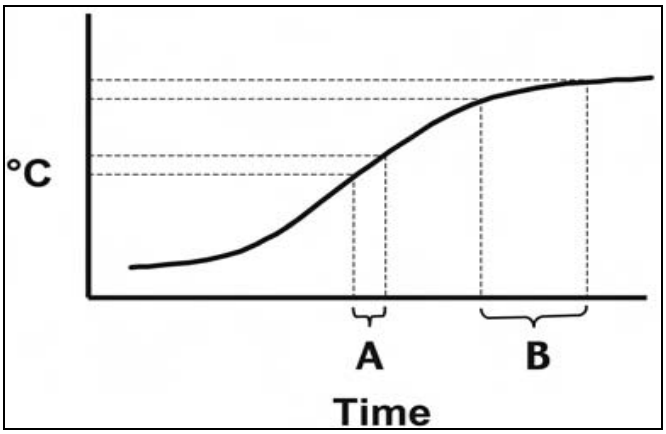


Fig.1.2 Graph depicting product temperature increase during cooking time. Time period A is less than time period B, even though both achieve the same amount of product temperature increase.

The rate of product heating is primarily determined by the temperature difference between the product surface and its core temperature (Hanson 1990). Humidity, or more correctly, the wet-bulb temperature setting, is generally increased in the early stages of heating since it will increase the cooler product's surface temperature by condensation and thereby increase the surface-to-core temperature difference. The humidity is then reduced in the latter stages so that the dry-bulb temperature becomes the driving force at the product's surface. Moisture migration and evaporation at the surface result in product "shrink" or weight loss. Although the yield is reduced, the drying effect during the latter stages of cooking is usually beneficial for enhancing the product's appearance. The cured color appears slightly brighter at the dried surface as compared to the moister, interior of the product.

The final temperature of cooking is specified by the USDA and depends on whether the product is cured or uncured. The regulatory requirement insures that these products are "ready-to-eat" without the necessity of further heating by the consumer. Federal regulations specify an internal temperature of 71°C for uncured and 68°C for cured poultry (Code of Federal Regulations 1999) when heat-processed by any method. However, many processors use an internal temperature of 73 to 74°C for products that are to be sliced, particularly if they are prepared with dark meat. In effect, the cooking temperatures assure pasteurization in the cooking of these products and thus special care is required post-cooking to prevent product contamination prior to packaging. A special case for heating during processing applies to poultry breakfast strips, similar to bacon, which are cured and smoked products that require additional cooking by the consumer prior to consumption. The USDA requires these products to be heated to an internal temperature of 60°C, and then cooled to 26°C within an hour and a half, and attaining 4°C or less within 5 h (Code of Federal Regulations 1999).

Vegetative microorganisms can all be destroyed at temperatures below 100°C, basically in the temperature range of 60°C to 85°C (depending on the type of microorganisms). Microorganisms are sensitive to heat and are killed at certain temperatures, which may be below or, in the case of spore forming microorganisms, above 100°C. Each species of microorganisms reacts differently to heat treatment, due to their different heat resistance. Microorganisms are quickly killed when they are exposed to relatively high temperatures. Microorganisms can also be killed at relatively low hot temperatures, but longer heat treatment periods will be necessary in such cases (Gunter and Peter, 2007).

Heat treatment at temperatures below 100°C, mostly in the temperature range of 60 to 85°C, also called "pasteurization" or simply "cooking". Core temperatures of +65°C would be sufficient to achieve the required texture through protein coagulation. However, for hygienic reasons and in order to eliminate a major part of the micro-organisms present in the batter, core temperatures of +70 to +72°C must be reached (Gunter and Peter, 2007).

In small and medium scale operations the sausages are transferred to a cooking vat and submerged in hot water of +74 to +80°C for a certain period of time until a core temperature of at least +72°C is achieved(Gunter and Peter, 2007).

Smoking, provided through the controlled oxidation and combustion of wood, contributes to product flavor and aroma, aids in development of surface color, and deposits several bacteriostatic compounds that can aid in extending product shelf-life. With natural smoke generators attached to processing ovens, the generated smoke is metered into the oven as a step in the cooking cycle. In order to avoid streaking while maximizing penetration and deposition, smoke is generally applied to the moist product surface after the early surface condensate on the product has evaporated. It is primarily the phenolic and carboxylic acids of smoke that provide flavor and bacteriostatic effects whereas color enhancement is related to the carbonyl (aldehydes and ketones) content. A noticeable browning effect, in addition to cured color development, results from products of the Maillard reaction between free amino groups of proteins or other nitrogenous compounds and the carbonyls of the smoke. The phenols, as antioxidants, also reduce oxidative rancidity (Schmidt 1986).

Liquid smoke flavorings applied through sprays or aerosols in the oven or added during mixing or tumbling with other ingredients, are manufactured from entrapment and concentration of smoke volatiles into water or oils. With a more consistent composition, and with removal of polycyclic hydrocarbons having carcinogenic properties, liquid smoke utilization has increased over the past decade (Pearson and Gillett 1999). To mimic natural smoking, smoke regenerators are available to heat and volatilize liquid smoke to vapor, which is then transferred to the oven and deposited at the product surface in the same manner as normal smoke vapor.

1.2.7.3. Physical Treatment:

Physical treatments are available to processors for pathogen control in products. In fact, temperature manipulation is the primary means by which pathogens in poultry products are controlled or eliminated. Besides temperature control, the application of ionizing radiation (irradiation) has recently been approved for raw fresh or frozen poultry, and has the potential to emerge as a means of eliminating pathogenic microorganisms from raw poultry.

Because most poultry-associated bacteria reproduce by binary fission, each growth cycle results in a doubling of bacterial population. Under optimal temperature and other environmental conditions, bacterial growth is characterized by a short lag phase in which bacterial numbers remain relatively constant over time, followed by a rapid growth phase in which cell numbers increase exponentially over a relatively short period of time. Under such conditions, bacteria can double in number in as little as 15 minutes. As the temperature moves below or above the optimum, bacterial growth rate will decrease. The further away from the optimum, the slower the growth rate. At some point, which is dependent on bacterial type, the organism will not be able to reproduce.

Thus, a basic tenet of food safety is that the rate at which bacteria multiply is temperature dependent, and temperature can be a useful tool to control bacteria on food products. Bacteria that occur on poultry products can be classified Table 1.9 according to the temperature range in which they can grow (Ayres et al., 1980; Banwart 1989).

Table.1.9 Grouping of bacteria based on temperature effects on growth.

	Temperature ranges that allow growth (°C)		
Bacterial group	Minimum	Optimum	Maximum
Psychrophilic	-15-5	5-30	20-40
Psychrotrophic	-5-8	20-30	30-43
Mesophilic	5-8	25-43	40-50

Source: Adapted from Ayres et al., 1980; Banwart 1989.

Therefore, the temperature at which poultry products are held can affect bacterial growth and influence the types of bacteria that will predominate. The predominant poultry spoilage bacteria are psychrotrophic or psychrophilic, while the primary food-borne pathogens associated with poultry are mesophilic. Refrigeration in addition to retarding microbial spoilage can be an effective means for preventing pathogens from increasing in number on poultry products. An exception would be *L. monocytogenes,* which has the ability to proliferate, albeit slowly, at refrigeration temperatures.

Because most of the pathogens of concern can proliferate between 5 and 50°C, this temperature range is often referred to as the danger zone. To prevent proliferation of bacteria, poultry products should be brought to 4°C or below as quickly after processing as possible according to USDA guidelines (9 CFR Part 381.66).

After processing, raw products must be stored either at or below 4°C. Products should be taken through the danger range as quickly as possible when a temperature change is necessary (e.g., cut-up and deboning, cooking and chilling, etc.). During cut-up and deboning, it is impractical to keep product at or below 4°C; however, product should not exceed 10°C, and processing time during these operations should be minimized. Storage of products at temperatures below the critical zone (refrigerator or freezer) does not kill bacteria; rather, bacteria are prevented from growing. When products are subsequently held within the danger zone, bacteria can increase in number, thereby increasing the risk of disease.

In the manufacture of ready-to-eat poultry products, vegetative bacteria cells are expected to be destroyed in the cooking process. Moreover, processors of ready-to-eat poultry products are required by the USDA-FSIS to meet lethality performance standards to ensure that these products are free of vegetative pathogens. Thermal processes should be designed and validated, within a reasonable margin of safety, to eliminate vegetative pathogens. Such processes are referred to as safe harbors and should provide a 5–7 $\log_{10}$ reduction in *Salmonella* serotypes and other vegetative pathogens. Although there are many factors that can influence the rate at which bacteria are killed by heat, it is generally accepted that internal product temperatures of at least 71.1°C will provide the safe harbor lethality to ensure elimination of non-spore-forming pathogens such as the *Salmonella*

serotypes, *C. jejuni, L. monocytogenes,* and *S. aureus (*Food Safety and Inspection Service, *Appendix A* 1999).

The spores of *C. perfringens* are typically not eliminated by such heat treatments, as spores of this organism have been demonstrated to survive, at least in part, an exposure to 80°C for 10 min. Because spores of *C. perfringens* and other bacteria can survive typical cooking processes, products must be cooled quickly following cooking. This is referred to as product stabilization. USDA requires producers of fully cooked, ready-to-eat products to meet stabilization performance standards to ensure the spores of *C. perfringens* will not germinate and grow (Food Safety and Inspection Service, *Appendix B* 1999).

As general safe harbor guidelines for product stabilization, during post-cook cooling, product temperature should not remain between 54.4 and 26.6°C for more than 1.5 h nor between 26.6 and 4.4°C for more than 5 h. Other cooling cycles are acceptable if the processor can validate that they prevent the outgrowth of *C. perfringens* spores.

1.2.8. Microbial Growth of Final Product.

There were many scientific studies on all aspects of the chicken meat products in this literature, so that was addressed to methods of storage for mainly raw material which used in the manufacture of sausage (Mortadella), also in this literature we review the relationship b/w the storage of frozen mechanical deboned meat (MDM) and the microbial growth related with changes in surrounding conditions.

The primary causes of spoilage are as follows: (1) prolonged distribution or storage time, (2) inappropriate storage temperature, and (3) high initial bacterial counts. If fresh poultry products are held long enough at refrigerator temperatures, they will spoil as a result of the growth of bacteria that are able to multiply under cold conditions. This situation may be improved by proper rotation of stock. Product that has remained on the shelf for the longest period of time should be sold first and product that is to be sold in locations far from the processor should be transported at temperatures that are near freezing (i.e., -3.3°C), but not sufficient to freeze the muscle tissue (deep chill) (Alan, 2001).

Inappropriate storage temperatures or fluctuations in storage temperature are the most avoidable causes of spoilage. Temperature abuse can occur during distribution, storage, retail display, or handling of the product by the consumer. The only means by which processors can determine whether their product has been temperature abused is to monitor temperature or evaluate bacterial populations throughout the distribution system. Initial bacterial counts on broiler carcasses may have a direct effect on the shelf-life of fresh product as well. The initial number of bacteria on poultry is generally a function of grow out procedures, production practices, and plant and processing sanitation (Alan, 2001).

Most processed meat products are heat treated during manufacture and cooled down in a next step, as they undergo shorter or longer cold storage periods for distribution and

sales. Hence, processed products must have an adequate shelf life, which can only be achieved if their microorganism content is low enough to perform the determined shelf life. During slaughtering, subsequent meat cutting and initial processing steps, numbers of microorganisms in meat are steadily increasing. The thermal treatment at the end of the processing stage is therefore important for microbial control. It is the effective tool to reduce or eliminate the contaminating microflora (Gunter Heinz and Peter Hautzinger, 2007).

Microbial contamination of carcasses is a natural result of procedures necessary to produce retail products from live animals (Anderson et al., 1977; Dickson and Anderson 1992). Contamination of poultry meat products can occur throughout initial processing, packaging, and storage until the product is sufficiently cooked and consumed. Heavy loads of bacteria enter the processing plant with the live bird, and these bacteria can be disseminated throughout the plant during processing. Most of the bacterial contaminants are non-pathogenic, and are associated with meat spoilage. However, poultry serve as reservoirs for a number of pathogens including, *Salmonella* serotypes, *Campylobacter jejuni, Listeria monocytogenes, Clostridium perfringens,* and *Staphylococcus aureus.*

Epidemiological reports indicate that more than 95% of all food-borne illnesses are the result of activities occurring after the product has left the plant ;(Bean et al., 1990) that is, illness is generally the result of temperature abuse and improper handling or preparation. However, when contamination and illness occur, investigators tend to look at raw product (how it was produced, processed, and handled), and press for elimination of pathogens before the product reaches the consumer (Shackelford 1988). This creates a challenge to the poultry industry to improve the microbiological safety and quality of its products.

As stated above, processed raw poultry meat naturally harbors bacteria. Most of these bacteria are responsible for the spoilage of poultry meat, but are not pathogenic to humans. However, poultry products can harbor bacteria capable of causing human disease (i.e., pathogens). A number of food borne pathogens have been isolated from processed poultry (Waldroup 1996).

Poultry meat and its derivatives are among the food products that cause the most concern to public health authorities, owing to the associated risks of bacterial food-poisoning (Baeumler et al., 2000; Beli et al., 2001).

1.2.9. Types of Microorganisms.

1.2.9.1. *Salmonella* Serotypes:

Salmonella are mesophilic, faculative, Gram negative bacteria of the family Enterobacteriaceae. There are three human disease syndromes caused by *Salmonella* spp.: typhoid fever, paratyphoid fever, and gastroenteritis. Typhoid and paratyphoid fever, which are rare in developed countries, are transmitted human to human by the fecal-oral route, and humans are the only reservoir.

In contrast, gastroenteritis (non-typhoidal salmonellosis) is caused by *Salmonella enteric* serotypes, which are found in the intestinal tract of both humans and non-human animals. Poultry has been identified as a primary reservoir for these salmonellae ((Bryan and Doyle 1995). There are >2300 serotypes. Of these, Typhimurium, Enteritidis, and Heidelberg are the most frequently isolated serotypes from human cases, and these are common poultry-borne serotypes, also. Human disease ranges from mild to severe and is characterized as a selflimiting infection of the lower intestinal tract. The infectious dose ranges from 10,000–1,000,000 cells. Symptoms typically appear 12–36 h after consumption of a contaminated food, and include nausea, vomiting, severe diarrhea, fever, abdominal cramps, and malaise.

While *Salmonella* serotypes ultimately originate from contaminated feces, a wide variety of environmental and food sources can harbor these pathogens. This widespread distribution demonstrates the ability of *Salmonella* to survive well in the environment. These bacteria are introduced into the processing plant with the live birds, which can harbor these pathogens in skin and feathers, as well as in the gastrointestinal tract. Consequently, *Salmonella* can persist on final raw products. Disease can result when these products are handled without good hygienic practices, not properly cooked, and/or subjected to temperature abuse.

K.L. Mattick et al. (2002) *detected that Salmonella spp.* was found in a significant proportion of sausages and are not always killed during the cooking process. The risk assessment model developed may provide useful quantitative data relevant for risk management initiatives, ultimately aiming at controlling the risk of salmonellosis from raw, frozen chicken products. (Dominguez Risco and Silvia A., 2009).

Salmonella bacteria are commonly found in poultry carcasses and their presence after processing may lead to food borne disease outbreaks as reported by Dominguez Risco and Silvia A. (2009), also Consumption of frozen chicken products containing raw poultry has been recently identified as a risk factor for salmonellosis and Survival of the pathogen after freezing and frozen storage may lead to infection if the food product is insufficiently cooked. Vijay K. Juneja, et al. (2010) resulted that lactate and oregano oil may be used to render *Salmonella spp.* more susceptible to the lethal effect of heat and to inhibit growth of *Salmonella spp.* that survives heat treatments.

1.2.9.2. *Listeria monocytogenes*:

L. monocytogenes is a psychrotrophic, Gram positive bacillus. This is an opportunistic pathogen, infecting primarily the immunocompromised. Pregnant women and their fetuses, AIDS patients, alcoholics, and the elderly are the most often affected. In these patients, listeriosis can progress to meningitis and therefore can be life threatening. In the immunocompetent, listeriosis is often a mild, flu-like illness. *L. monocytogenes* tends to be an environmental contaminant, and can persist in cool, damp areas of a poultry processing plant. Drains, refrigeration-freezing equipment, and other fomites are known to harbor *L. monocytogenes.* Post-process contamination of fully cooked, ready-to-eat

poultry products has emerged as a major food safety issue. Therefore, processors of such products must take steps to prevent contamination.

In this term Silvia Dominguez and Donald W. Schaffner (2009) indicated that pathogens such as *Listeria Monocytogenes* possessed a significant cross-contamination risk during further meat and poultry processing, distribution, and storage. It was found that increasing the NaCl concentration protecting *L. Monocytogenes* against the lethal effect of heat, while high sodium pyrophosphate (SPP) concentrations and low pH values increased heat sensitivity of *L. monocytogenes* (Juneja et al., 1999). The addition of Lactobacillus *Sakei* strains significantly decreased the stimulating of *L. monocytogenes* concentration of ready-to-eat fermented sausages at the time of consumption (Drosinos et al., 2006).

Meijun Zhu et al. (2006) found that formulating meat products with antimicrobial additives is another common approach to control *L. monocytogenes* in Ready-To-Eat (RTE) mortadella meat, also determined that Irradiation is an effective technology to eliminate *L. monocytogenes* but can influence the quality of RTE meat products significantly.

1.2.9.3. *Clostridium perfringens*:

C. perfringens is an anaerobic, spore-forming, Gram positive bacillus. If large numbers of *C. perfringens* spores ($>10^6$) are consumed, a toxicoinfection can occur. The toxicoinfection results when *C. perfringens* attaches and colonizes the lower GI tract where it will enter into a spore-vegetative cell cycle. An enterotoxin, which produces a profuse watery diarrhea in the host, is produced during this growth cycle. The disease is generally mild and limited to one to two days in duration.

This pathogen is found in the soil and is carried in the fowl's GI tract. Therefore, *C. perfringens* can be introduced into the processing plant with live birds. Because the organism produces spores, it can survive in harsh environments. Thus, it can spread during processing and persist into final product. Cooked poultry products, particularly those cooked in large batches are of greatest risk. Such products can be difficult to heat thoroughly and subsequently cool quickly. If spores are not destroyed by the cooking process, they can grow to high numbers if the product is not cooled at a sufficient rate to prevent spore germination. In meat products that provide an anaerobic environment and are held at improper temperatures, this pathogen can enter a growth cycle in which numbers of spores will double every 15 min. For this reason, cooked meat products must be cooled rapidly to prevent the germination and outgrowth of *C. perfringens.* This requirement for rapid cooling of cooked products is often referred to as "product stabilization."

Also Ellin Doyle (2002) determined that the critical temperature range for growth of *B. Cereus, C. Perfringens*, and some *C. Botulinum* strains was approximately 10–50°C although some psychrotrophic *C. Botulinum* strains could grow at refrigerator temperatures

On the other hand, mechanically deboned chicken meat is made from the deboning and cutting of parts with lower commercial value, such as the back and the neck although deboning process can be applied to whole part of poultry carcasses (Barreto, 1995).

This undesired way of deboning process decreases the quality of mechanically deboned poultry meat with the other reasons and also makes it short shelf-lived product with high microbial load (Field, 1998). The composition and storage stability of the final product is affected by the raw materials and conditions used for mechanical deboning.

According to Regulation (EC) 853/2004, mechanically deboned poultry meat must be produced from the feet, neck skin and head bones of the poultry. Raw poultry surface and the other parts were known as high microbial load. Many decontamination methods were reported.

Ellin Doyle (2002) found that bacterial spores were not only very heat resistant but also heat actually stimulate the spores to start growth, so generation times as short as 7 min have been recorded; therefore, it was important to cool cooked meats quickly to temperatures below the minimum that allows germination and growth of spores.

A variety of cooling methodologies exist, and the decision on which to use depended on the type of meat product as well as on economic costs (Ellin Doyle 2002).

Ellin Doyle (2002) also studied another very important consideration was the avoidance of post-processing contamination during cooling and packaging. The actual cooling rate and safety of cooling processes should be investigated for each product because various factors such as sodium chloride concentration, fat percentage, and the presence of some inhibitory compounds may make a specific cooling regime safe for one product but not for another.

Contamination of sliced cooked meat products with a *Lactobacillus sake* starter strain was suspected to cause spoilage in the products before the end of the expected shelf life. Results indicated that handling the fermented product in the refrigerating and packaging rooms together with cooked products was not the major cause of spoilage in these products (Bjorkroth et al. 1996).

Lammerding (2006) reported that quantitative modeling of the sources, prevalence, numbers, and behavior of food chain pathogens, such as *Salmonella*, and of the host/pathogen interaction and consequences provided scientific information and insights useful for decision makers in managing the safety of the food supply.

The role and accuracy of predictive food microbiology would increase as understanding of the complex interactions between microorganisms and food becomes clearer, the reliance of food microbiology on laboratory techniques and skilled personnel to determine process and food safety is still necessary (Karl McDonald and Da-Wen Sun, 1999). Leistner L. (2000) resulted that the food safety hurdle concept is an approach that

combines several inhibitory hurdles or stress factors that together can act synergistically to inhibit microbial pathogens.

Human infection is usually attributed to cross-contamination in the kitchen, inadequate cooking, and improper storage temperatures (Silliker and J. H, 1980). TG Lee et al. (1997) studied the relationship between the storage time and psychrotrophic organisms for refrigerated sausage and frozen sausage and showed that psychrotrophic organisms increased with increased storage time for the refrigerated sausage, and remained static for the frozen sausage.

1.2.9.4. *Staphylococcus aureus*:

S. aureus are aerobic, Gram positive cocci. Certain strains, typically referred to as coagulasepositive *S. aureus,* produce enterotoxins as a byproduct of their growth. These enterotoxins can cause a generally mild gastroenteritis in humans. Food-borne illness results from the ingestion of enterotoxin(s) that have been pre-formed in a food product. Therefore, conditions must exist that allow the organism to grow to high populations ($>10^6$cfu/g).

While *S. aureus* is part of the natural microflora of poultry, poultry-associated strains of *S. aureus* can be differentiated from human strains. The poultry-associated strains do not seem to be involved in food-borne disease (National Advisory Committee on Microbiological Criteria for Foods 1997). In terms of coagulase-positive *S. aureus,* these typically originate from humans. Therefore, employees (food handlers) are the primary source of *S. aureus* contamination in the processing plant. Most staphylococcal intoxications involving poultry products are related to recontamination of cooked product by food handlers, followed by improper holding temperatures (National Advisory Committee on Microbiological Criteria for Foods 1997).

1.2.9.5. *E. Coli*:

The combination of sulphur dioxide preservative and low temperature demonstrated the best efficiency against *E. coli O157:H7* survival. The study showed that *E. coli O157:H7* can be thermally decreased at 60 min by a temperature of 60 °C, 80 s at temperature, and 65 °C and 60 s at 70 °C (Charimba, C.J. Hugo and A. Hugo, 2009).

Nahed M. Ahmed et al. (2006) determined that the effect of fat level and low fat formulation on survival of *Escherichia coli O157:H7* isolate 204P (cell strain) heated in ground beef, pork sausage, chicken, and turkey, it resulted that higher fat levels in all products resulted in higher D-values so product composition affected lethality of heat to *E. coli O157:H7.*

1.2.10. Chemical Composition of Final Product.

The technical Regulations of the Identity and Quality of Mortadella and sausages were approved, establishing the limits to the addition of MDM. Table 1.10 presents data on

some of the characteristics of the products for which the use of MDM is allowed. This regulation allows the use of up to 60% of MDM in substitution of the meat raw material in some types of emulsified sausages. The problem with the use of large ratios of MDM in meat products is the low stability of this raw material, which is very prone to lipid and pigment oxidation as well as microbial growth.

Table.1.10 Some identity and quality characteristics of meat products containing MDM.

Products	Humidity[1] (max.) %	Fat[1] (max.) %	Calcium[2] (max.) %	MDM[3] (max.) %
Bologna	65	30	0.9	60
Bologna	65	30	0.3	20
Poultry bologna	65	30	0.6	40
Cooked sausages	60	30	0.3	60
Sausages	65	30	0.9	60
Vienna or Frankfurt sausages	65	30	0.9	60
Poultry sausage	65	30	0.6	40
Cooked hamburger	---	23	0.45	30
Cozida meat balls	---	18	0.45	30

Source: Beraquet (2000). [1]Fresh weight basis; [2]Dry weight basis; [3]Mechanically deboned meat of (animal species).

The content of the amino acid hydroxyproline is an index of the amount of connective tissue in a meat has been considered by several researchers. According to Babji et al. (1980), hydroxyproline contents was higher in the MDM of cooked hen (0.76g/100g of tissue) in comparison to the MDM of chicken back and neck (0.37g/100g of tissue), because cooking facilitates the removal of collagen. This report was confirmed by Hamm and Young (1983) who, next to other raw materials, compared the MDM of raw and cooked hens. There was a small elevation in the content of hydroxyproline for the cooked hen's MDM (0.38g/100g of tissue) in comparison to the raw hen's MDM (0.36g/100g of tissue). However, even with this increase, the content of hydroxyproline was close to that found for the MDM of chicken (0.30g/100g of tissue), unlike the values found by Babji et al. (1980).

FSIS decided that cholesterol was not an issue in mechanically separated poultry because this meat is primarily used as an ingredient in further processed products where cholesterol levels must be declared on the label. It was suggested that people needing to limit cholesterol in their diets would be able to make educated decisions based on product labels.

Sausage formulations use a bind index as an indirect measure of the amount of myofibrillar or salt soluble protein available for binding and are often expressed in arbitrary units of 0.0 to 1.0, 0.0 to 30.0, 0 to 100, or 0 to 1000. Irregardless of the number, bind indices are based on pre-rigor bull meat being assigned the highest bind index value (i.e., 1.0, 30, 100, or 1000) (Saffle 1968; Labudde 1993). Whole-muscle poultry and turkey have bind indices of approximately 90 (pre-rigor bull meat = 100) while MDP

may be only 50 to 60. The bind index along with color (the amount of myoglobin in lean tissue, 0.5 to 4 mg/g) and collagen (the amount of collagen in lean tissue, 2%) indices are combined with compositional characteristics (moisture, crude fat, total protein), processing constraints (fat content, moisture content, minimum bind, color or collagen value, etc.), and price constraints into least cost regression programs to formulate specific products such as frankfurters or bologna.

The two factors that affect WHC and binding ability of muscle tissues most are final pH (net charge of the myofibrillar proteins) after resolution of rigor mortis and the degree of contraction of the muscle tissues (steric effect) ((Hedrick et al., 1994). At ~pH 5.1 myofibrillar proteins have a net charge of 0 and retain the least amount of water. Ingredients and muscle conditions or treatments which tend to increase the muscle pH also increase the tissue's WHC. However, in muscle tissue exhibiting PSE characteristics, the denatured proteins do not respond well (no increase in WHC) to the increase in pH. Salt (NaCl) levels normally used in further processed or cured products (2 to 3%) increase protein solubilization and swelling to allow increased fluid retention. Alkaline phosphates in combination with salt and mechanical agitation in a mixer/blender, vacuum tumbler, or massager increase pH and myofibrillar protein extraction and solubilization.

The physical and chemical characteristics of poultry fat affect the processing characteristics of emulsified sausages and product stability. During the emulsification phase of processing, poultry batter temperature and chopping times should be monitored to avoid melting the fat globules. Skin content has influenced the functional properties of mechanically separated poultry (Froning et al., 1973; Schnell et al., 1973). Higher skin levels decreases emulsion stability and emulsion capacity, which are largely related to the higher fat content contributed by skin. However, Schnell et al. (1973) reported that higher skin content increased organoleptic tenderness of frankfurters.

There have been attempts by some researchers to texturize mechanically separated poultry. Acton (1973) forced mechanically separated chicken through a grinder path (cutting blade omitted) with a 4-mm orifice. The meat strands were heat set at 100°C for 1, 3, 5, 7.5, and 10 min. longer heating times and higher skin content increased the shear resistance. Although heating caused a loss of extractable protein, emulsion stability of extruded strands was improved. Lampila et al. (1985) also texturized mechanically separated turkey meat by extrusion and heat setting. They proposed its utilization in restructured meat products. Modifying mechanically separated poultry by centrifugation has been investigated (Froning and Johnson, 1973; Dhillon and Maurer, 1975). Centrifugation reduced the fat content and improved the water-holding capacity and emulsifying capacity. Commercial-scale centrifugal separators are available.

Several additives have been observed to affect functional attributes of mechanically separated poultry meat. Froning and Janky (1971) reported that salt preblending improved the emulsifying stability of mechanically separated poultry meat. Schnell et al. (1973) found that the addition of 3% sodium caseinate or 0.5% Kena (polyphosphate) increased the viscosity of frankfurter emulsions made from mechanically separated chicken meat. Froning (1973) chilled spent fowl in 6% polyphosphate prior to deboning

and observed improved emulsification stability and emulsion capacity of the resultant mechanically separated fowl meat.

McMahon and Dawson (1976) reported that a combination of 0.5% polyphosphate and 3% sodium chloride improved extractable protein, water-holding capacity, and emulsifying capacity of mechanically separated turkey meat.

The use of structured soy protein in combination with mechanically separated poultry improved textural attributes (Lyon et al., 1978a, b, and c). However; others have observed decreased emulsion stability of mechanically separated poultry with added soy protein (Janky et al. 1977).

During food processing and storage, polyunsaturated fatty acids tend to oxidize. Cholesterol can be oxidized as a result of the same mechanism as fatty acids. Therefore, lipid radicals formed during processing and storage of food, and also meat products, can accelerate formation of oxysterols (Chan et al., 1993; Ohshima et al., 1993). As previously shown different researchers oxidative changes of lipids are closely associated with cholesterol oxidation (Grau et al., 2001; De Vore V.R., 1988). Similar increases in oxysterol contents during refrigerated storage were reported by Kesava et al. (1996).

Zofia et al. (2001) resulted that Storage time influenced level of sum of total oxysterols, cholesterol, 7 β -OHC, 7 keto-C and peroxide value. Also, lipids peroxidation was significantly correlated with cholesterol, 7 β -OHC, 7 keto-C and total oxysterols, and acid number and TBA value was not correlated with cholesterol and oxysterols contents.

Free fatty acid (FFA), peroxide and TBA values are the most common chemical methods determining the storage stability and quality of a product. Oxidation of fatty acids primarily affecting the storage stability is mainly influenced by factors such as storage time, vacuum treatment, raw materials, conditions used for mechanical deboning and even by the particular carcass parts used (Love and Pearson 1971; Orr and Wogar 1979; Froning 1981; Crosland *et al.* 1995; Ertaş 1998). Although the effect of carcass part on the quality of further processed products containing MDM has been reported in other studies (Baker and Kline 1984; Pizzocaro *et al.* 1998).

Emulsified (comminuted) poultry products such as frankfurters, bologna, or loaf items are typically manufactured from chilled or frozen mechanically deboned poultry or turkey (MDP, MDT). These fully cooked products are more cost effective than their red meat counterparts, contain approximately half the maximum fat (30%) allowed by the USDA-FSIS, and are convenient to prepare. The USDA-FSIS permits up to 15% MDP or MDT in comminuted red meat products with the appropriate label declaration, but poultry products may contain 100% MDP or MDT as long as these conform to the CFR ((United States Department of Agriculture, 1999) specifications found in 381.173 and 381.174.

Red meat comminuted products, which are defined in the CFR (United States Department of Agriculture, 1999) 319.180 have a Standard of Identity with a maximum fat content of

30%. Added water in the finished product is limited to no more than 4 x meat protein content % +10% while the combination of added water + fat may not exceed 40% (this allows for varying amounts of fat and added water in the product, i.e., 5% fat + 35% added water = 40%). Additional binders such as soy protein concentrates and isolates are allowed at levels of 3.5 and 2.0% (dry weight basis, DWB), respectively, with appropriate label declarations. In comparison, poultry products do not have these limitations and have greater formulation flexibility with regard to meat type and content. If a product contains 50% or more poultry meat it may be labeled as a poultry item.

The main use of the MDM, a soft texture material, is in the production of a meat batter as, sausages and Mortadella. In annexes II, III and IV of Normative Instruction no. 04, of March 31, 2000 (Brasil, 2000).

The use of mechanically deboned poultry meat (MDM) has increased recently in the food industry because of the strong tendency to replace red meat for healthier white meat in industrialized countries, and the lower price of the latter compared with other kinds of meat (Daros *et al.* 2005).

Poultry production in Turkey has made great progress in the last decade with the increased production of cut-up parts for sale. In the formulation of comminuted meat products, MDM is widely used owing to its fine consistency and lower cost. The incorporation of MDM into emulsified meat products as well as into nonemulsified meat products in relatively low proportions has provided extra markets for meat from chicken frames and necks (Mielnik *et al.* 2002).

1.2.11. Sensory Evaluation for Final Sausage Product

In general, differences in the flavor (aroma and taste) of products added up to 20% of MDM are not noticed, making them even more acceptable at times for being softer and juicier. However, with storage, the change in the flavor is more quickly detected than in products with no addition of MDM. This change has been described by Baker et al. (1984) as a residual flavor of liver and has been carried out a sensorial evaluation of the global acceptance in a 9-point scale (9 = excellent and 1 = poor) for hamburgers elaborated with manually deboned meat of chicken thigh (GrTh) and mechanically separated Leghorn hens meat (MDSL), and reported that the sensorial panel rated 7.0; 6.0 and 5.2 the products elaborated with 100% GrTh, 50%/50% GrTh/MDSL and 100% MDSL, respectively.

As to the texture, products with over 30% of MDM are generally considered to present graininess (Field, 1988), due to bone particles.

However, there may be some drawbacks in the usage of MDM. A rapid onset of oxidative stability resulting in off-flavors and off-odors is a major problem for products manufactured with MDM (Macneil *et al.*, 1973; Lee *et al.* 1975; Mielnik *et al.* 2002).

Stability of turkey meat is also influenced by cooking, grinding and storage, and the combination of these factors results in considerable lipid oxidation. More sensory quality problems may be expected from cooked ground turkey than from other product forms if held under equal temperature and packaging conditions (Dawson and Schierholz, 1976; DeFremery et al., 1977; Klose et al., 1959).

The thawing process also has an effect upon the quality of muscle foods. When muscle foods are frozen prerigor, slow thawing seems to produce less drip. In flesh products, where texture is important, it appears that a slow thawing process at not too low a temperature is to be preferred. Thus, the practice of cooking unthawed, frozen muscle food may be convenient, but it is not preferred from a quality point of view (Jul, 1984). However, Fulton et al. (1967) reported no important difference between shear force values, "palatability" scores, and yield of cooked meat from whole turkeys cooked from the frozen state and those cooked from the thawed state. Ibbetson et al (1968) found that dark meat was tenderer when thawed before being cooked, but found no difference in flavor desirability or juiciness between turkey cooked from the frozen state and that thawed before being cooked. Engler and Bowers (1975) reported that turkey meat had similar amounts of moisture when cooked to an internal temperature of 80 C from the frozen, partially frozen and thawed states.

Stephens (1977) conducted an extensive survey on consumer acceptance of turkey cooked in roasting bags from the frozen state. The cooking bags used were manufactured from oriented polyethylene terepthalate, heat-shrinkable polyester having a melting point of 218-232 C. He reported that 1180 questionnaires were returned. More than 80% rated juiciness, tenderness, flavor, and texture of the turkey roasted in the bag "good" or "very good", and 90% who cooked the turkey from the frozen state found the roasting bag method more convenient than cooking methods previously used. Main objections to the cooking method were inability to stuff the turkey cooked from the frozen state, and inaccessibility of giblets until after the turkey was cooked.

The most significant visual result in curing is the development of a characteristic pink to pinkish-red coloration in the cooked end product. The native pigment myoglobin and its oxidized form metmyoglobin (as well as the corresponding pigments of hemoglobin) are converted initially to nitrosylmyoglobin, and with sufficient heating, to nitrosylhemochrome Fig.1.3. The intensity of color formed depends on the pigment concentration in the raw material. Poultry white meat contains from 0.1 to 0.4 mg of myoglobin per gram tissue whereas the dark meat has a concentration range from 0.6 to 2.0 mg/g tissue. Because mechanically deboned meat will contain some bone marrow, it will have higher pigment levels than the respective hand-deboned tissue (Froning and Johnson 1973) and thus yield higher color intensity. Because the USDA requires cured poultry products to reach an internal temperature of at least 68°C (155°F), the final pigment form is nitrosylhemochrome.

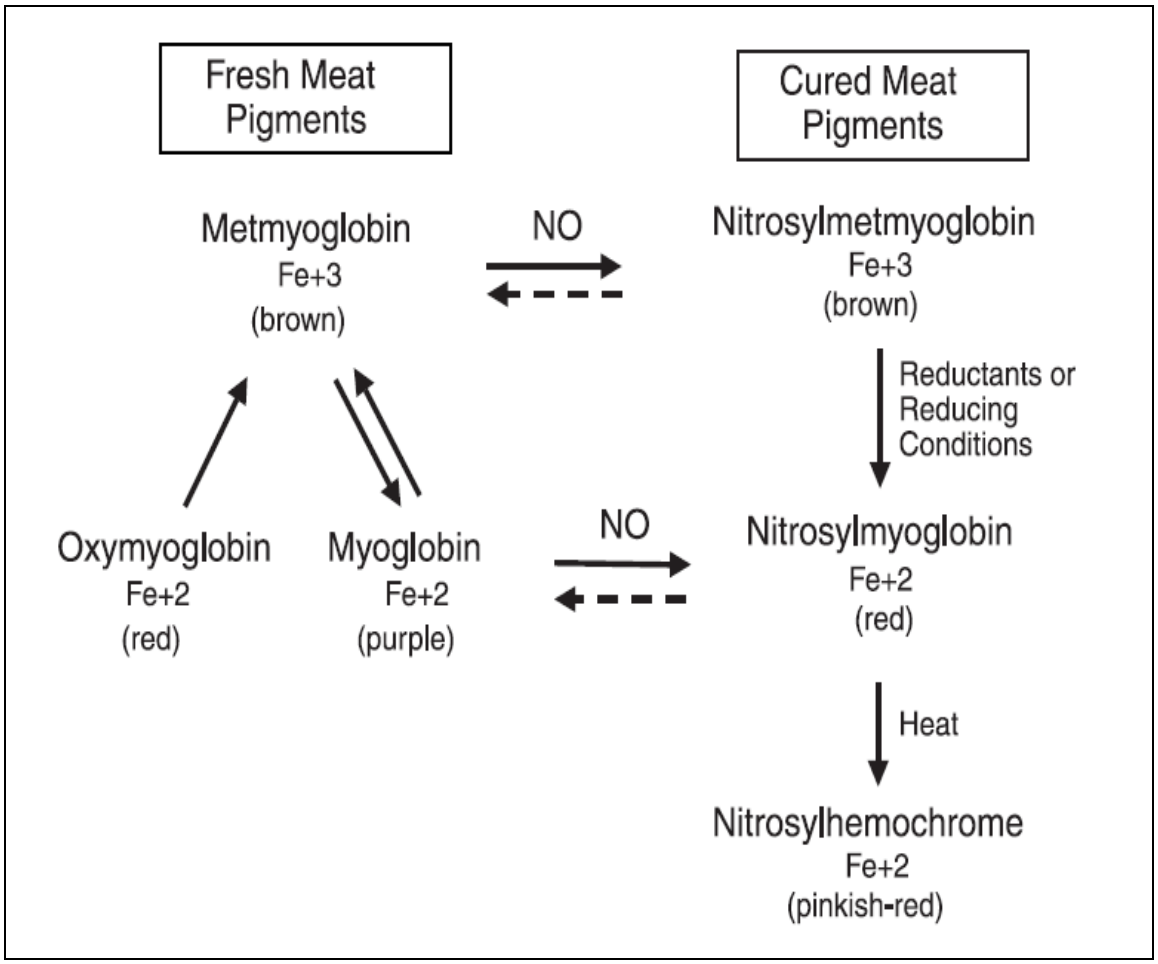


Fig.1.3 Nitric oxide (NO) reaction pathways from fresh meat pigments to form cured meat pigments leading to the final cooked pigment form known as nitrosylhemochrome.

The nitric oxide (NO) which reacts with myoglobin and metmyoglobin is formed from nitrite through a series of reactions Fig.1.4. Important factors in the rate of NO formation and its ultimate reaction with the globin pigments are meat pH and the presence of reducing conditions. The pH of post-rigor poultry ranges from approximately 5.7 to 5.9 for breast and 6.4 to 6.7 for dark meat (Bryan 1980).

Initially, equilibrium is established between the concentration of the nitrite ion (NO_2-) and its undissociated conjugate acid, nitrous acid (HNO^2). The slightly acidic environment of the meat keeps the equilibrium shifted toward NO^2- and provides only a low concentration of HNO^2. However, HNO^2 rapidly decomposes yielding NO. The decomposition thereby allows for the continual slow formation of HNO^2 and consequently, more NO formation over time. NO production from nitrite is enhanced by the addition of reductants, such as sodium ascorbate or erythorbate. The interaction pathway of nitrite and ascorbate is significant and complex (Skibsted 1992), involving several intermediates. The compound 2,3-dehydroascorbic acid is believed to be formed and provides for more rapid formation of nitrosylmyoglobin. In addition, the reductants serve to reduce metmyoglobin to myoglobin, which also accelerates the rate of curing.

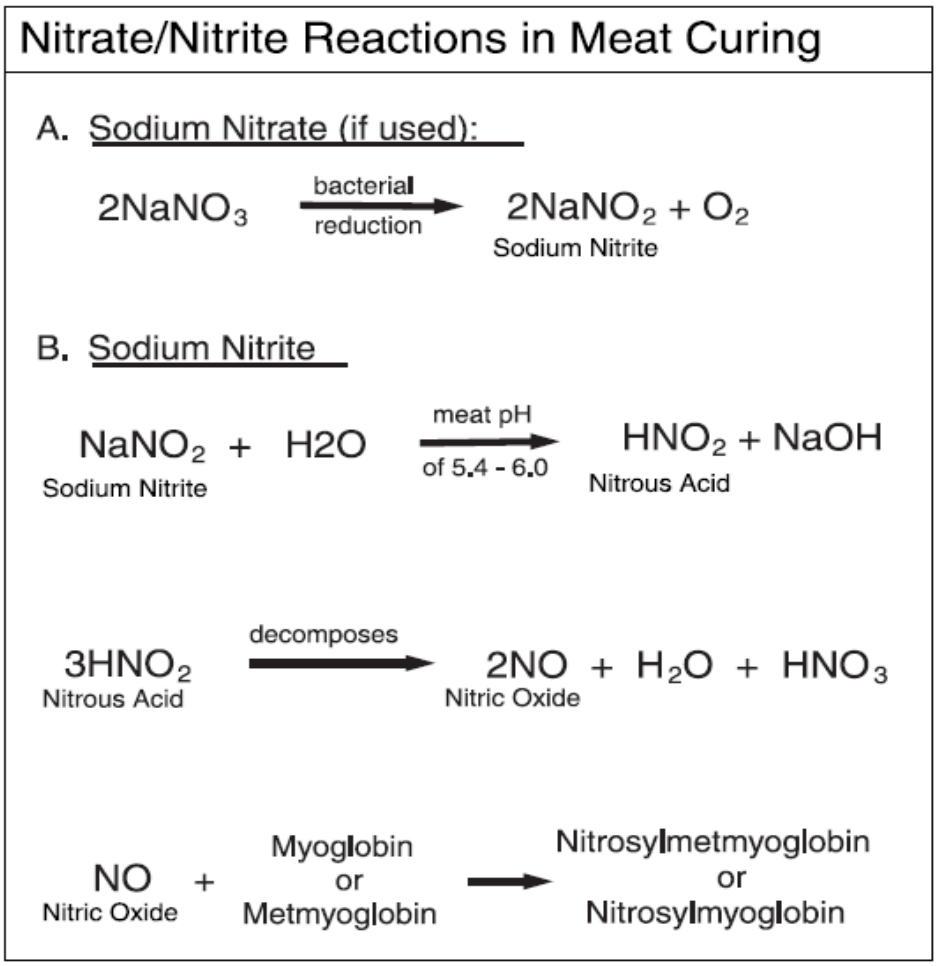


Fig.1.4 Reaction sequence from either sodium nitrate or sodium nitrite leading to the formation of nitric oxide (NO).

Nitrosylhemochrome undergoes rapid light-induced dissociation if the product is not properly packaged, resulting in "fading" or surface discoloration to a light gray appearance. Light and oxygen play a central role in a sequence of reactions initially involving NO dissociation from the central iron atom of the hemochrome's porphyrin structure Fig.1.5.

The dissociated NO is then free to recombine with the porphyrin, reforming the pigment and thus maintaining the pink to pinkish-red cured product color.

The reestablishment of the pigment is favored by vacuum-packaging with films of low oxygen transmission rate. The dissociated NO can also be oxidized by oxygen and thus not be available to recombine to form the original pigment (Kartika et al., 1998).

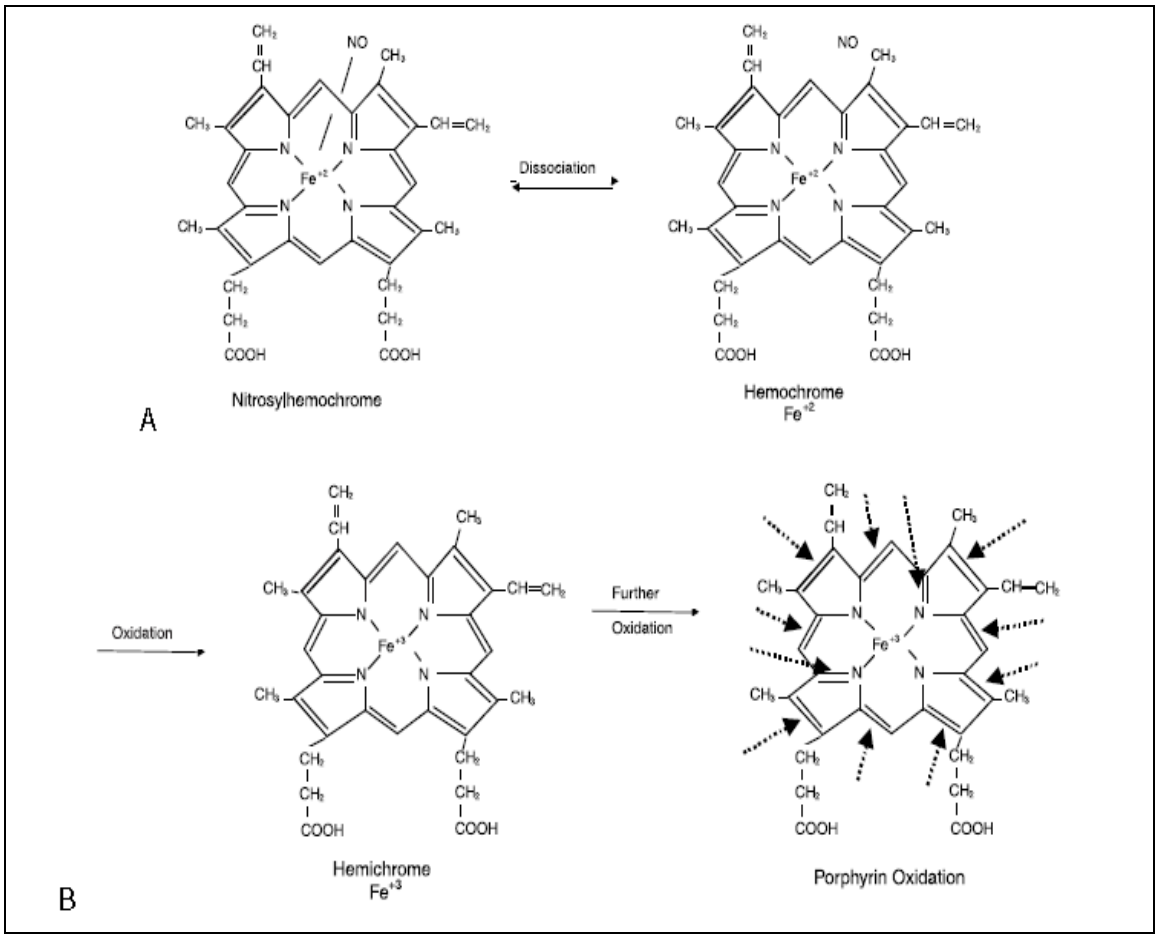


Fig.1.5 The dissociation of nitric oxide from the cured pigment nitrosylhemochrome and its sequential oxidation leading to "fading" of the cured product. (Reprinted from Kartika, S., Dawson, P. L., and Acton, J. C., Act. Rep. Res. Dev. Assoc., 51, 293-299, 1998. With permission of Research & Development Associates for Military Food and Packaging Systems, Inc., San Antonio, TX).

In extensive cases of fading, additional oxygen permeating the film is then free to oxidize the central iron of the hemochrome (Fe^{+2}) resulting in production of a hemichrome (Fe^{+3}). This loss is visually detectable as lightening of the product with a distinct loss of the red hue.

Under conditions of extreme oxidation, sites of the hemichrome's structure are oxidized, and the product appears severely bleached. Visual light-induced fading can be minimized by selection of packaging films with oxygen transmission rates generally in the range of 15 to 17 cc/m^2/24h (at 23°C, 0% RH, 1 atm); Fig.1.6.

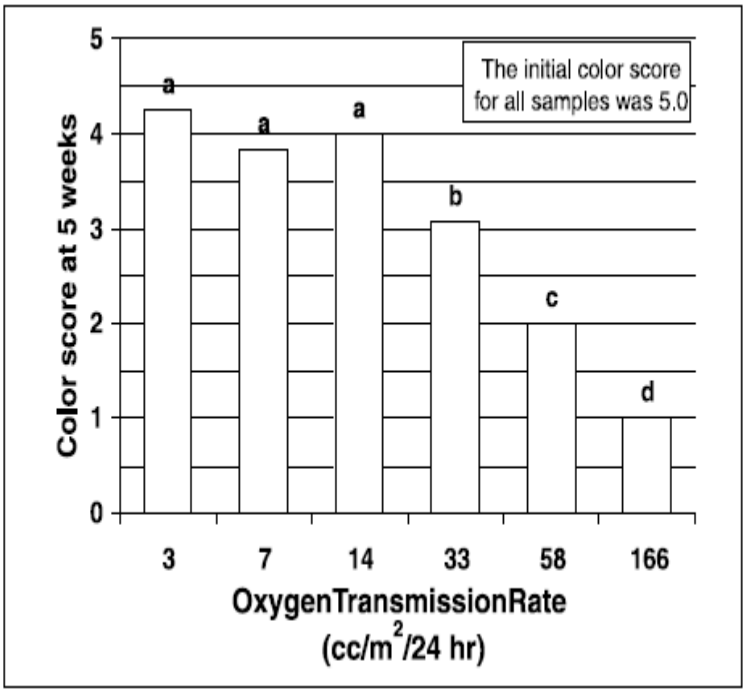


Fig.1.6 Visual color scores at five weeks of lighted display for turkey bologna vacuum-packaged in films differing in oxygen transmission rate. (Bars having a different letter are significantly different at $p < 0.05$).

In most cured, deli-style poultry products, adequate packaging is utilized for color retention, whether the product is vacuum-packaged or modified atmosphere packaged (MAP) with oxygen exclusion. In some MAP systems, the oxygen absorber is placed in a sachet within the package and fastened underneath the label or at the bottom of the package.

With in-going nitrite concentrations of 156 ppm, the average residual nitrite level found in products after a week or so of storage is of the order of 10-fold lower than the initial concentration. The small residual level also provides a potential source for slow NO generation which aids in cured color maintenance.

There is a significant difference between flavors of most cured products when compared to their uncured counterparts. Although cured meat flavor development attributable to nitrite has defied complete definition, a simple test of tasting a slice of cooked breast meat and comparing it to a slice of cured, cooked breast meat reveals its unique sensory characteristics. The elucidation of the volatile and non-volatile compounds that are responsible for the cured flavor is still on-going. Part of the flavor difference appears to relate to the decrease in the rate lipid oxidation that occurs post-heating. For example, hexanal is an oxidation product that was reported at a concentration of 9.84 mg/kg in cooked, uncured chicken and at only 0.11 mg/kg when cured (Ramarathnam, 1998). The main hypothesis for nitrite's antioxidant activity is that it prevents release of Fe^{+2} from heme-containing pigments during cooking since it has reacted in the formation of nitrosyl-derivatives, such as nitrosylhemochrome.

Other mechanisms possibly include nitrite reaction directly with non-heme Fe^{+2} and nitrite stabilization of unsaturated lipids of muscle cell membranes (Skibsted, 1992). Although the safety of nitrite has been questioned in the past, there is no known substitute that can impart the characteristic flavor associated with curing (Gray et al., 1981). In some cured products, particularly those flavored by seasonings such as salamis and frankfurters, nitrite does not seem to be so clearly related to flavor as long a salt is used in the formulation. However, nitrite's importance in preventing lipid oxidation during storage is clearly evident in that poultry frankfurters and other cured luncheon-type products do not readily develop rancidity.

CHAPTER TWO

MATERIALS AND METHODS

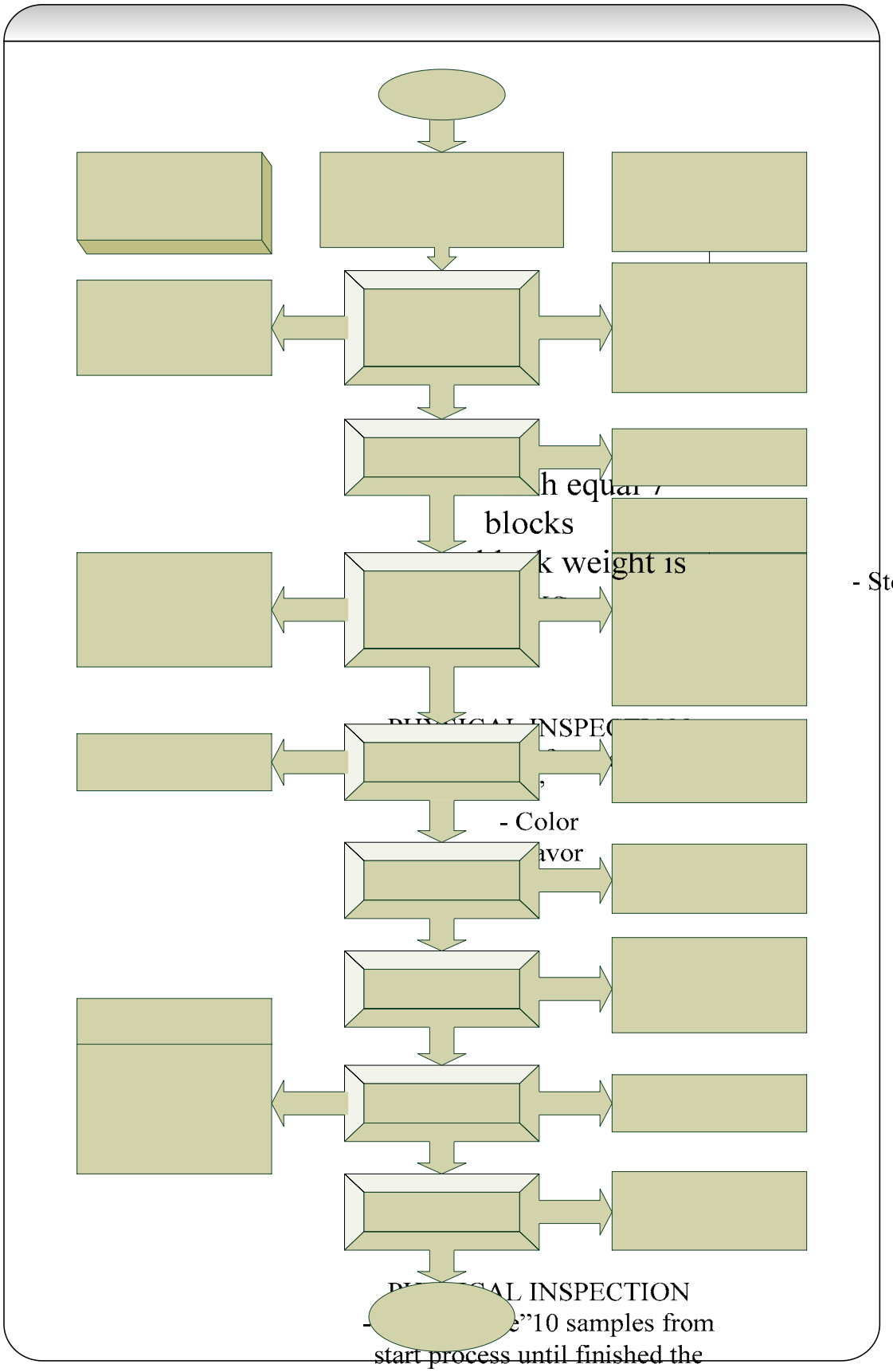
Chapter Two Materials & Methods

2. Introduction

To study and evaluate the quality and safety factors of frozen mechanically deboned meat, frozen chicken skin, pasteurization process for samples, and final product. Investigation under storage in term of; number of microbial, chemical, and sensorial analysis were used. PH indicator for peroxidation of lipids in the MDM and chicken skin stored under freezing condition, microbial inspection which determines the important factor on stability of raw meat, was also used. Microbial inspection for thermal process with changing in temperature and time. Sensory panel test was also investigated for final product through storage.

All analytical procedures were carried out according to: AOAC, FDA (BAM), COFRAC – (AFNOR Norms) this chapter will consider the experimental design (including sampling, materials used and other methods used in this investigation).

To obtain high quality results for this investigation, process description must be within high quality and under strict control and this is applied as described in Fig.2.1.


blocks
- Color
- Flavor
start process until finished the
batch quantity ”
- Color
- Flavor

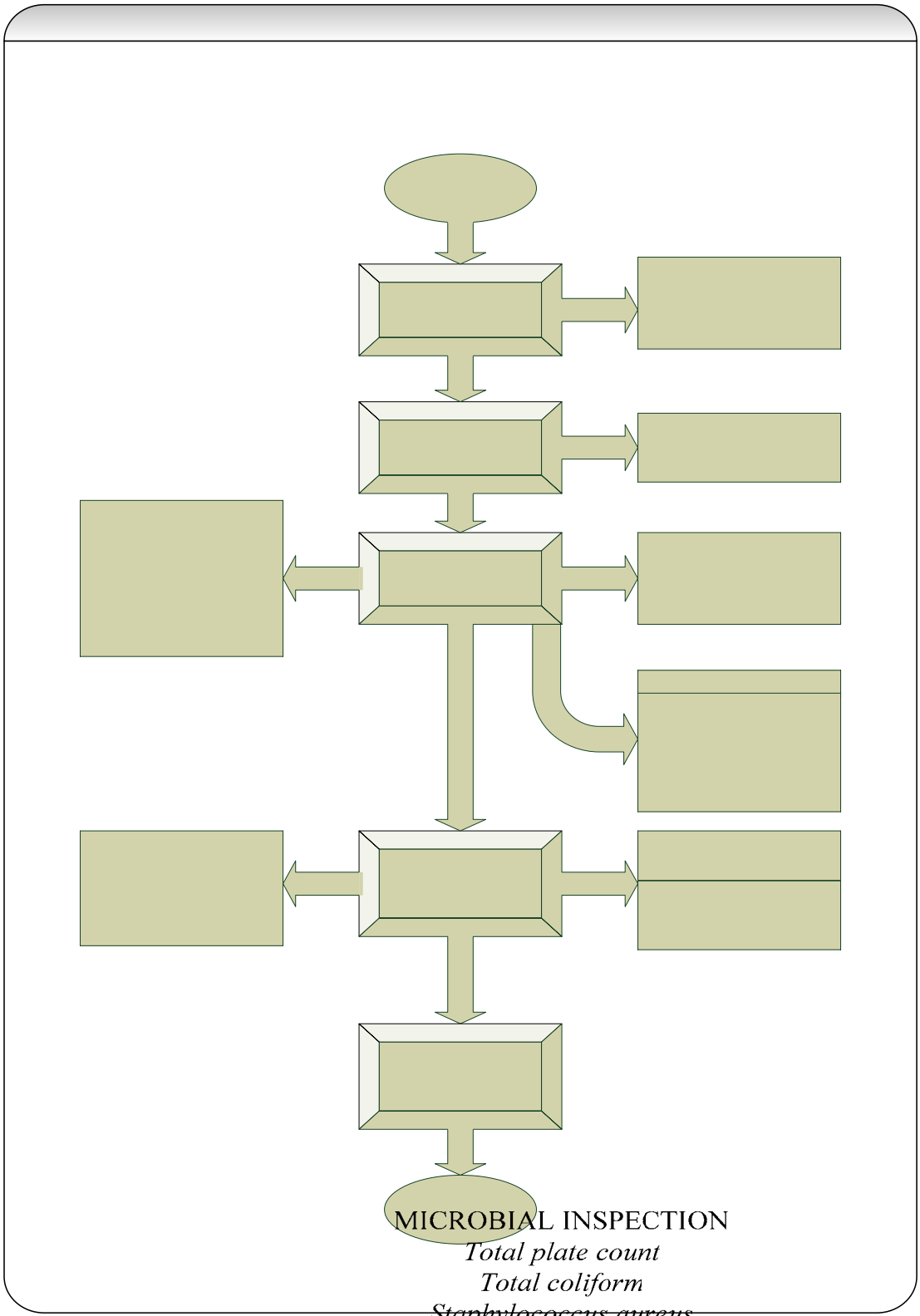


Fig.2.1 Flow chart for experimental design and sampling.

2.1. Experimental Design

The experimental design of this investigation was based on four categories as follows:

First: Frozen minced mechanically deboned meat and frozen chicken skin samples were taken under aseptic conditions, and then stored under different temperatures (-1, -8, -14, and -17 °C) through 15 days in intervals of 3 days (3, 6, 9, 12, and 15).

Second: Final product stored at factory refrigeration temperature 0-4°C after cooking. Different factors like oven temperature (74, 77, 80, 83, 86, and 89°C), core temperature (64, 67, 70, 73, 76, and 79°C), and holding time (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 minutes) were used as investigated factors.

Third: The work of analytical test were periodically done "monthly" (1, 2, 3, and 4 months) through storage at factory refrigeration temperature 0-4°C to determine the shelf life as approved by Palestinian standards for meat sausages (PS1-2006).

Fourth: Through storage the sensory panel test was applied for investigated samples every 15 days by standard procedure during the life time of the investigation (4 months).

2.2. Sampling

Samples were taken after mincing and filled in sterilized plastic bags with frozen samples from minced mechanically deboned meat and frozen chicken skin under aseptic conditions and directly stored at different freezing temperature during 15 days, after that the manufacturing steps under good manufacturing practices and personal hygiene conditions to reach the cooking process was followed.

Through pasteurization different factors were determined, like; oven temperature, core temperature, and holding time for samples. 30 sample were taken for microbial and analytical procedures tests.

Through storage at 0-4°C sensorial analysis performed by special five experienced persons from Palestinian Standards Institute according to standards limits. And also chemical analysis for storage samples were taken every month during 4 months.

2.3. Materials

Different materials used in formulation of sausage (Mortadella) for this study as mentioned in Table 2.1, (p.66) part of investigation was applied mainly on mechanically deboned meat (MDM) and chicken skin.

Mechanically deboned meat MDM were obtained from a local poultry slaughterhouse, and were processed by using an extraction mincer. The analytical and microbial limits for the MDM was adopted depend on Palestinian standards (PS1-1031).

Table.2.1 Ingredients used in formulation of mortadella emulsion.

Ingredients	Percentage %	Supplier
MDM	70	Palestine – Beer Nabala region - HALAL Company
Chicken skin	7.8	Palestine – Beer Nabala region - HALAL Company
Water or Ice	11	Ramallah station
Tapioca Starch	5	KMC TAPIOCA STARCH CO. LTD – Vietnam
Soya Protein 70	3.5	SOLBAR Company -Israel
Spices	1.2	Israel –Frutarom Company
Salt	1.162	Israel –Kallia Company
Sodium Nitrite	0.018	S. C. Deli Company - Romania
Beet root Color	0.17	Deli international S . R . l. Iasi. Romania
Smoke Solution	0.1	Red Arrow Products company LLC–Manitowoc-U.S.A.
Sodium Erythrobate	0.072	PMP Fermentation product. INC. – Peoria - USA

2.4. Methods

All methods used and practical work were carried out in laboratory and production unit of Salwa Food Company /Ramallah-Palestine and chemical analysis was applied in Food Technology Lab/ Food Technology Department/ Al-Quds University.

2.4.1. Mortadella Emulsion Formulation

Frozen (MDM) blocks were cut in slices by meat breaker, Table 2.2 describe models for all machines used for mortadella emulsion formulation. The frozen MDM pieces (2-10 cm thickness) chopped in bowl cutters without previous thawing thus avoiding drip losses, bacterial growth and discoloration which would happen during thawing.

Chicken skin was cut in cubes of 2-4 cm thickness to facilitate the Subsequent chopping in mincer. Pieces of frozen slices "MDM" and chicken fat were reduced in size by passing them through meat mincer machine; minced meat was chopped by mixer with all ingredients.

Emulsion was passed through emulsifier machine to reduce the size of the meat particles, also to achieve a very fine texture. The pH of emulsion was between 5.85 - 6.3 and moisture content not exceeded the 65%, with temperature lower than 0˚C.

Polyamide plastic casing were soaked in cold water, (max. 18˚C) to prevent shrinkage, wrinkling of casing, giving favorable stuffing, and prevent losses of product seam (not hermetic condition) during cooking process.

The emulsion was stuffed under vacuum of 90% around 1kg weight into impermeable plastic casings (115mm-wide). Emulsion meat was filled in hopper and extrude to internal polyamide plastic casing, then, the casing was sealed by metal clips in each side by clipper machine.

Filled washed casings were cooked directly by steam oven until temperature reached 73°C (approx. 130 minutes for1kg) and this was controlled by calibrated thermocouple to monitor the core temperature during the cooking process. Product then cooled under a shower until the internal temperature dropped to room temperature (within approx. six hours) to prevent shrinkage and wrinkling of the product casing also to reduce probability of *Clostridium perfringens* growth. Samples then stored at 4ºC in dry condition, this process enhanced the flavor, color, texture, produced the desired final product and inhibiting the bacterial growth which is responsible for spoilage. Fig.2.2 describes the main steps with additional ingredients used to process meat sausage (mortadella) manufacturing.

Table.2.2 Machines used for manufacturing of meat emulsion.

Machine Name	**Model**
Breaker	MAVURIT-554
Mincer	SIMO-300
Mixer	AMFEC-510
Emulsifier	KS-225
Stuffer	VEMAG-DP10
Clipper	POLY CLIP FCA-3462
Steam Oven	FESSMAN-T3000
Thermocouple	HI-145

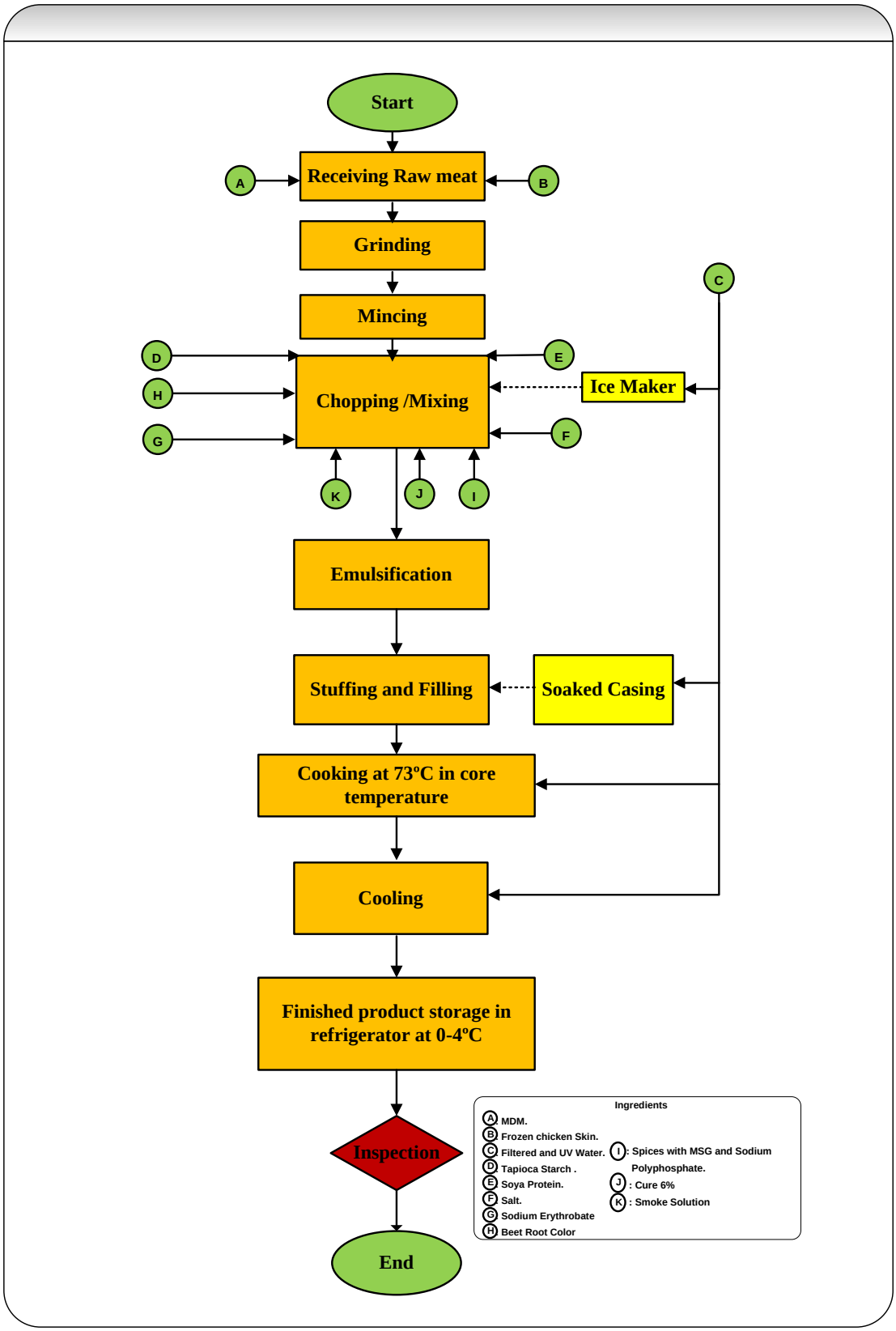


Fig.2.2 Flow chart of processed meat sausage (mortadella) production unit operation.

2.5. Analytical Procedures

2.5.1. Chemical Analysis.

Through this investigation samples were examined for moisture content, fat content, ash content, protein content, peroxide value, and residual nitrite. All the chemical and analytical procedures are shown in Appendix 1.

2.5.1.1 Moisture Content:

The determination of the moisture content is done by drying an appropriate amount of the sample. The difference in weight between the fresh and dried samples represents the moisture content. For rapid determination of moisture content a microwave oven was used (AOAC No. 985.14).

2.5.1.2 Fat (Crude fat) in Meat and Meat Products:

Determination of the fat content is the most complicated component of simple meat and meat product analysis, as analytical equipment is needed. Samples for fat analysis are semi-dried before being subjected to ether-extraction using the Soxhlet apparatus. After complete extraction, the fat is obtained by evaporating and recovering the ether (AOAC No. 985.15).

2.5.1.3 Ash in Meat:

The defatted samples are then used for ash analysis by subjecting it to a temperature of around 600°C in a muffle furnace for two hours. The weight of the ash is used to calculate the minerals content in percentage (%) (weight of ash, divided by total sample weight, multiplied by 100) (AOAC No. 920.153).

2.5.1.4 Crude Protein:

The protein content is determined at laboratory level by using the Kjeldahl method, where meat products are digested by acid to obtain the nitrogen compounds and then distilled and titrated to determine nitrogen quantitatively, with which the protein component can be calculated. In a simplified approach protein is not chemically determined, but can be calculated (approximately) as the remaining component, after water, fat and ashes content has been determined and subtracted from 100%. This simple mathematical method should only be applied for pure meat and meat products as it is not accurate for highly extended products containing non-meat ingredients such as grains, starches or vegetables. In the case of using meat extenders and/or fillers, the result reflects the organic non-fat component (protein and carbohydrates in %) of the product (AOAC No. 981.10).

2.5.1.5 Peroxide Value:

Peroxide value is an indicator for evaluating the early stages of the degradation of triglycerides by oxidation which causes the appearance of odorous compounds (aldehydes, alcohols, ketones) these compounds alter the flavor of oil and lead to rancidity (Boskou, 2006). Oxidation of oil is caused firstly by contact with atmospheric oxygen, then light (UV) and heat which act in the initiation stage of oxidation. Finally, metals such as iron and copper operate at extremely low levels in catalyzing the oxidation process (Baldassari, 2008), (AOCS-AOAC No. 965.33).

2.5.1.6 Residual Nitrite:

Level of nitrite (mg $NaNO_2$/kg sample) was determined in agreement with standards (Codex-Adopted-AOAC No.973.31).

2.5.2 Physical Analysis.

Physical agents were examined, namely; temperature and pH values, throughout the investigation. All the physical procedures are shown in Appendix 2.

2.5.2.1 Temperature:

Temperature measurement by using thermo-elements/thermocouples which based on the thermo-electrical effect was used to determine temperature tested.

2.5.2.2 PH:

PH measurement is useful for evaluation of meat quality for further processing, in particular acidity and the water holding capacity.

2.6. Microbiological Inspection

The major factor in this investigation was the ability of microorganisms on growth. This is in correlation related the time and temperature. For the final product enumeration of *Total Plate Count*, enumeration of *Total Coliforms*, enumeration of *Clostridium perfringens*, detection and enumeration of *Staphylococcus aureus*, detection of *Salmonella, and* detection of *Listeria monocytogenes* were taken into consideration. While for frozen mechanically deboned meat and chicken skin, enumeration of *Total Plate Count*, enumeration of *Total Coliforms*, and detection of *Salmonella* were suggested, this is in accordance with PSI recommendations, All the microbiological procedures are shown in Appendix 3. According to COFRAC – (AFNOR Norms).

2.7. Sensorial Analysis

The organoleptic properties of mortadella (color, flavor, smell, and texture), were determined by a five experienced persons from the Palestinian Standards Institute. They evaluated the product according to the hedonic scales (Appendix 4).

2.8. Chemicals and Apparatus:

Although the Appendices show the procedures used for either tests applied in this investigation, however, the major chemicals and apparatus used are shown in Table 2.3 and Table 2.4, respectively.

Table.2.3. Major Chemicals used for microbial tests.

No.	Media	Source	Specifications
1	Nutrient Agar Total Plate Agar	Oxoid or HY-Lab, HI-Media	*Total plate count*
2	Violet Red Bile Agar	Oxoid or HY-Lab, HI-Media	*Total Coliform*
3	Baird-Parker Agar with Egg Yolk Tellurite emulsion	Oxoid or HY-Lab, HI-Media	*Staphylococcus aureus*
4	Palcam agar with PALCAM Selective Supplement plates	Oxoid or HY-Lab, HI-Media	*Listeria monocytogenes*
5	Hektoen with Rapapport-Vassiliadis Enrichment Broth	Oxoid or HY-Lab, HI-Media	*Salmonella*
6	Sulphite Polymixin Sulphadiazine Agar	Oxoid or HY-Lab, HI-Media	*Clostridium perfringens*

Table.2.4. Major Apparatus used for microbial tests.

Instrument Name	Model
pH meter	HI-221
sterilizer	EA-653 AUTOCLAVE
stomacher	BAG MIXER-400
Water Bath	WATER BATH P-SELECTA
Incubator	INCUBATOR P-SELECTA
Hy-enterotest	Hy-Labs

CHAPTER THREE

RESULTS AND DISCUSSIONS

Chapter Three Results and discussion

3. Introduction:

The obtained results were presented below; however each studied factor is discussed separately in order to determine how these factors are affected by good manufacturing practices, namely; personal hygiene, environmental conditions, and prerequisite programs available for production (mainly include mechanically deboned meat and chicken skin) also to verify the effect of thermal processing on final quality, safety, and susceptibility of end products.

Results shown of this investigation were based on the four categories composing this research sectors, as follows:

First: Frozen minced mechanically deboned meat and frozen chicken skin samples taken under aseptic conditions, and then stored under different temperatures (-1, -8, -14, and -17 °C) through 15 days in intervals of 3 days (3, 6, 9, 12, and 15).

Second: Final product stored at factory refrigeration temperature 0-4°C after cooking. Different factors like oven temperature (74, 77, 80, 83, 86, and 89°C), core temperature (64, 67, 70, 73, 76, and 79°C), and holding time (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 minutes) were used as investigated factors.

Third: The work of analytical test periodically done monthly (1, 2, 3, and 4 months) through storage at factory refrigeration temperature 0-4°C to determine the shelf life as approved by Palestinian standards for meat sausages (PSI-2006).

Fourth: Through storage the sensory panel test was applied for investigated samples every 15 days by standard procedure during the life time of the investigation (4 months).

3.1. Influence of Storage Time of MDM on Microbial Growth:

Each factor of storage time and temperature was studied separately for individual type of microorganism, namely; *Total Plate Count, Total Coliforms*, and *Salmonella* growth. These results will be used as good indicator for quality, safety, and good hygiene.

3.1.1. Influence on *Total Plate Count* Growth:

Results in Fig.3.1 showed that, the *Total Plate Count* growth in MDM increased significantly with increasing the period of storage time under sequential temperatures. This is due to the fact that, the storage time and temperature for MDM act as the main factor for increasing the potential pathogen risk of MDM and directly effect the final product quality.

These results were in agreement with results previously obtained by Kumar et al., (1986) who concluded that, the large surface area, the higher release of cellular fluids and the heat generated during mechanical deboning all enhance bacterial count and growth. Also as reviewed by Pooni and Mead (1984); Daud et al. (1978); Barnes (1976); and Ayres et al. (1950) who investigated that, the most important factor affecting psychrotrophic bacterial growth and hence, the shelf-life of poultry products as MDM is holding temperature. As storage temperatures were reduced, the shelf-life of carcasses was extended. Moreover, Baker et al. (1956) reported that the temperature and time of storage are related to shelf-life.

As reviewed by European Union (1997) meat quality can be judged very well by the quality of microbes. Generally it can be said that if the number of bacteria on the surface of meat exceeds 1×10^{8} cfu/g, the meat is unfit for human consumption. If the microbe count is 1×10^{7} cfu/g the meat is of poor quality. Even that kind of raw material should not be used as raw material for MDM.

Furthermore Fig.3.1, shows sharply increase in *Total Plate Count* growth in MDM by 12 days of storage at -1°C storage temperature.

This finding was in agreement with results of Elliot and Straka (1964); Spencer et al. (1961); Spencer et al. (1955); and Newell et al. (1948) who observed that, there were no major increases or decreases in shelf-life and spoilage between unfrozen and thawed chicken meat.

This finding is also in agreement with results previously obtained by Dainty and Mackey (1992) who determined that proteolysis and slime production under aerobic conditions begins when bacterial numbers reach 10^{7}-10^{8} cfu/g.

At the same time, a slow growing level indicated at 3, 6, and 9 days of storage temperatures at -8 and -14°C. This is due to the exponential bacterial growth with time under constant temperature. Therefore, MDM storage must be controlled under strict conditions of time and temperatures.

These results were in agreement with Brown (1957) who found that, Initial bacterial load immediately after processing has also been shown to affect shelf-life; also Brown (1957) demonstrated that an increase in the initial bacterial load results in a concomitant dramatic decrease in shelf-life. This effect is due to the fact that much less time is required for bacterial populations to reach numbers that are high enough to produce spoilage defects when bacteria are high in number initially.

The best conditions for storage of MDM were under temperature -17°C, as noted in Fig.3.1 regardless specific times. This finding was scientifically justified, as the higher temperature of freezing, the lower total number of bacterial growth.

This finding was conformity with Ayres et al. (1950) who found that, when fresh poultry is placed in a cold environment, conditions for replication of most species of bacteria are

no longer optimal, this decrease was due to the followings: (1) the unsuitability of the temperature for reproduction and survival of chromogenic bacteria (pigment producers) and mesophilic bacteria, and (2) insufficient time for psychrotrophic bacteria to begin the exponential phase of growth.

This is in agreement with Mazur (1984); Hartsell (1951); and Sair and Cook (1938) who reviewed that, when cells are frozen rapidly, both intra and extracellular fluid freezes. However, when cells are frozen at a slow rate, an intra- and extracellular osmotic gradient occurs due to freeze concentration. This may result in cellular disruption.

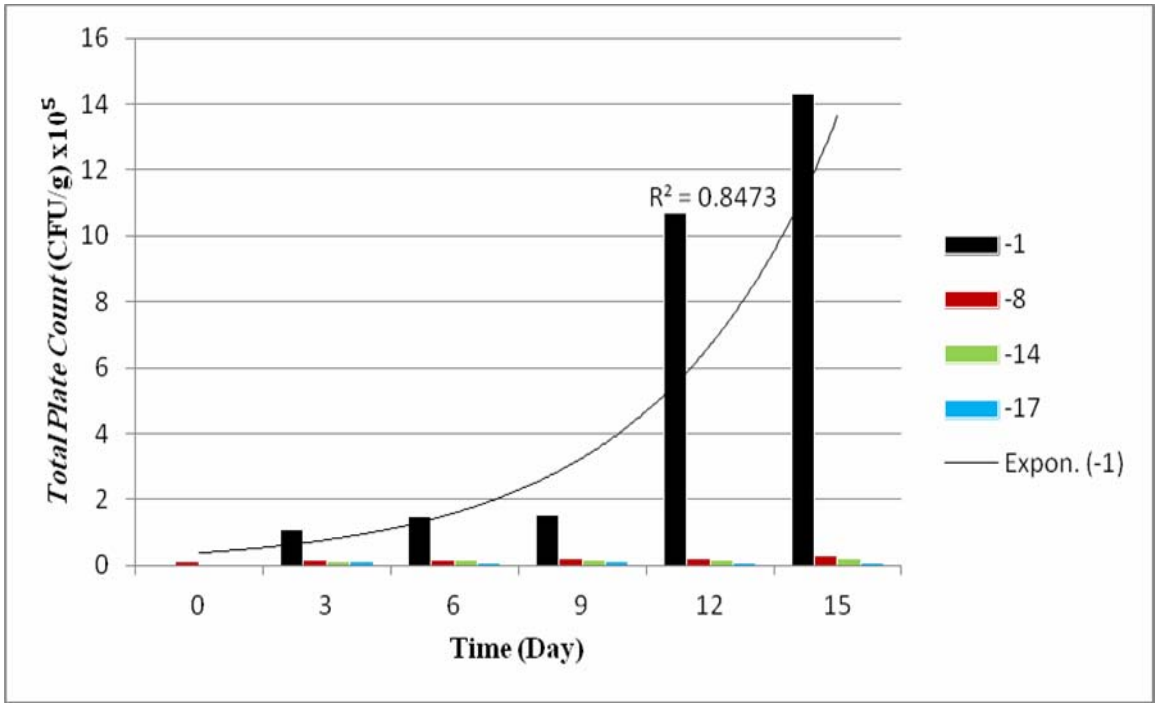


Fig.3.1 *Total Plate Count* growth of MDM storage under different temperatures.

3.1.2. Influence on *Total Coliforms* Growth:

Results obtained in Fig.3.2 show that the *Total coliforms* growth in MDM increased normally with increasing the period of storage time under sequential temperatures. Storage time and temperature of MDM work as main factors for increasing the potential pathogen risk for MDM which is directly related to the quality and environmental conditions of MDM manufacturing.

This result was confirmed by Alan (2001); Ayres et al. (1980); Greene and Jezeski (1954); and Olsen (1947) who observed that, the higher receiving temperature means a potential decrease in shelf-life of the product, and when considering the relationship of temperature to microbial life, two factors must be considered: the holding temperature of the microorganism and the time the microorganism is exposed to that temperature. Generation time indicates the speed of cell division; whereas, maximum cell population takes into account cell death as well as cell production.

Results plotted in Fig.3.2 also showed that at -1 and -8°C storage temperature, the *Total coliforms* growth was increased gradually in MDM for all times investigated. This increase was much more than other storage temperatures (i.e. -14 and -17°C) at the same storage times, this is in comparison to the related initial number of *Total coliforms* at temperatures of -1 and -8°C.

This finding was in accordance with Alan (2001); Hedrick et al. (1989); Ingraham (1958); Zobell and Conn (1940); and Muller (1903) who investigated that, materials should not be above 4.4°C when received and frozen materials should be below -17.8°C. The least desirable temperature for holding frozen meat trimmings is -11.1 to -10°C, which is the point of phase transition between intercellular crystalline ice and a combination of ice and water.

While, many bacteria responsible for spoilage they are able to survive and multiply at low temperatures. The temperatures that are optimal for growth are well above freezing.

As also noted in Fig.3.2, the best conditions for storage of MDM in term of *Total coliforms* growth was under temperature -17°C, regardless storage time. This finding was approved scientifically as, the lower storage temperature, the large increasing in microbial growth.

This finding was in agreement with Hedrick et al. (1989) who reported that, Poultry muscle that is frozen and held at -17.8 to -28.9°C should retain its quality for 6 to 10 months, moreover Berry and Magoon (1934) observed that, under specific conditions, moderately cold storage temperatures (-2 to -4°C) may negatively impact bacteria to a greater degree than storage at -20°C. Finding of this investigation was agreement with Mazur (1984); Hartsell (1951); and Sair and Cook (1938) who reviewed that, when cells are frozen rapidly, both intra and extracellular fluid freezes. As a result slower freezing resulted in higher growth.

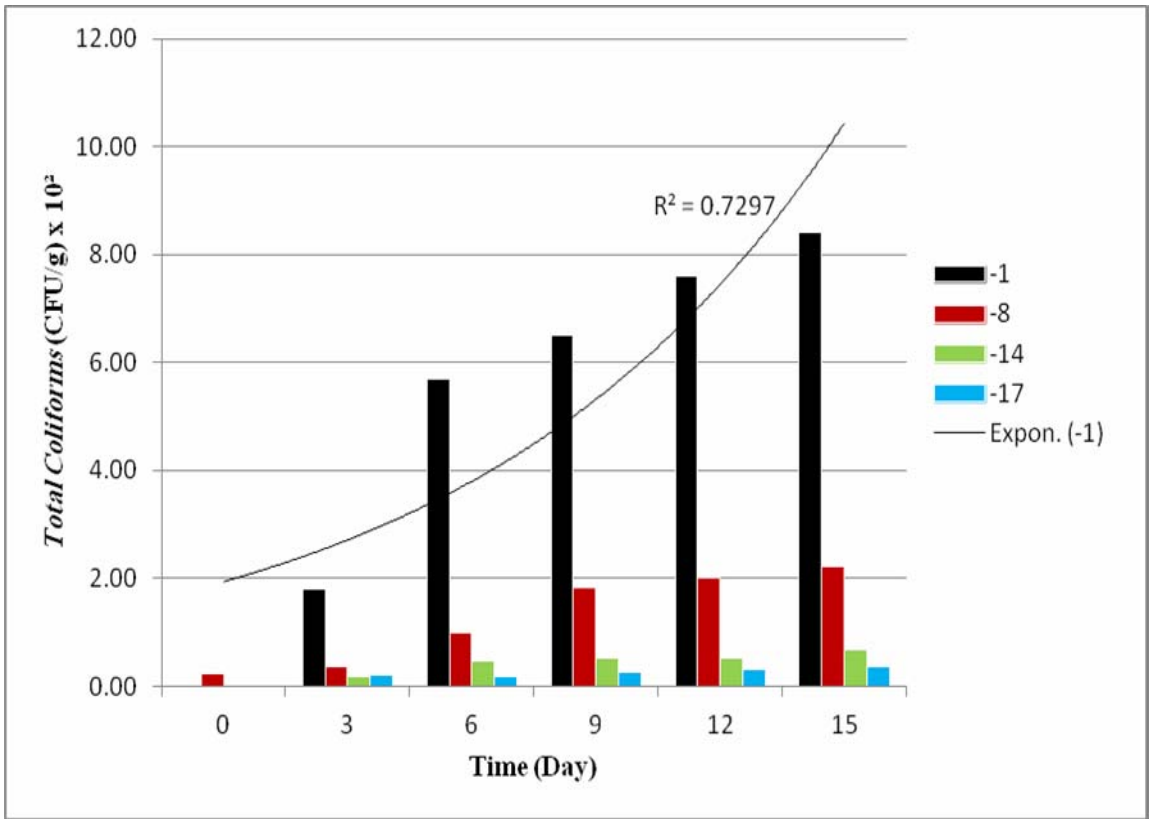


Fig.3.2 *Total coliforms* growth of MDM storage under different temperatures.

3.1.3. Influence on *Salmonella* Growth:

The presence of pathogen *Salmonella* in MDM was shown in Table (3.1). It was found that all storage conditions under investigated times and temperatures was the reason behind the presence of *Salmonella.* This result is explained as, the specified times and temperatures are not suitable and not enough to kill or to inhibit the *Salmonella* growth. However, there is a need for addition preventive control steps for killing *Salmonella* at the stage of final products in term of thermal application.

Table.3.1 Influence of storage time of MDM under different temperatures on *Salmonella* growth.

	Storage temperature (°C)			
Storage time (Day)	-1	-8	-14	-17
	Results			
0		+		
3	+	+	+	+
6	+	+	+	+
9	+	+	+	+
12	+	+	+	+
15	+	+	+	+

positive result; presence of *Salmonella*.

This result as shown in Table 3.1 was in agreement with results previously obtained by Wakefield (1999); Byrd et al. (1998); National Advisory Committee on Microbiological Criteria for Foods (1997); European Union (1997); Bailey et al. (1987); and Rigby et al. (1980) who concluded that, the probability of presence of pathogen *Salmonella* in MDM was very high and this return for different sources through and before processing.

3.2. Influence of Storage Time of Chicken Skin on Microbial Growth:

3.2.1. Influence on *Total Plate Count* Growth:

Results shown in Fig.3.3 review that the *Total Plate Count* growth in chicken skin increased normally as exponential with increasing the period of storage time under sequential temperatures. This relationship depend on mainly the initial number of microorganism in addition to Storage time and temperature of chicken skin which work as main factors for increasing the potential pathogen risk for chicken skin which is directly related to the quality and environmental conditions of chicken skin manufacturing.

This result was in accordance with results previously obtained by Kumar et al., (1986) who concluded that, the large surface area, the release of cellular fluids and the heat generated during chicken processing, all enhance bacterial count and growth, also as reviewed by Pooni and Mead (1984); Daud et al. (1978); Barnes (1976); and Ayres et al. (1950) who investigated that, the most important factor affecting psychrotrophic bacterial growth and hence, the shelf-life of poultry products is holding temperature. As storage temperatures were reduced, the shelf-life of carcasses was extended. Moreover, Baker et al. (1956) reported that the temperature and time of storage are related to shelf-life, and hence, as reviewed by European Union (1997) that, meat quality can be judged very well by the quality of microbial status.

Fig.3.3 revealing the best results for *Total Plate Count* growth of chicken skin which is clearly appeared at 3, 6, 9, 12, and 15 days of storage time at temperatures of -14 and -17°C. This is due to the exponential bacterial growth with time under high freezing temperatures. Therefore, chicken skin storage must be controlled under strict conditions of time and temperatures. This finding was scientifically justified, as the higher temperature of freezing, the lower total number of bacteria.

This finding is in agreement with results previously obtained by Mazur (1984); Hartsell (1951); and Sair and Cook (1938) who reviewed that, when cells are frozen rapidly, both intra and extracellular fluid freezes. However, when cells are frozen at a slow rate, an intra- and extracellular osmotic gradient occurs due to freeze concentration. This may result in cellular disruption, also Straka and Stokes (1959) observed that some nutrients that are required by bacteria for growth are rendered inaccessible by the freezing process, thereby preventing bacterial multiplication.

This finding was conformed to results obtained by Ayres et al. (1950) who found that, when fresh poultry is placed in a cold environment, conditions for replication of most species of bacteria are no longer optimal.

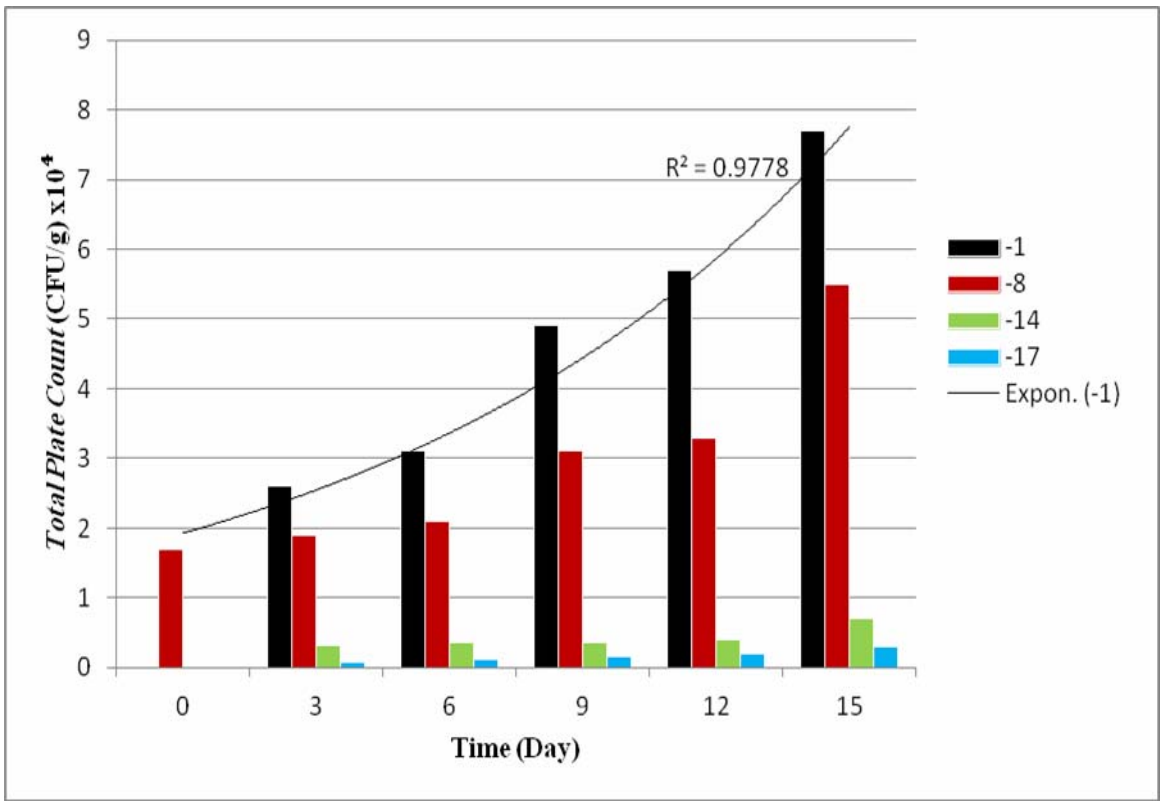


Fig.3.3 *Total Plate Count* growth of chicken skin storage under different temperatures.

3.2.2. Influence on *Total Coliform* Growth:

Results shown in Fig.3.4 review that the *Total coliform* growth in chicken skin increased normally with increasing the period of storage time under sequential temperatures. Storage time and temperature of chicken skin work as main factors for increasing the potential pathogen risk of chicken skin which is directly related to the quality and environmental conditions of chicken skin manufacturing.

This result was in agreement with results previously obtained by Alan (2001); Ayres et al. (1980); Greene and Jezeski (1954); and Olsen (1947) who observed that, the higher receiving temperature means a potential decrease in shelf-life of the product, and when considering the relationship of temperature to microbial life. However, the following needed to be considered: the holding temperature of the microorganism and the time the microorganism is exposed to that temperature. Generation time indicates the speed of cell division; whereas, maximum cell population takes into account cell death as well as cell production.

Results plotted in Fig.3.4 also shown that at -1 and -8°C storage temperature of the *Total coliform* growth was increased gradually in chicken skin for all times investigated. This

increase was much more than other storage temperatures (i.e. -14 and -17°C) at the same storage times, this is in comparison to the related initial number of *Total coliform* at temperatures -1 and -8°C.

This finding was in agreement with results obtained by Alan (2001); Hedrick et al. (1989); Ingraham (1958); Zobell and Conn (1940); and Muller (1903) who investigated that, materials should not be above 4.4°C when received and frozen materials should be below -17.8°C.

Thus, many bacteria responsible for spoilage are able to survive and multiply at low temperatures, the temperatures that are optimal for growth are well above freezing.

As also noted in Fig.3.4 the best conditions for storage of chicken skin in term of *Total Coliforms* growth was under temperature -17°C, regardless storage time. This finding was approved scientifically as, the lower storage temperature, the large increasing in microbial growth.

This finding was in agreement with results previously obtained by Hedrick et al. (1989) who reported that, Poultry muscle that is frozen and held at -17.8 to -28.9°C should retain its quality for 6 to 10 months, also Berry and Magoon (1934) observed that, under specific conditions, moderately cold storage temperatures (-2 to -4°C) may negatively impact bacteria to a greater degree than storage at -20°C. Straka and Stokes (1959) also observed that some nutrients that are required by bacteria for growth are rendered inaccessible by the freezing process, thereby preventing bacterial multiplication.

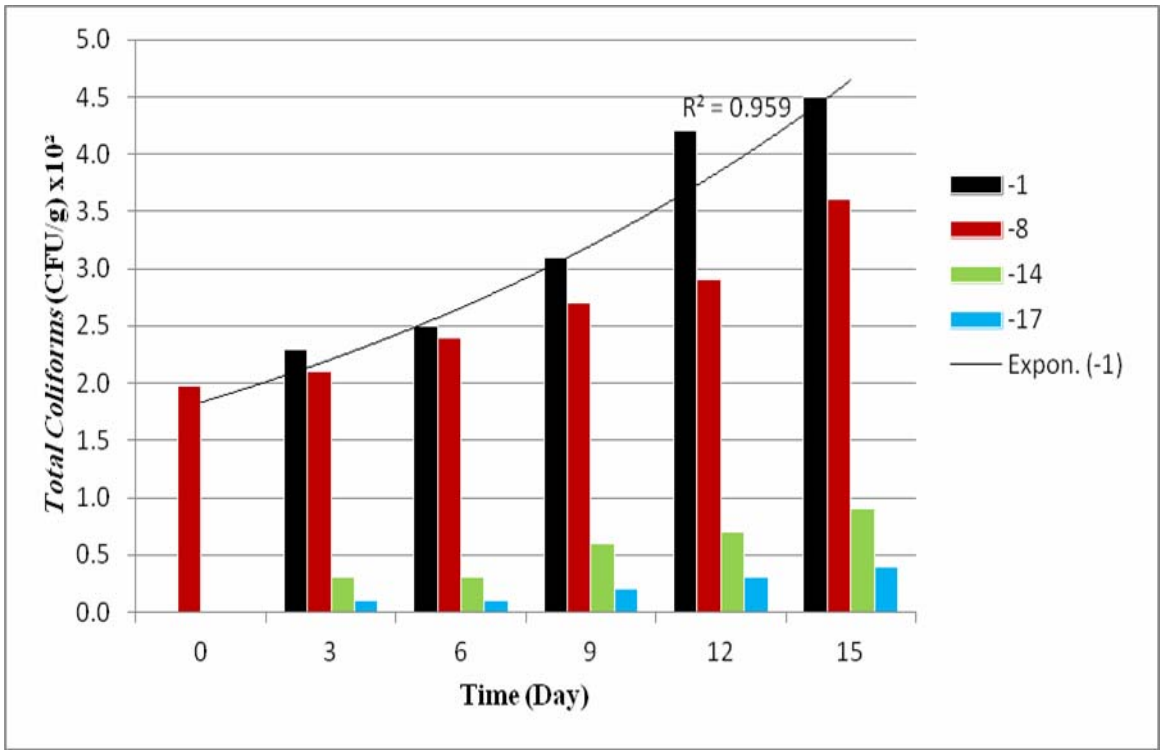


Fig.3.4 *Total Coliform* growth of chicken skin storage under different temperatures.

3.2.3. Influence on *Salmonella* growth:

Table 3.2 shown that the probability of presence of pathogen *Salmonella* in chicken products was conducted and this return for different sources before processing. This result is in agreement with result obtained by Wakefield (1999); Byrd et al. (1998); National Advisory Committee on Microbiological Criteria for Foods (1997); European Union (1997); Bailey et al. (1987); and Rigby et al. (1980).

Table.3.2 Influence of storage time of chicken skin under different temperatures on *Salmonella* growth.

	Storage temperature (°C)			
Storage time (Day)	-1	-8	-14	-17
	Results			
0		+		
3	+	+	+	+
6	+	+	+	+
9	+	+	+	+
12	+	+	+	+
15	+	+	+	+

positive result; presence of *Salmonella*.

As well as completely in agreement and conformity with Ryser and Marth (1999); Byrd et al. (1998); National Advisory Committee on Microbiological Criteria for Foods (1997); Hargis et al. (1995); Mulder et al. (1978 who make asured that all processing units prior to chicken-product freezing like slaughtering, scalding, defeathering, rupture of the intestinal tract, and freezing equipment (i.e.water, air, personnel, and all product contact surfaces) can be considered as a major contributor to cross contamination of important pathogens, and increase the total microbial growth for end products (i.e. mechanically deboned meat and chicken skin) of chicken.

3.3. Influence of Freezing Temperature on pH during Time:

3.3.1. Influence of Freezing Temperature on pH of MDM during Storage Time.

Results investigated in Fig.3.5 showed that pH values of MDM at storage temperatures (i.e. -1, -8, -14 and -17°C) was decreased gradually for the investigated times. This is due to great diversity content of MDM and hence increases the potential of oxidation of MDM which is related to storage temperatures and so results were unrelated with each other based on a specific reason.

This finding is in agreement with results obtained by Ozkececi et al. (2008); Aurelia et al. (2003); Beraquet (2000); Munch et al. (2000); Kesava et al. (1996); Pie et al. (1990); Yan and White (1990); and Field (1988) who presented that high pH ranges of MDM from 6.8 to 7.4 was due to the incorporation of red marrow. This high pH values favor the water holding capacity. On the other hand it contributed to increase in the bacterial load, as well as speeding up the spoilage process. The stability of MDM is influenced by the oxidation

reactions that degraded the lipids and proteins and deteriorate the aroma, texture and color. MDM is also rich in hemoglobin and myoglobin in oxidized form, thus catalyze the oxidation poultry lipids that are rich in phospholipids having a high content of polyunsaturated fatty acids. Moreover, cholesterol rapidly undergoes oxidize in the presence of oxygen, light, metal ions, radiation and other compounds, which could generate free radicals. The pH, FFA and peroxide values for mechanically deboned chicken meat were affected by the prolonged storage time.

At the same time Alan (2001); European Union (1997); Peterson and Gunderson (1960); and others reported that changes in quality of MDM related with proteolytic activity of bacteria at low temperatures so that production of proteolytic enzymes by *P. fluorescens* was higher when this bacterium was cultured at lower temperatures and this directly affect on pH values and other characteristics of MDM during storage time.

Fig.3.5 revealed that the best conditions for storage of MDM in term of pH changes are to store under temperature -17°C. This finding was approved scientifically as, the lower storage temperature, the slow decrease in pH values.

This finding was in agreement with results previously obtained by R.B. Ozkececi et al. (2008); Aurelia et al. (2003); Bailey (1986); moreover, Labuza (1982) established time-temperature relationships that can be used for predicting the extent of shelf life of refrigerated or frozen muscle food. As general, the freezing temperatures (-12 to -18°C) reduce the rate of proteolytic processes. After five months of storage at -12°C, the accumulation of proteolysis compounds and amino acids deamination and decarboxylation were situated at low level.

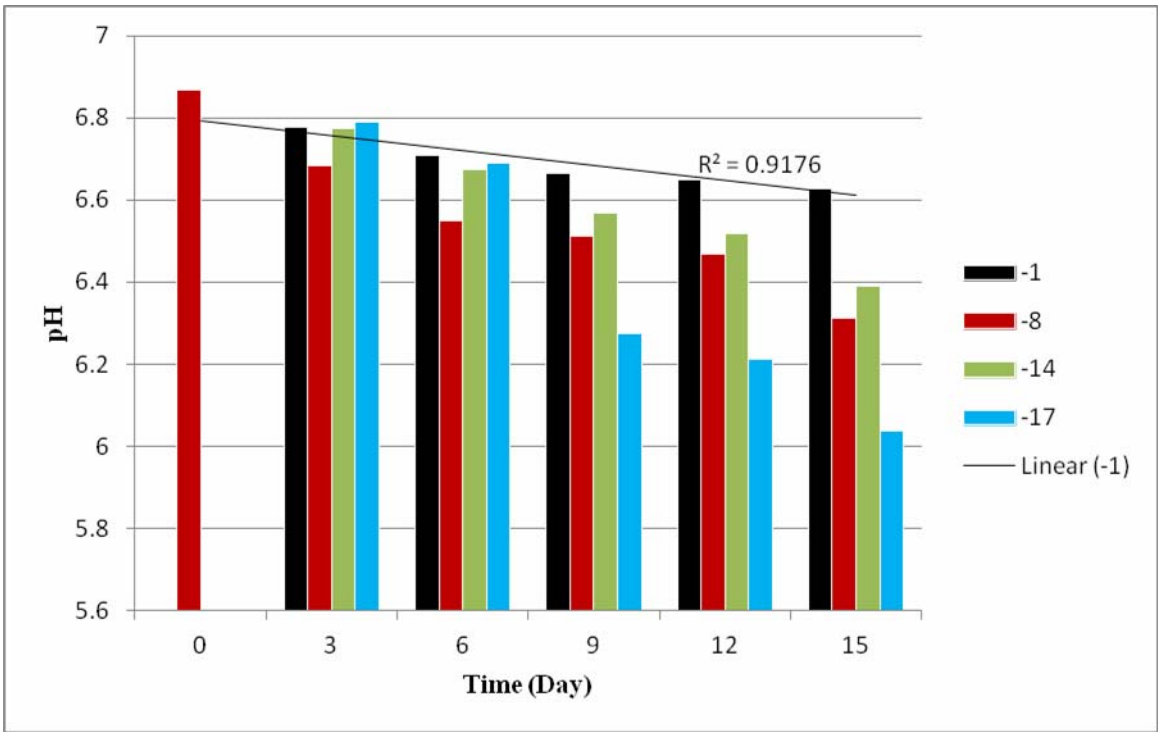


Fig.3.5 pH changes of MDM storage under different temperatures.

3.3.2. Influence of Freezing Temperature on pH of Chicken Skin During Storage Time:

Results plotted in Fig.3.6 show that pH value of chicken skin at storage temperatures (i.e. -1, -8, -14 and -17°C) was gradually decreased linearly. This is due to the lack of content and basic components in the composition of chicken skin. This decrease is major due to oxidation of fatty acids under suitable temperatures during a certain time.

This finding was agreement with results previously obtained by Linseisen and Wolfarm (1998); Hwang (1991); Higley et al. (1986); Bailey (1986); and Labuza (1982) who proven that oxysterols associated with lipid oxidation in meat arise from heating, storage, various stages of processing, and type of meat product.

Fig.3.6 also showed that the best storage temperature for decreases pH rate is at temperature -17 °C. This is due to higher freezing temperature than other temperatures.

This finding was in agreement with previous results obtained by Alan (2001); and Hedrick et al. (1989) who reported that the lower the temperature and the more protection from atmospheric oxygen, the greater the reduction in oxidative rancidity and extension of storage life, since most of the cellular water molecules are fixed in a crystaline structure, but reactions may continue slowly.

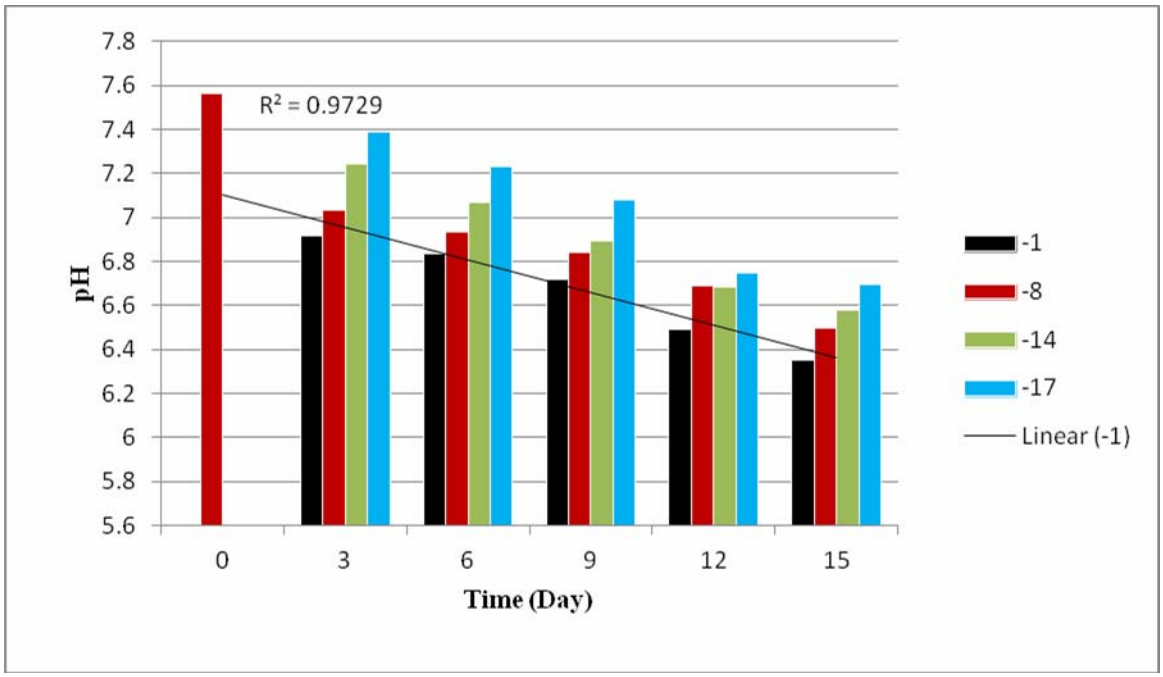


Fig.3.6 pH changes of chicken skin storage under different temperatures.

In addition to previous results as reviewed in Fig.3.5 and Fig.3.6 it is noted that changes in pH for MDM at temperature -1°C was higher for chicken skin. This is due to the fact that the main component of chicken skin is lipid which is in contrast with MDM that

contain less amount fat in their composition. Also this result is related to high quantity of water in MDM compared with chicken skin that minimizes the pH effect.

This finding was scientifically justified, as the higher quantity of fat, the higher oxidation when exposure to higher temperature, also pH for water around 7 , thus the higher water content the higher pH values.

3.4. Chemical Composition of Mechanically Deboned Meat.

As shown in Table. 3.3 The composition of MDM came in accordance with Palestinian Standards (No. PSI-1031).

Table.3.3 Proximate composition of mechanically deboned meat of chicken.

Content	Average
Moisture	67.51%
Fat	17.24%
Ash	2.03%
Protein	13.70%
Peroxide Value	156 mg/Kg
PH	6.89

This analysis is in agreement with previously result obtained by authors as presented in Table 1.5 (page 23).

Meats originating from local source were characterized by higher fat content; lower protein content as well as higher ash content as compared with international resources as shown in Table 1.6 (page 23) which previously obtained by Aurelia et al. (2003).

This comparison indicated that MDM of Palestinian sources gave suitable characteristics which influence on several functional properties as variations in the emulsifying capacity of MDM which may result from its composition, quality and amount of proteins, protein denaturation, freezing and storage. The content of fat in MDM is the main factor that affects its capacity of emulsification as previously obtained by Froning et al. (1973); and Swift et al. (1961).

3.5. Influence of Thermal Treatment on Microbial Growth of Emulsified Meat:

3.5.1. Influence of Oven Temperature.

Results plotted which investigated in Table 3.4 showed that there is no vegetative microbial growth for any specific bacteria, except for *Total Plate Count*, and this is good indicator for sufficient cooking and good manufacturing practices.

Table.3.4. Influence of oven temperature and total time on microbial growth of emulsified meat.

No.	Oven temperature (°C)	Cooking time (h)	Core temperature (°C)	Holding time (min)	Microorganism (cfu/g)					
					T.P.C	*Total Coliform*	*Clostridium Perfringens*	*Staphylococcus aureus*	*Salmonella*	*Listeria Monocytogenes*
1	74	02:35	73	5	170	Nil	Nil	Nil	Nil	Nil
2	77	02:05	73	5	130	Nil	Nil	Nil	Nil	Nil
3	80	01:41	73	5	110	Nil	Nil	Nil	Nil	Nil
4	83	01:35	73	5	550	Nil	Nil	Nil	Nil	Nil
5	86	01:15	73	5	810	Nil	Nil	Nil	Nil	Nil
6	89	01:05	73	5	930	Nil	Nil	Nil	Nil	Nil

In addition to previous result it is found also that the oven temperatures were related directly with time under constant core temperature and holding time whereas increasing oven temperature until reach 80 °C, *Total Plate Count* growth decreased. But when oven temperature increased more than 80 °C, *Total Plate Count* growth increased under constant core temperature and holding time. This return to insufficient time to minimize the microbial growth of *Total Plate Count*, whereas the time decreased from 01:41h to 01:35h for following oven temperatures (i.e. 86 and 89°C).

Gunter and Peter (2007) reported that microorganisms can also be killed at relatively low hot temperatures, but longer heat treatment periods will be necessary in such cases. Heat treatment at temperatures below 100°C. Temperature ranges between 60 to 85°C, also called "pasteurization" or simply "cooking".

As noted previously, the cooking time is very critical for completely pasteurization although of high oven temperatures.

3.5.2. Influence of Core Temperature

Table 3.5 shown that there is no vegetative microbial growth for any specific bacteria, except for *Total Plate Count*, and this is good indicator for sufficient cooking and good manufacturing practices.

The temperature measured inside the processed meat sausage (Mortadella) sample shown that, the total microbial count was decreased.

Table.3.5. Influence of core temperature and total time on microbial growth of emulsified meat.

No.	Core temperature (°C)	Cooking time (h)	Oven temperature (°C)	Holding time (min)	Microorganism (cfu/g)					
					T.P.C	*Total Coliform*	*Clostridium Perfringens*	*Staphylococcus aureus*	*Salmonella*	*Listeria Monocytogenes*
1	64	01:15	80	5	300	Nil	Nil	Nil	Nil	Nil
2	67	01:22	80	5	100	Nil	Nil	Nil	Nil	Nil
3	70	01:30	80	5	100	Nil	Nil	Nil	Nil	Nil
4	73	01:43	80	5	10	Nil	Nil	Nil	Nil	Nil
5	76	02:05	80	5	10	Nil	Nil	Nil	Nil	Nil
6	79	02:50	80	5	10	Nil	Nil	Nil	Nil	Nil

In addition to previous result it is found that the core temperatures was related directly with time and microbial growth under constant oven temperature and holding time whereas increasing core temperature until reach 79 °C *Total Plate Count* growth decreased. This was clear in correlation with the increasing time of cooking.

As noted previously, the core temperature is very critical for completely pasteurization although of increasing in cooking time.

3.5.3. Influence of Holding Time.

Table 3.6 shown that there is no vegetative microbial growth for any specific bacteria, except for *Total Plate Count* with change holding time of cooking under constant oven temperature and core temperature, and this is good indicator for sufficient cooking and good manufacturing practices.

Table.3.6. Influence of holding time on microbial growth of emulsified meat.

No.	Holding time (min)	Cooking time (h)	Oven temperature (°C)	Core temperature (°C)	Microorganism (cfu/g)					
					T.P.C	*Total Coliform*	*Clostridium Perfringens*	*Staphylococcus aureus*	*Salmonella*	*Listeria Monocytogenes*
1	1	01:37	80	73	700	Nil	Nil	Nil	Nil	Nil
2	2	1:38	80	73	400	Nil	Nil	Nil	Nil	Nil
3	3	01:39	80	73	330	Nil	Nil	Nil	Nil	Nil
4	4	01:40	80	73	210	Nil	Nil	Nil	Nil	Nil
5	5	01:41	80	73	90	Nil	Nil	Nil	Nil	Nil
6	6	01:42	80	73	60	Nil	Nil	Nil	Nil	Nil
7	7	01:43	80	73	20	Nil	Nil	Nil	Nil	Nil
8	8	01:44	80	73	10	Nil	Nil	Nil	Nil	Nil
9	9	01:45	80	73	10	Nil	Nil	Nil	Nil	Nil
10	10	01:46	80	73	10	Nil	Nil	Nil	Nil	Nil

In addition to previous result it is found that the holding time was related directly with time of cooking and microbial growth under constant oven temperature and core temperature whereas increasing holding time until reach 10 minute, *total Plate Count* growth decreased. This was clear in correlation with the increasing time of cooking.

These findings were in accordance with previous results obtained by FSIS (1999) who reported that internal product temperatures of at least 71.1°C will provide the safe harbor lethality to ensure elimination of non-spore-forming pathogens such as *Salmonella* serotypes, *C. jejuni, L. monocytogenes,* and *S. aureus*. USDA standard for commercial cookers reported that the end point temperature required by law for pasteurization of poultry products labeled as fully cooked are 71°C or 74°C according to FDA standard for retailer cooking. Beside safety, it is important in maintaining yield not to exceed the end point temperature too much. The rate of product temperature increased during cooking gradually decreases the product finishing up cooking.

These finding were clearly in agreement with Gunter and Peter (2007) who reported that core temperatures of 65°C would be sufficient to achieve the required texture through protein coagulation. However, for hygienic reasons and in order to eliminate a major part of the micro-organisms present in the batter, core temperatures of 70 to 72°C must be reached.

In small and medium scale operations the sausages are transferred to a cooking vat and submerged in hot water of 74 to 80°C for a certain period of time until a core temperature of at least 72°C is achieved.

Gunter and Peter (2007) reported that microorganisms can also be killed at relatively low hot temperatures, but longer heat treatment periods will be necessary in such cases. Heat treatment at temperatures below 100°C. Temperature ranges between 60 to 85°C, also called "pasteurization" or simply "cooking".

3.6. Microbial Growth of Final Product.

As shown in Table 3.7 the result of microorganism population of final product was in agreement with Palestinian standards regulations (No. PSI-2006) under standard storage conditions.

Table.3.7 Influence of storage time on microbial growth of final meat product.

No.	Storage time (monthly)	Humidity %	Refrigerator temperature (°C)	Core temperature (°C)	Microorganism (cfu/g)					
					T.P.C	*Total Coliform*	*Clostridium Perfringens*	*Staphylococcus aureus*	*Salmonella*	*Listeria Monocytogenes*
1	0	58	3.0	2.8	90	Nil	Nil	Nil	Nil	Nil
2	1	62	3.1	3.0	110	Nil	Nil	Nil	Nil	Nil
3	2	58	3.4	3.0	110	Nil	Nil	Nil	Nil	Nil
4	3	61	3.4	2.8	150	Nil	Nil	Nil	Nil	Nil
5	4	59	2.8	2.9	210	Nil	Nil	Nil	Nil	Nil

Results in Table 3.7 shows that there is no vegetative microbial growth for any specific bacteria, except for *Total Plate Count* during storage time of final processed meat sausage (mortadella) sample.

Investigated result shown in Fig.3.7 showed that an exponential increase of *Total Plate Count* growth during all storage periods. This is in agreement with result obtained by Gunter and Peter (2007); Alan (2001); Dickson and Anderson (1992); and Anderson et al. (1977) who reported that if fresh poultry products are held long enough at refrigerator temperatures, they will spoil as a result of the growth of bacteria that are able to multiply under cold conditions. The initial number of bacteria on poultry is generally a function of grow out, production practices, and plant and processing sanitation. Processed products must have an adequate shelf life, which can only be achieved if their microorganism content is low enough to perform the determined shelf life.

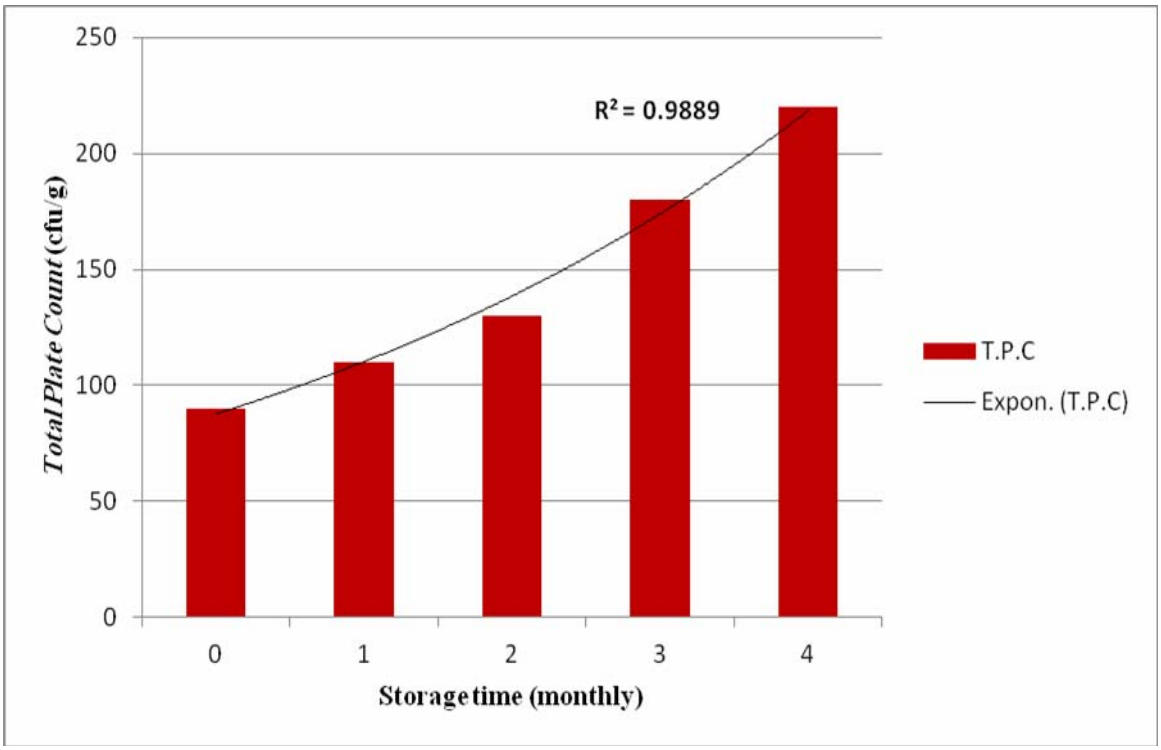


Fig.3.7 Influence of storage time on *Total Plate Count* growth of final meat sample (Mortadella).

Last mentioned researchers concluded that, heat treatment is the effective tool to reduce or eliminate the contaminating microflora. Thus microbial contamination of meat is a natural result of procedures necessary to produce retail products from live animals. So contamination of poultry meat products can occur throughout initial processing, packaging, and storage until the product is sufficiently cooked and consumed.

3.7. Chemical Composition of Final Product.

As shown in Table. 3.8 The composition of final product was in agreement with Palestinian standards regulations (No. PSI-2006).

These findings are in agreement also with previously results obtained by Beraquet (2000) as shown in Table 1.10 who reported some identity and quality characteristics of meat products containing MDM include humidity and fat.

Table.3.8 Proximate composition of final meat sample (mortadella).

Content	Average
Moisture	64.4%
Fat	11.5%
Ash	3.5%
Protein	12.1%
Peroxide Value	Not detected
Nitrites as $NaNo_2$	83 mg/kg
PH	6.2

As shown in Fig.3.8 there was no clear and significant changes to moisture, fat, ash, and protein during storage time (0, 1, 2, 3, and 4 month), on the contrary of changes and degradation of peroxide value, nitrites, and pH as shown in Fig. 3.9, 3.10, and 3.11. These changes were return to storage time and temperature also, which work on acceleration the oxidation of fatty acids and followed increasing peroxide value, thus decreased pH values to be more acid. Moreover, decreasing of nitrites related to temperature and oxygen during time.

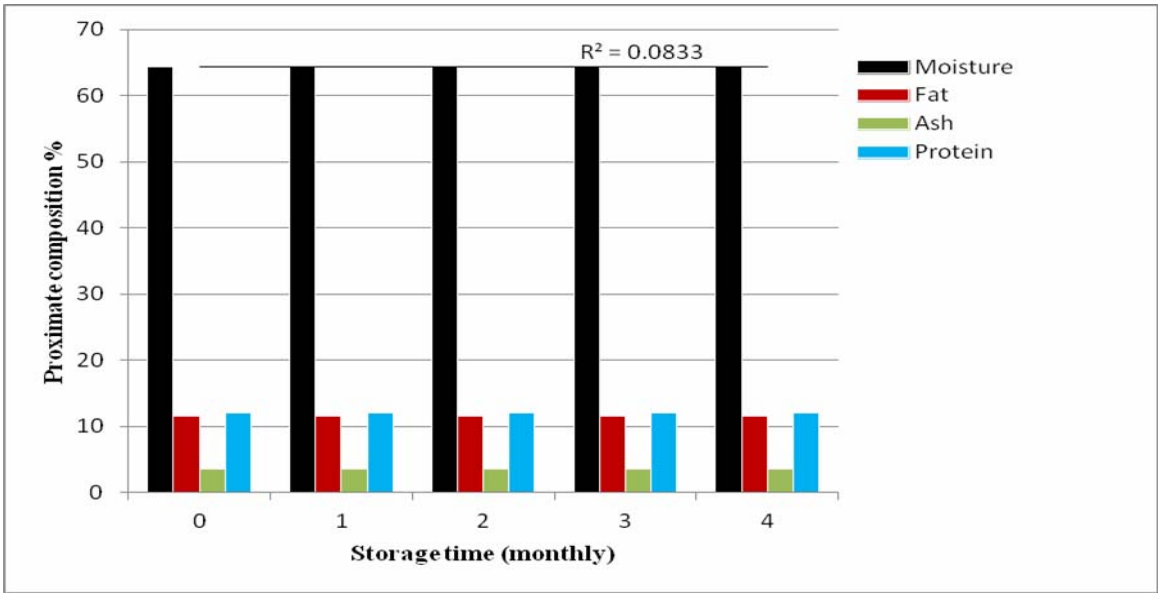


Fig.3.8 Influence of storage time on proximate composition of final meat sample (Mortadella).

These investigation were in agreement with previously result obtained by Grau et al. (2001); Zofia et al. (2001); Ertas (1998); Kesava et al. (1996); Crosland et al. (1995); Chan et al. (1993); Ohshima et al. (1993); De Vore V.R. (1988); Froning (1981); Orr and Wogar (1979); and Love and Pearson (1971) who agreed on lipid radicals formed during processing and storage of food, and also meat products can accelerate formation of oxysterols (Chan et al., 1993; Ohshima et al., 1993). Also oxidative changes of lipids are closely associated with cholesterol oxidation (Grau et al., 2001; De Vore V.R., 1988). Similar increases in oxysterol contents during refrigerated storage were reported by Kesava et al. (1996).

Moreover Zofia et al. (2001) found that storage time influenced level of sum of total oxysterols, cholesterol, 7 β-OHC, 7 keto-C and peroxide value. Also, lipids peroxidation was significantly correlated with cholesterol, 7 β-OHC, 7 keto-C and total oxysterols, moreover acid number and TBA value was not correlated with cholesterol and oxysterols contents.

Free fatty acid (FFA), peroxide and TBA values are the most common chemical determining the storage stability and quality of a product. Oxidation of fatty acids primarily affecting the storage stability is mainly influenced by factors such as storage time, vacuum treatment, raw materials, conditions used for mechanical deboning and even by the particular carcass parts used (Love and Pearson 1971; Orr and Wogar 1979; Froning 1981; Crosland *et al.* 1995; Ertaş 1998).

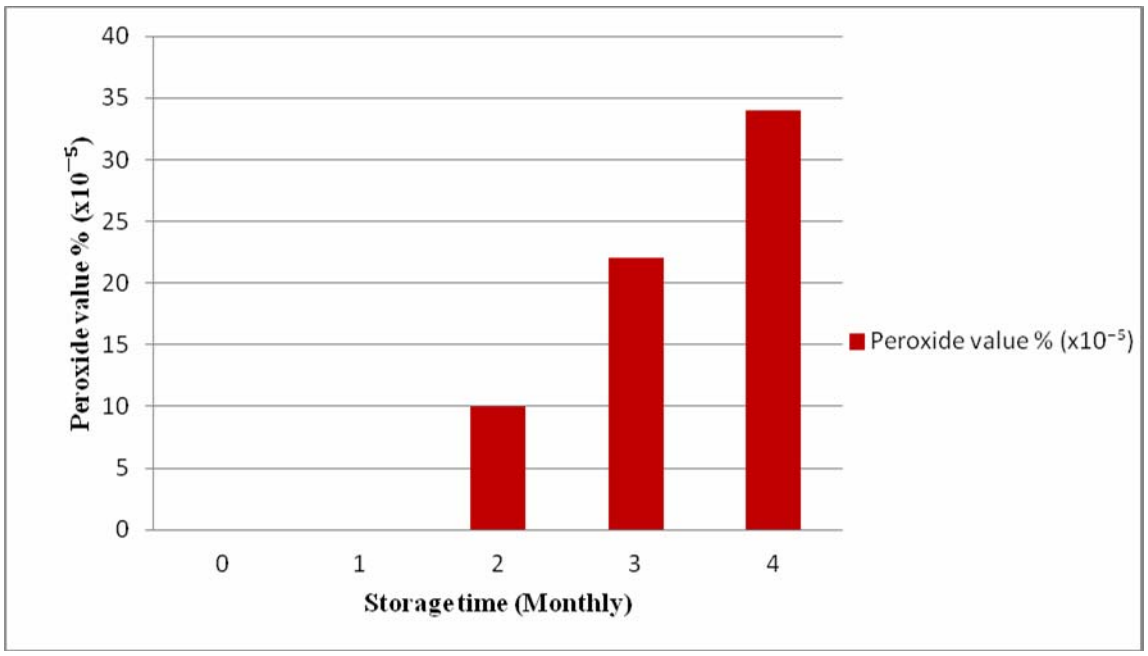


Fig.3.9 Influence of storage time on peroxide value of final meat sample (Mortadella).

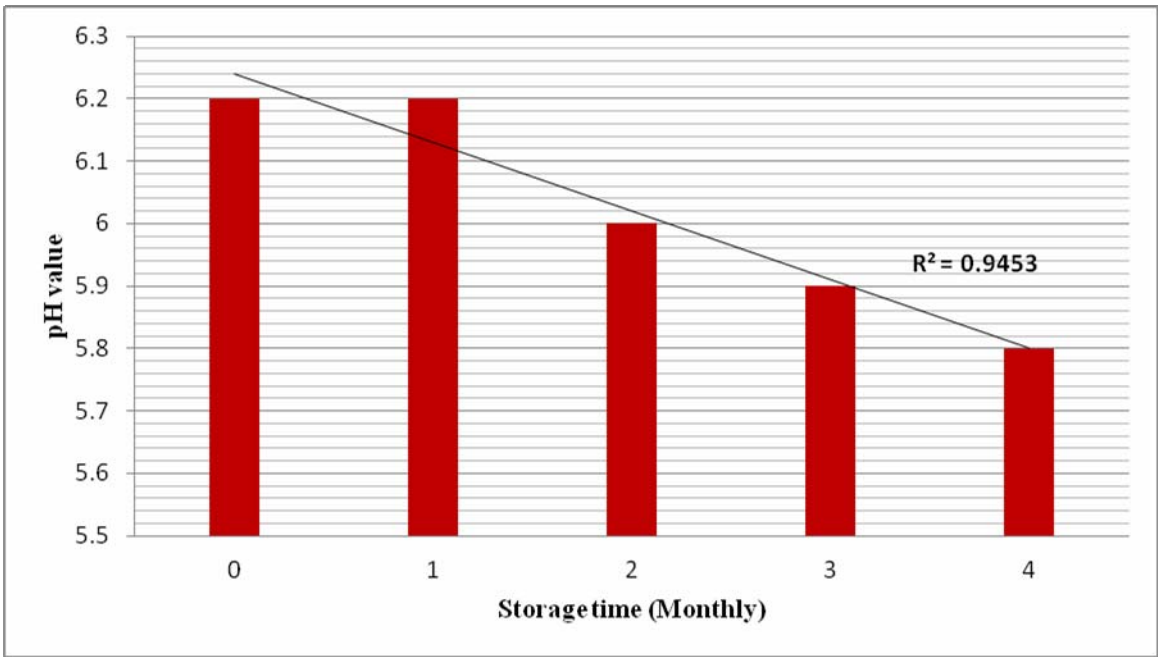


Fig.3.10 Influence of storage time on pH value of final meat sample (Mortadella).

Nitrites was clearly degraded and decreased during storage time as shown in Fig.3.11 and this return to light and temperature with time which work on decreasing the active material of mortadella coloring.

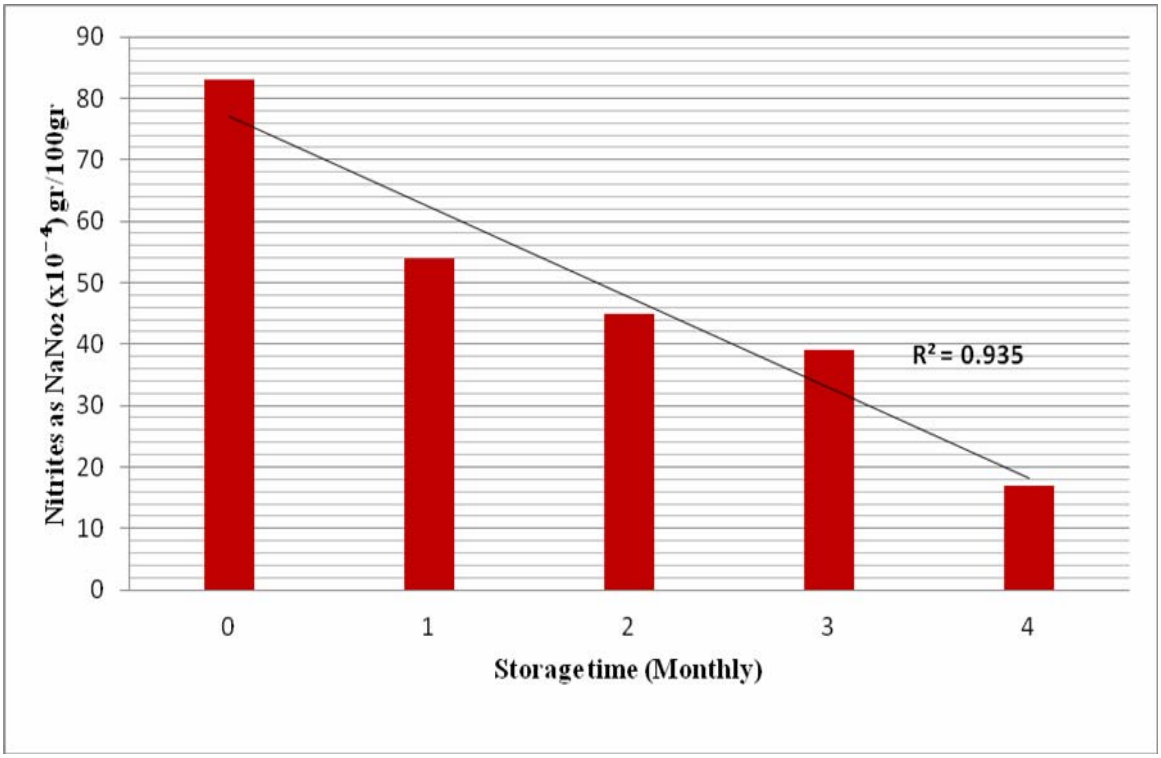


Fig.3.11 Influence of storage time on nitrites as $NaNo_2$ of final meat sample (Mortadella).

3.8. Sensory Evaluation of Final Product through Storage Time.

The sensory test was done for sample subjected to storage conditions after (0, 1, 2, 3, and 4 months) of storage time, to determine the effect of storage condition with respect to time on the sensory characteristics of the product.

Table 3.9 showed that, the sweet, salty, smell (odor), and color characteristics of sample was gradually less than other characteristics during the fourth months of storage, and this return mainly to the effect of temperature and oxygen on product color and lipid oxidation, directly. These factors work on degradation quality of final product through storage time.

Table.3.9 Average score of sensory evaluation for final mortadella sample.

Storage time (day) / Character	0 day	15 day	30 day	45 day	60 day	75 day	90 day	105 day	120 day
Color	8.8	8.6	8.6	8.4	8.4	8.2	7.5	7	6.2
Sour	8.4	7.8	7.8	7.6	7.6	7.4	7.2	7	7.2
Sweet	8.8	8.8	8.6	8	8	7.8	7.6	7.2	6.6
Bitter	9	9	9	9	9	8.4	7.8	7.4	7.2
Salty	9	9	8.8	8.8	9	8.6	7.4	7.2	6.6
Smell (odor)	8.8	8.6	8.4	7.6	7.6	7.6	7.4	7	6.4
Softening	8.6	8.6	8.6	8.6	8.6	8	8	7.6	7.2
Coarse (rough)	8.2	8	7.8	7.8	7.8	7.6	7.8	7.8	7.4
Hard particles	8.2	8.2	8	7.8	7.8	8	8	7.8	7.4
Slicing	8.4	8.4	8.2	8	8.2	8	7.8	7.6	7

This finding on color degradation was in agreement with previously results obtained by Froning and Johnson (1973) who observed that the most significant visual result in curing is the development of a characteristic pink to pinkish-red coloration in the cooked end product. The native pigment myoglobin and its oxidized form metmyoglobin (as well as the corresponding pigments of hemoglobin) are converted initially to nitrosylmyoglobin, and with sufficient heating, to nitrosylhemochrome.

Bryan (1980) reported that important factors in the rate of NO formation and its ultimate reaction with the globin pigments are meat pH and the presence of reducing conditions.

Also Kartika et al. (1998) reported that light and oxygen play a central role in a sequence of reactions initially involving NO dissociation from the central iron atom of the hemochrome's porphyrin structure (Figure 1.5). The dissociated NO is then free to recombine with the porphyrin, reforming the pigment and thus maintaining the pink to pinkish-red cured product color. The reestablishment of the pigment is favored by vacuum-packaging with films of low oxygen transmission rate. The dissociated NO can also be oxidized by oxygen and thus not be available to recombine to form the original

pigment. In extensive cases of fading, additional oxygen permeating the film is then free to oxidize the central iron of the hemochrome (Fe^{+2}) resulting in production of a hemichrome (Fe^{+3}). This loss is visually detectable as lightening of the product with a distinct loss of the red hue. Under conditions of extreme oxidation, sites of the hemichrome's structure are oxidized, and the product appears severely bleached.

Others characteristics (sweet, salty, and smell) degradation were in agreement with previous results obtained by Nychas and Tassou (1997) who reported that the concentration of glucose decreased progressively and more rapidly toward the end of the storage period and occurred to a greater degree for samples held at higher temperatures. Similar observations were made for concentrations of L-lactate. Concentrations of free amino acids increased as proteolysis occurred throughout the storage period.

Also Schmitt and Schmidt-Lorenz (1992) demonstrated that there was an increase of low molecular weight peptides and free amino acids on chicken carcasses stored at 4°C.

Otherwise, as bacterial populations' increase and glucose availability begins to decrease. These bacteria begin utilizing other substrates, such as protein, which yields much more odorous end products as reported by Pooni and Mead (1984).

3.9. General Discussion

- **Influence of Storage time of MDM on Microbial Growth**

The *Total Plate Count* growth in MDM increased significantly with increasing the period of storage time under sequential temperatures; sharply increase in *Total Plate Count* growth in MDM after 12 days of storage at -1°C storage temperature. At the same time, a slow growing at (3, 6, and 9 days) of storage at temperatures -8 and -14°C. Results of the investigation also showed that the best conditions for storage of MDM were under temperature -17°C.

The *Total coliforms* growth in MDM increased normally with increasing the period of storage time under sequential temperatures. Results also revealed that at -1 and -8°C storage temperature the *Total coliforms* growth was increased gradually in MDM for all times investigated, his increase was much more than other storage temperatures (i.e. -14 and -17°C) for the same storage times. The best conditions for storage of MDM in term of *Total coliforms* growth was under temperature -17°C, regardless storage time.

It was found that all storage conditions under investigated times and temperatures were the reason behind the presence of *Salmonella.* This result is explained as, the specified times and temperatures are not suitable and not enough to kill or to inhibit the *Salmonella* growth.

- **Influence of Storage time of chicken skin on Microbial Growth**

The *Total Plate Count* growth in chicken skin increased normally in exponential patern with increasing the period of storage time under sequential temperatures. At the same time, the best results for *Total Plate Count* growth of chicken skin that clearly appeared at 3, 6, 9, 12, and 15 days of storage at temperatures -14 and -17°C.

The *Total coliform* growth in chicken skin increased normally with increasing the period of storage time under sequential temperatures. As also noted the best conditions for storage of chicken skin in term of *Total coliform* growth was under temperature -17°C.

It was found that all storage conditions under investigated times and temperatures was the reason behind the presence of *Salmonella.* This result is explained as, the specified times and temperatures are not suitable and not enough to kill or to inhibit the *Salmonella* growth.

- **Influence of Freezing Temperature on pH during Storage Time**

PH values of MDM at storage temperatures (i.e. -1, -8, -14 and -17°C) were decreased gradually for all times investigated related with storage time. As also noted that the best conditions for storage of MDM in term of pH changes were under temperature -17°C.

PH values of Chicken skin at storage temperatures (i.e. -1, -8, -14 and -17°C) was gradually decreased as linear as possible for all times investigated. Also results which

showed that the best result for decreases rate of pH values was at temperature (-17 °C) this is due to high freezing temperature than other temperatures during same times investigated.

- **Chemical Composition of Mechanically Deboned Meat**

This comparison indicated that MDM of Palestinian sources gave suitable characteristics which influence on several functional properties as variations in the emulsifying capacity of MDM which may result from its composition, quality and amount of proteins, protein denaturation, freezing and storage**.**

- **Influence of thermal treatment on microbial growth of emulsified meat**

The investigation showed that there was no vegetative microbial growth for any specific bacteria, except for *Total Plate Count*, and this is excellent indicator for sufficient cooking and good manufacturing practices.

In addition to previous result, it is that the oven temperatures was related directly with time under constant core temperature and holding time whereas increasing oven temperature until reach 80 °C *Total Plate Count* growth decreased. When oven temperature increased more than 80 °C, *Total Plate Count* growth increased under constant core temperature and holding time. This return to insufficient time for minimizes the microbial growth of *Total Plate Count* whereas the time decreased from 01:41h to 01:35h and also for followed degrees.

Results obtained showed that there was no vegetative microbial growth for any specific bacteria, except for *Total Plate Count*, and this is excellent indicator for sufficient cooking and good manufacturing practices.

In addition to previous result it is found that the core temperatures was related directly with time and microbial growth under constant oven temperature and holding time whereas increasing core temperature until reach 79 °C, the *Total Plate Count* growth was decreased. This was clear in correlation with the increasing time of cooking.

Results also showed that there was no vegetative microbial growth for any specific bacteria, except for *Total Plate Count* with change holding time of cooking under constant oven temperature and core temperature, and this is excellent indicator for sufficient cooking and good manufacturing practices.

In addition to previous result it is found that the holding time was related directly with time of cooking and microbial growth under constant oven temperature and core temperature whereas increasing holding time until reach 10 minute, thus *Total Plate Count* growth decreased. This was clear in correlation with the increasing time of cooking.

- **Microbial Growth of Final Product**

Result of microorganism population of final product was in agreement with Palestinian standard (No. PSI-2006), under standard storage conditions. Results showed that there was no vegetative microbial growth for any specific bacteria, except for *T.P.C* during storage time of final mortadella sample. Result also showed that an exponential increase of *Total Plate Count* growth during all storage periods.

- **Chemical Composition of final product**

The composition of final product was in agreement with Palestinian standard (No. PSI-2006). There were no clear or significant changes to moisture, fat, ash, and protein during storage time (0, 1, 2, 3, and 4 month). Changes and degradation of peroxide value, nitrites, and pH, these changes were return to storage time and temperature. Also, an acceleration of oxidation for fatty acids and followed increasing peroxide value thus decreased pH values to be more acid. Moreover, decreasing of nitrites related as well to temperature and oxygen during time.

- **Sensory Evaluation of Final Processed meat sausage**

Results obtained namely; the sweet, salty, smell (odor), and color characteristics of sample were gradually less than other characteristics; sour, bitter, softening, coarse (rough), hard particles, and slicing during the fourth months of storage.

CONCLUSION AND RECOMMENDATIONS

Conclusion

- The stability of MDM and chicken skin during storage at different temperatures is influenced by the oxidation reactions and microbial growth which is related to temperature and time.

- Freezing conditions represent a reliable indicator for estimating the quantity of peroxidated lipids and pH values in the MDM.

- The freezing temperatures (-14 to -17°C) reduce the rate of proteolytic processes during 4 months.

- Cooking, curing, and refrigeration of final processed meat sausage (Mortadella) are essential and very important for stability, safety, and quality during storage.

- Heat treatment methods cause various physical-chemical alterations in meat, which result in the beneficial sensory and hygienic effects on the processed meat sausage products.

- Prerequisite programs of based meat sausage manufacturing are important practices which must be controlled with critical regulations to minimize any possibility of contaminations.

- The microorganism population of final processed meat sausage related directly to the sufficient cooking factors namely; cores temperature, oven temperature, and the holding time.

- Quality, safety, and sensory evaluation of processed meat sausage (Mortadella) will continue to be important and drive the development of products for consumers.

- The primary causes of spoilage are as follows: prolonged distribution or storage time, inappropriate storage temperature, and high initial bacterial counts.

Recommendations

- Product should flow from the area of highest microbial load to the area of lowest microbial load (e.g., raw to cooked), and not “back track”.

- The processing plant must provide hygienic environmental and operating conditions (i.e., follow GMPs) such that products are produced in a safe, sanitary, and wholesome manner.

- The plant should have access to a good water source and be able to maintain water quality in the plant.

- The processing plant site should be well drained, landscaped with minimal shrubbery, and designed to facilitate waste management.

- Company management must work on support and assist the quality and safety team because they are the active and critical department in any food institute.

- Provide an intensive training courses specializing in food safety procedures and mechanisms of controlling production processes.

- Dependence on local sources of raw meat as being good quality and also conform to the local and global regulations.

- Provide all the necessary requirements for microbial testing laboratories because of their critical role in the success of the manufacturing of food products in our country.

Prospects

- Study the effect of the distribution conditions of meat products through distribution on the likelihood increasing of microbial contamination and chemical changes of the product.

- Study the commitment and follow-up of laboratory technicians for microbiology and chemical tests with conditions and health requirements required during inspection process on the results differences.

- Study the aspects of similarities and differences in commitment to health conditions and required regulations between local sources of mechanically deboned meat and imported from abroad.

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Al-Quds University

APPENDICES

Appendix One: Chemical Test

1. Chemical Test

1.1 Purpose

Chemical analysis to determine the content of moisture, fat, minerals (Ash), protein, peroxide value, and residual nitrites of processed meat products and establish the nutritive and economic value of the products. Samples of the meat product are finely ground and weighed accurately for each respective chemical analysis.

1.2 Sampling of Meat and Meat Products:

- Grind the cold meat sample, minimum weight 500 grams. Use food grinder with 3mm plate opening.
- Mix rapidly at a cold temperature.
- Keep ground sample in glass or similar containers which are air and liquid tight.
- Ready for analysis. If any delay occurs, chill the sample to inhibit decomposition.
- Weigh the sample as rapidly as possible to minimize loss of moisture.

1.3 Chemical Procedures:

1.3.1 Moisture Analysis (Microwave Drying)

1.3.1.1 General

Samples are dried in a microwave oven and the loss of weight upon drying is expressed as percent moisture content.

1.3.1.2 Application

This method may be used to determine the moisture content of fresh meat, semi-processed meat, meat mixes and processed meat products.

1.3.1.3 Equipment

- Mincer with 6mm plates or heavy duty food processor.
- Balance with at least 0.1g sensitivity.
- Microwave oven with 600-700 watt microwave energy output, turntable and time accurate to 15 seconds.

- Desiccators with silica gel.
- Beaker
- Filter papers, 7cm diameter or open weave disposable kitchen cloth.
- Silicon carbide (carborandum) finely ground.
- Sand or salt.

Table. 2.5 Approximate Drying Times for Sample Sizes of Meat

Sample size	**Approximate drying time**
3x10g	3.5-4.5 min.
3x25g	7.5-9.5 min.
2x50g	8.5-11 min.

1.3.1.4 Method

- Prepare the sample by mincing or chopping as described in sample preparation.
- Preheat the oven
- Dry the beakers and filter papers by heating them in a microwave oven for one minute.
- Determine the heating time necessary to completely dry the samples in the microwave oven.
- Weigh an empty beaker plus filter paper. Weigh about 10 grams of sample in the beaker. For meat samples, spread the samples into a thin layer around the lower wall of the container with spatula or spoon. Place the filter paper over the top of the beaker and fold to close and accurately weigh the beaker plus filter paper.
- Place the samples in the preheated oven. The samples should be spaced at equal distances around the turntable.
- Cool the samples in a desiccator and accurately weigh the beaker plus dried samples plus filter paper.
- Repeat drying until constant weight is obtained.

1.3.1.5 Calculations

Weight of beaker plus filter paper = A
Weight of beaker plus filter paper + Sample (Before drying) in grams = B
Weight of beaker plus filter paper + Sample (After drying) in grams = C

$$\%\text{Moisture} = \frac{(B-C)}{(B-A) = (\text{Weight of sample})} \times 100$$

1.3.2 Crude fat determination

1.3.2.1 Methods

- Get the weight of the dried sample.
- Put the dried sample inside the filter paper and fold to close.
- Place the dried sample inside the soxhlet extraction tube connected to the soxhlet flask.
- Pour enough ether into the extraction tube.
- Extract for 10 hours, at 3-4 drops per second.

- After extraction, take out the defatted sample from the extraction tube and air dry the sample for traces of ether. Dry further in an oven at 100°C and cool in a dessicator. Weigh the defatted cooled samples to constant weight.

1.3.2.2 Calculation

$$\%Fat = \frac{\text{Weight of Dried Sample} - \text{Weight of Defatted sample}}{\text{Original weight of the sample}} \times 100$$

- Weight of dried sample = weight of beaker + filter paper + dried sample minus weight of beaker + filter paper.

1.3.3 Ash determination

1.3.3.1 Methods

- The defatted sample is placed in a constant weight porcelain crucible with cover.
- The crucible is then placed in a muffle furnace, and at a temperature of 600°C the sample is ignited for two hours.
- After ignition the crucible is placed in the oven to bring down the temperature for about 30 minutes, and then cool in a dessicator for another 30 minutes.
- The sample is then weighed to constant weight.

1.3.3.2 Calculation

$$\%Ash = \frac{\text{(Wt. of crucible with cover + ash)} - \text{wt. of crucible with cover}}{\text{Original weight of the sample}} \times 100$$

1.3.4 Protein content determination

1.3.4.1 Calculation

Calculation of the approximate protein content for pure meat and meat products:

% Protein = 100% - (%water + % ash + % fat)

1.3.5 Peroxide value

1.3.5.1 General

Peroxide value measures the oxygen content expressed as oxygen milli equivalents per kg of oil. A high peroxide value (> 20 meq. O_2 kg^{-1}) indicates a strong potential towards rancidity.

1.3.5.2 Apparatus

- 100 ml beakers.
- 250 ml glass-stoppered conical flask.
- 50 ml graduated cylinder.
- 10 ml graduated cylinder.
- 100 ml graduated cylinder.
- 50 ml burette.
- Burette handle.

1.3.5.3 Reagents

- Glacial Acetic Acid-Chloroform (3:1).
- Potassium Iodide saturated solution.
- Sodium thiosulfate solution 0.1 N.
- Sodium thiosulfate solution 0.01 N prepared by diluting 100 ml of 0.1 N sodium thiosulfate solution to 1 L, using freshly boiled and cooled water. (It is stable for 1-2 days only).
- Starch Indicator solution (1% W/V).

1.3.5.4 Procedure

- About 5 grams of sample are weighed into 250 ml glass-stoppered conical flask.
- Acetic acid-Chloroform solution is added (30 ml) with swirling to dissolve sample completely.
- The saturated potassium iodide solution (1 ml) is added.
- The flask is quickly Stoppard and let to stand with occasional shaking for 1 minute.
- Thereafter, 30 ml of freshly boiled and cooled water are added and flask contents are titrated with 0.01 N sodium thiosulfate solutions with vigorous shaking until yellow color had almost gone.
- About 0.5 ml of starch solution is added and titration is continued with vigorous shaking, to release all iodine from chloroform layer, until the blue color just disappeared.

1.3.5.5 Calculation

Peroxide value is the number of milliequivalents of peroxide found in1000 grams of sample.

Peroxide value = (V1 – V2) x N x 1000W

Where:

V1 = Volume (in ml) of solution thiosulfate used in test.

V2 = Volume (in ml) of sodium thiosulfate used in blank test.

N = Normality of sodium thiosulfate solution.

W = Weight (in g) of sample.

1.3.6 Residual Nitrites (As $NaNO_2$)

Reagents and Apparatus

- NED reagents:

- Dissolve 0.2 g N-(1- naphthyl1) ethylendi-amin. 2HCl in 150 ml 15% (v/v) acid store in brown glass bottle.

- Sulfanilamide reagents:
  - Dissolve 0.5 g sulfanilamide in 15 ml 15% (v/v) acetic acid, and store in brown glass bottle.

- Nitrite standards solution:
  - Stock solution – 1000 ppm $NaNo_2$ dissolves 1.00 gm $NaNo_3$ in H_2O and dilute to 1 liter.
  - Intermediate solution- 100 ppm $NaNo_2$ dilute 100 ml stock solution to 1 liter with H_2O.
  - Working solution- 1 ppm $NaNo_2$ dilute 10 ml intermediate solution to 1 liter with H_2O.
  - Filter paper.

Procedure:

- Weight 5 gm finely comminuted and thoroughly mixed sample into 50ml beaker.
- Add care 40ml H_2O heated to 80°C.
- Mix thoroughly with glass rod, taking care to break up all lumps and transfer to 500ml volumetric flask.
- Thoroughly wash beaker and rod with successive portions of the hot water, adding all washings to flask.
- Add enough hot water to bring volume to 100ml, transfer flask to steam bath and let stand 2hr, shaking occasionally cool to room temperature, dilute to volume with water, and remix.
- Filter, add 2.5ml sulfanilamide reagent to adequate containing 5-50mg NED reagent, mix, dilute to volume, and let color develop in 15 minutes.
- Transfer portion of solution to photometer cell and determine absorbency (A) at 540mm against blank of 45ml H_2O. 2.5ml sulfanilamide reagent and 2.5ml NED reagent.
- Determine nitrite present by comparison with standard curve prepared as follow. Add 10, 20, 30, and 40 ml nitrite working standard solution to 500ml volumetric flask, add 2.5ml sulfanilamide reagent, mix, and proceed as above beginning after 5 minutes. Standard curve is straight line to 1 ppm $NaNo_2$ in final solution.

Principle:

Determination of nitrite in cured meat depends upon the formation of diazole compound by interaction of nitrite and sulfanilamide. Then diazole compound by react with N-(1-nophthyl1) ethylene dine 2HCl.
Producing red color, the intensity of the color is direct proportional to the concentration if nitrite present in the sample.

Appendix Two: Physical Test

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1. Physical Test

1.1 Purpose

With physical test methods important parameters such as temperature, acidity (pH), can be determined. All routine physical testing can be carried out with portable instruments.

1.2 Measurements:

1.2.1 Electronic thermometer (Temperature)

Temperature measurement with thermo-elements/thermocouples is based on the thermo-electrical effect.

1.2.1.1 Physical principle

The electronic thermometer functions according to this principle. On one welding point of the thermocouple the reference temperature is taken.

The other welding point is the tip of the metallic thermo-sensor of the instrument, which is exposed to the temperature to be measured.

Both welding points are of different temperature, which generates the electric current within the system. The electric tension (voltage) is equivalent to the temperature difference between the two points and can directly be translated into the temperature reading on the instrument.

The welding point for the reference temperature is located in the instrument. For the correct functioning of the system, the reference temperature must be at a constant level. The temperature of 0°C is taken for reference. Even though the instrument is exposed to various temperatures, the reference temperature is electronically set constantly at 0°C regardless the ambient temperature.

1.2.2 pH meters

Portable instruments are battery driven and have glass electrodes. The pH–value in meat and meat products can be measured by direct contact between the sensitive diaphragm of the electrode and the meat tissue.

Through the diaphragm differences in electrical load between the meat and electrolyte solution (e.g. Potassium chloride KCl) inside the glass electrode are measured and directly indicated as the pH-reading.

In raw fresh meat, it is recommended to spray small amounts of distilled water onto the tissue at the point of measurement (prior to inserting the electrode), because the operation requires some fluidity in the sample and the glass electrode should be thoroughly wet. The amount of water necessary will not appreciably alter the pH. For accurate pH readings the pH-meter should be calibrated before use and adjusted to the temperature of the tissues to be measured. The electrode must be rinsed with distilled water after each measurement.

1.2.2.1 Purpose

PH measurement is useful for evaluation of meat quality for further processing, in particular acidity and the water binding capacity.

Table 2.6 pH values for meat and meat products

Product	pH value (Range)
Meat mixes in jelly + vinegar added	4.5-5.2
Raw fermented sausage	4.8-6.0
Beef	5.4-6.0
Pork	5.5-6.2
Canned meats	5.8-6.2
Curing brines	6.2-6.4
Blood Sausage	6.5-6.8
Muscle tissues, immediately after slaughter	7.0-7.2
Blood	7.3-7.6

1.2.2.2 Physical principle

The pH is a measure of the acidity or alkalinity in solutions or water containing substances. PH values lower than 7 are considered acidic, while pH values higher than 7 are considered alkaline. A pH of 7 indicates neutrality. pH values are related to the concentration of hydrogen ions (H+) in the substance.

Appendix Three: Microbiological Tests

1. Microbiological Tests

1.1 Enumeration of *Total Plate Count*.

Fig.2.3 describes the method of enumeration of *Total Aerobic Microorganisms* in meat and meat products, according to the COFRAC method.

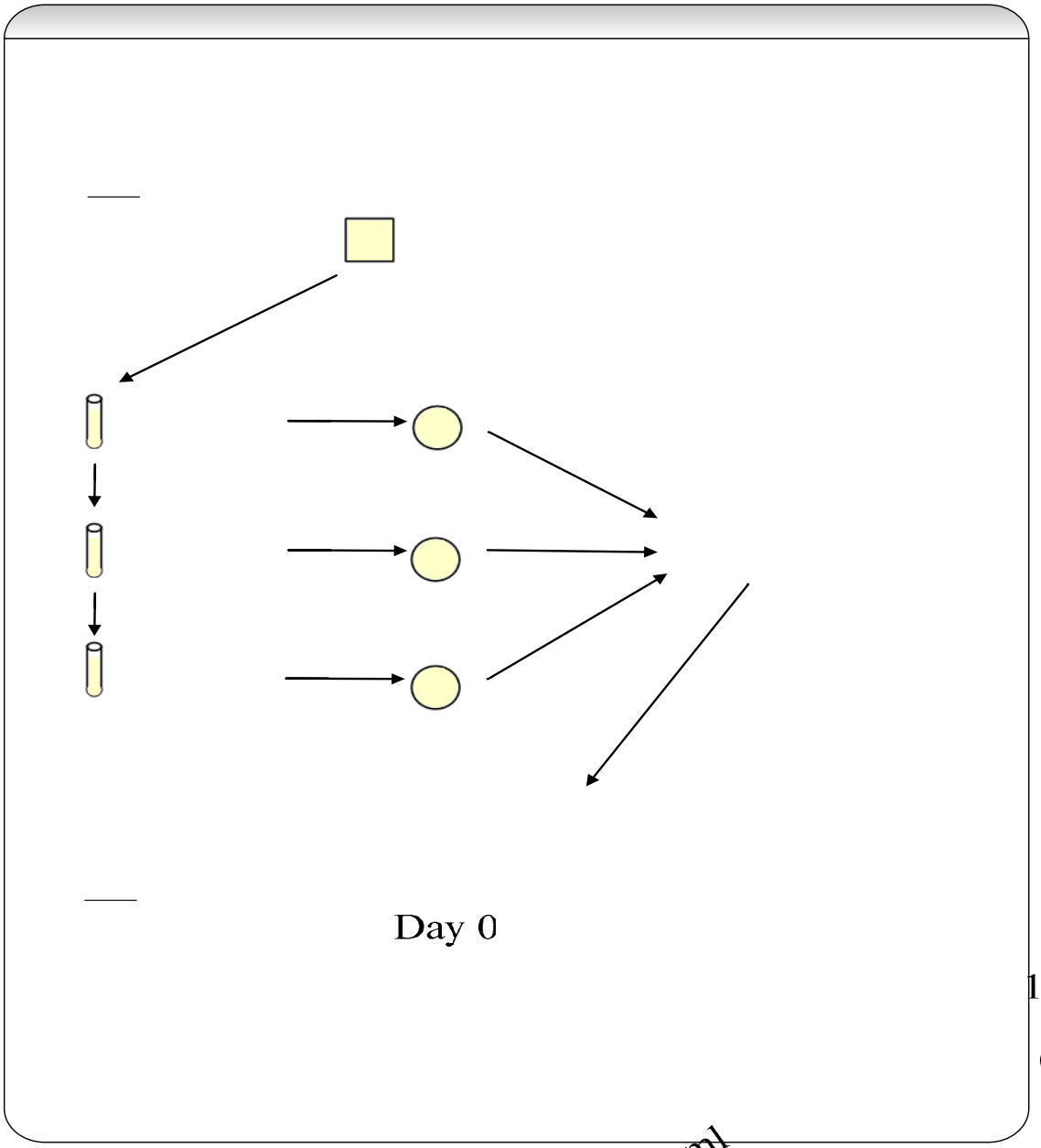

Fig.2.3 Enumeration of aerobic microorganisms (*Total Plate Count*).

1.2 Enumeration of *Total Coliforms*.

Fig.2.4 describes the method of enumeration of *Total Coliforms* in meat and meat products, according to the COFRAC method.

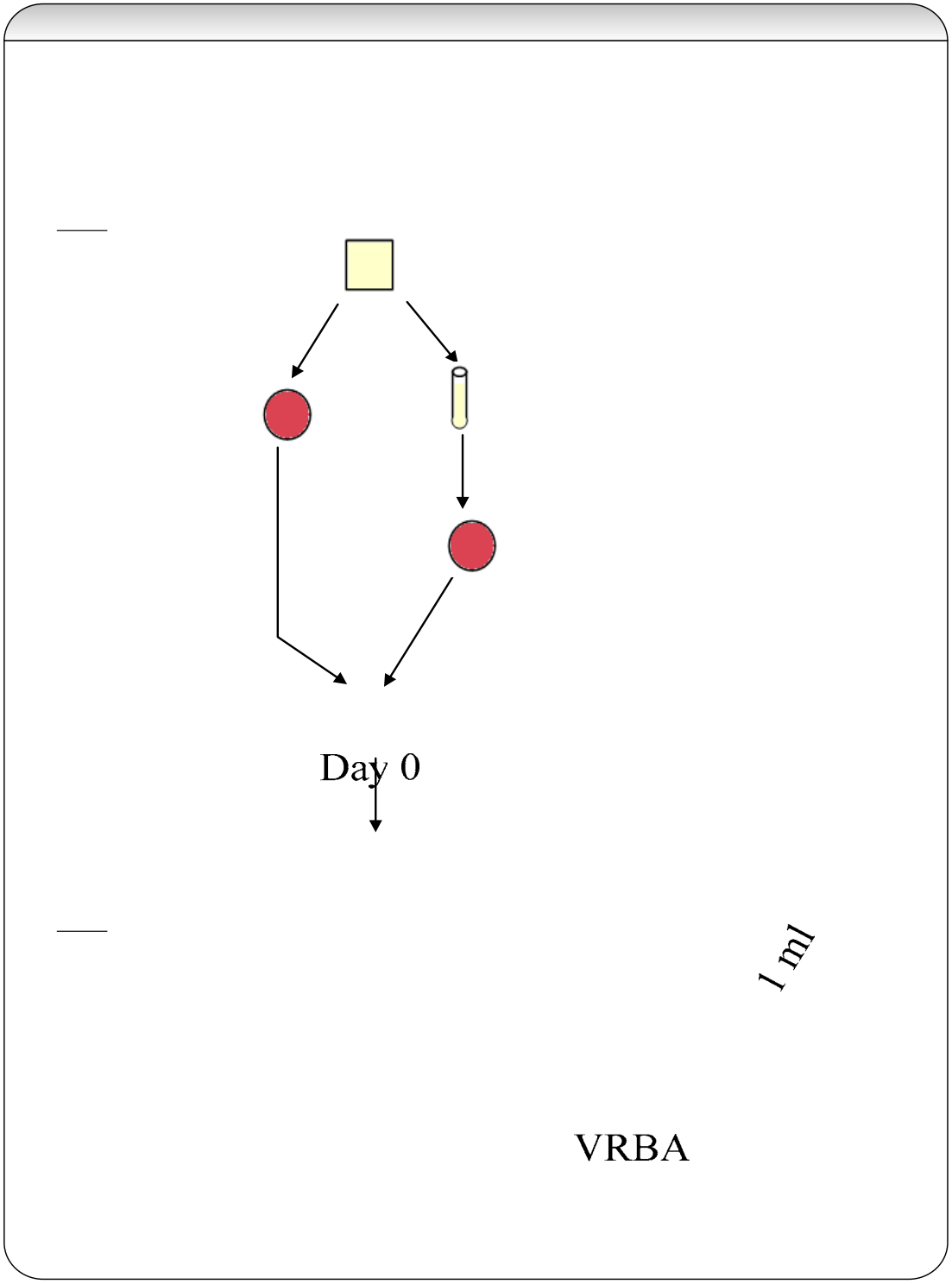

Fig.2.4 Enumeration of *Total Coliforms*.

1.3 Enumeration of *Clostridium perfringens*.

Fig.2.5 describes the method of enumeration of *Clostridium perfringens* in meat and meat products, according to the COFRAC method.

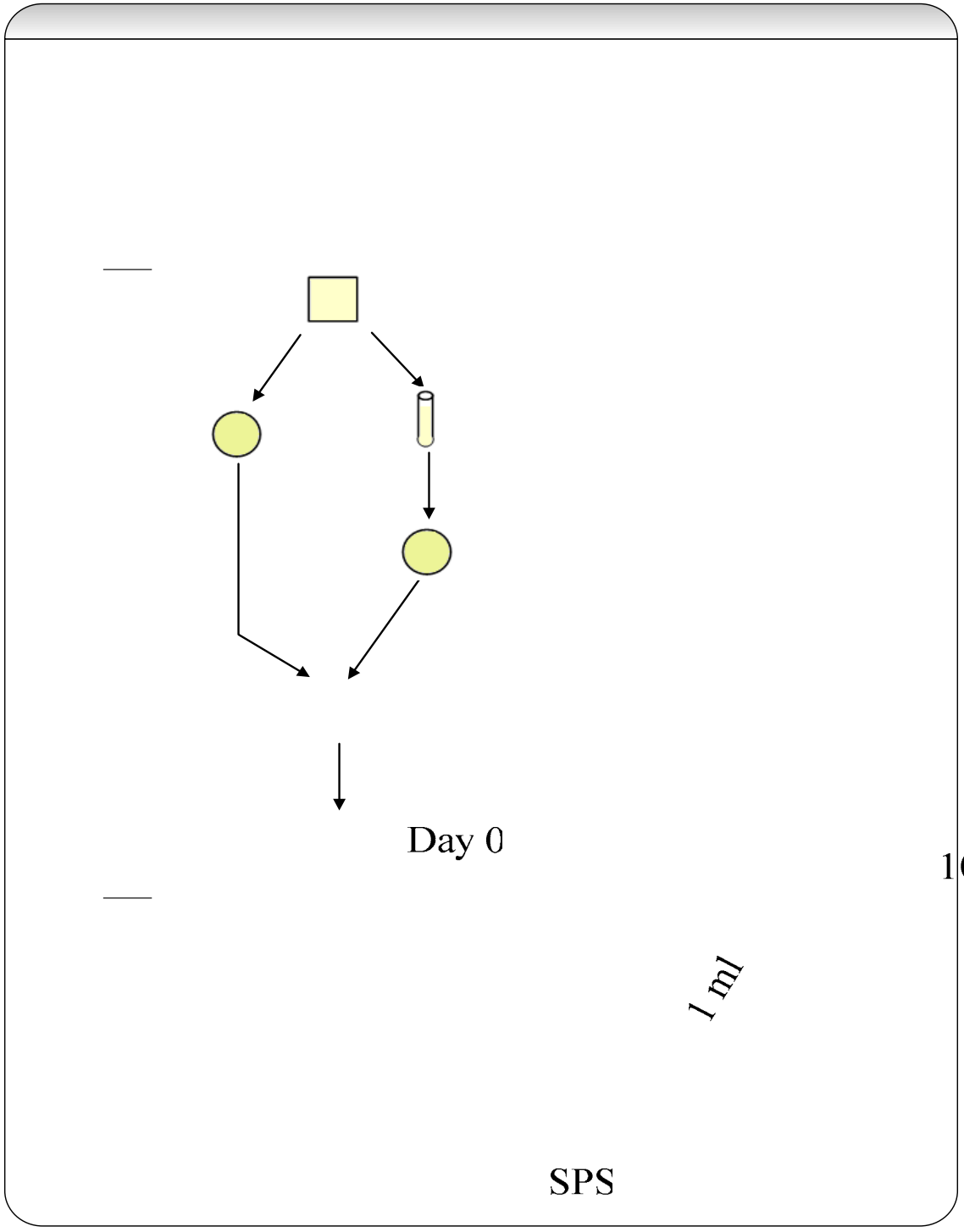

Fig.2.5 Enumeration of *Clostridium perfringens* (Anaerobic Sulphito-Reductors)

1.4 Detection and enumeration of *Staphylococcus aureus*.

Fig.2.6 describes the method of enumeration of *Staphylococcus aureus* in meat and meat products, according to the COFRAC method.

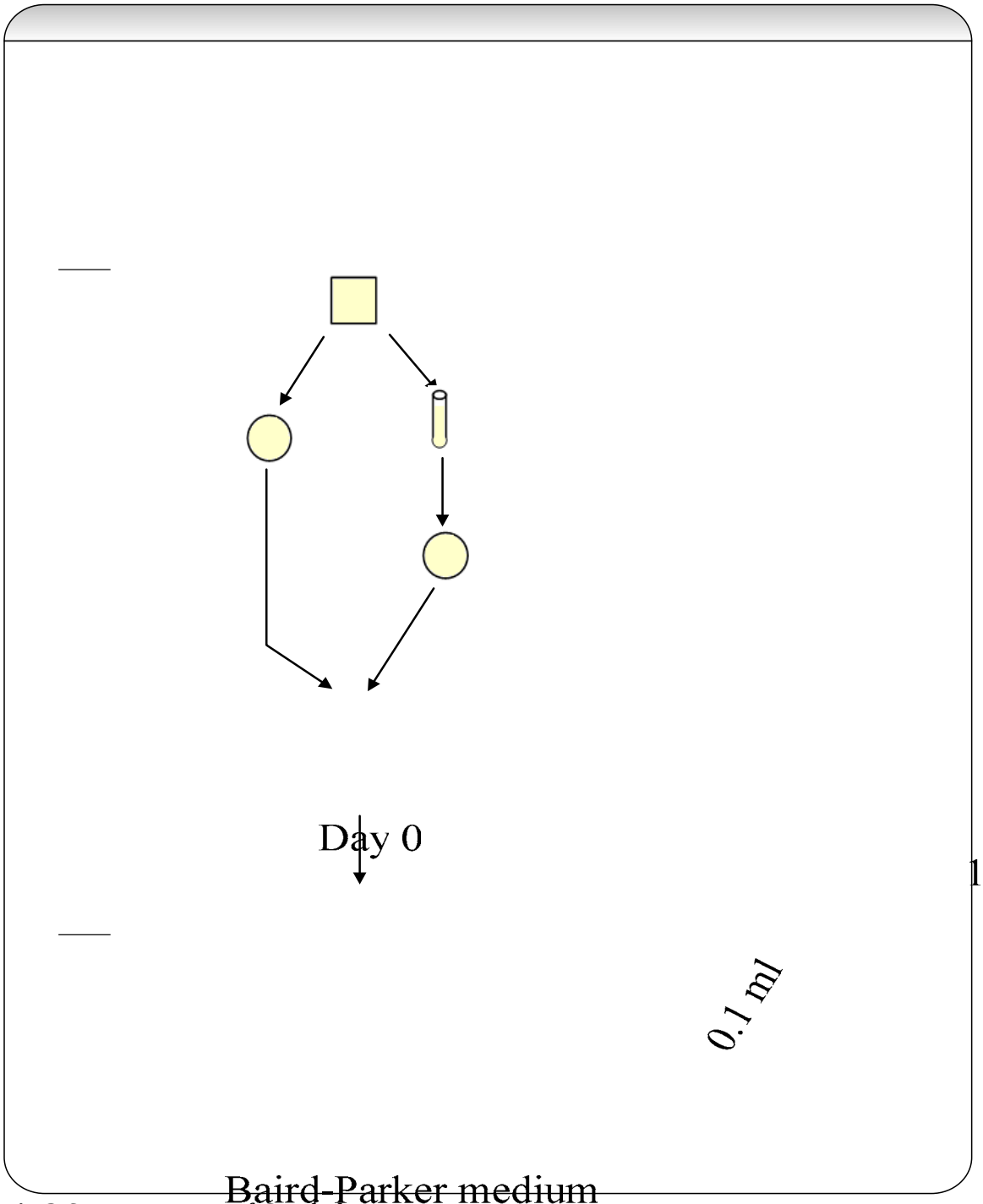

Fig.2.6 Enumeration of *Staphylococcus aureus*

1.5 Detection of *Salmonella.*

Fig.2.7 describes the method of detection of *Salmonella* in meat and meat products, according to the COFRAC method.

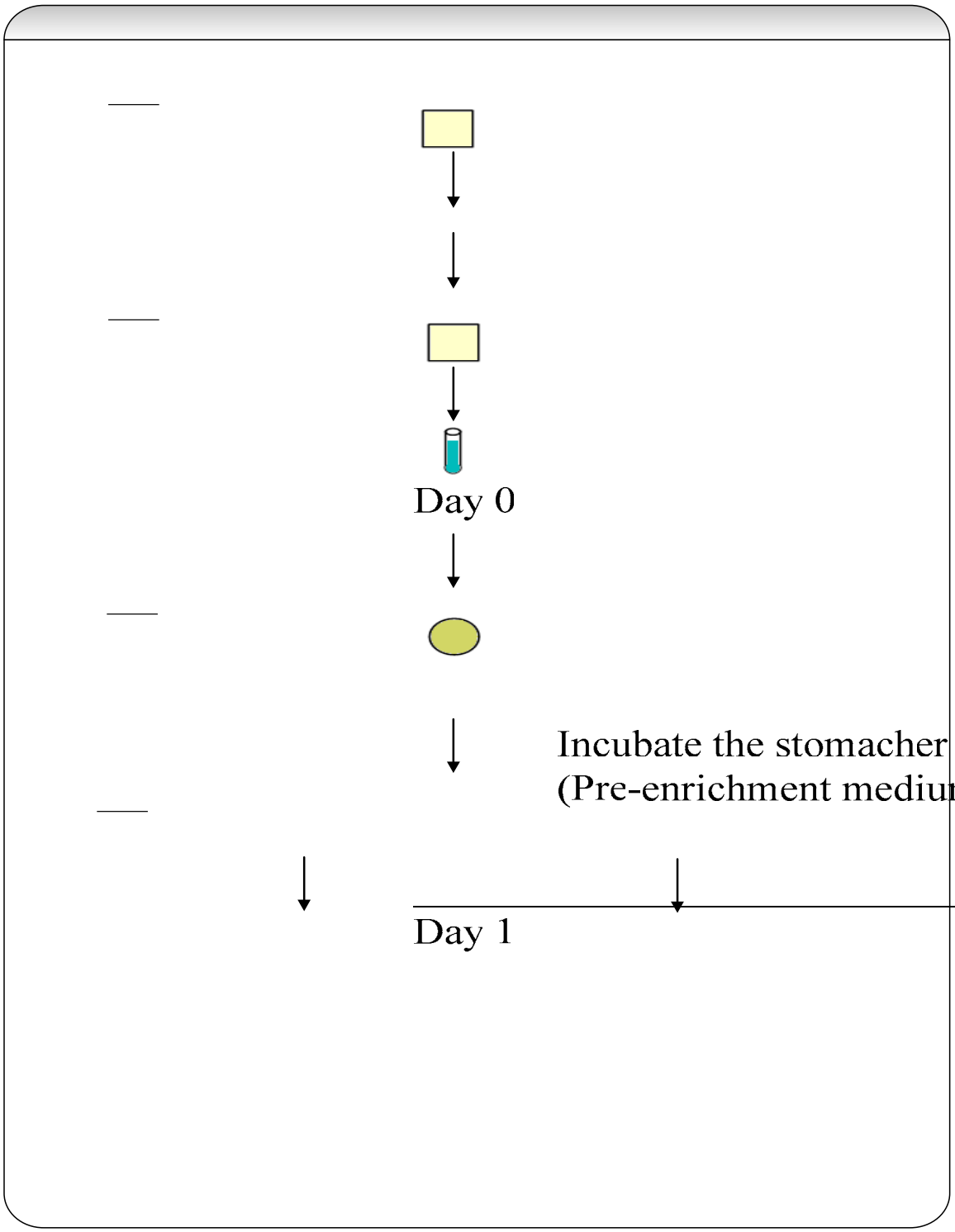

Fig.2.7 detection of *Salmonella*

Incubate the test tube for 24 hours at 37

1.6 Detection of *Listeria monocytogenes*.

This procedure as shown in fig.2.8 describes the method of detection of *Listeria monocytogenes* in meat and meat products, according to the COFRAC method.

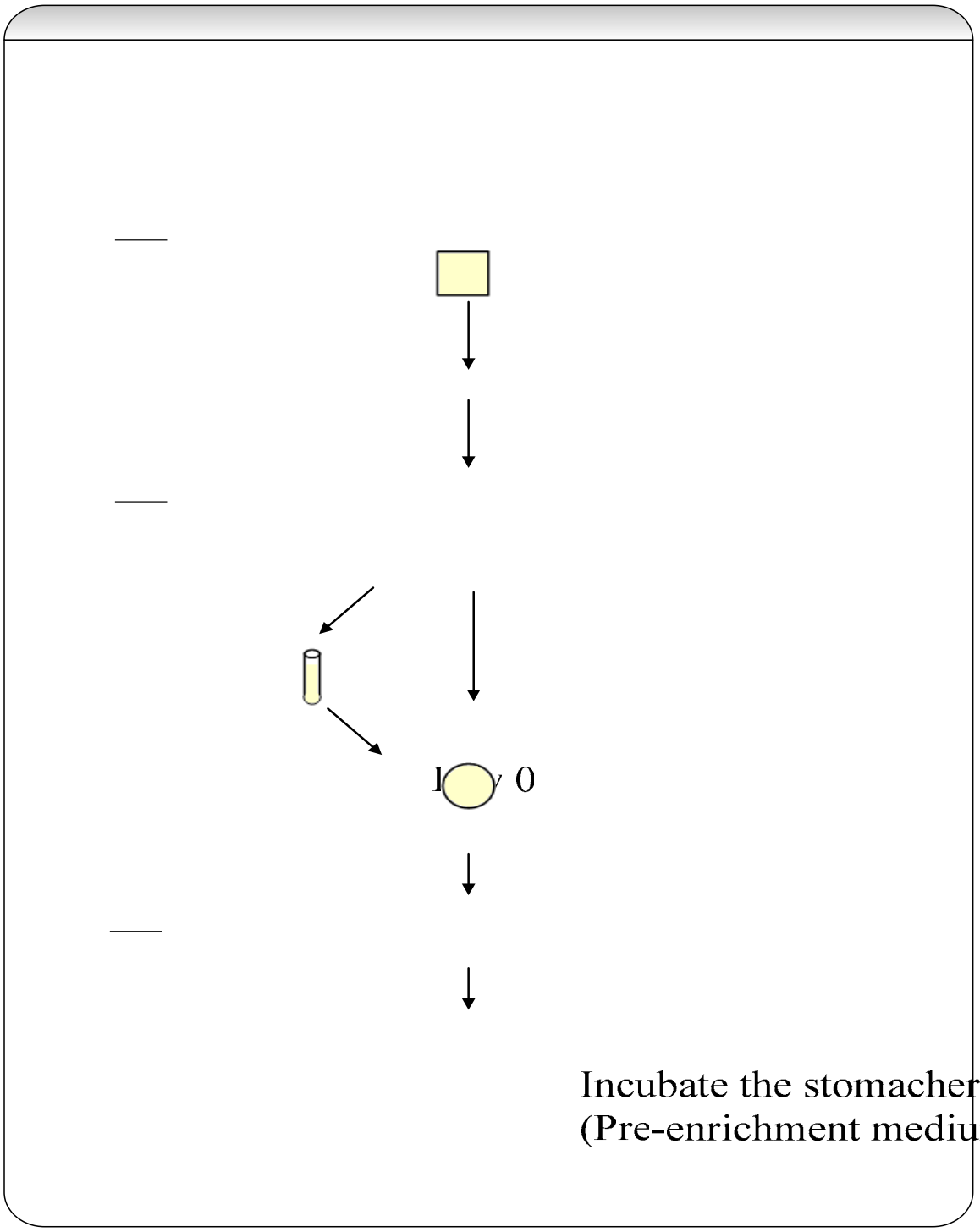

Fig.2.8 Detection of *Listeria monocytogenes*

Day 1

Inoculate 0.1 ml on Palcam pla
(Enrichment medium)

Appendix Four: Organoleptic Test

1. Organoleptic Test

1.1 Purpose

Sensory evaluation is a common and very useful tool in quality assessment of processed meat products. It makes use of senses to evaluate the general acceptability and quality attributes of the products.

1.2 Fields of Application:

- Sense of sight is used to evaluate the general appearance of the product such as color, size, shape etc.
- Sense of smell for the odor.
- Sense of taste for the flavor which includes the four basic tastes sour, sweet, bitter and salty.
- Sense of touch for the texture either by mouth feel or finger feel.

1.3 Principle and General Specifications for Installation:

The premises, however large they are, shall meet the following specifications:

They shall be pleasant and suitably lighted but neutral in style. For this purpose, a soothing, plain, light color is recommended for the walls so that a relaxed atmosphere is created.

The premises shall be such that they are easily cleaned and shall be separated from any source of noise; consequently, they shall preferably be sound proofed. They shall also be kept free from extraneous odors for which purpose, if possible, they shall be fitted with an effective ventilation device. If the fluctuations in ambient temperature so warrant, the test room shall be equipped with air conditioning to keep the atmosphere close to 20-22º C.

In the simplest way of sensory testing, we assisted by special staff, will test a product's color, smell, taste and texture upon manufacture. We used sophisticated approach a team of trained panelists in order to make the results as objective as possible. For this purpose we went to the use of an appropriate testing room available with lights, temperature and seating arrangements with individual testing compartments so as not to distract the members of the panel.

As an ideal arrangement the panel is composed of ten trained panelists usually employed in the meat processing plant. It is obvious that for reliable test result the panelist need relevant instructions and some experience in the food sector.

Only people with good sensory capability should be chosen in order to find out differences in color, texture, flavor and taste.

All panelists must use proven and identical test methods in order to make their results comparable. Each panelist involved in such tests is given a score sheet, where they mark their findings. Score sheets of the team of panelists are evaluated and a test result for each individual product is produced based on multiple observations.

1.4 Common test method

Hedonic scale rating test or acceptability test where samples are tested to determine their acceptability or preference (Fig.1.1).

The hedonic scale rating can be used for internal factory testing, and this method is also suitable for market research by determining the consumer's acceptance or preference for certain products.

Table.2.7 Score sheet for hedonic-scale rating test.

Name:	Product:		
Panelist No.:	Date:		
Instructions: Taste the given samples, and then place an (X) mark on the point in the scale which best describes your feeling.			
SCORE*	SAMPLE CODE		
(9) Like extremely			
(8) Like very much			
(7) Like moderately			
(6) Like slightly			
(5) Neither like nor dislike			
(4) Dislike slightly			
(3) Dislike moderately			
(2) Dislike very much			
(1) Dislike extremely			

* Note: Numbers in parentheses are to be assigned during data analysis and are not to appear in the score sheet.