METHODOLOGIES SUGGESTED FOR REDUCING DAMAGES OF THERMOFORM VACUUM PACKAGES AND IMPROVING METHODOLOGIES FOR THE ABOVE.

By

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00/AS/033

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DECLARAION

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AFFECTIONATEY DEDICATED TO
MY EVER LOVING PARENTS
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ABSTRACT

Thermoform vacuum packages of pre-cooked meatballs get non-vacuuming conditions in different stages from packaging to consumption. Due to the low resistance of packaging material for stress conditions, humidity, and temperature variations, damages occur during storage, transportation and handling. Because of that vacuum leakage problem arises. Meatball is a processed meat product, which is packed in a polythene polyamide lamination. The objectives of the study were to decrease the physical damage percentage of the thermoform vacuum packages and to find the correct combination of material layering to restrict air and water vapor migration between in and out.

Randomly selected 250 thermoform vacuum packages were filled by using a special air pump and immersed in to water basket. Using this method, the damage areas of the thermofom vacuum packages were identified. Then the main factors, which caused the non-vacuuming conditions, were identified and listed out. After that non-vacuumed trade returns were analyzed by using Pareto analysis. The major cause was identified as the punctures of the bottom material. The available bottom material for Skinless chicken and meatball was laminated LDPE nylon. So the bottom material was changed and new material was used. That new material was co extruded LDPE nylon.

Under the same condition, using both packaging materials, 500 packets of meatballs were produced Those packets were bundled in to 20 bundles. Those bundles were sent to out station area by using a company truck and received through the same vehicle without unloading. The number of non-vacuumed packets was listed out. Finally overall damage percentages of available material and the changed material were listed out. Those data were statistically analyzed.

Then microbiological tests were carried out to check whether it was microbiologically accepted or not. Such tests were carried out to determine the presence of *Staphylococcus aureus* and *Escherichia coli* . And also Total Plate Count (TPC) was determined. *Staphylococcus aureus* and *Escherichia coli* were not present at initially and after three weeks. The total plate count was initially 300 cfu/g and after three weeks 300 cfu/g. It is necessary to carry out further studies for microbiological tests. Finally it can be concluded that co extruded LDPE nylon packaging material has higher mechanical strength and resistivity than laminated LDPE nylon packaging material.

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CHAPTER 1
Introduction

1.1 Introduction:

Thermoform vacuum packages of pre cooked sausages and meatballs get non- vacuuming conditions at different stages from packaging to consumption. Sausage is a processed meat product. It is packed in a polyethylene nylon lamination, which is called commercially Nylon/LDPE laminate.

The simplest modified atmosphere package is known as vacuum package. There are three different types of vacuum packages which are cryovac method, chamber method, and thermoforming method. The vacuum packages are also extremely tight because of the pressure differential between the inside and surrounding atmosphere. There are many types of films used for thermoforming including acrylonitrile butadiene styrene, cellulose propionate, ionomer, polyethylene, polyprolene, polystyrene, polyamide combinations.

Oxygen permeability of packaging material is an important variable and has significantly greater effect on the sensory shelf life of the vacuum packed meat products than that of gas flushed meat products.

Inside of the vacuum package a vacuum is created by evacuation of air. Since there is no oxygen present, aerobic microbe like pseudomonas, lactic acid bacteria, salmonella cannot grow under this condition. But anaerobic microbes if present (eg- clostridium sp) will create good environment for their growth. But the addition of nitrite salt into processed meat products confirms the destruction of *Clostridium botulinum* like anaerobic microorganism.

The other important variable is the sealing strength of material. Even use of more oxygen impermeable material cannot get expected results if there are leak indicating sealing. Sealing strength mainly depends on the 3 factors which are types of the material, sealing temperature, time period taken for the sealing.

1.2 Objectives:

1. To decrease the physical damage percentage of thermoform vacuum packages

2. To find the correct combination of material layering to restrict air and water vapor migration between in and out.

CHAPTER 2
Review of literature

2.1 History:

Animals particularly rodents, insects and microorganisms (moulds, yeast, & bacteria), all cause wastages at various stages in the growth, harvesting, processing, storage, transport & sale of food. If microorganisms are permitted to flourish in food, they will make it unattractive. The provision of food, which is good and safe to eat, is therefore a duty of the food industry. The earliest packaging materials were probably leaves from larger plants .In early days when man lived by hunting & gathering, their nomadic existence necessitated some means of keeping food "fresh" while they travelled. Animal's skin was used for water &woven baskets were also employed.

It is perhaps inevitable that some of the landmarks in the fight to preserve food should concern the provision of armies. At the beginning of 19th century Napoleon was finding it increasingly difficult to do this. The "scorched earth" policy of his adversaries meant that it was not possible to live off the conquered territories, and the blockade by the British fleet deprived France of the sugar needed to preserve such produce as fruit. He needed packaged foods that could be taken along with his armies, and he offered a prize of 12 000frances for a suitable method of preservation. Frenchman, Nicolas Appert, won this in 1810 for the development of canning process, although he at that time used glass jars. (Paine and Paine, 1992).

Marketing is relatively modern invention and it has become intricately involved with the packaging function. In early times products were produced principally by hand for very limited market areas and readily found purchasers through the simplest of advertising techniques with the invention of printing, it became easier to put manufacturers identification or brand on to the product and thus allow the label to remind the customer where he purchased the product.

The purchaser now has a choice among competitive products. It became necessary for the manufacturer to seek methods for influencing that choice Thus, simultaneously with the development of mass production and mass distribution of goods came the development of what is now known as the marketing function(Paine and Paine,1992).

2.2 What is packaging:

Packaging has been defined in several ways.

1. A coordinated system of preparing goods for transport, distribution, storage, retailing and end use.

2. A means of ensuring safe delivery to the ultimate consumer in sound condition at minimum overall cost

3. A techno economic function arrived at minimizing cost of delivery while maximizing sale.

To ensure delivery the package must at least provide information as to the address of recipient, describe the product and perhaps explain how to handle the package and use the product. In addition we recognized that packaging is part of the marketing process.

Marketing may be defined, as the identification, anticipation and satisfaction of customer need profitably. Packaging has also been described as a complex, dynamic, scientific and controversial segment of business.

Packaging is certainly dynamic and is constantly changing. New materials need new methods, new methods demand new machinery, and new machinery results in better quality, opens up new markets, which require changes in packaging (Paine and Paine,1992).

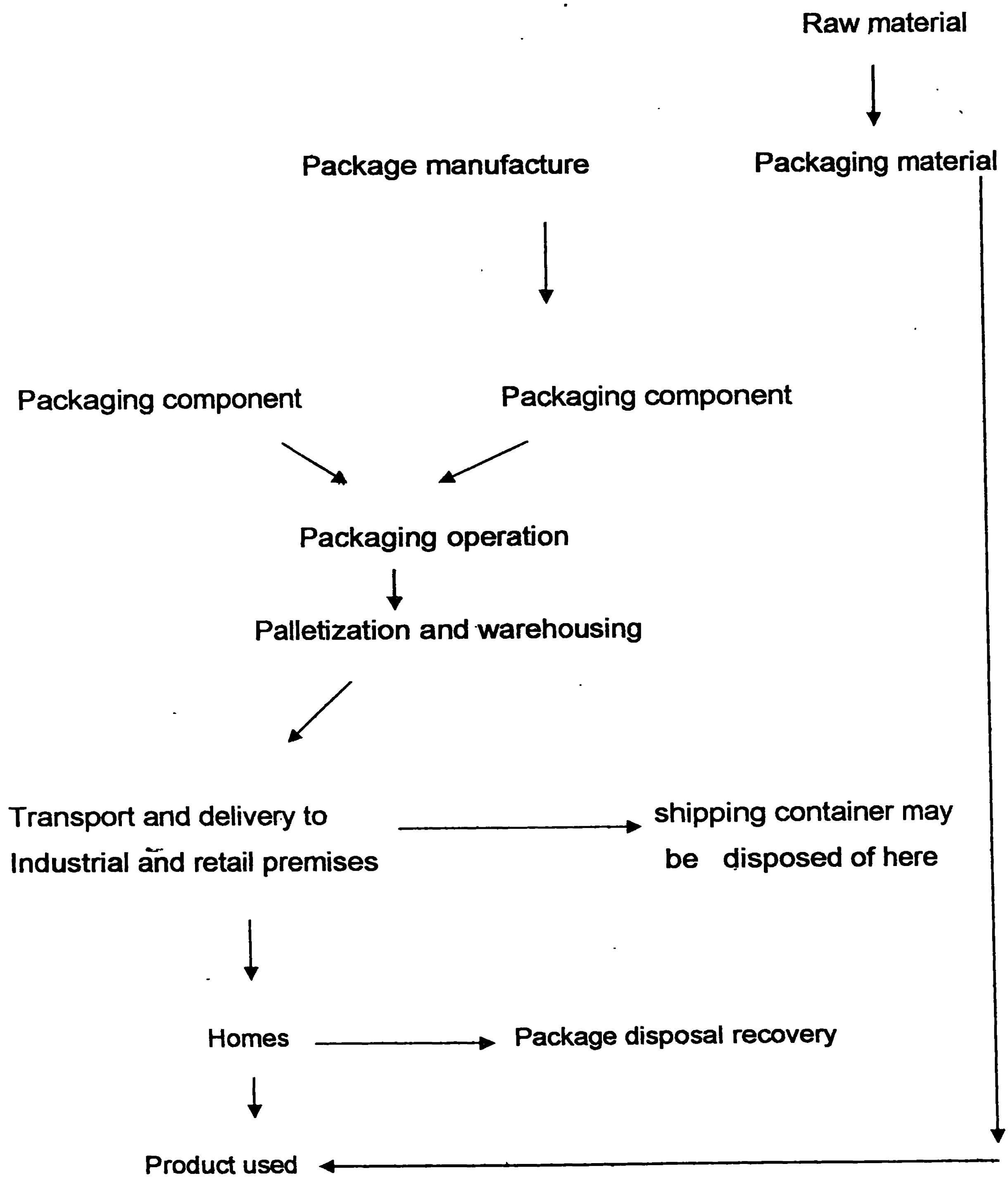


Figure 2.1 The marketing process and the packaging (Paine & Paine,1992).

2.3 Functions of packaging:

The role of packaging in food preservation is essentially one of protection against extraneous agents, and dirty handling. These include all the spoilage agents mentioned previously. If food is already contaminated before packing, the latter can have little preventive effect. However most foods are subjected to some form of preservation before packing. The packing material may however, further extends the shelf life of the product. Also packing is used to present the commodity in an attractive form to the buyer.

Packaging embraces both the art and science of preparing goods for storage, transport and eventually sale. Hence packaging has other functions in addition to the protection. The successful application of packaging film in the field of packaging may, therefore, require the collaboration of the food manufacturer, the packaging film producer and converter, the designer and ultimately the consumer. If we produce a new package it should be evaluated economic basis also. The consumers are interested only in the product and not its package. Hence, the package should be as simple and as inexpensive as possible.

Further constraints may be imposed by the need to use machinery during production process, so that qualities such as flexibility, printability, ease of working on wrapping machines, heat sealability and suitability for forming by blow-moulding, extrusion, vacuum or thermal techniques may also influence initially the material to be used. Other importances in the food industry include transparency and permeability to water vapor and to gases such as CO2, O2, and N2 (Tamine, 1999).

2.4 Requirement for effective food packaging:

1.Be nontoxic
2.Protect against contamination of microorganisms
3.Act as a barrier to moisture loss or gain and oxygen ingress
4. Protect against ingress of odors or environment toxicants
5.Filter out harmful uv light
6.Provide resistance to physical damage
7.Be transparent
8.Be tamper-resistant or tamper-evident
9.Be easy to open
10.Have dispensing and resealing features
11.Meet size, shape, weight requirements
12.Have appearance, printability features
13.Be low cost
14.Be compatible with food

2.5 Problems encountered in food packaging:

Whilst many technological problems encountered in the use of plastics for food-packaging applications are those that are common to the packaging industry as a whole, some other considerations arise solely through the nature of the product to be packaged. Most foods are perishable, having a useful life, which can be as short as only a day or two in the case of some fresh foods, depending on the conditions of storage. Hence requirements for a food packaging material are generally more stringent than for many other retail and household commodities.

The principle intrinsic requirements of a food packaging material can be classified as follows.

1.Transparency and surface gloss.
2.Control over transfer of moisture.
3.Control over transfer of other gases.
4.Wide temperature range in storage and use.
5.No toxic constituents.
6.Low cost.
7.Protection against crushing.

Customers like to see what they are buying and, hence transparency (with gloss) is an essential requirement in many food-packaging applications. Opaque materials such as trays, although widely used, prevent the customer seeing both sides of the product.This can engender distrust and consequently sales resistance. Control over changes in the moisture content of a food during storage is most important. Some products, e.g. Snack foods, biscuits, or boiled sweets, must be packed in a material with a very low permeability to water vapor to preserve cryspness

It is also essential to reduce moisture losses with many foods and again a low permeability film would be required. In other cases, some losses of moisture concomitant loss of transparency as well as increased risk of mould growth.

In addition to transfer of water vapour in and out of the packaging material, control over permeability to other gases such as oxygen and carbon dioxide is also very important. Most fresh foods need to 'breath'; hence the packaging material used for these products must allow ingress of oxygen and respiration of carbon dioxide, where the material chosen does not permit sufficient gas transfer, the problem can often be solved by incorporation of a few holes punched into the film. Fresh meat also requires ingress of oxygen to maintain a satisfactory surface colour. On the other hand, foods with a high fat content become rancid on exposure to oxygen and are often vacuum packaged, or in an inert atmosphere, using a material of very low permeability (Crosby, 1981).

2.6 The principal polymers found in the food industry:

Most polymers used for food packaging are thermoplastic. They can be softened by heating and hardened on cooling, repeatedly provided that no chemical decomposition occurs. Thermosets, on the other hand, undergo an irreversible change on heating and do not soften. They char at high temperatures since their molecular structure is a complex 3 dimensional bonded network.

Thermoplastics are preferred in food industry since the basic polymer may have to be subjected to several heating and cooling cycles during manufacture into formed containers. Thermosets could not be processed in this way. Most thermoplastics are derivatives of ethylene (CH2=CH2). They are also often referred to as the vinyl plastics since they all contain an olefin linkage before polymerization.

2.7 Basic films:

2.7.1 Polyolefilms:-

They have generally similar properties, being extensible, heat sealable and good water vapour but poor gas barriers. Polyethylene has been the most widely used material, mainly in its low-density form. LDPE(low density polyethylene) has low melting point with high heat sealability.

2.7.2 Copolymers:-

As the name suggested these differ from the homo polymers in that they are formed by the copolymerisation of two dissimilar molecules.

-Ethylene vinyl Alcohol

A material with extremely good gas barrier properties, moved unfortunately by a high sensitivity to water vapour.

-Vinylidene chloride-Vinyl chloride

A copolymer which is used as a self supporting film in order to utilize the very high barrier performance that poly (vinyldene) can provide, but which is not suitable for use as a straight film (Crosby,1981).

2.7.3 Other plastics :-

-PolyVinyl Chloride

It is used in two distinct forms.

1.Unplasticised-Rigid material for thermoforming.
2.Platicised-Very flexible high transparent and with pronounced blocking tendencies, with high gas permeability characteristics. Especially suitable for stretch wrap applications.

-Polystyrene

Used as a thermoformable sheet in food packaging. Thermoformable sheet material is normally modified by the incorporation of synthetic rubber compounds to improve the impact resistance. It is very permeable to water vapour and moderately so to gases.

-Polyamides

Good gas barrier performance, grease resistance and mechanical strength are very useful. Polyamides are resistant to higher temperatures than most other common plastics. The polyamides are rather moisture sensitive and quite permeable to water vapour. Their major form of use is as a co-extrusion with low-density polyethylene, thus both protecting the nylon and providing the barrier to water vapour.

-Acrylonitriles

These have very high gas barrier properties and have been used in sheet form for thermoforming.

2.7.4 Modified cellulosics

Derived form naturally occurring cellulose, are chemically modified in to true thermoplastics. The major areas of application are thermoformed blisters, windows and for cartons and rigid transparent cartons.

2.7.5 Regenerated cellulose

These films are made from selected wood chips in which the cellulose is taken in to solution and reprecipitated as a continuous transparent film. The basic material is an excellent barrier to gases, but is sensitive to, and very permeable to water vapour.

2.7.6 Non-plastics:

Materials used in combination with plastics for food packaging include paper in wide range of types and Aluminium foil. The former contribute stiffness and printability.
The Aluminum foils provide an almost perfect barrier to gasses and light. Both provide folding properties and rigidity. (Crosby,1981).

2.8 Multilayer packaging:

Simply combination of different layers/films on different polymers are processed by extrusion process or lamination process.

1.Lamination: -Wet bond lamination
-Dry bond lamination
-Wax lamination
-Thermal or pressure lamination

2.Extrusion coating

3. Extrusion lamination

2.8.1 Lamination:

2.8.1.1 Wet bond lamination

Any process where liquid adhesive is applied to substrate that is then immediately combined with a second ply to create a laminate. The adhesive is water born one of the webs must be permeable to the solvent vapour. Laminated film can be delaminated.

2.8.1.2 Dry bond lamination

Solvent born adhesive is applied to a substrate that passes through a drier to evaporate the carrier solvent. This then combined with a second substrate in a heated pressure nip. Laminated film can be de laminated.

2.8.1.3 Wax lamination

Bonding of paper-to-paper, paper to board or paper to foil with continuous wax film. Micro waxes, which may be blended with paraffin wax, are used as the bonding agent. Sometime special resins are added to improve adhesion. Laminated film can be de laminated.

2.8.1.4 Thermal or pressure lamination

It is difficult to separate some thermal bonding from dry bonding. Previously applied thermoplastics adhesive, which are activated by the heat and pressure of the thermal laminating is called Thermal or pressure lamination. Laminated film can be de laminated.

2.8.2 Extrusion coating

Extrusion coating is a process in which an extruder force melted thermoplastic. Though horizontal slot die into moving web material the rate of application control the thickness of the continuous film deposited on the surface of the paperboard, film or foil. The melt stream extruded in one or several layers can be used as a coating or as an adhesive to sandwich two webs together. This foil can't be delaminated.

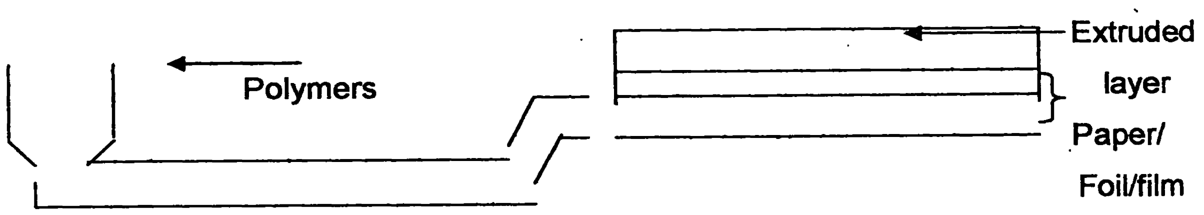


Figure 2.2 Extrusion Coating process

2.8.3 Extrusion lamination

In extrusion laminating a film of molten polymer is deposited between two moving webs in a nip created by a rubber pressure roll and a chrome plated steel chill roll. In this continuous operation chemical priming or other surface treatment to make it receptive the extrusion coating and to help develop adhesion between the two materials prepares the surface of the substrate. This foil can't be delaminated (Abstract of packaging institute)

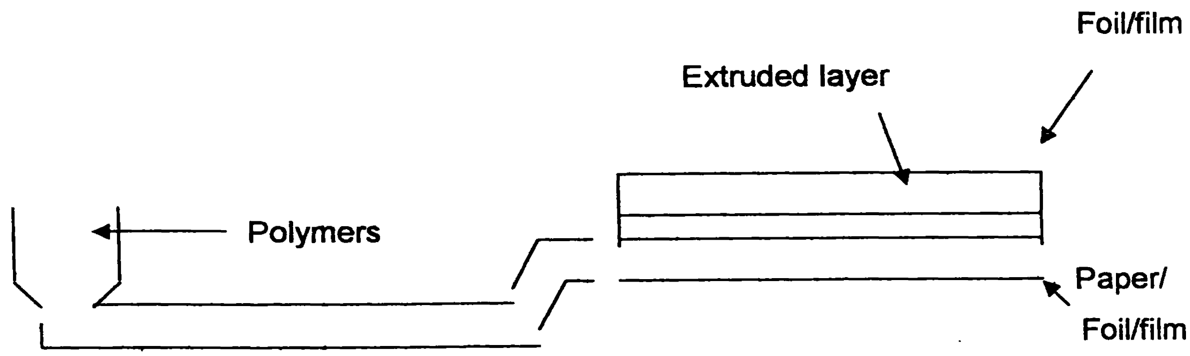


Figure 2.3 Extrusion Laminating process

2.9 What are thermoformed packages?

In recent years, several European companies and their American affiliates, as well as some American companies, have introduced thermoform, fill and seal equipment. Thermoform fill and seal machines built by the packagers of portion-pack jams, jellies, and condiments, have been used in the United States for many years but have not been available on the commercial market.

These machines heat a web stock consisting of a plastic such as impact polystyrene and then form the sheet with pressure in to a female cavity mold. The cavities are moved under a filling head, where the contents are filled. Contents may be fluid (eg; cream, yogurt, juice), viscous (eg; jam), or solid (eg; roasted peanuts). After filling, a film or laminated flexible membrane is heat sealed to the flat flanges formed between the successive cavities at another station. A die cutting device separates the individual cavities. Almost all thermoform, fill and seal machines in the world are made in Western Europe countries like France and West Germany.

In recent years solid phase pressure forming machine was introduced, which has been used in the United states to permit use of polypropylene sheet to make performed cups(Anon,1969A).

2.10 The shelf life in the vacuum:

The difference in the quality and shelf life between vacuum & gas packed meat products depended mainly on the initial microbial load and the gas permeability of packaging material. Many scientific researches proved that the O_2 permeability of packaging material is an important variable and has significantly greater effect on the sensory shelf life of vacuum packed meat product. When we develop a new package for processed meat products it should be accepted both microbiologically and physically. In vacuum packages O_2 permitting through the packaging material is immediately in contact with the surfaces of the products and promote the growth of aerobic microbes. Microbial growth in vacuum packed, cooked meat products mostly occur in the surface layer not at the center of the product.

Even a quite small decrease in the O_2 permeability of packaging films can substantially improve the shelf life of the vacuum packed meat products. The O_2 permeability of packaging films has a very great effect on the growth of obligatory aerobic spoilage organisms in vacuum packed freshly comminuted beef.

One possible reason for the shorter shelf life of vacuum-packed products even with gas-tight materials is the residual air in the headspace. The initial oxygen concentration in vacuum packages is near to 21%(v/v), whereas in gas packages the residual oxygen concentration is usually below 1%(v/v) and in addition carbon dioxide has been added. Consequently aerobic spoilage flora will more likely start multiplication in a vacuum that in a gas atmosphere.

2.11 Processed meat and poultry packages:

The processed meat and poultry segment include sausages, cured meats, smoked meats, meat specialties such as luncheon meat, and processed poultry. Compared to the fresh meat and poultry category the sales volume of these products is somewhat smaller, about $18 billion in the US. But they consume an important quantity about$250 million, of plastic packaging materials. Processed meat products are sold in much larger volume than processed poultry but most of the requirements and structures are the same for the two product lines.

2.11.1 Package requirements:-

Centralized packaging of processed meat requires a barrier packaging material, which will prevent discoloration and rancidity due to O_2.This adverse reaction, is accelerated by light. These requirements have made processed meat packaging a major market for sophisticated plastic structures For example, for to days application, these structures must have very low oxygen permeability in the range of 0.2cc/100in2.atm.24 hr at50%R.H.

2.11.2 Early processed meat packages:-

Until the 1940,processed meat was sold in butcher shops, where the meat was sliced to order and wraped in butcher paper, frankfurters were prepacked in natural casings in the form of long cylinders and cut off order. As with so many other foods, the advent of the supermarket required pre-packing so the product could be selected directly by the customer, by passing the butcher.

The only packaging material then available for this purpose was uncoated cellopane. It kept the meat clean and visible in the showcase but could not protect it from air long enough to allow shipment of prepackaged product from the manufacturer to the store. Thus the butcher still prepared the product and packaged it in the back room.

2.11.3 The development of modern packages:-

The development in the 1950s of PVDC-coated cellopane, which provided an oxygen barrier below 1cc/100in2 atm.24 hr enabled producers to package processed meat in automated facilities in their packing plants and ship the product directly to the display case. Eliminating the store butcher from the distribution chain yielded major cost savings and allowed the manufacturer much better control over product quality.

Cellopane tends to be brittle and lacks puncture resistance at low temperatures. This problem, coupled with the high cost of cellopane as compared to plastic structures, gradually led to its displacement by plastics, beginning in the 1960s.At first the structures used were cellopane plastic laminations, but as time went on lower cost, all plastic structures became the material of choice.

2.11.4 Material requirement of current packages:-

Among the various categories of processed meat there are general requirements that apply to all products.

Oxygen barrier:

The barrier to oxygen must be sufficient to provide the desired shelf life, which will vary among the different products. Bacon, frankfurters, luncheon meats, for example, are fast selling items that rarely require shelf lives longer than 6 days except in distant markets. Not only must the material itself have sufficient barrier, but the seal must also be hermatic.

Clarity:

The product must be seen and judged for suitability by the buyer.

Resistance to cracking:

This is particularly impotent at low temperatures.

Resistance to puncture and pinhole formation:

Frequent product handling makes this a requirement.

Good sealability:

The seal must be hermetic even in the presence of fat grease. In addition, high hot tack is important.

Easy opening:

This characteristic, while desirable, is often sacrificed if the producer feels that an easy opening feature will compromise the O2 barrier characteristics that are needed.

Reclosability:

The comments above also apply to this desirable characteristic.

2.11 Cured meat:

Meat that is treated with salts such as NaCl, $NaNo_2$, and $NaNo_3$ differ from untreated fresh meat. The most apparent differences are in the color and flavor of fresh and cured meat. However the intrinsic factors of cured meat, such as PH, water activity and the presence of air, play and important role in the microbial ecology of these products. Sausage and meatball are cured and pre cooked processed meat product.

The water activity of cured meat (0.88to 0.95) is lower than that of fresh meat (0.98to1.00) and the specific inhibitory effects of the added salts may account for the differences in the spoilage patterns of fresh and cured meats (Banwart, 1987).

2.12 Microbiological effect on cured meat:

2.13.1 Spoilage:-

Spoilage of these products is generally of three types; ***sliminess, souring***, and ***greening, Slimy*** spoilage occurs on the out side of casings, especially of frankfurters, and may be seen in its early stages as discrete colonies, which may later coalesce to form a uniform layer of gray slime. Yeast, lactic acid bacteria of the genera lactobacillus, Enterococcus, Weissela, and B.thermosphacta may be isolated from the slimy material. W.viridescens produces both sliminess and greening. Slime formation is favoured by a moist surface and is usually confined to the outer casing. Removal of this material with hot water leaves the product essentially unchanged.

Souring generally take place underneath the casing of these meat and result s from the growth of lactobacilli, Enterococi, and related organisms. The usual sources of these organisms to processed meats are milk solids .The souring results from the utilization of lactose and other sugars by the organisms and the production of acids. Sausage usually contains a more varied biota than most other processed meats due to the different seasoning agents employed, almost all of which contribute their own biota. B.thermosphacta has been found by many investigators to be the most predominant spoilage organism for sausage.

Although mold spoilage of these meats is not common, it can and does occur under favorable conditions. When the products are moist and stored under conditions of high humidity, they tend to undergo bacterial and yeast spoilage. Mold spoilage is likely to occur only when the surfaces became dry or when the products are stored under other conditions that do not favor bacteria or yeasts.

Two types of greening occur on stored and processed red meats .One caused by H_2O_2 and the other by H_2S.The former occurs commonly on frankfurters as well as on other cured and vacuum packaged meats. It generally appears after an anaerobically stored meat product is exposed to air. Upon exposure to air, H_2O_2 forms and reacts with nitrosohemochrome to produce a greenish oxidized porphyrin. H_2O_2 may accumulate when heating if nitrite destroys catalase, and the peroxide reacts with meat pigments to form choleglobin, which is green. Greening also occurs from growth of causative organisms in the interior core, where the low oxidation-reduction potential allows H_2O_2 to accumulate. *Weissella viridescens* is the most common organism in this type of greening, but leuconostics, Enterococus are capable of producing greening of products.

Greening also can produce by H_2O_2 producers such as Lactobacillus jensenii, is resistant to >200ppm $NaNO_2$, and it can grow in the presence of 2-4%Nacl but not in 7%. W.viridescens has been recovered from anaerobically spoiled frankfurters and from both smoked pork loins and frankfurter sausage stored in atmosphere of CO_2 and N_2. Inspite of the discoloration, the green product is not known to be harmful when eaten (Jay,2000)

Table-2.13.1 Genera of Bacteria and Fungi most frequently found on Processed meat:-

Table-2.13.1.1 Bacteria:

Genus	Gram reaction	Relative prevalence
Acinetobacter	-	*
Aeromonas	-	*
Alcaligenes	-	*
Bacillus	+	*
Brochothix	+	*
Carnobacterium	+	*
Enterobacter	+	*
Enterococcus	-	*
Hafnia	+	*
Kocuria	+	*
Kurthia	+	*
Lactobacillus	+	**
Leuconostoc	+	*
LIsteria	+	*
Microbacterium	+	*
Micrococcus	+	*
Moraxella	-	*
Paenibacillus	+	*
pediococcus	+	*
Pseudomonas	-	*
Vibrio	-	*
Serratia	-	*
Staphylococcus	+	*
Weissella	+	*
Yersinia	-	*

Table-2.13.1.2 Fungi:

Genus	Relative prevalence
Yeasts: *Candida*	*
Debaryomyces	**
Saccharomyces	*
Trichosporon	*
Molds: *Alternaria*	*
Aspergillus	**
Botrytis	*
Cladosporium	*
Fusarium	*
Geotrichum	*
Monilia	*
Mucor	*
Penicillium	**
Rhizopus	*
Scopulariopsis	*
Thamnidium	*

(Note: *= known to occur, **=most frequently reported)

(Source-Jay, 2000)

2.13.2 Cooked cured meats as an environment for micro-organism:-

The major sources of organism present on meat are the animal hide and intestine. Both have their own natural micro flora but the hide is also contaminated with organisms that originate from faeces, soil and animal feed. During processing organisms present are transferred to previously sterile tissues. As already indicated with the exception of the liver and lymph glands, the tissue of the live healthy animal is essentially sterile. After slaughter, contamination can occur at any processing stage with skinning and eviscerstion being the most critical. Other sources of contamination are the slaughter house environment, processing equipment and people; failure to clean and sanitize equipment can lead to unacceptable levels of contamination. Contamination is confined to the meat surface except when meat is comminuted.

Cooked cured meats vary considerably as a medium for microbial growth .In the case of many modern products which are moist and of low levels of Nacl and NO2, there is little difference from uncooked cured meat and only the more sensitive Gram-negative bacteria are inhibited .In such cases extrinsic factors (refrigeration and vacuum packaging) are responsible for stability. More traditional hams have a higher level of stability as a consequence of higher levels of curing ingredients and a lower water content (Varnam & Sutherland,1995).

2.13.3 Cooked cured meats and food poisoning:-

(a)Staphylococcus aureus

Cooked cured meat such as sausages, ham are classic vehicles of staphylococcal food poisoning .In the past number of cases resulted from growth of Staphylococcus aureus during two stages of ham boiling. This process is considered inherently unsafe but is now carried out on only a very small scale and for a number of years the most common cause has been contamination at slicing followed by storage at high temperatures. Staphylococcus aureus, favoured by relatively high NaCl content, is able to grow very rapidly in air at high temperatures and critical levels of enterotoxin can be synthesized in as little as 6-8 hrs. General use of refrigeration at retail level and the use of vacuum packaging for pre-cooked products have reduced the incidence of *Staphylococci*_intoxication due to ham, sausage. Although a substantial number of cases still occur. These are largely associated with catering. Staphylococcal food poisoning has also been associated with the ham filling of commercially prepared sandwiches, which had been stored at room temperature on a hot day.

Staphylococcus aureus is only rarely involved in food poisoning due to consumption of centrally sliced and vacuum-packed cooked cured meats. This may be attributed in part, to the lower level of handling associated with automated packing and to improved refrigeration. There is also an effect of vacuum packaging that may be due to the inability of Staphylococcus aureus to synthesize enterotoxins in conditions of reduced oxygen tension. The organism produces an intoxication cased by enterotoxin secreted into the food during growth. The presence of the live organism in ingested food is irrelevant to production of the disease. Vacuum packaging also favors the growth of competitive lactic acid bacteria, especially in relatively low Nacl products, and this likely to be another significant factor. Canned hams were involved in a number of out breaks of Staphylococcus aureus food poisoning due to incorrect seam sealing led to recontamination in a processing environment of generally poor hygienic standards

(b)Salmonella

Salmonella has been responsible for a number of out breaks of food poisoning involving cooked cured meats .On some occasions; uncured cooked meats have also been involved, problems arising from under cooking or cross-contamination. Cross contamination can be a particular problem in small operations where separation of cooked and uncooked meats is physically difficult. Salmonella is unable to grow Wiltshire-type and more traditional products. But multiplication is possible on at least some types of sweet-cure ham if temperature abuse occurs. Any food that contains Salmonella is a potential hazard to the consumer (Vamam & Sutherland,1995).

Salmonellosis

Salmonella enteritis or Salmonella food poisoning is called salmonellosis. The organism penetrates epithelial cells of villi in the lower part of the small intestine entering the connective tissue below, where they stay and multiply. Endotoxins are released giving rise to the disease syndrome(Garbutt,1997).

(c) Listeria monocytogenes

In 1990, major product recall was necessary due to a high level of contamination by Listeria monocytogenes on vacuum packaged sliced ham. No cases of illness were known to have resulted, but political controversy followed allegations of unnecessary delay by the United Kindom department of health. Listeria monocytogenes is relatively resistant to NaCl and NO_2 and can grow on some types of cooked ham held at low temperature. There are no known cases of listeriosis due to consumption of cooked cured meats and the risks are not specific to cured products
(Varnam & Sutherland, 1995).

Listeriosis

The disease is thought to be rare, but mild symptoms may not be reported and diagnosed. High risk groups are pregnant women, the very young, the elderly and individuals with suppressed immunity (Garbutt,1997).

(d)Other bacteria

A number of other bacteria have been implicated in food poisoning following consumption of cooked cured meat. Most frequent were members of the *proteus-providencia* group, Yersinia enterocolitica and Enterococcus. Symptoms tended to be mild and poorly defined and the validity of many reports has been doubted (Varnam & Sutherland,1995).

CHAPTER 3
Materials & Method

3.1 Materials:

Dry sterilization-hot air oven range(0-250°C)
Wet sterilization-automatic and portable autoclaves
Pipettes (1ml/10ml)
Test tubes (16 mm diameter)
Petri dishes
Standard plate count agar
Buffered peptone water
Incubator-35 ± 0.5°C
Laboratory consumables
Mac conkey agar
Electronic balance-0 to 2 Kg range, 0.01g sensitivity
Water bath-44°C± 0.5°C
Glass spreaders (sterilized)

3.2Method:

Randomly selected 250 thermoform vacuum packages were filled by using a special air pump and immersed in to a water basket. Using this method, the damage areas of the thermoform vacuum packages were identified.

Then the main factors, which caused the non-vacuuming conditions, were identified and listed out. After that non-vacuumed trade returns were analyzed by using Pareto analysis. The major cause was identified as the punctures of the bottom material. The available bottom material for Skinless chicken and meatball was laminated LDPE nylon. So the bottom material was changed and new material was used. That new material was co extruded LDPE nylon.

Under the same condition, using both packaging materials, 500 packets of meatballs were produced. Those packets were bundled in to 20 bundles. (Each one contained 25packets).

Those bundles were sent to Anuradhapura area by using a company truck and received through the same vehicle without unloading. The number of non-vacuumed packets was listed out. Finally overall damage percentages of available material and the changed material were listed out. Those data were statistically analyzed.

Then microbiological tests were carried out to check whether it was microbiologically accepted or not. Such tests were carried out to determine the presence of *Staphylococcus aureus* and *Escherichia coli*. And also Total Plate Count (TPC) was determined.

3.2.1 Determination of total plate count:

3.2.1.1 Media preparation:

20.00g of buffered peptone water was dissolved in 1000.00ml of distilled water.
The mixture was heated until the solids were dissolved. Then it was autoclaved at 121^{0}C for 20 minutes.

3.2.1.2 Sample inoculation and preparation of dilution:

90ml of pre-sterilized buffered peptone water was taken in to a sterilized stomacher bag. 10g of the test sample was introduced aseptically. The sample was blended by using stomacher blender at high speed for 30 seconds. 1/10 diluted, 1ml of the blended sample was introduced into a test tube. 9ml of peptone water were added aseptically. Dilution series were prepared as above for the requirement.

3.1.1.3 Preparation of the total plate count (TPC) agar plates and inoculation:

The sterilized agar bottles were liquefied, using steam for 5 minutes. 1ml of 1/10 dilution was introduced aseptically in to the sterilized plate and 15-20ml of TPC agar was poured at below 45^{0}C. It was closed with a lid and gently mixed for even distribution and kept for 30 minutes at room temperature for solidifying.

3.1.1.4 Incubation of the TPC Agar plates:

The solidified plates were placed upside down in the incubator and set the temperature 33-35^{0}C and incubated for 48 hours.

3.1.1.5 Counting the colonies and recording the results:

The colonies were counted by using the colony counter and results were recorded.

3.2.2 Determination of *Escherichia coli*

3.2.2.1 Media preparation:

7.4g of Mac conkey broth powder was dissolved in 100ml of distilled water.
The mixture was heated until the solids were dissolved. It was autoclaved at 121^0C for 20min.

3.2.2.2 Estimation of presumptive *Coliforms:*

1g of original sample was dispensed to each 3 tubes containing 10ml of Macconkey broth. These tubes were mixed by gentle rotation. Tubes were incubated at 36 ± 1^0C for up to 48hrs.

3.2.2.3 Confirmation of *Coliform* and *E.coli*:

Each of the positive tubes was subcultured into 10ml of Brilliant Green Bile broth tubes.
And also these positive tubes were inoculated into 20 ml of Tryptone water tubes and incubated at 44^0C for 24-48hrs.
Two or three drops of Indole acetic acid were mixed with tryptone water tube.

3.2.3 Determination of *staphylococcus aureus:*

3.2.3.1 Media preparation:

Baird-parker medium :-

6.3g of Baird- parker powder was dissolved in 100ml of distilled water.
Then it was heated until dissolved and autoclaved at 121°C for 15min.It was cooled to 50°C and 50ml of eggyolk-tellurite emulsion was aseptically added. It was mixed well before pouring into the sterilized plates.

3.2.3.1 Sample inoculation & preparation of dilutions:

100ml of sterilized buffered peptone water was taken in to a sterilized stomacher bag Then 10.0g of the test sample was introduced aseptically. The sample was blended by using stomacher blender.

3.2.3.2 Introducing the samples to baird parker plates and incubation:

0.1ml of sample was introduced aseptically into two Baird parker plates. This quantity was carefully spreaded as possible over the surface of the Baird parker plates. Plates were incubated at 35^{0}C for 24-48hrs.

3.2.3.3 Counting colonies and recording results.

The colonies were counted using the following standards. Standards were shown in Table 3.1.

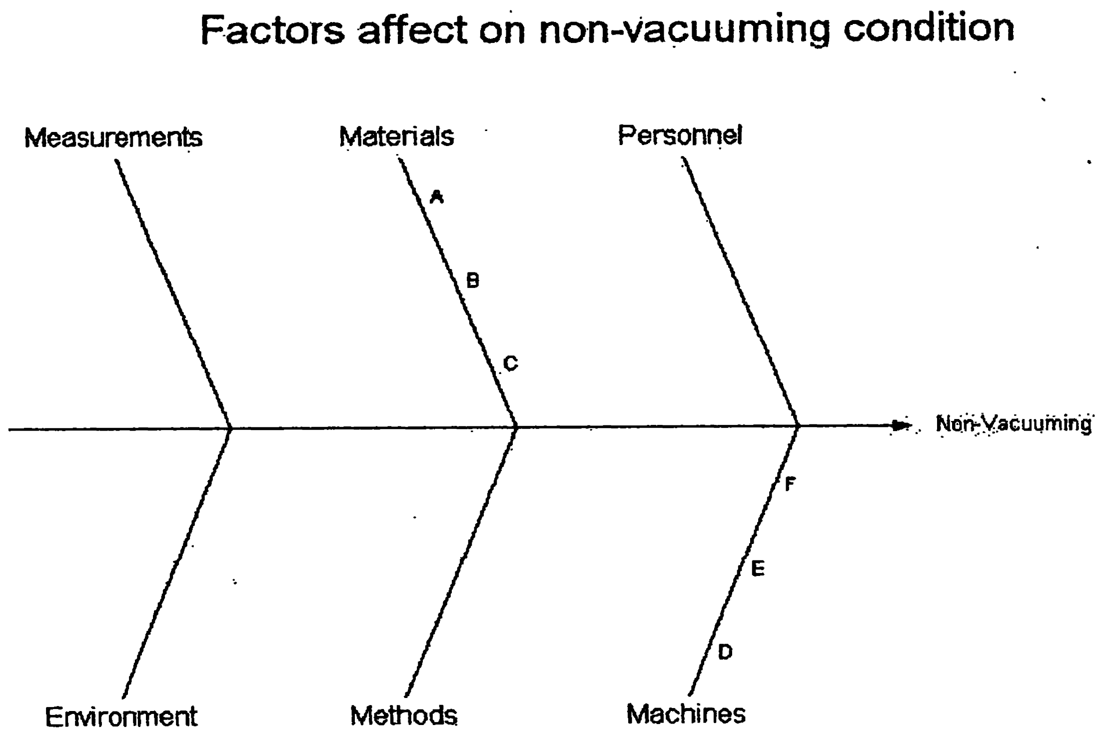
Table 3.1: Characteristics of colonies

Organism	Characteristics
Staphylococcus aureus	Grey black shiny convex1-1.5mm diameter(in 18hrs) Narrow white entire margin surrounded by clear zone
Staphylococcus epidermitis	Not shiny black and seldom produces a clear boundary
Staphylococcus saprophyticus	Irregular colony and may produce a clear Boundary with wide opaque zones.

CHAPTER 4
Results and Discussion

Packaging is a very important step when consider large-scale industrial food manufacturing. Processed cured meat are highly perishable foods and susceptible for the growth of spoilage and disease causing organisms such as *Salmonella*, *Staphylococcus aureus*, *Escherichia coli*. Thermoform vacuum packages of pre cooked sausages and meatballs get non-vacuuming conditions in different stages from production to consumption. This damaged sausage and meatball packages have short shelf life period and unsuitable for human consumption. Because of this reasons returned percentage had increased.

When problem was observed there are six factors affect on this non- vacuuming condition including poor film, vacuum sealability, punctures on the top film, punctures on the bottom film, leak indicated packets, inaccurately separated packets, piercing of top film due to hot foil printing. The cause and effect diagram is a method of expressing this type of problem easily. The out put or result of a process can be attributed to a multitude of factors and cause and effect relation can be found among those factors. The structure of problem can be identified by observing it systematically. It is difficult to solve complicated problems without considering this structure, which consists of a chain of causes and effects.



A-Poor sealability,

B- punctures on the top film

C- punctures on the bottom film

D- vacuum leak indicated packets,

E- inaccurately separated packets,

F- piercing of top film due to hot foil printing

Figure 4.1: Factors affects on non-vacuuming conditions

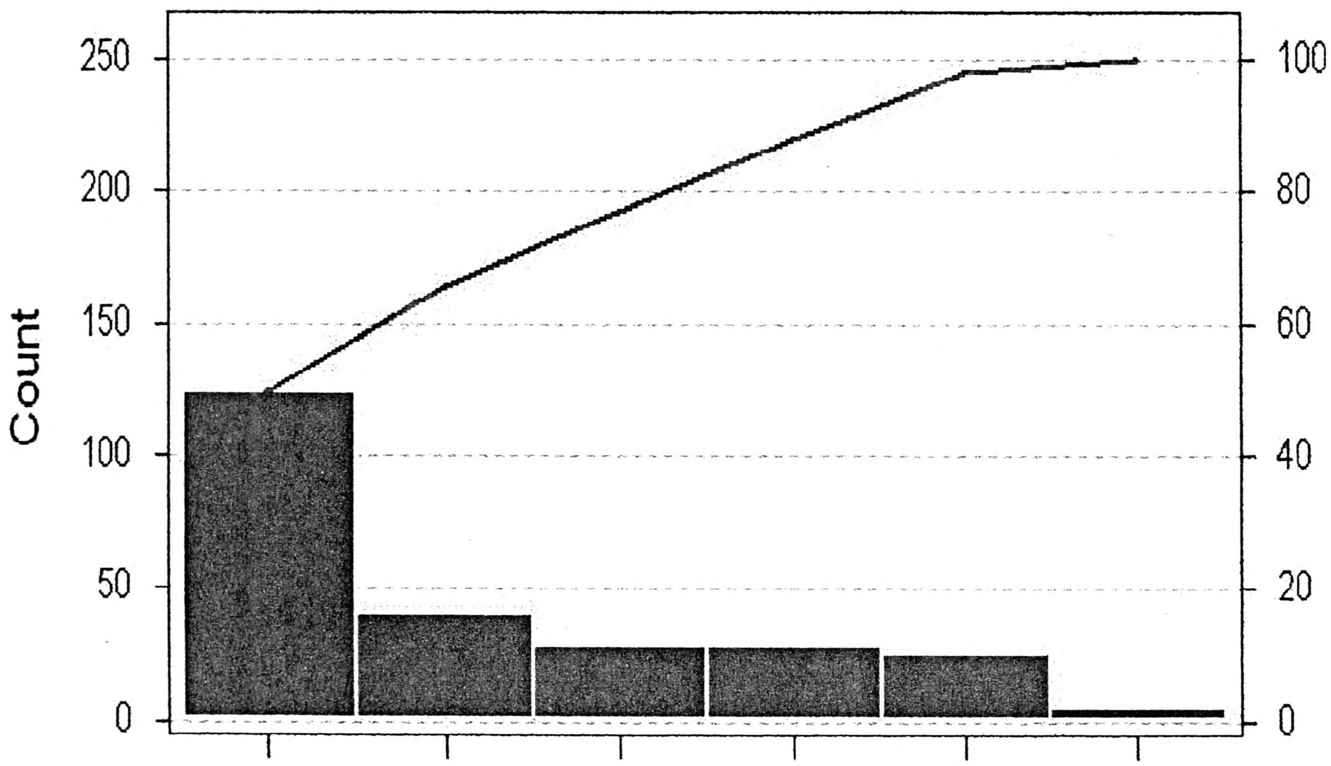
These six factors were attributed on mainly two areas, which were material and machines. These identified factors are illustrated and explained in figure 4.1.

Table 4.1 Analysis of the causes in non vacuumed trade returns:

Poor sealing indicate packets	Punctures on the top film	Punctures on the bottom film	Vacuum leak indicate packets	Inaccurately separated packets	Piercing of top film due to hot foil printing
6	5	24	5	9	2
5	6	26	5	8	0
7	5	23	7	9	0
4	5	26	5	7	1
3	7	25	6	7	2

Quality problems occur due to defective items and it makes losses. It is extremely important to clarify the distribution pattern of the loss. Most of the losses occur due to very few types of defects. These defects can be attributed by very small number of causes. Thus if the causes of these vital few defects are identified, We can eliminate Almost all the losses can be eliminated by concentrating on these particular causes, leaving aside the other trivial many defects for the time being. By using the Pareto diagrams, These types of problems can be solved efficiently by using the pareto diagrams.

Analysis of the causes in non vacuumed trade returns



Defect	C	E	B	D	A	Others
Count	124	40	28	28	25	5
Percent	49.6	16.0	11.2	11.2	10.0	2.0
Cum %	49.6	65.6	76.8	88.0	98.0	100.0

A-Poor sealability
B- punctures on the top film
C- punctures on the bottom film
D- vacuum leak indicated packets
E- inaccurately separated packets
F- piercing of top film due to hot foil printing

Figure 4.2: Analysis of the causes in non vacuumed trade returns

By using Pareto analysis punctures on the bottom material was selected as the major factor causing non- vacuuming condition of thermoform packages (Figure 4.2). LDPE /Nylon laminated is the available material of the company which has poor mechanical strength. Because of that during storage, transport and handling the packages can be damaged. The changed material was LDPE/nylon co extruded material.

Table 4.2 Analysis of the damage percentages of samples (In Control condition transportation)

Bundle no	1	2	3	4	5	6	7	8	9	10
LDPE/nylon laminated material	4	5	4	7	5	4	3	7	3	3
LDPE/nylon co extruded material	2	1	0	1	0	3	0	0	1	1

Statistically analyzed data of overall damage percentages of available material and changed material is shown in Table 4.2. Under controlled transportation conditions damage percentages of samples are shown in Table 4.3.

Table 4.3: Damage percentages of samples

Material	Percentage of Damages
LDPE / nylon laminated material	18%
LDPE / nylon co extruded material	3.6%

Low damage percentages of the samples indicate the higher strength of the packaging material. Those damages were statistically analyzed as follows using MINITAB.

Ho: Damage percentage of both materials is same.

H1: At least damage percentage of one material is different from the others.

P=0.000,So P<0.05

Reject Ho at 5% significant difference level (See appendix 1)

According to the statistically analyzed data, available packaging material containing samples have higher mean value (4.500) for damage percentages than changed packaging material containing samples mean value (0.900). Due to that physical strength of LDPE /nylon co extruded packaging material (changed packaging material) is higher than LDPE/nylon laminated material (available packaging material).

When consider the microbiological effects, shelf life and quality of vacuum packed foodstuffs, appropriate packaging materials are more important. When selecting a packaging material for vacuum packing, it is necessary to consider resistance of packaging material for stress conditions, humidity and temperature variations. Due to low resistance of packaging material for above factors, during storage, transportation and handling can occur damages in packages and it causes vacuum leakage.

Under the freezer conditions initial microbial counts of the samples are shown in the Table 4.4 and microbial counts after three weeks also shown in the Table 4.5.

Table 4.4: Initial microbial counts

	LDPE/nylon lamination	LDPE/nylon co extrusion
Escherichia coli	Absent	Absent
Staphylococcus	Absent	Absent
Total Plate count	300 cfu/g	300 cfu/g

Table 4.5: Microbial counts after three weeks

	LDPE/nylon lamination	LDPE/nylon co extrusion
Escherichia coli	Absent	Absent
Staphylococcus	Absent	Absent
Total Plate count	300 cfu/g	300 cfu/g

Microbiological tests were carried out for both packaging materials, initially and after three weeks. When samples were subjected to Escherichia coli testing procedure Escherichia coli was absent in the both samples in the freezing conditions. Although Escherichia coli is generally a harmless part of the normal micro flora of the gut of humans and other warm blooded animals, a number of groups of Escherichia coli are pathogenic for humans and have been associated with food born diseases.

The cooked cured meat such as meat ball, sausages,are classic vehicle of staphylococcal food poisoning. *Staphylococcus aureus* favoured by relatively high NaCl content ,is able to grow very rapidly in air at high temperatures and critical levels of enterotoxin can be synthesized in as little as 6-8 hours.

The results of total plate count were observed 300cfu/g initially and after 3 weeks. In cooked products total plate count is lower than permissible level. (10 6 cfu/g) So the product is within the microbiological safe level. (Sri lanka standards institute (SLS) standards of microbiological counts are shown on appendix 2.)

CHAPTER 5
Conclusion & Recommendations

5.1 Conclusion:

Co extruded LDPE/nylon packaging material has higher mechanical strength and resistivity than laminated LDPE/nylon packaging material.

5.2 Recommendations:

It is necessary to carry out further studies for microbiological tests.

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Appendix 1

One-way ANOVA: C2 versus C1

Analysis of Variance for C2

Source	DF	SS	MS	F	P
C1	1	64.80	64.80	39.67	0.000
Error	18	29.40	1.63		
Total	19	94.20			

```
                              Individual 95% CIs For Mean
                              Based on Pooled StDev
Level    N    Mean    StDev   ---------+---------+---------+------
1       10   4.500    1.509                      (-----*-----)
2       10   0.900    0.994   (-----*-----)
                              ---------+---------+---------+------
Pooled StDev =  1.278                1.5       3.0       4.5
```

Appendix 2

Microorganism	SLS standard
Staphylococcus aureus	For pre cooked products should not be more than100 per gram
Escherichia coli	For pre cooked products Should be absent in 1g
Total plate count	For pre cooked products >1000 000 unsatisfactory

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