DIELECTRIC HEATING FOR ANTIMICROBIAL TREATMENT OF FRESH MEATS

by

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ABSTRACT

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DIELECTRIC HEATING FOR ANTIMICROBIAL TREATMENT OF FRESH MEATS

The present study focussed its energy on the evaluation of a dielectric pasteurization for fresh meat. This research investigates ways to reduce the bacterial load on raw beef surfaces with microwave or RF energy in combination with different packaging methods and a natural antimicrobial combination.

Sterilized raw beef cores were inoculated with *Escherichia coli* biotype 1, *Pseudomonas* D17 and *Carnobacterium* "845" of a known inoculum. Treatments were imposed to the cores and packaged in either retail or vacuum packaging. The treatments for the full experiment were RF1 (600W–30s, 400W–30s, 200W–60s), RF2 (600W–30s, 400W–30s, 100W–60 s), Nisin-lysozyme alone, Nisin-lysozyme/RF1 and Nisin-lysozyme/RF2. Positive and negative control treatments were added to facilitate the comparison. Microbial analysis, pH measurement, L*a*b* colour measurement and sensory evaluation were performed during the storage period to follow the evolution of the meat samples.

The results obtained in this study showed us the difficulty of the RF technology to increase the surface temperature to a killing level using our combination of power and time of exposure. All microbiological analyses for either retail or vacuum packaging indicated a higher log number over time compared to control ones except for *E. coli* which experienced a reduction over time probably due to competition among bacterial types.

Measurement of pH indicated an increase in pH level for samples in retail packaging and a fairly constant pH level for samples under vacuum packaging. Colour measurements for most treatments revealed lower L* value, higher a* value and fairly

constant b* value. The trend over time was to get darker and more discoloured samples as confirmed by the sensory evaluation. The off odour intensity was judged unacceptable at day 2 or week 1 which is early in the storage period.

Microwave trials showed that the temperature reached on the surface was higher compared to RF treatments but still no significant differences were obtained with this technology.

Finally, no significant reductions ($P<0.05$) in bacterial numbers were observed in this study and none of the treatments showed positive results. Therefore the treatments used would not be considered as a good pasteurization treatment for keeping the quality of raw beef.

RÉSUMÉ

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M. Sc. Génie Agricole et des Biosystèmes

CHAUFFAGE DIÉLECTRIQUE POUR LE TRAITEMENT ANTIMICROBIEN DE LA VIANDE FRAÎCHE

La présente étude concentre son énergie sur l'évaluation du chauffage électrique comme technique de préservation pour la viande fraîche. Cette recherche étudie des méthodes de traitement visant à obtenir une réduction du niveau de bactérie sur la surface de la viande fraîche à l'aide de micro-onde ou de fréquence radio en combinaison avec différentes méthodes d'emballage et une solution antimicrobienne.

Des échantillons de viande fraîche ont été inoculés, à un niveau connu, avec des bactéries *Escherichia coli* biotype 1, *Pseudomonas* D17 et *Carnobacterium* "845". Les échantillons ont été soumis à des traitements et emballés soit avec une simple pellicule de plastique ou en emballage sous-vide. Les traitements utilisés pour l'expérience sont les suivants : RF1 (600W–30s, 400W–30s, 200W–60s), RF2 (600W–30s, 400W–30s, 100W–60 s), Nisin-lysozyme seulement, Nisin-lysozyme/RF1 et Nisin-lysozyme/RF2. Des échantillons témoins positif et négatif ont aussi été ajoutés à l'expérience afin de faciliter la comparaison. Des analyses microbiologiques, des tests de pH, de mesure de la couleur L*a*b* et des évaluations subjectives ont été accomplis tout au long de la période d'entreposage afin de suivre l'évolution des échantillons de viandes.

Les résultats obtenus dans cette recherche nous démontrent la difficulté d'atteindre, avec la radio-fréquence, une température à la surface des échantillons adéquate pour le traitement anti-microbien. Tous les tests microbiologiques des emballages sous pellicule plastique ou sous vide ont indiqué une augmentation du nombre de bactéries en fonction du temps comparé aux échantillons témoins. Seul le *Escherichia coli* a présenté une réduction en fonction du temps probablement dû à une compétition entre les types de bactéries.

La mesure du pH a démontré une augmentation du niveau de pH pour les échantillons emballés sous pellicule de plastique et une constance dans le niveau du pH pour les échantillons emballés sous vide. La mesure de la couleur a révélé une diminution de la valeur de L*, une augmentation de la valeur de a* et un maintient constant de la valeur de b* pour la plupart des traitements. La tendance dans le temps, qui est confirmé par l'évaluation subjective, est d'obtenir des échantillons plus foncés et décolorés. L'intensité des odeurs dégagées à été jugée inacceptable à partir du jour 2 ou la semaine 1 ce qui est très tôt dans la période d'entreposage.

Les essais avec des ondes micro-ondes ont démontré que la température atteinte à la surface des échantillons était plus grande comparée à l'expérience avec les radio-fréquences mais aucune différence significative n'a été obtenue avec cette technologie.

Finalement, aucune réduction significative ($P<0.05$) n'a été observée dans cette recherche et aucun des traitements n'a démontré de résultats positifs. Par conséquent, les traitements utilisés ne peuvent être considérés comme de bon traitement de pasteurisation pour maintenir la qualité de la viande fraîche.

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I. INTRODUCTION

Today's consumer demands low fat, safe, healthy, fresh meats to which no artificial preservatives have been added. Extra care at slaughtering and processing time is taken in order to preserve high quality products. However, foodborne illness incidences from fresh meats or meat products are occurring worldwide and are of great concern for public health. Usually there are two types of foodborne illness. They can be caused by infection with the organism with resulting symptoms or by ingestion of a toxin produced by the organism. Only the latter would be considered food poisoning.

Safe raw meat or longer shelf life raw meat requires that the product have very low numbers of bacteria on its surface. The inner part of the meat is considered as a sterile environment (Gill, 1979). Bacterial contamination of the external surface occurs on the exposed surfaces of the meat during slaughtering and processing. Growth of these bacteria during storage and retail display results in spoilage and can compromise safety. Spoilage of raw meat accounts for annual losses to processors and retailers of at least $200 million in Canada. Beef flesh is ranked as one of the top agri-food products exported from Canada. Fresh beef, boneless and slaughter cattle represent all together an average of 2 billion dollars or near 10% of the total revenue in Canada for agri-food exportation of goods (Statistics Canada, 2001). After the United-States, Japan is the country importing the highest amount of Canadian beef. In order to ship beef as far as Japan and to improve product safety, it is important to develop new techniques to reduce spoilage losses.

Meat is defined as "the edible part of the skeletal muscle on an animal that was healthy at the time of slaughter" (Canadian Food and Drug Act, 1990). The oxygen within the animal's muscles is rapidly exhausted after exanguination and the changes produce the conversion of muscle to meat. Like many food products, meat progressively deteriorates over time primarily due to some factors like temperature, atmospheric oxygen, indigenous enzymes, moisture, light and microorganisms (Lambert *et al.*, 1991). Controlling and understanding the interactions between these factors has lead to the development of new techniques to improve product shelf life and

quality. Research has shown that the growth of microorganisms is by far the most important factor to consider while developing such techniques (Gould, 1996).

Although special care is taken at the processing plant, there are always some bacteria sticking on the surface which cause spoilage and sometimes foodborne illness problems. Many techniques are available so far to help preserve meat attributes. They may be used alone or in combination with others. Methods used to reduce meat spoilage caused by bacteria that are unavoidably deposited on its surface can be divided into two categories: (a) Reduction or inhibition of growth, (b) Inactivation of microorganisms. The first category includes temperature control by, placing the product in a refrigerated environment, which will slow down bacterial multiplication or freezing the product. Quality attributes such as colour and taste of the product experience some changes with the frozen samples. Vacuum packaging is a processing method involving the removal of air (O_2), which inhibits the growth of aerobic microorganisms. Modified atmosphere packaging (MAP) is described as an enclosure in which the gaseous environment has been changed (Young *et al.*, 1988). By keeping oxygen in MAP, the product is visually more attractive for consumer than with vacuum packaging. The second category is comprised of pasteurization methods like spray washing, steam pasteurization, dielectric heating and irradiation. Spray washing and steam pasteurization treatments consist of reducing the initial load of bacteria at the meat surface, process principally done at the slaughtering plant. Recontamination of the produce with subsequent handling or processing is often reported. Irradiation provides a high reduction in the numbers of foodborne pathogens to extend meat shelf-life (Thayer, 1993). Pathogens are more radiosensitive than most bacteria so that they are killed by irradiation, although those that cause spoilage persist (Mussman, 1996). A combination of irradiation and packaging method can improve shelf life of meat. However, the consumer acceptance of irradiated products is very low which forces the industry to develop other alternatives.

Interest in the possibility of controlling pests with high frequency electric energy dates back to 70 years (Fabian and Graham, 1933; Fleming, 1944; Nyrop, 1946; Brown and Morrison, 1954; Carroll and Lopez, 1969). Concern about the health hazards of chemical pesticides and food safety has stimulated further studies on the possible uses

of dielectric heating (at radio-frequency (RF) or microwave frequency) for pasteurization and food safety. In dielectric heating, the material to be heated is placed in an alternating electromagnetic field which causes internal friction within the material, thus generating heat. The rise in temperature is rapid and can offer advantages when compared to conventional methods.

Past research using either RF or microwave heating as a pasteurization method shows inconsistent results. Obtaining the right combination of time and power for raw beef product while preventing protein denaturation has never been done before. This research investigates ways to reduce bacterial load on raw beef surface with microwave or RF energy in combination with different packaging methods and natural antimicrobials.

II. HYPOTHESIS AND OBJECTIVES

2.1 Hypothesis

The overall theory relies on killing bacteria at the surface of the meat. It has been demonstrated that high level of heat can kill bacteria. However, as the heat increases, the quality of the product (raw beef in this case) decreases. The hypothesis with dielectric heating is that heat can be generated fast enough by the electric field, that it minimizes the negative thermal impact on the quality. Our goal is to reach a point where the bacterial killing at the surface of the raw meat is at its maximum without altering the quality of the product.

2.2 Objectives

The overall objective of this study was to examine whether it is possible to use microwave, radio-frequency (RF) and a combination of RF heating and nisin-lysozyme solution as antimicrobial treatments on vacuum packed and retail packed beef in order to extend storage and shelf life while maintaining the quality of the raw meat product. Experimental treatments were imposed on meat samples to determine the optimum level of treatment necessary to maintain the meat quality. When the samples were inoculated with bacteria, the bacterial load during anoxic storage and aerobic conditions on raw beef was examined for all treatments. Finally, an evaluation of the efficiency of the treatments compared to the preservation of the meat quality is provided, and improvements in the methods used are proposed.

III. LITERATURE REVIEW

3.1 Economical Aspect of Red Meat

The Canadian beef industry has undergone extensive structural change during the past ten years. There has been a significant westward shift of production, primarily from Ontario and Manitoba to Alberta. Cattle feeding and slaughter activities have concentrated in Alberta with production units becoming larger in size and fewer in number (Young *et al.*, 1997).

In 1997, the meat and meat products industry (excluding poultry) was the largest sector of the Canadian food manufacturing industry with $10.9 billion shipments sales. This sector employed 37 377 employees in 1997 in a total of 477 establishments. Meat and meat products industry is placed in the fourth place among Canada's leading manufacturing industries behind motor vehicle (first place), petroleum products (second place) and sawmill and planning mills (third place) (Canadian Meat Council, 2000).

The Canadian red meat production includes beef, pork and lamb. Processing companies make also frozen, smoked, canned and cooked meats, as well as sausage and deli meats. The total production of beef in Canada was reported to be around 830 kt (kilo–tonnes) in 1999. High quality cuts in 1999 made up 49% of the total beef production, grinding beef accounting for 43%, and the remainder 8% for manufacturing cuts. High quality refers to "beef which generally comes from youthful, grain fed cattle and is predominantly used for high quality table cuts" (Young *et al.*, 1997). The total production of red meat in Canada in 1999 was 2 785 000 metric tonnes. Around 43% of this production is due to beef and nearly 55% for pork (Figure 3.1) (Canadian Meat Council, 2000).

Total beef consumption in Canada in 1999 was around 730 kt with 368 kt only for high quality cuts. The kg per capita total of meat consumption increased from 58.3 in 1997 to 64.5 in 1999. Beef consumption accounted for 32.8 kg per capita (carcass weight basis) in 1999 compared to 30.9 in 1997. International trade has become an increasingly important component of the Canadian market with beef imports almost

doubling in the 1988-1994 period while beef exports essentially tripled during the same time span. Imports of fresh/chilled and frozen beef make up more than 90% of Canada's dressed beef imports with the remainder being processed and cured beef. Beef imports from the U.S. are mostly high quality cuts with 66 kt importation compared to 70 kt for all countries combined together (for high quality cuts). Since 1990, red meat and live animal exports have increased from $1.9 billion to $4.5 billion. Between 1994-1999, red meat exports have increased by 84%. Canada's beef exports rose about 7% to 421 935 tonnes in 1999 (Figure 3.2). Sales to the United States decreased 3%, while shipments to Japan rose 17% to 24 541 tonnes. Canada's other major beef markets include South Korea, Mexico, Russia, Taiwan and Hong Kong. Exports of beef and beef products to all countries are estimated at $1.68 billion.

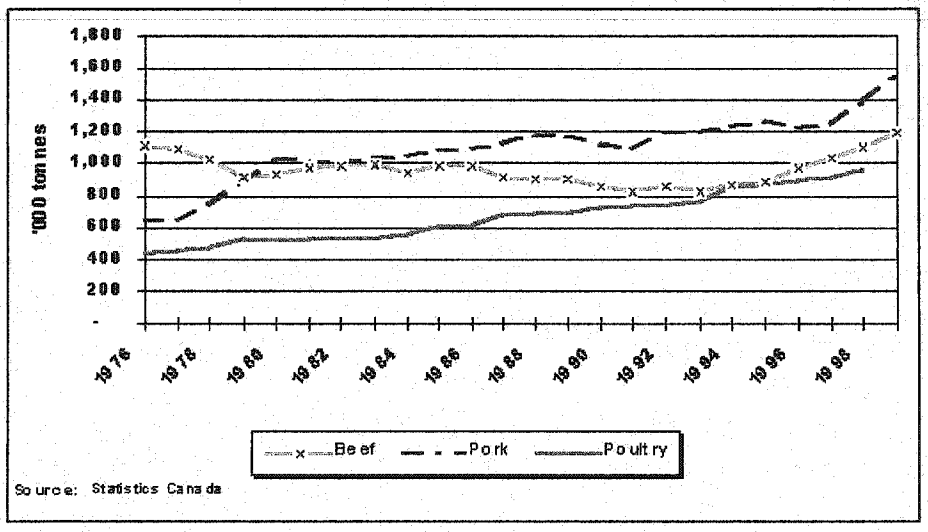


Figure 3.1: Meat production in Canada

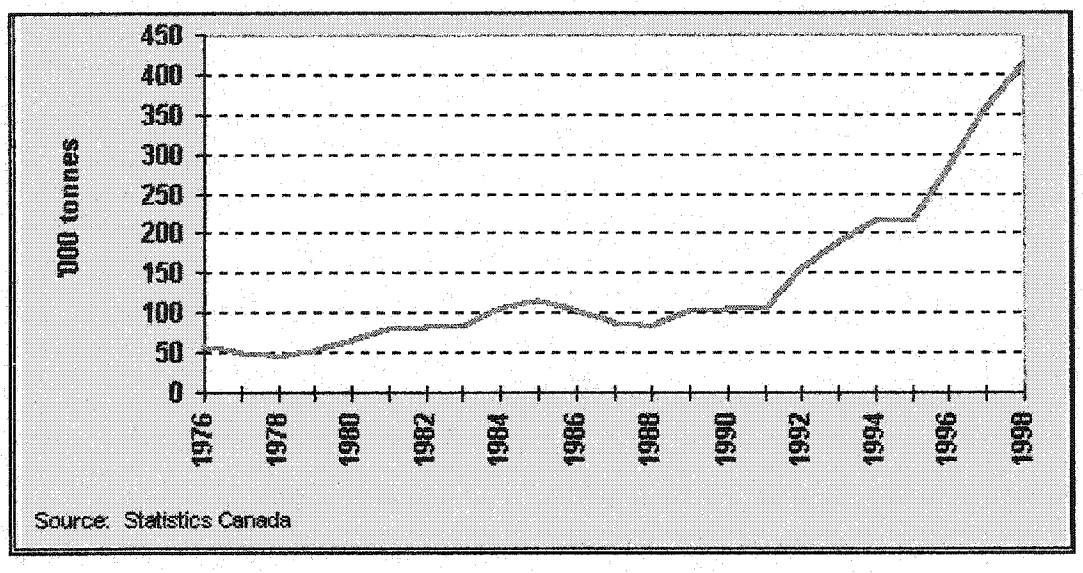


Figure 3.2: Canadian beef exports (Dressed carcass basis)

3.2 Meat Quality

Meat has been part of our diet for a long time. The Canadian Food and Drug Act (1990) defined meat as "the edible part of the skeletal muscle of an animal that was healthy at the time of slaughter." Meat is composed primarily of water, protein, lipid and carbohydrate as well as other minor components such as vitamins, enzymes, pigments and flavour compounds. An interaction between these constituents gives meat its particular structure, texture, flavour, colour and nutritive value (Lambert *et al.*, 1991). Lean muscle tissue is composed of 73% water, 21% protein, 6% lipid and around 1% soluble, non-protein substance.

Many changes in the postmortem muscle occur once the life of an animal ends. The oxygen within the muscles is rapidly exhausted after exsanguination and the changes produce the conversion of muscle to meat. Biological products, such as meat, can undergo a deterioration process when affected by some specific factors. They may be due to microorganisms or simply to biochemical degradations. Factors are storage temperature, oxygen availability, enzymes, moisture level, light and microorganisms (Britannica, 2001). Potential of microbial contamination is influenced by the condition

of animals prior to slaughter, abattoir practices, extent of handling and subsequent storage conditions (Jackson *et al.*, 1997; McDonald and Sun, 1999). When conversion of muscle to meat begins, microorganisms are more susceptible to attach to meat surfaces since the immune system of the dead animal does not function. Meat quality is highly affected by the microbial populations on the meat surface. Meat spoilage has been defined as "any single symptom or group of symptoms of overt microbial activity, manifested by changes in meat odour, flavour or appearance" (Gill, 1986). Food spoilage microorganisms found on meats are molds and bacteria. These organisms are responsible for detrimental quality changes in meat. The changes include discolouration, unpleasant odours, and physical alterations. Molds usually appear dry and fuzzy and are white or green in colour. Common molds in meat include the genera *Cladosporium, Mucor*, and *Alternaria*. Slime molds produce a soft, creamy material on the surface of meat. Common aerobic spoilage bacteria include *Pseudomonas, Acinetobacter*, and *Moraxella*. Under anaerobic conditions, such as in canned meats, spoilage can include souring, putrefaction, and gas production. This is a result of anaerobic decomposition of proteins by the bacteria.

Microorganisms have been implicated in many outbreaks of foodborne illness. In fact, in 1982, *Escherichia coli* O157:H7 was identified as a cause of foodborne illness (Wells *et al.*, 1983). Intoxication occurs when food-poisoning microorganisms produce a toxin that triggers sickness when ingested. Several different kinds of toxins are produced by the various microorganisms and may cause vomiting and diarrhea. Microorganisms capable of causing food-poisoning intoxication include *Clostridium perfringens* (found in temperature-abused cooked meats--*i.e.,* meats that have not been stored, cooked, or reheated at the appropriate temperatures), *Staphyloccocus aureus* (found in cured meats), and *Clostridium botulinum* (found in canned meats). However, the most important pathogens on raw meats are *Campylobacter* and *Salmonella*. *Listeria monocytogenes* is a very important organism in processed meats, much more prevalent than *Clostridium* or *Staphylococcus*. Consumers have become more suspicious when buying meat. Improvement in processing and packaging methods are needed in order to reduce the initial number of bacteria on the meat surface. Also, an increased demand in meat exportation is accelerating the research in meat conservation

for increased storage and shelf life. Shelf life is the time required for a food to become unacceptable from a sensory, nutritional, microbiological, or safety perspective (Labuza, 1996). When purchasing fresh meat, consumers judge the acceptability of the product largely on the appearance of the exposed muscle tissue (Gill, 1996).

Many researchers are involved in extension of meat shelf life. The goal is always to keep the meat quality (colour, texture, odour) while reducing the microbial population on the surface of the piece of meat (spoilage control). Some techniques use high temperature treatment and others use chemical compounds for controlling microbial population.

3.3 Meat Conservation Techniques

A lot of research has been done in the past years to develop new conservation techniques for preserving meat quality. Meat can deteriorate rapidly if it is mishandled. The shelf life of meat at room temperature is less than a day but can be extended while preserved at refrigeration temperatures (Lambert *et al.*, 1991). Microorganisms are the most important factor causing such deterioration in raw meat. Control of the environment temperature will have an effect on the bacterial growth. Although some bacteria can grow at low temperature, the general rule is to conserve fresh meat at chiller temperatures (-1.5°C to 5°C). Microbial growth on chilled meat is inhibited but not prevented (McMeekin, 1981). Combination of low temperature environment with other preservation techniques can improve shelf life of meat.

Product type, dimension and initial bacterial load are important to know for appropriate selection of conservation methods. The chemical and microbiological content is different from one type of meat to another. Beef muscle, for example, has a lower pH level than lamb, which results in an unfavourable environment for bacterial growth. Surface area available for gaseous exchange is related to the size of the product (Church and Parsons, 1995). Conservation techniques are strongly linked to the initial bacterial load at the surface. Research has shown that shelf life is inversely proportional to initial microbiological load (Sutherland *et al.*, 1975; Christopher *et al.*, 1979; Kraft 1986).

Several preservation techniques are available right now in order to increase food shelf life. They may be used alone or in combination with others. Methods used to reduce meat spoilage caused by the unavoidable deposit of bacteria on the surface can be divided into two categories: 1- Inhibition of growth and 2- Inactivation of microorganisms. The first category is mainly comprised of chill and frozen storage, vacuum and modified atmosphere packaging, and use of additives. The inactivation of microorganisms can be achieved by pasteurization and ionizing irradiation application. Table 3.1 summarizes the techniques used as general food preservation technologies. However, any technique available does not eliminate the necessity for proper, safe manufacturing procedures nor the needs for careful handling at all stages from factory to table (Phillips, 1996).

Table 3.1: Existing and emerging antimicrobial techniques to preserve foods and to achieve desired shelf lives (Gould, 1996)

Objective	Preservation factor	Method
Reduction or inhibition of growth	Low temperature	Chill and frozen storage
	Low water activity	Drying, curing and conserving
	Restriction of nutrient availability	Compartmentalization in water-in-oil emulsions
	Lowered oxygen	Vacuum and nitrogen packaging
	Raised carbon dioxide	Modified atmosphere packaging
	Acidification	Addition of acids; fermentation
	Alcoholic fermentation	Brewing; vinification; fortification
	Use of preservatives	Addition of preservatives: inorganic (sulphite, nitrite); organic (propionate, sorbate, benxzoate, parabens); antibiotic (nisin, natamycin)
Inactivation of microorganisms	Heating	Pasteurization and sterilization
	Irradiating	Ionizing irradiation
	Pressurizing	Application of high hydrostatic pressure
	Electroporating	High voltage electric discharge
	Manothermosonication	Heating with ultrasonication at slightly raised pressure
	Cell lysis	Addition of bacteriolytic enzymes (lysozyme)

Preservation techniques are important to keep the quality and extend the shelf life of produce. A red piece of meat is more attractive for consumers than a brownish one. Extending the shelf life of meat is economically profitable for the industry. Increasing the time between slaughtering and consumption allows the local market to export their product farther.

Appropriate selection of the conservation techniques is thus related to the product itself, the quality of the end-product desired as well as the minimal amount of time the product needs to be conserved. The economic figures may also count in the process of choosing the right techniques for your product.

3.3.1 Freezing

Refrigeration has probably been the first technological meat preservation method. It is, still today, the main method used for fresh produce preservation. At chilled temperature, red meat product stored aerobically can be kept for a maximum of one week to be acceptable for human consumption. Preservation of food products for a longer time period is achieved by reducing the environment temperature even lower. Freezing has been investigated as a method to extend the shelf life of food products. Comparison of storage time for different frozen food product is given in Table 3.2.

The quality of frozen food is related to the process used and the storage conditions. Shorter freezing time generally allow a better end-product quality. Freezing systems can be of indirect or direct contact. Indirect contact is defined as any system without direct contact where the packaging material can also be considered as a barrier (Singh and Heldman, 1993). Plate freezers, air-blast freezers and liquid food systems are examples of indirect contact systems. Direct contact can be obtained from an air blast system or direct immersion of the produce.

Reduction of temperature below 0°C causes the water content in the product to be converted to the solid state. Spoilage rate is reduced due to the reduction of water availability. Also, the effect of temperature is not the same on all types of microorganisms. In fact, the rate at which bacterial growth decreases with decreasing temperatures varies according to species and strains of microorganisms (Lambert *et al.*, 1991; Ayres, J.C., 1960).

Table 3.2: Practical storage life of frozen foods at several storage temperatures (Singh and Heldman, 1993)

Product	Storage time (months)		
	-12°C	-18°C	-24°C
Meats and poultry			
Beef carcass (unpackaged)	8	15	24
Beef steaks/cuts	8	18	24
Ground beef	6	10	15
Veal carcass (unpackaged)	6	12	15
Veal steaks/cuts	6	12	15
Lamb carcass, Grass fed (unpackaged)	18	24	> 24
Lamb steaks	12	18	24
Pork carcass (unpackaged)	6	10	15
Pork steaks/cuts	6	10	15
Sliced bacon (vacuum packed)	12	12	12
Chicken, Whole	9	18	> 24
Chicken, parts/cuts	9	18	> 24
Turkey, Whole	8	15	> 24
Ducks, Geese, Whole	6	12	18
Liver	4	12	18
Seafood			
Fatty fish, Glazed	3	5	> 9
Lean fish	4	9	> 12
Lobster, Crab, Shrimps in shell (cooked)	4	6	> 12
Clams and Oysters	4	6	> 9
Shrimps (cooked/peeled)	2	5	> 9

Animal products are especially subjected to microbiological deterioration; to preserve quality, some 75-80% of all commercially purchased, fresh red meat is later frozen at home (Bruce, 1987). However, freezing and frozen storage are known to produce or result in deleterious changes, which can significantly reduce meat quality depending on storage conditions (Miller *et al.*, 1980; Reid, 1983). Product quality is highly influenced by pigments and lipids oxidation in frozen meat storage (Greene and Price, 1975). Ground pork has been reported to lose redness over time in frozen storage (Brewer and Harbers, 1991). The same research found that combining a packaging method excluding oxygen and light to the frozen storage could reduce the loss of red colour. Quality deterioration in frozen storage is mostly noticeable on the external surface of the piece of meat.

Experiment done by Sage and Ingham (1998) showed that "the death of *E.coli* O157:H7 in frozen-thawed ground beef patties ranged from 0.62 to 2.52 $\log_{10}$CFU/g". They concluded that freezing and thawing could not be considered as a significant intervention strategy for prevention of infection in ground beef. Frozen seafood under vacuum packaging showed a slight reduction of the *Listeria monocytogenes* population after storage (Harrison *et al.*, 1991). It was suggested that frozen storage may be used as an alternative treatment.

3.3.2 Vacuum Packaging

Vacuum packaging involves placing a product in a film of low oxygen permeability, the removal of air from the package and the application of a hermetic seal (Smith *et al.*, 1990). Vacuum packaging is often used in combination with other techniques in order to affect different types of spoilage organisms. The main characteristic of this processing method is the removal of oxygen (O_2) principally, which inhibits the growth of aerobic organisms. This method may be considered as a subdivision of the MAP technique. Vacuum-packaged meat can be stored for several weeks in a low temperature environment while keeping meat quality. This processing technique is very useful for meat exportation.

Proper selection of the film permeability is required. The gas environment will change with time as the oxygen is consumed by the product and the gas concentrations in the package are diffused through the film. Different plastic packaging films with their oxygen permeability are shown here: 1) a double layer film composed of a Nylon film (Dartek N-201, Dupont Canada) and a polyethylene film (Sclairfilm A-332, Dupont Canada) has an oxygen permeability (at 20°C) of 60 cc/m^2/24h; 2) a polypropylene film, PP (Cryovac, Canada) has an oxygen permeability (at 20°C) of 26 cc/m^2/24h; 3) a high density polyethylene film, PE (Cryovac, Canada) has an oxygen permeability (at 20°C) of 2500 cc/m^2/24h (Orsat, 1999). Newton and Rigg (1979) showed a decrease in the shelf life of vacuum packaged meat as the film O_2 permeability increases.

By removing air in vacuum pack products, the concentration of CO_2 is getting higher. Change in the atmospheric package has an impact on the microbial types and

populations. Low concentration of oxygen means that the environment is under anaerobic conditions.

Many research proved that "lactic acid bacteria (LAB) are the prevailing micro organisms on chill-stored fresh meat packaged under vacuum" (Egan, 1983; Dainty and Mackey, 1992). Seideman *et al.* (1976) said that vacuum package alters the product microbiota so that spoilage of fresh beef is usually caused by lactic acid producing bacteria often resulting in a sour flavour. Lactic acid bacteria predominated on vacuum skin packaging samples on which other spoilage bacteria grew slowly, if at all; resulting in a long odour-free shelf-life (Taylor *et al*, 1990). Predominance of Lactobacilli could also be explained by the antimicrobial effect it has on competing microorganisms.

Chicken meat under modified atmosphere or vacuum packaging can inhibit mesophilic bacteria, like *Salmonella* [at chill (<10°C) temperatures] (D'Aoust, 1991). A study done on iced catfish shows that vacuum packaging with high oxygen permeable film may retard psychrotrophic bacterial growth and improve the retail quality. However, they do not consider this method as a way to extend shelf life (Huang *et al.*, 1992).

Metmyoglobin formation due to residual O_2 in vacuum packaging created an undesirable brown discolouration of the meat. Bright red colour is more associated with good meat quality. The expansion of vacuum packaging of retail cuts has been slow in North America because of the negative consumer perception of the purple colour of vacuum-packaged meat (Young *et al.*, 1988).

Meat cuts are subjected to deformation due to the high packaging pressure and bone-in product can puncture the package. Longer term colour stability provided by vacuum skin packing gives the opportunity to pack the meat at an early stage after slaughter, and allows it to become more tender by ageing during distribution and retailing. This could be particularly useful if long transportation distances were involved (Taylor *et al.*, 1990).

3.3.3 Modified Atmosphere Packaging (MAP)

By definition modified atmosphere packaging is "the enclosure of food products in gas-barrier materials, in which the gaseous environment has been changed in order to inhibit spoilage agents and therefore either maintain a higher quality within a perishable food during its natural life or actually extend the shelf-life (Young *et al.*, 1988 and Church & Parsons, 1995).

Modified atmosphere packing is designed primarily to preserve the bright red appearance of meat, whereas vacuum skin packing is an anaerobic system, and therefore cannot present meat in the bright red state, which depends on the presence of oxygen (Taylor *et al.*, 1990). MAP or gas packaging exists in the same family as vacuum packaging. It has been developed to alleviate some problems encountered with vacuum packaging. Since the principle of MAP is to change the concentration of the packaging atmosphere, it contributes to inhibit a broader range of microorganisms. Also, MAP prevents compression problems associated with vacuum packaging.

Combination of oxygen (O_2), nitrogen (N_2) and carbon dioxide (CO_2) is used for MAP. Concentration of each element is adjusted as a function of the product type. The oxygen and carbon dioxide sensibility and colour stability as well as the bacterial species able to grow on the product are important factors to consider while making the choice (Phillips, 1996). Some food products with their suggested atmosphere compositions are shown in Table 3.3.

Oxygen is important in meat packaging for its maintenance of the bright red colour associated with meat freshness. Consumers considered freshness in meat at a level of metmyoglobin (MetMb) lower than 20% (MacDougall, 1982). Nitrogen is used as package filling to prevent the package from collapsing. Carbon dioxide effect is mainly known to be an antimicrobial agent. Reddy *et al.* (1992) suggested that the microbial growth is reduced at high level of carbon dioxide.

Table 3.3: Atmosphere combinations for different food products (Farber, 1991, Church, 1993 and Phillips,1996)

Product	Atmosphere
White fish	40% CO_2: 30% O_2: 30% N_2
Fatty fish	40-60% CO_2: 40-60% N_2
Bacon	20-35% CO_2: 65-80% N_2
Cooked poultry	30% CO_2: 70% N_2
Poultry	100% CO_2
	25-30% CO_2: 70-75% N_2
	20-40% CO_2: 60-80% O_2
	60-75% CO_2: 5-10% O_2: 20% N_2
Cured meat	20-50% CO_2: 50-80% N_2
Fresh meat	30% CO_2: 30% O_2: 40% N_2
	15-40% CO_2: 60-85% O_2
Pasta (with meat)	50-80% CO_2: 20-80% N_2
Cheese	0-70% CO_2: 0-30% N_2
Bakery	100% N_2
	100% CO_2
	20-70% CO_2: 20-80% N_2
Fruits and vegetables	3-8% CO_2: 2-5% O_2: 87-95% N_2
Pasta	100% N_2

Good combination of gases does not mean that all bacteria are inhibited to grow. In vacuum packages, lactic acid bacteria (LAB) are able to grow and proliferate to become dominant in the package. In MAP, the competition between organisms is reduced which allow *B. thermosphacta* to grow to a higher number than in vacuum packs. The later bacteria produces odorous compound under aerobic conditions (Dainty & Hibbard, 1980). The resulting effect is that off-odours developed much more rapidly in MAP packs than in vacuum skin packaging. Lactic acid bacteria may also produce odorous compounds under aerobic conditions (Taylor *et al.*, 1990; Kandler, 1983); this could contribute to the more rapid development of off-odours in the MAP packs.

Pathogenic bacteria can survive and grow under improper refrigerated temperatures and even under MAP conditions. Use of 25-50% CO_2/20% O_2 /balance N_2 may extend the shelf-life of turkey only when proper refrigeration is used in order to eliminate the risk of *C. perfringens* food poisoning (Juneja *et al.,* 1996). Another research done by Nychas and Tassou (1996) said that pathogen growth was inhibited in MAP compared to aerobically stored product. However, in this research also the final suggestion was to properly keep the product at chilled temperature.

Potential advantages of MAP for the consumer are an increased shelf-life, high quality product, clear view of the product and little or no need for use of chemical preservatives. For the food producer centralized packaging and the reduction in distribution costs due to fewer deliveries over longer distances are considered as advantages. Specialized training and equipment necessary for the food producer can add some disadvantages for the consumer such as an increase in the price, a required temperature control, and increase in pack volume leading in an increased retail display space and transport cost (Phillips, 1996). A summary of the advantages and disadvantages is given in Table 3.4.

Table 3.4: Advantages and disadvantages of MAP (Farber, 1991)

Advantages
Potential shelf-life increases of 50 to 400%
Reduced economic loss
Products can be distributed over longer distances and with fewer deliveries, leading to decreased distribution costs.
Provides a high quality product
Easier separation of slices
Disadvantages
Visible added cost
Temperature control necessary
Different gas formulation needed for each product type
Special equipment and training required
Less environmental friendly, more packaging equal more waste (Cocotas, 1991)

3.3.4 Decontamination

Beef products pass under many steps before reaching our table. The slaughtering process is composed of many steps (Figure 3.3). During these steps, many opportunities are available to contaminate the surface of beef carcasses. It includes the processing equipment, workers, the environment and the animal itself. The hide, hooves, intestinal contents, and milk have the potential to harbor not only large numbers of bacteria but also pathogenic bacteria (Phebus *et al.*, 1997; Dickson & Anderson, 1992).

Many decontamination process were studied for beef processing including physical removing of fecal material by knife trimming, water washing, hot water/steam spot vacuuming, and applying various antimicrobial compounds (Phebus *et al.*, 1997).

3.3.4.1 Natural antimicrobials

Nisin is an antimicrobial peptide produced by lactic acid bacteria and it is non-toxic (Hurst, 1981). Henning *et al.* (1986) suggested that the antimicrobial effect of nisin is due to a decrease in pH (Smulders, 1987) and to the interaction with the phospholipid components of the cytoplasmic membrane which interfere with the membrane function. Many food products have been tested successfully with nisin as a preservative agent. Some examples are processed cheese, fresh and canned evaporated milk, canned vegetables, soups and cereal pudding to name only a few (Fowler, 1979; Gregory *et al.*, 1964; Heinemann *et al.*, 1965).

Results of the research done in 1989 by Chung *et al.* indicated that nisin could delay growth of gram-positive bacteria (*Listeria monocytogenes, Staphylococcus aureus,* and *Streptococcus lactis*) attached to meat. However, the same research showed that gram-negative bacteria (*Serratia marcescens, Salmonella typhimurium* and *Pseudomonas aeruginosa*) were not efficiently inhibited by nisin treatment. Similar results were obtained on cooked pork where *Pseudomonas fragi* was unaffected by nisin and *Listeria monocytogenesa* (Fang and Lin, 1994). Evaluation of the antibacterial effects of a 3% solution of lactic acid at 55°C on the growth of spoilage bacteria and cold tolerant pathogens on pork fat and lean tissue was done by Greer and Dilts (1995).

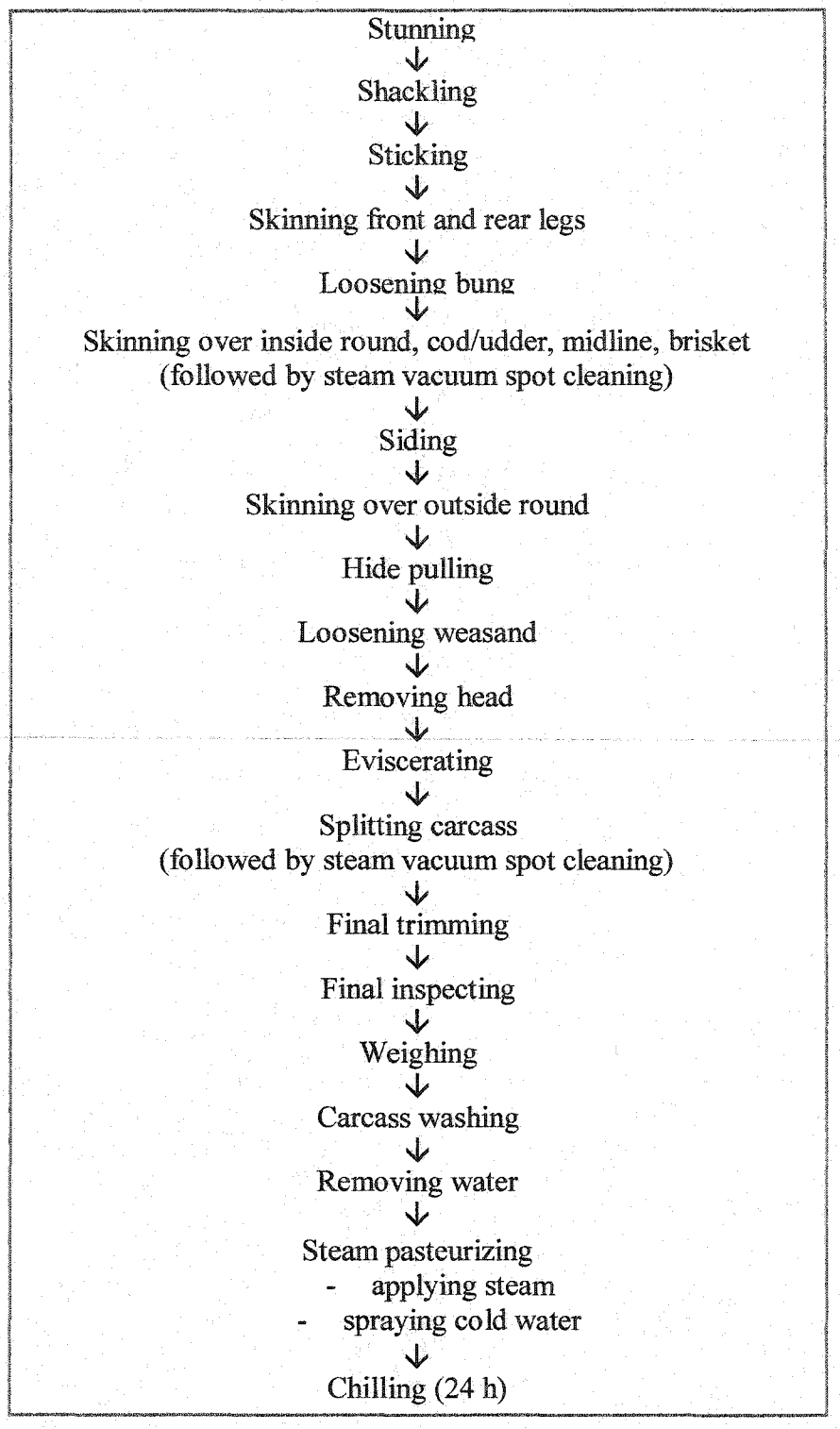


Figure 3.3: Flow of slaughter operations in a commercial testing facility (Nutsch et al., 1997)

They found a reduction of 7 to 8 logs in the numbers of both *P. fragi* and *B. thermosphacta* on fat tissue treated with lactic acid compared to water-treated controls. Cold tolerant pathogens (*L. monocytogenes, Y. enterocolitica* and *A. hydrophila*) were all reduced by the acid treatment by 6 to 7 logs cycles compared to water treatment.

Combination of MAP with a buffered lactic acid (pH 3.0) seems to give positive results on the shelf life of poultry legs. In fact, a treatment with 10% lactic acid/sodium lactate (pH 3.0) combined to MAP (90% CO_2/10% O_2) resulted in a 2.35 $\log_{10}$ units reduction compared to legs only packaged in MA after 13 days of storage at 6°C (Zeitoun and Debevere, 1992). A similar research using a lactic acid buffer (10%) on fresh chicken carcasses showed an increased shelf-life to 13 days compared to 6-7 days for untreated carcasses when stored at 4°C, and 10 days compared to 4-5 days for untreated carcasses when stored at 7 °C. Combination of MAP to the latter treatment as a pre-treatment extended the shelf-life to more than 36 and 35 days compared to 22 and 13 days for untreated MAP carcasses (Sawaya *et al.*, 1995). Combination of MAP (100% CO_2, 80% CO_2 and 20% air) with nisin (10^3, 10^4 IU/ml) resulted in a diminution of the growth of *L. monocytogenes* and *P. fragi* on cooked pork. However, the inhibitory effect was higher at 4°C than at 20°C (Fang and Lin, 1994).

Further investigations show that nisin in combination with a thermal treatment produces greater bactericidal effect than either nisin or heat used alone against gram-positive and gram-negative bacteria (Kalchayanand *et al.*, 1992). Speculation on the cause of this occurrence were advanced by Henning *et al.* (1986) and could be attributed to the fact that the sublethal heat increases the permeability of the cell wall, which permits an easier access for nisin into the cytoplasmic membrane. A reduction of 3 to 5 logs of *L. monocytogenes* was observed on cans of cold-pack lobster when both nisin and heat were applied. When used separately, nisin or heat resulted in decimal reductions of 1 to 3 logs (Budu-Amoako *et al.*, 1999).

3.3.4.2 Spray washing

Spray washing is used to reduce the bacterial load on carcass surfaces before further processing of meat. The washing can be applied with hot or cold water, mild solutions of hypochlorite, acetic acid or lactic acid (Lambert *et al.*, 1991). The

effectiveness of the treatment will depend on the type of product, numbers and type of microorganisms, as well as the temperature and strength of the washing solutions, the time of application, line pressure, volume of water and speed of travel of the meat through the spray (Anderson *et al.*, 1975).

Brackett *et al.* (1994) reported that warm and hot acetic, citric and lactic acids sprayed on raw beef against *E. coli* were not significantly effective in reducing the bacterial populations. However, Hardin *et al.* (1995) obtained opposing results where they concluded that washing treatment with organic acid reduced significantly the level of pathogens such as *E. coli*. Also, they found that lactic acid was performing more efficiently than acetic acid on *E. coli*. Other chemicals were used by Delazari *et al.* (1998) for *E. coli* decontamination on beef lean, fat and connective tissues. Hydrogen peroxide (3%), chlorhexidine (0.1%) and acetic acid (5%) were found to reduce *E. coli* by 4 log CFU/cm^2, 5 log CFU/cm^2 and 1 log CFU/cm^2 respectively compared to a normally washed control. Beef carcasses dipped in acetic acid (1.2%) showed 37.5% more reduction of the initial level of *Pseudomonas* spp. compared to pieces dipped in water (Bell *et al.*, 1986). Research done by Dorsa *et al.* (1998) confirmed the results of Bell *et al.*(1986) by finding the lowest levels of *pseudomonas* on samples treated with acetic acid (2%). Also, in the same research done by Dorsa *et al.* (1998) they used washed treatments of lactic acid (2%), acetic acid (2%), trisodium phosphate (12%), hot water (74°C) and warm water (32 °C). The results showed that when treated with chemicals, the samples had low levels (<1 CFU/g) of *E. coli*, *Listeria innocua*, *Salmonella typhimurium*, and *Clostridium sporogenes* compared to water washed samples. Spray washing using decontamination chemicals effectively reduced the bacterial load on carcasses, which can extend the shelf life of meat. However, organic acids were found to bleach the meat even at low concentration (Smulders *et al.*, 1986). Also, recontamination of the meat product can occur prior to packaging.

3.3.4.3 Steam pasteurization

Steam pasteurization can be defined theoretically as the process of applying a "gaseous steam vapour", which would reach all surfaces of a carcass uniformly, resulting in consistent bacterial destruction over the entire carcass surface.

Steam pasteurization system basically consists of three steps: water removal, steam application, and surface cooling. In a study conducted by Nutsch *et al.* (1998), the pasteurization treatment is applied as the final step in the slaughter process just after the final carcass wash (see Figure 3.3 for details). It is important to remove excess water on the carcass surface after standard washing, since that residual water may protect bacteria from the steam treatment and thus obstruct the pasteurization process. An example of a commercial steam pasteurization unit is shown in Figure 3.4 from the web site of the Frigoscandia Equipment Group, FMC FoodTech Corporation. The three process steps are within one piece of in-line equipment.

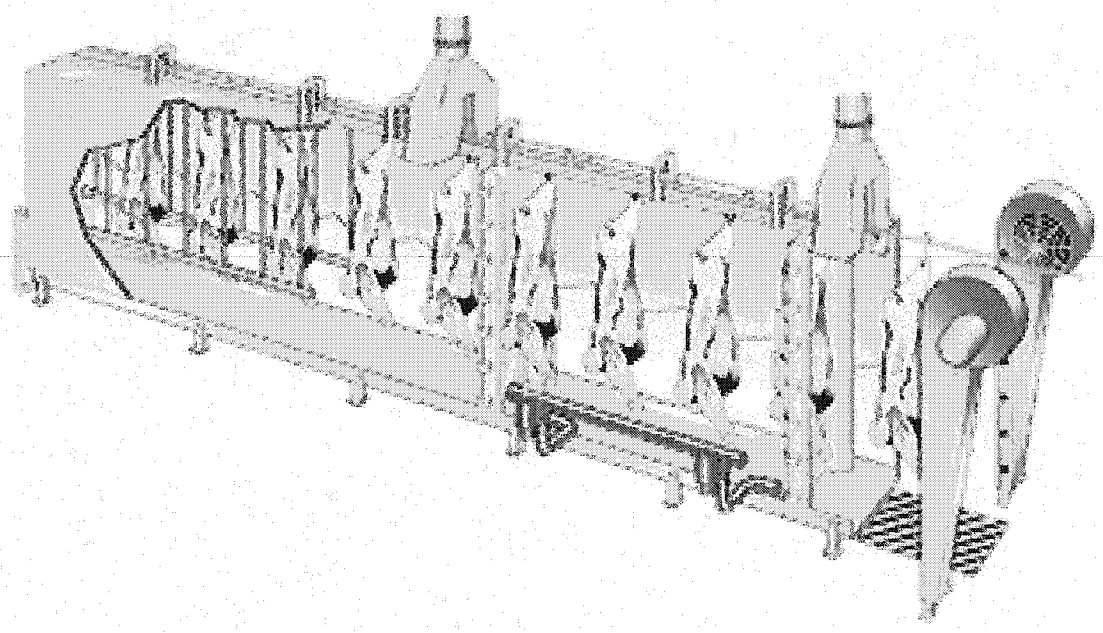


Figure 3.4: Diagram of a commercial steam pasteurization unit (Frigoscandia Equipment Group, FMC FoodTech web site)

A study on precooked vacuum packaged beef loin chunks was done in 1993 (Cooksey *et al.*, 1993) and demonstrated a reduction of *C. perfringens* spores and vegetative cells using pasteurizing. In a research done by Nutsch *et al.* (1997) the temperature inside the pasteurization chamber ranged from 90.5°C to 94.0°C. After the 8 seconds of pasteurization, the carcass temperature on its surface ranged between 17.5 °C and 22.4 °C. The results of this experiment indicated that before the pasteurization

treatment, a level of 16.4% of carcasses were infected with *E. coli* bacteria, 37.9% with coliforms and 46.4% were positive for *Enterobacteriaceae*. After the treatment, 0% of carcasses were positive for E. *coli,* 1.4% were positive for coliforms, and 2.9% were positive for *Enterobacteriaceae*. This group of researchers did similar research a year later where the steam pasteurization treatment (82.2°C for 6.5 s) showed also an effective decrease of the bacterial load on carcasses during slaughter (Nutsch *et al.*, 1998).

So far, steam pasteurization demonstrates good results in reducing the bacterial load on beef carcasses. The advantages found with this treatment over other decontamination methods are that stream vapour can uniformly cover irregular shaped surfaces. Also, the water used does not require to be treated since no chemicals are employed. The system is automatically operated, thus reduces the possible mishandling error by the worker (Phebus *et al.*, 1997). However, since the pasteurization treatment is done prior to processing and packaging, the risk for recontamination is quite high.

3.3.5 Ionizing Irradiation

Radiation has been described by Radomyski *et al.* (1994) as "a physical phenomenon in which energy travels through space or matter". Radiant energy has the potential to break chemical bonds, destroy cell walls and cell membranes and break down the DNA chain of microorganisms, pathogens and insects. Food irradiation uses ionizing radiation in the process of food preservation. Compared to non-ionizing radiation like microwaves and radio waves, ionizing radiation has higher energy and is capable with this high energy to transform atoms into ions. However, ionizing radiation has not enough energy, to split atoms, which causes radioactivity. In food processing, the ionizing radiation used is mostly cobalt-60, cesium-137, accelerated electrons, and X-rays.

Isotopes such as Cobalt-60 and Cesium-137 produce gamma rays energy. Cobalt-60 radioactive material is enclosed in two sealed stainless steel tubes called "source pencils". Accelerated electrons (electron beams) are produced by an electron beam linear accelerator machine (Figure 3.5), which concentrates and accelerates electrons to 99% of the speed of light. The electron beam accelerator machine produces

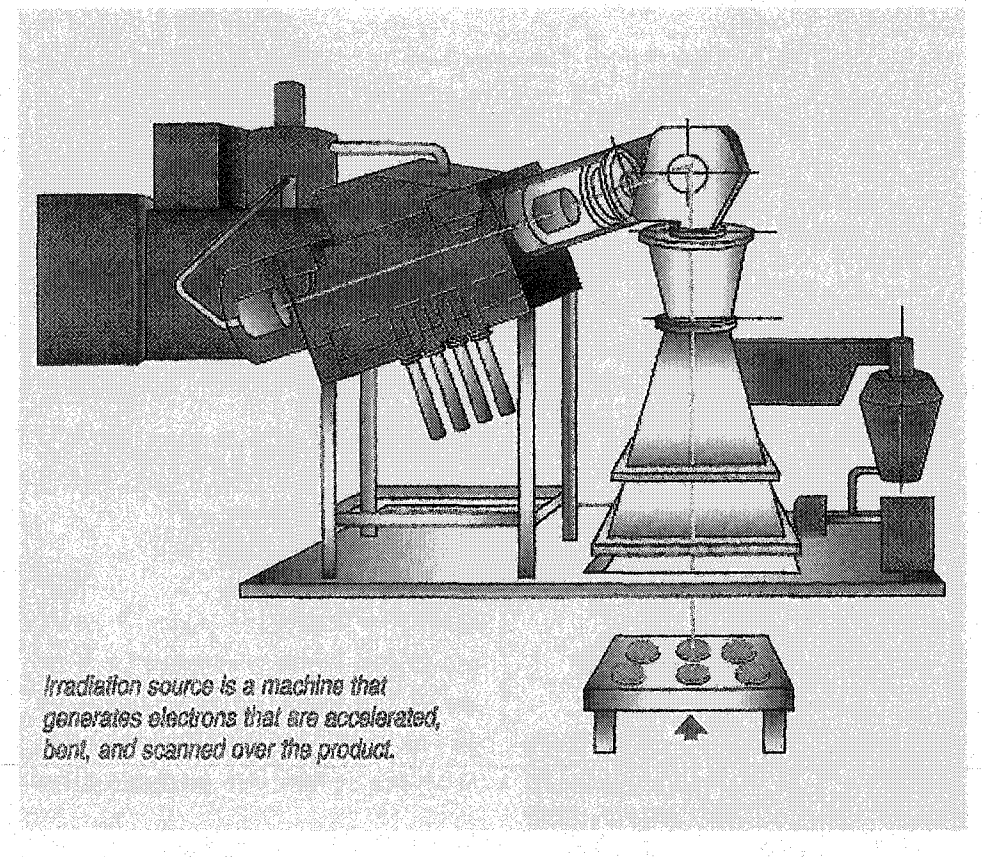


Figure 3.5: Meat treated by an electron beam linear accelerator machine (Center For Consumer Research)

X-rays where the electrons are projected on a metal plate. Some energy is absorbed and the rest is converted to X-rays (Center For Consumer Research).

Irradiated food products only absorb a small portions of radiation applied during the treatment. The absorption (dose) depends on the intensity of radiation and the length of time the product is exposed to the treatment. The ancient unit used to describe the amount of energy absorbed was the **rad** (radiation absorbed dose) and represented 100 ergs absorbed by 1 gram of matter. The International System of Units developed a new term called the **gray** (Gy), which is defined as 1 joule of absorbed energy per kilogram of irradiated material (Lambert *et al.*, 1991). The relationship between the rad and the gray is 1 Gy equals 100 rads (Radomyski *et al.*, 1994).

Many factors affect the determination of the dose required to apply for food preservation. It depends on the type of food, the number and type of organisms to be treated and the expected shelf life of your product. Low doses (up to 1 kGy) are designed to control insects, control parasites in meat, inhibit sprouting in vegetables and inhibit decay in fruits and vegetables. Medium doses (1-10 kGy) can control microorganisms in meat, poultry and fish or delay mold growth on fruits. High doses (greater than 10 kGy) have the potential to kill microorganisms and insects and sterilize food (Andress *et al.*, 1998).

Food irradiation can increase the shelf life of meats and poultry by effectively reducing foodborne pathogens (Thayer, 1993). The use of ionizing radiation has been investigated by Drake *et al.* (1960) on canned hams. Application of one megarad of gamma rays was sufficient to destroy most of the bacterial load in canned hams. However, they have reported undesirable odour and flavour of the product. D_{10} values for *L. monocytogenes* were reported to be in the range of 0.42 to 0.55 for poultry meat and in the range of 0.51 to 1.0 kGy for raw ground beef (Patterson *et al.*, 1989; El-Shenawy *et al.*, 1989). Results from Gürsel and Gürakan (1997) show a reduction in the growth of *L. monocytogenes* when raw chicken or beef were irradiated with 2.5 kGy and stored at 4°C. Inhibition of bacterial growth was also observed when chub-packed ground beef were irradiated with a medium dose (2.2 to 2.4 kGy) of X-ray and stored at 2°C. The shelf life was 27 days for irradiated meat and 13 days for non-irradiated chubs (Gamage *et al.*, 1997). The shelf life of ground beef patties can be extended using gamma radiation of 5.0 and 7.0 kGy. The initial bacterial load on the samples is a really important factor, which will affect the acceptable length of storage (Roberts and Weese, 1998).

Combinations of processes are always popular and quite successful. The sensory quality of the treated product can be affected by severe irradiation treatments. Combining radiation with other treatments can assure the microbiological quality of the food while preserving its sensory properties (Urbain, 1986; Fielding *et al.*, 1997). The level of heat and irradiation required when used in combination in order to sterilize canned hams has been reported to be so high that the product loses its quality (Drake *et al.*, 1960). Lee *et al.* (1996) investigated the combined effects of electron-beam

irradiation and modified atmosphere packaging (25% CO_2, 75% N_2). They used steaks placed in MAP, irradiated with 2 kGy and stored at 15°C or 30°C. Compared to nonirradiated vacuum packaged samples stored at 2°C, on the basis of tenderness, chemical, visual and microbiological effects, they suggested that MAP combined with irradiation could be used for an accelerated aging process of beef at 30°C for 2 days. Combination of electron beam irradiation with acetic acid has been explored by Fielding *et al.*(1997) on *E. coli* and *Lactobacillus curvatus*. Cultures, in a liquid medium with the presence of acetic acid (0.02-2.0%) at pH 4.6, were irradiated at a level of 0-1.8 kGy. *E. coli* load was reduced by the combined treatment of irradiation and acetic acid (0.02-1.0%). *L. curvatus* was not affected by irradiation up to 1.8 kGy and combined with acetic acid up to 2%.

The Food and Drugs Act regulates food irradiation in Canada. It has been stated by the Codex Alimentarius Commision that foods irradiated below 10 kGy present no toxicological hazard. Food irradiation or so-called "cold pasteurization", can process food products without any significant increase in the food's temperature. This cold treatment minimizes the nutrient losses and changes in food texture, colour and flavour of the treated product (Center For Consumer Research). Research done on the quality of meat after irradiation shows promising results. Fu *et al.* (1995 a) evaluated raw beef steaks and ground beef as well as pork chops irradiated with 2.0 kGy (1995 b). For both type of meats, there was no colour difference. Some off-odours were detected at the opening of the package which dissipated. Rodríguez *et al.* (1993) also reported no odour detection from trained panelists for irradiated beef at 2.0 kGy.

The advantages of food irradiation are that it can control or inhibit insects, pests, and pathogens and can delay ripening of fruits and sprouting of vegetables. The disadvantages of food irradiation are that irradiation is ineffective against viruses, that new technology is relatively expensive, it can only treat a limited range of foods, and can affect some constituents of foods. A survey on consumer acceptability towards irradiated food demonstrated that 45% of consumers would buy irradiated food, 19% would not buy it and the remaining have no opinion (Resurreccion *et al.*, 1995). In general, the consumers are aware of the technology but they don't have enough information to make up their minds. The same survey indicated that "87.5% of

consumers heard about irradiation but do not know much about it". A better consumer's education on irradiated food would probably increase the popularity of this technique.

3.3.6 Dielectric Heating

Dielectric heating has gained a lot of popularity for industrial application purposes. Such techniques are well implanted in heating or drying foods, woods, textiles, papers, ceramics and many other materials. Compared to conventional heating where heat penetrates into the product by conduction, dielectric heating has the ability to generate heat within the product preventing overheating of surfaces (U.I.E., 1992). Radiation which can cause changes in the body is classified as ionizing. Dielectric heating is recognized in the non-ionizing radio wave of the spectrum. Dielectric heating is composed mainly of two bands of frequencies. On the electromagnetic spectrum (Figure 3.6), the portion between 300 kHz to 300 MHz is called Radio-Frequency (RF). Microwaves (MW) belong in the frequency range of 300 MHz to 300 GHz. Industrial frequencies used are 0.915, 2.45, 5.8 and 22.125 GHz for microwaves and 13.58, 27.12 and 40.68 MHz for radio-frequency. RF applications are well known for plastics welding, wood glueing, plastics pre-heating and moisture removal (drying/baking). On the other hand, microwave applications are categorized by pre-heating and vulcanizing of rubber, tempering of frozen products, pasteurization and ceramics (U.I.E, 1992).

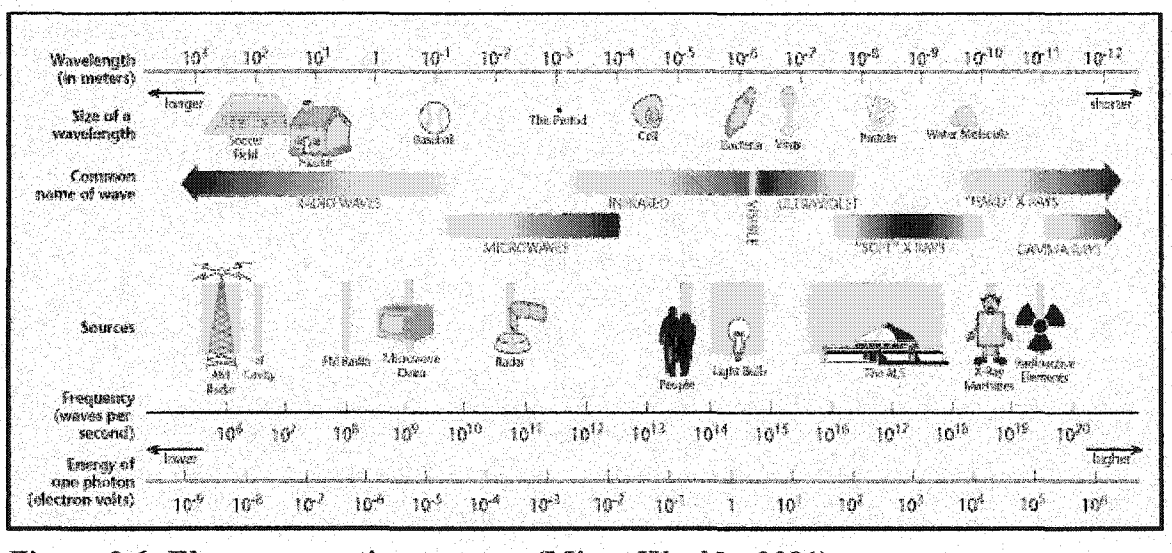


Figure 3.6: Electromagnetic spectrum (Micro Worlds, 2001)

The technique of radio-frequency heating relies on the electric field created between two electrodes. Two plates connected to an alternating voltage source generate the electric field. The *field strength* E is equivalent to the *voltage* V applied by the alternating current divided by the *distance* d between the plates. The number of times the current alternates in one second is called the *frequency* and is expressed in Hertz (Hz) with the symbol f. The distance between two oscillations λ is called the *wavelength*. When the material is placed in the alternating electric field, the heating is generated by the rapid rotation and movement of molecules within the material

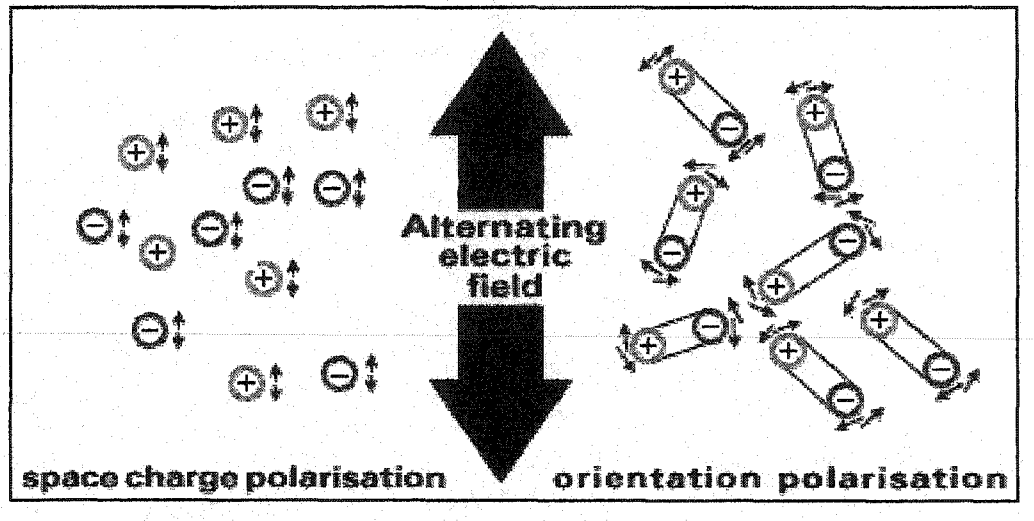


Figure 3.7: Space charge and orientation polarisation under an alternating field (U.I.E., 1992)

attempting to reorient themselves due to the alternating electromagnetic field (Figure 3.7). Dielectric materials exhibit the property of polarisation because their molecular structure has strongly bound electrons unlike that of conductive materials, which have free or loosely-bound electrons. Polarisation can take place at both the atomic and molecular level. The energy produced in the form of dipolar polarisation is found under both radio-frequency and microwave. Dipolar (orientation) polarisation is the realignment of molecules by the influence of an alternating field. Space charge polarisation can also be found under radio-frequency and is defined as the migration of some charge carriers induced by an electromagnetic field. However, the ability of the material to be heated depends on the moisture and ionic content of a food, the specific

heat of various food constituents, product density, shape, load volume, its temperature and the frequency applied (U.I.E., 1992; Heddleson and Doores, 1994).

The material property known as the *loss factor* ε'' is the ability of the dielectric material to convert the applied electric field into heat. Some examples of loss factors are given in Table 3.5. The higher the *loss factor* is, the easier the dielectric material is to be affected by dielectric heating. Materials with a *loss factor* greater than 0.02 are generally considered for dielectric heating (U.I.E., 1992). However, temperature can sometimes increase the loss factor of some products. The *permittivity* denoted by the symbol ε is the ability of a dielectric material to be polarized. Dividing the *permittivity* by the *permittivity of free space* (ε_0 = 8.85 x 10^{-12}F/m) resulted in the *relative permittivity (dielectric constant)* ε'.

$$\varepsilon' = \frac{\varepsilon}{\varepsilon_0} \tag{1}$$

Table 3.5: *Loss factors* (ε'') for common materials (U.I.E., 1992)

	10 MHz	13.56 MHz	27.12 MHz	900 MHz	2450 MHz
Suet	<0.5	<0.5	<0.5	<0.3	<0.3
Pork fat	110	94	51	17	2.7
Pork meat	950	775	420	20	18
Fruit	620	510	275	13.5	15.5
Polyethylene	<0.1	0.0004	<0.1	<0.1	0.001
Ice (at –20°C)	0.4	0.3	0.2	0.1	0.1
Salt water	2350	1750	900	29	19.6
Pure water	0.8	0.6	0.4	3.9	10.7
Asphalt	0.7	0.5	0.4	0.2	0.2

The permittivity of a material can be expressed as a complex quantity, the real part of which is associated with the capability of the material for storing energy, and the imaginary part is associated with the dissipation of electric energy in the material by conversion of electric energy to heat (Nelson, 1995). The complex permittivity is shown here where j represents the complex operator $\sqrt{-1}$:

$$\varepsilon = \varepsilon' - j\varepsilon'' \tag{2}$$

In polarized materials, the friction between molecules is generated in reaction to the electric field applied and yields an increase in temperature of the material. However, the delay between the penetration of the electric field and the production of heat is called the *loss angle* δ. The ac electrical conductivity associated with the dielectric loss in the material is $\sigma = \omega\varepsilon_0\varepsilon''$ siemens/m (S/m) where ω is the angular frequency, 2πf. The *loss angle* can be expressed as a component of the *loss factor* as follows:

$$\varepsilon'' = \varepsilon' \tan\delta \tag{3}$$

Both the *loss tangent* and the *dielectric constant* vary with the frequency applied and the temperature of the material. The *power* absorbed by the material is the value of heat generated through the material and is represented as follows:

$$P = E^2\sigma = 2\pi f E^2 \varepsilon_o \varepsilon'' \tag{4}$$

where,

P = power density (W/m^3)

E = rms electric field strength in the material (V/m)

σ = conductivity (1 Ω m)

f = applied frequency (Hz)

ε_o = permittivity of free space (F/m)

ε'' = loss factor

The rate of temperature increase (°C/s) in the material is given by (Nelson, 1995):

$$\frac{dT}{dt} = \frac{P}{c\rho} \tag{5}$$

where,

c = specific heat of the material (kJ/kg °C)

ρ = density of the material (kg/m^3)

The penetration depth is defined as the depth at which the power has decayed to 0.368 (1/e) of its maximum value (U.I.E., 1992). It may vary depending on the *loss factor* and the *frequency* used. Usually, the higher the *loss factor* is, the lower the penetration depth will be. As wavelength increases, the penetration depth increases as well. The relationship between wavelength and penetration depth is expressed as follows:

$$D = \frac{\lambda_0}{2\pi}\sqrt{\frac{2}{\varepsilon'\left[\left(1+\tan^2\delta\right)^{1/2}-1\right]}} \tag{6}$$

where,

D = penetration depth (cm)

λ_0 = wavelength in free space (cm)

The penetration depth of microwave radiation has been shown to be reduced by the presence of dissolved salts (ionic compounds) (Heddleson *et al.*, 1993; Lentz, 1980). The water content influences the penetration depth also. Generally, at high moisture content in the material, the microwave absorption will be higher but with a decrease in the penetration depth. High moisture content in the material refers to larger dielectric loss factor, thus efficient heating. Products with low moisture content can also be

efficiently heated if the specific heat capacity of the product is low, since lower moisture content allows an increase in the penetration depth (Mudgett, 1982).

The size and shape of the material subjected to heating is really important. If the product has sharp corner (90° edges), the heating has a tendency to be concentrated on that specific point. The result is a less uniform heating which may cause undesirable cooking in areas while the rest of the material is still raw. Specific heat and thermal conductivity of the product are important to consider. Also, the temperature achieved by the material within the heating process needs to be considered. Increasing temperature means an evaporation of the moisture in the food. As shown before, the moisture content influences the dielectric loss, dielectric constant and the loss tangent, thus the heating capacity of the product (Jones and Rowley, 1996).

An enormous controversy is battling around dielectric heating process to find out if the possible athermal (non-thermal) effect of the process on microorganisms is real or is the killing effect is due to heat. Selective heating theory proposed that a microorganism absorbs the electromagnetic energy. The solid microorganisms get hotter than the surrounding fluid and reach the temperature required for pasteurization. Both the material and the microorganisms are exposed to the same frequency. However, the dielectric loss factor of each of them may not be the same, thus the intensities of the electric field may affect them differently (Kozempel *et al.*, 1998). However, non-thermal effects are stated to be due to the lack of precise measurements of the time-temperature history and its spatial variations (Heddleson and Doores, 1994).

The primary advantage of dielectric heating is the rapidity in reaching the desired temperature for pasteurization and sterilization when compared to conventional heating. The large savings in process time is very important for the industry. The high-temperature short time processing is possible with this technique and allows bacterial destruction with minimal undesired product deterioration. Heating is done more uniformly with microwave or radio-frequency heating. Also, the heating systems can be turned on and off instantly, treated throughout the package and the process is more energy efficient (U.S. Food and Drug Administration, 2000).

3.3.6.1 Microwave

Microwave operates with shorter wavelengths than radio-frequency technology. Common domestic microwave ovens operate at 2450 MHz frequency. Microwave technology is schematically shown in Figure 3.8. Power is emitted by magnetrons, carried by waveguides and beamed into tuned cavities. Waveguides are used to carry the energy from the generator to the applicator with little loss and no radiation hazard. The magnetron is an oscillator electronic tube. Magnetrons working at a frequency of 2450 MHz generate an output power ranging from 0.6 to 6 kW. The overall efficiency of such microwave generator is 50% to 60% due to losses in the magnetron, which is air or water-cooled.

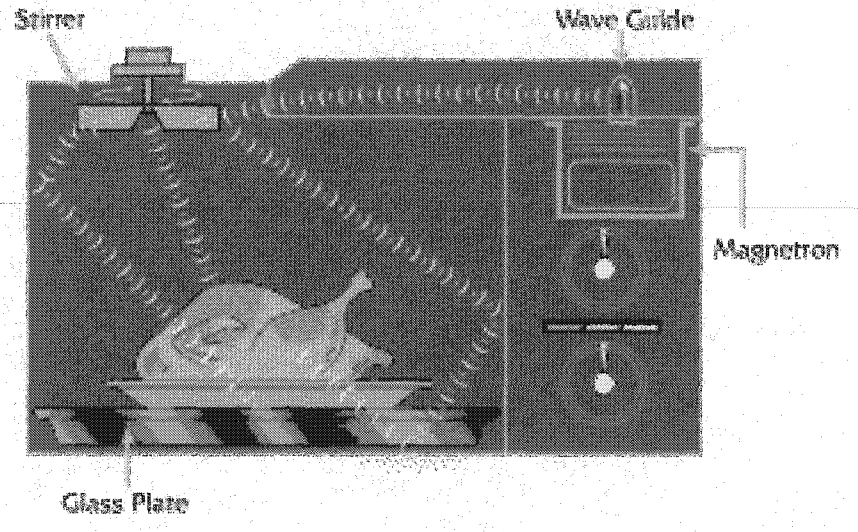


Figure 3.8: Domestic microwave oven (Petrie Technologies)

Many microwave generators are equipped with a circulator (or insulator) device to protect the magnetron against reflected power. When the magnetron is started, the electro-magnetic waves emitted are carried via the waveguides to the material placed in the microwave. Those waves are called the incident power and are categorized by a optimal power transfer. The material is called a "matched-load" when the material absorbs all the incident power. However, when the material reflects a part of the incident waves, it produces a situation of "mismatched-load". The reflected waves

bounce back to the waveguides and into the magnetron, which may cause overheating of the magnetron.

The applicator is the environment where the product enters in contact with the electromagnetic waves. Usually, it has a form of a metallic box. The design of the applicator is really important to ensure both safety and efficiency. The metallic structure does not allow waves to escape and is able to reflect the waves with low energy losses.

Domestic microwave ovens use multi-mode cavity applicators. The power of the magnetron is emitted from one side of the cavity via a waveguide link. The electric field pattern in the cavity produces many successive reflections of the wave on the metallic walls. As a result, the whole applicator environment is filled with electric field allowing the product to be heated throughout. However, the reflected waves interfere with each other causing local differences in the electric field strength leading to uneven temperature distribution. Rotating turntable and rotating mode stirrer are provided to obtain a more uniform heating. A mode stirrer acts as a moving reflector to periodically change the electric field pattern in the cavity.

3.3.6.2 Microwave and food products

Microwave heating has been investigated to pasteurize food products. The popularity of microwave oven has rapidly increased in the heart of customers. It is well known for its rapid heating compared to conventional heating. Industrial frequencies used are 2450 MHz and 915 MHz while microwave ovens at home use only 2450 MHz. At a frequency of 2450 MHz, the heat is applied to the food without deep penetration. Most of microorganisms are found on the external surface of meat pieces. The process of high temperature and short time treating will allow heating on the surface without major perturbation of the product.

Microwave heating has been investigated to demonstrate its potential to reduce bacterial loads on meat surfaces. Raw chicken patties were exposed to 0, 10, 20 and 40 seconds microwaves at a frequency of 915 MHz. This research, done by Cunningham (1978), reduced total counts from 10^4 to nearly 10^2 after 40 sec. However, signs of cooking were found on samples treated for 40 sec. Paterson *et al.* (1995) did research

on microwave treated vacuum packaged beef and its surface bacterial load. At 50°C, they obtained a reduction of one log. While increasing the surface temperature, the bacterial counts decreased. At 60°C, the reduction was up to 4 log but the product experienced signs of cooking. On the other hand, sublethal temperatures have been investigated to inactivate microorganisms with microwave radiation on fluids. Bacteria are more readily destroyed in water, glucose solutions, and apple juice than in apple cider or tomato or pineapple juice, and none were killed in skim milk with a product temperature below 40°C (Kozempel *et al.*, 1998). Temperature differences of up to 60 and 80°C were found between different points on the surface of the same sample after 30 s and 3 minutes of heating respectively with a standard microwave oven (2450 MHz). A research done by Göksoy *et al.* (1999) finally concluded that non-even temperature distributions were found in a domestic microwave, which could not allow reduction of bacterial numbers without causing cooking on the surface of poultry meat. The same researchers (2000) obtained confirming results by applying a short time microwave exposure (up to 30 s) on chicken meat. Again, no significant bactericidal effect and no effect on subsequent growth of microorganisms at refrigerated temperatures were obtained. Also, cooking signs were apparent starting with microwave exposure of 20 s.

Many studies so far have been carried on the subject, however no unanimous conclusions on the pasteurization potential of microwave radiation have been pronounced. Some researchers state that microwave radiations contribute to kill microorganisms with non-thermal phenomena, which means that there is an effect attributable only to the intrinsic nature of microwaves and unrelated to lethality caused by heat (Chipley, 1980). In 1975, Culkin and Fung inoculated soups with *S. typhimurium* and *E. coli*, exposed the soups to microwaves and determined the survival rate at three different regions. The coolest part was at the top and the hottest spot was found in the middle of the soups. They found out that the percentage survival was the lowest at the coolest spot. From those results, it is assumed that heat alone is not responsible for destroying microorganisms (Cunningham, 1978).

3.3.6.3 Radio-frequency

Radio-frequency technology for industrial purposes is divided into two systems. The first one is called the conventional RF heating equipment while the other one, and more recent called the 50Ω heating system. RF equipments are composed of a power generator, an electrode system and coupling devices between the generator and the electrodes. The power generators are most of the time "free running oscillators": an oscillator circuit is coupled to a triode valve, which is fed by a DC high voltage power source (U.I.E., 1992). The oscillator circuit is composed of an inductor and a capacitor connected in parallel. When a pulse is applied in the circuit it oscillates and vanishes progressively. In order to keep the oscillations, the oscillator circuit is connected to a triode valve, which acts as a power switch. The triode valve is air or water cooled, which is where the majority of the generator losses may occur. The electrode systems are really important in the design of the RF equipment. The RF high voltage coming from the power generator is transferred to the electrodes, also called applicators where an electric field is created between the two plates (electrodes). The product to be heated is placed between the plates and subjected to the electric field. For protection purposes, the electrodes and the products are enclosed in a cabinet to prevent electric field leakages. Three common types of applicators are available for RF industrial uses. They are shown in Figure 3.9 and called the through field electrodes, the stray field electrode system and the staggered through field electrode system.

The through field electrodes configuration consists of two flat metal plates between which the product is placed. In the stray field electrode system, the field is produced horizontally and produces a non-uniform field. The electrodes are shaped as rods, bars or strips. This applicator is suitable for continuous processing and for thin materials. Staggered through field electrode system is similar to the stray field electrode system except that the electrodes are arranged above and below the material to be heated. This is useful for treating thicker materials (U.I.E., 1992).

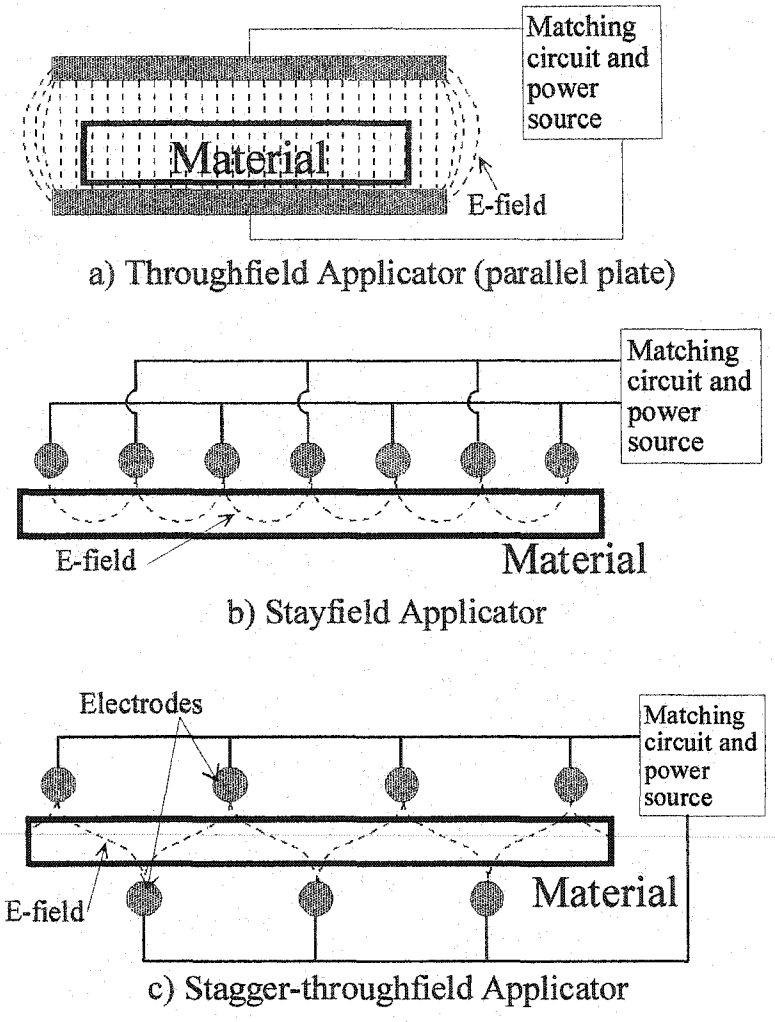


Figure 3.9: Electrode configurations (Metaxas, 1988)

The RF voltage source is connected to the electrodes where the RF electric field is created. This electric field will vary in the space between the electrodes depending on the shape and dielectric properties on the heated product. When the material fills out all the space between the parallel plates (in the case of through field electrodes), the electric field distribution is homogenous except close to the edge. The electric field magnitude is equal to the voltage applied on the electrodes divided by the distance separating the two electrodes. However, it is possible to have an air gap between the material and the electrodes as shown in Figure 3.10.

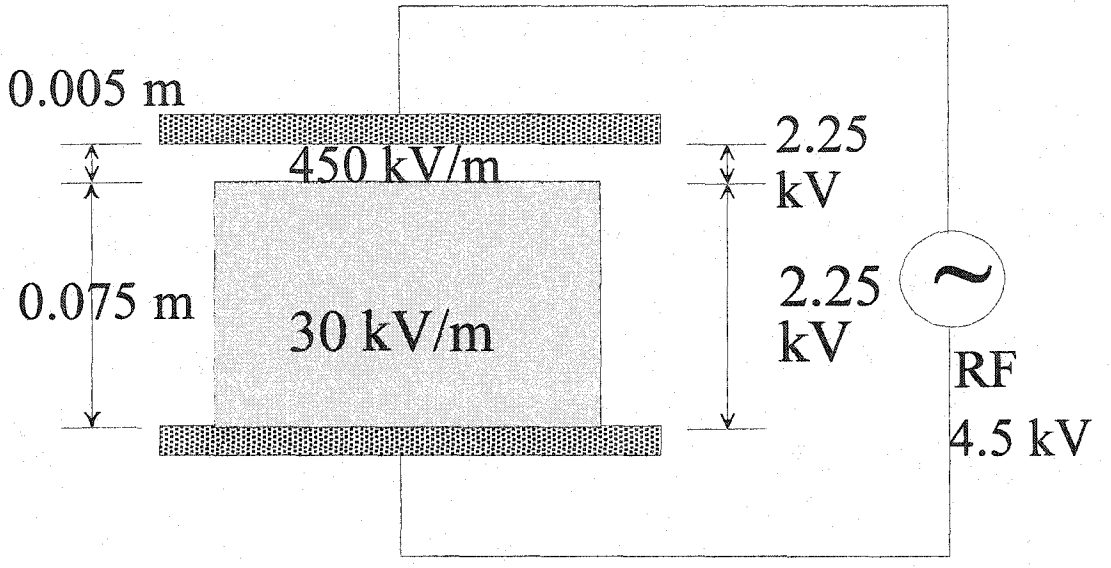


Figure 3.10: Effect of an air gap (Orsat, 1999)

There are two homogeneous electric field distributions, in each medium, but the corresponding values are not independent: the electric field in the air is equal to the electric field in the product multiplied by its dielectric constant (usual values for dielectric constants are from 2 to 15). The voltage applied on the electrodes is then the sum of two voltages: one creates the electric field through the product, the other through the air. In Figure 3.10, the voltage is 2250 V in the product (30 kV/m * 0.075 m) and 2250 V for the air gap (450 kV/m * 0.005 m). The total is 4.5 kV applied to the electrodes instead of only 2.25 kV for the same heating effect but without an air gap. With the air gap, there is a waste of energy associated with 2.25 kV going directly through air and not in the product. Air gap should be minimized for this reason (U.I.E., 1992; Orsat, 1999).

Efficient power transmission and control from the generator to the product via the electrode system is obtained using additional coupling devices. Coupling devices tune the applicator to the operating frequency of the generator, and then adjust the power load of the generator to obtain the suitable heating rate. The coupling elements are usually adjustable capacitors or inductor coils, which are located close to the electrode system, or in the generator, or in specific "matching boxes" between the

applicator and generator. The RF generator is physically separated from the RF applicator by a high power coaxial cable which characterizes the recent 50Ω RF heating systems (Figure 3.11). The generator uses a fixed frequency controlled by a crystal oscillator. Frequencies are usually fixed at exactly 13.56 MHz or 27.12 MHz. The purpose of fixed frequency is to decrease the interference with radio communication services. With fixed frequency, the output impedance of the RF generator is easily set up to a convenient value (50Ω). An impedance matching box is included in the system to adjust the impedance of the RF applicator to 50Ω. Both the RF generator and the RF applicator need to operate under the same impedance for efficient power transfer.

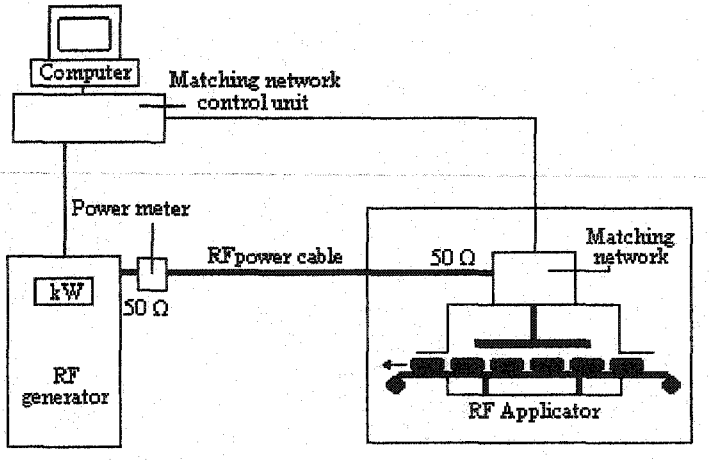


Figure 3.11: 50Ω RF system (EA Technology, 2001)

3.3.6.4 Radio-frequency and food products

High frequency electric heating on biological products have been investigated to demonstrate the potential of this energy other than only heating the product. In 1946, Nyrop published an article on positive results obtained using radio-frequency (RF) against bacteria where the treated product stayed at lower temperature than product treated with heat. He applied RF energy of 10-100 kHz to *E. coli* in broth suspensions. The results observed under field strength of 205 V/cm were 99.6% kill for a 5 seconds

exposure and for 10 seconds the kill when up to 99.98%. Temperature of 60°C and 600 seconds would have been required to obtain the same percentage. He also treated foot-and-mouth disease virus with 260 V/cm for 10 seconds. Brown and Morrison (1954) studied the effect of RF energy at 50 Hz, 190kHz, 25 MHz on *E. coli*. The bacteria were irradiated in nutrient broth by means of a capsule electrode assembly. Results showed no significant effect of high electric field on bacterial destruction rate. However, they did some tests and found out the temperature at which the bacteria were starting to be killed. At a temperature higher than 50°C, the bacterial cells were decreasing considerably. Since the RF treatment used on *E. coli* were always around this temperature range, they have concluded that no destructions were related to the high electric field used but only by the thermal effect on the cells.

Pasteurization of foods with radio-frequency has not been really popular compared to microwave heating. So far, research has not given consistent results. Experiments at 35 and 60 MHz were made with a generator of 1kW output and a conveyer feeding arrangement on cured lean and fat hams (Bengtsson *et al.*, 1970). Bacteriological examination was made by surface sampling, using a cork-bore technique, plating on APT-agar and incubating at 30°C for 3-4 days. Lower juice losses were obtained with RF-processing than in hot water processing and treatment time was less than half. Microbiological examination after prolonged storage showed considerably higher total counts for RF-processed hams, indicating a need for higher final temperatures or supplementary heat treatment. Total counts decreased with increasing salt content and final water temperature. Fat hams, however, showed microbial counts about 10 times higher than in hot water processing. Dielectric pasteurization of lean hams at 60 MHz resulted in acceptable temperature distribution and substantially reduced heat treatment time and juice losses with indication of an advantage in sensory quality. On the other hand, the shortened heat treatment, in combination with a lower surface temperature than in conventional hot water processing, gave a higher surface infection.

Houben *et al.* (1991) performed research on dielectric heating for a continuous and flexible pasteurization process of sausage emulsion. They chose to use 27 MHz ISM band for the experiment. The system treated sausage emulsions from 15°C to 80°C

at a mass flow of 120 kg/hr in about 2 minutes. The rapid heating rates resulted in considerably reduced Cook Values as compared to conventional heating methods. Product's appearance presented only minor differences between the two heating methods. Radio-frequency pasteurization of moving sausage emulsions demonstrated very promising results.

IV. MATERIALS AND METHODS

4.1 Experimental Apparatus

4.1.1 RF Heating

Our design is based on the 50 Ω generator technology as described in section 3.3.6.3. The generator operates at a fixed frequency of 27.12 MHz, which is one of the frequency bands approved by the ISM (Industrial, Scientific and Medical). The maximum power output with our machine is 600 W for a maximum applied voltage around 5 kV. The RF applicator is shown in Figure 4.1 as a schematic drawing and as a photograph in Figure 4.2. The electrodes are square in shape, 0.2 m x 0.2 m in dimensions and made of aluminum. They are designed with the parallel plate configuration and the electrodes are separated from each other by Teflon columns. The distance between the two plates is 4.5 cm and the plates allow a holding container of around 9 cm in diameter. The matching box is placed under the applicator system. The lower electrode was thus chosen to be the high voltage one to ensure the shortest connection between the matching box and the electrode. They are connected via silver plated copper strips. To prevent radiation leakage an aluminum perforated plate cabinet surrounds the applicator. Also, it provides proper grounding under high voltage conditions since the cabinet is in contact with the frame at numerous points around the openings. A small blower is mounted on the applicator cabinet to pass an air stream across the electrodes to carry water vapour away from the electrodes. Water vapour may cause flash overs between the electrodes or between the high voltage electrode and the material being heated.

The full system is represented schematically in Figure 4.3 and the photographic representation is also available in Figure 4.4.

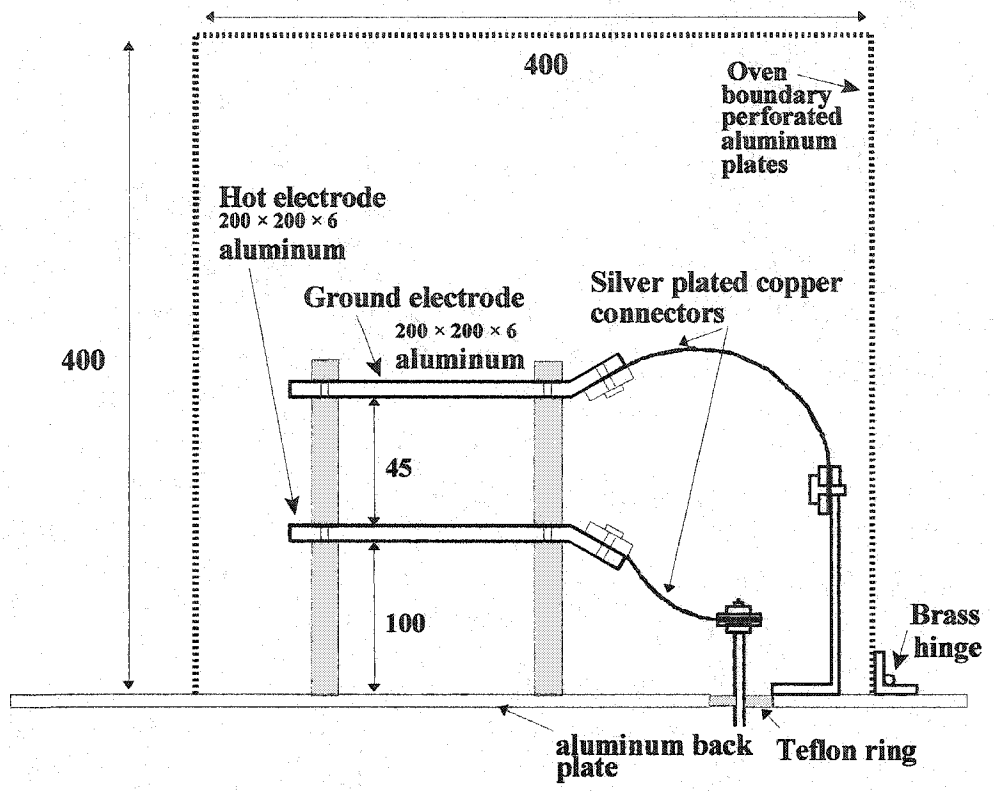


Figure 4.1: Schematic of RF applicator

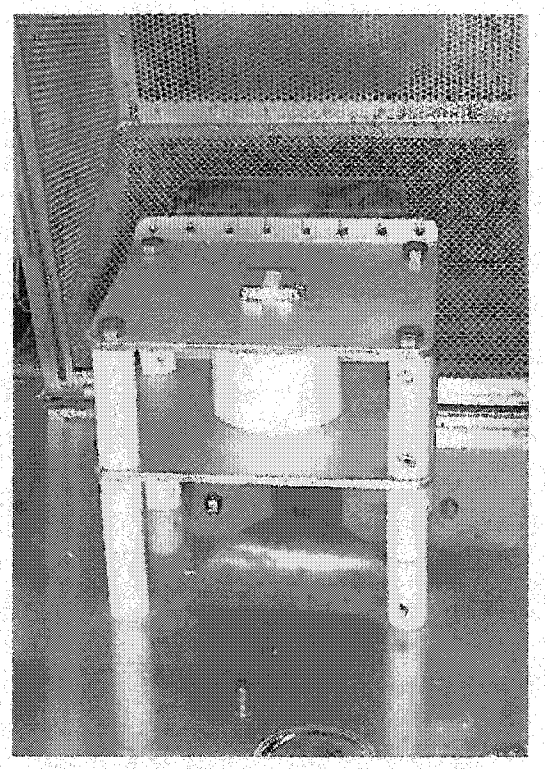


Figure 4.2: Photograph of the RF applicator

The matching box is required to match the impedance of the applicator with the impedance of the RF generator, which in our case is 50 Ω. Proper tuning is crucial. The matching network is mainly composed of automatically controlled tuning with motorized variable capacitors with phase and amplitude discriminators. The active incident and reflected powers between the generator and the matching are measured using simple couplings on the coaxial cable and the readings are made from monitors placed on the generator control board. The control board of the matching box corrects automatically the impedance tuning of the system to ensure optimal energy transfer to the material. To prevent thermal overload, the matching box is cooled by cold water circulation through the fixed resistor coil.

The RF power generator is a free running oscillator circuit coupled to a triode valve, which is fed by a high voltage power source (220 V). The oscillator circuit produces the oscillations, which are sustained by the triode valve. The output power from the generator is indicated and adjusted by a potentiometer placed on the front the generator. There are two galvanometers located on the front of the generator. One displays the incident power supplied by the generator and the other one displays the reflected power, which comes back to the generator when the power is not adequately absorbed by the load in the applicator. If the amount of reflected power is too great, the life of the generator will be significantly reduced. The generator is thus equipped with a safety feature that automatically shuts off the generator when reflected power is above 10% of the incident power.

The temperature measurement system is composed of optical fiber sensor probes, which are transparent to electromagnetic interferences in comparison to traditional sensors. The probes can be directly inserted in the material to be heated or placed on the surface. They have rapid response time and the measurements are easily interfaced to a data acquisition system.

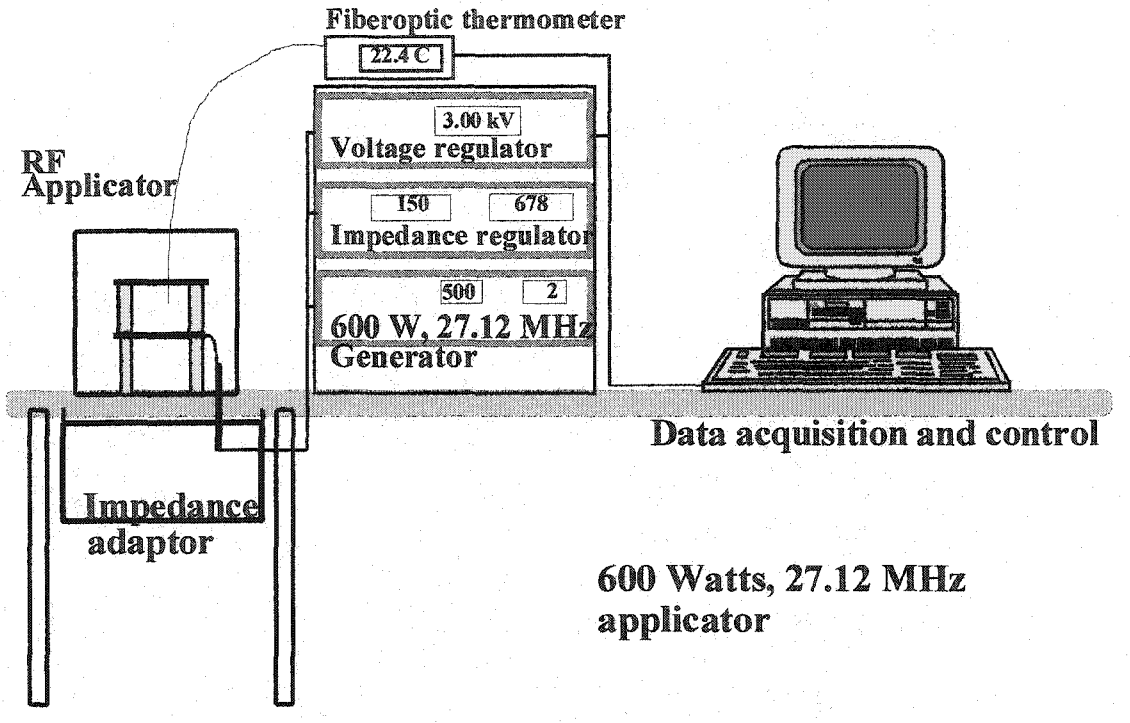


Figure 4.3: Schematic view of complete RF system

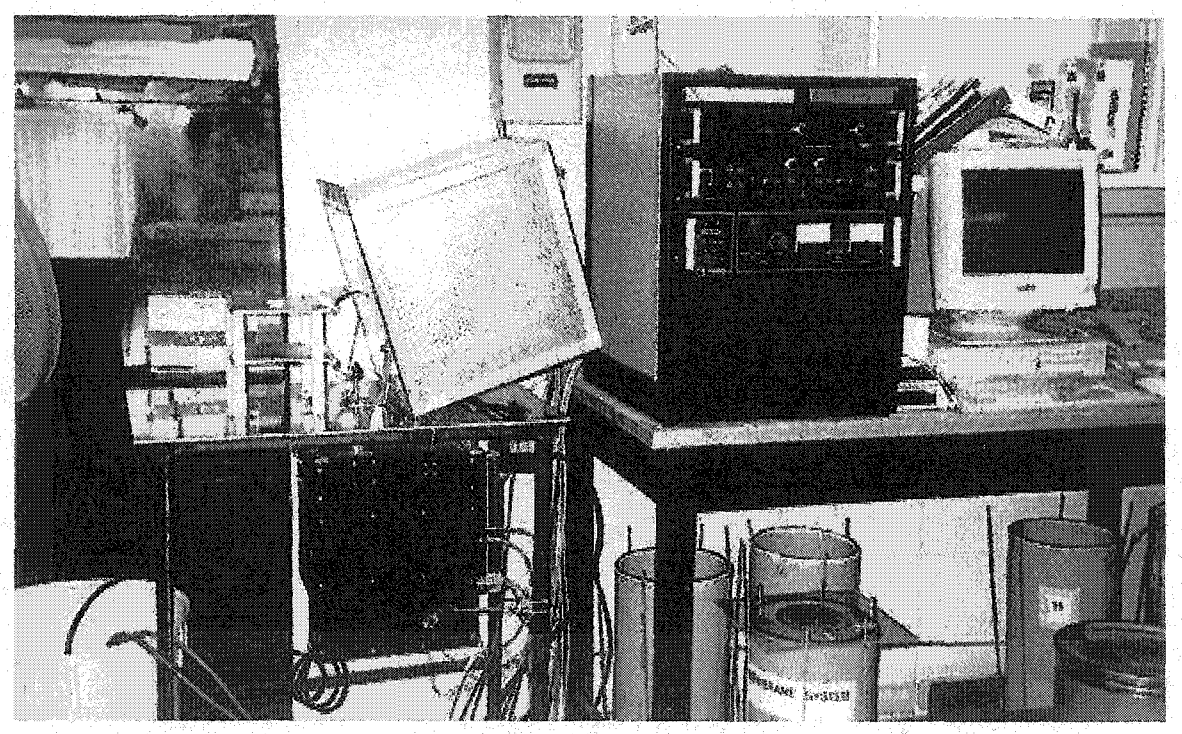


Figure 4.4: Picture of the complete RF system

To fit the applicator system, meat samples were cut cylindrically to minimize heating at edges. Also, to minimize the air gap, the length of samples was set at 4 cm with a diameter of 3 cm and contained in an aseptic glass petri dish. The system was tested in the fall of 1999 with preliminary meat samples in order to determine the time and power application possible without cooking of the meat surface. Microwave applications were also examined at since the RF preliminary results were not giving satisfactory results.

4.1.2 Microwave Heating

The microwave setup is shown in Figure 4.5 and consists of a variable power microwave generator (2450 MHz, 750 W). The microwave cavity measures 40 x 35 x 25 cm and is equipped with an access door for sampling. Fiber optic sensors are introduced in the cavity for temperature monitoring.

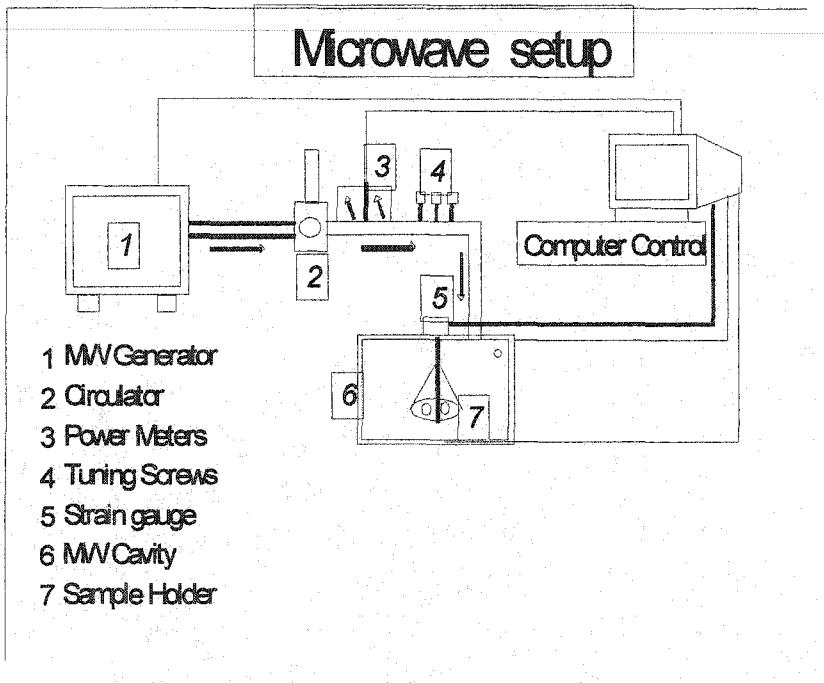


Figure 4.5: Microwave heating system

Three series of microwave heating trials were conducted operating under a combination of pulsing modes of 30 s on/off and 15 s on/off with applied microwave powers of 0.3 W/g up to 1 W/g for treatment times ranging from 1.5 minutes up to 4 minutes.

4.2 Preliminary Trials

Beef loins were supplied from cattle slaughtered on site at the Lacombe Research Centre in Alberta. The loins were sterilized to ensure that the surface of the meat was initially bacteria free. Sterilization was done by dipping each loin, in 95% ethanol, and flaming the piece of meat, which procedure was repeated three times. The loin was put down on a sterile cutting board. The flamed surface of the meat was removed carefully with sterile scalpels and forceps to keep only the sterile raw meat. Beef cores were removed from the loins with a coring devices (10 cm^2 diameter) to obtain uniform cores. The cores were packed in sterile bags and frozen or refrigerated (2°C). Then, they were shipped in a cooler to Montreal by air within 6-7 hours. Duplicate cores were used for each treatment studied.

Experiments were performed using *Escherichia coli* biotype 1, *Pseudomonas* D17 and *Carnobacterium* "845" obtained from the Lacombe Research Centre. To obtain the needed inoculum the microorganisms were inoculated separately into 5 ml of tryptic soy broth (TSB) (Fisher Scientific, Montreal, Canada) and incubated at room temperature for 48 hours. Each micro-organism was transferred using 100 µl of the cultures into 5 ml of TSB and incubated overnight at room temperature. The next day, bacteria were transferred again individually by using 2 ml of the cultures into 100 ml of TSB and incubated overnight at room temperature. The cultures were washed by centrifuging (12 500 rpm, 3 min) with 50 ml peptone (0.1 %). A dip suspension for the inoculation of the cores was prepared to introduce approximately log 3.5 cfu/cm^2 bacteria on the cores. The inoculation process was done in the coldroom at 2°C. Each core sample was dipped into the bacterial solution for 15 s. The dipped samples were suspended and allowed to drip dry for 15 min to allow the cells to adhere before they were treated.

Two types of samples were used: (a) Controls were sterile meat cores dipped in sterile 0.1% peptone and used as negative control and packed without any treatment, (b) Cores to be heated with RF were placed on sterile petri dishes to be placed between the two aseptic electrode of the radio-frequency set-up.

Inoculated samples were used for positive control, radio-frequency 1 (RF1) and radio-frequency 2 (RF2) treatments and then packed by one of the methods described in the experimental design. RF1 and RF2 samples were exposed to a treatment of 600 Watts for 45 s and 60 s respectively. Each core was then divided in half with sterile scalpels prior to packaging with a careful identification of which was the top half of the core and the bottom half of the core. The top part of the core was shipped by air to the Lacombe Research Centre for analysis. The bottom part of the core was kept in Montreal. Both the top and the bottom part of the samples were analysed as described in the experimental design. All preliminary trial combinations were conducted in the fall 1999. Other combinations were tried before starting the full experimental design with the RF heating system and the microwave system.

4.3 Experimental Design

All the trials following the experimental design were conducted in February and March 2000 and consisted of treating duplicate inoculated beef cores with the best two RF treatments obtained from our preliminary fall trials. The treatments for the full experiment were: 1) RF1 treatment consisting of 600 Watts RF incident power for 30s, followed by 30s at 400 Watts RF incident power and 60s at 200 Watts RF incident power; 2) RF2 treatment consisting of 600 Watts RF incident power for 30s, followed by 30s at 400 Watts RF incident power and 60s at 100 Watts RF incident power; 3) an antimicrobial treatment alone; 4) the antimicrobial treatment in combination with RF1; and 5) the antimicrobial treatment in combination with RF2.

The antimicrobial solution consisted of a 1:3 mixture of Nisin (2.5% nisin 1000 IU/mg) and lysozyme (Canadian Inovatech Inc.) suspended in sterile water to obtain a concentration of 600 mg/g on the surface of the core. Cores were dipped in antimicrobial for 30 sec prior to exposure to RF heating. Antimicrobial samples were

dipped into the antimicrobial solution following the bacterial inoculation of the cores. Antimicrobial positive controls were packaged as described below.

All samples were packed and stored by one of the following methods: (a) Samples were placed onto Styrofoam trays (Scott National, Calgary, AB, Canada) and overwrapped in an oxygen permeable (8000 cc / m^2 / 24 h) polyvinyl chloride film (Vitafilm Choice Wrap, Goodyear Canada Ltd., Toronto, Ontario) and stored for up to 7 days in a 7°C incubator; (b) Samples were placed in vacuum bags (Winpak, Winnipeg, MB), vacuum packaged (Cryovac, Canada vacuum sealer) and stored for up to 6 weeks in a 2°C cold room. The oxygen transmission rate of the bags was 40-50 cm^3/m^2 in 24 h at 23°C.

The microbial population on the beef cores stored in retail packages (7°C) was determined at days 0, 2, 6 and 8 and in vacuum packages (2°C) at week 0, 1, 2, 3, 4, 5 and 6. Before sampling, each core was visually evaluated by five panelists for any discolouration or off-odour emanation. The surface pH was measured with an Oktron Digital pH meter (Model Wo-0060500-000, Anachemia Scientific, Calgary, AB) equipped with a flat surface polymer body combination electrode (Fisher Scientific, Nepean, ON). Meat colour reflectance coordinates (L*, a*, b*) were measured objectively, as recommended by the Commission Internationale de l'Éclairage (CIE, 1978) using a Minolta Chroma Meter II (Minolta Camera Co. Ltd., Japan). Sensory evaluation was done on the cores. It consisted of the assessment of muscle colour (9-point scale: 0 = completely discoloured; 1 = white; 8 = extremely dark red), surface discolouration (7-point scale: 1 = no surface discolouration; 7 = complete discolouration), retail appearance (7-point scale: 1 = extremely undesirable; 7 = extremely desirable), off-odour intensity (5-point scale: 1 = no off-odour; 5 = prevalent off-odour), and odour acceptability (5-point scale: 1 = acceptable; 5 = unacceptable) by an experienced, trained, 5-member sensory panel (Greer *et al.*, 1993).

Top surface of the core (10 cm^2) was removed carefully with sterile scalpels and forceps and placed into a double layer sterile stomacher bag. The samples were homogenized separately in 90 ml of sterile 0.1 % peptone for 2 min (Difco laboratories, Inc., Detroit, MI, USA). in a Colworth stomacher in McGill laboratories (Baxter

Diagnostics Corp.) or with the Stomacher®Lab-Blender Model '400' (Seward Laboratory, London, England) in the Lacombe Research Centre.

The bacterial population was determined by preparing serial dilutions of the rinse suspension and plating portions onto 1) deMan Rogosa and Sharpe agar, amended with streptomycin sulfate (MRSS) for enumerating the *Carnobaterium*; 2) Cephaloridine-fucidin-cetrimide agar (CFC) to enumerate *Pseudomonas* D17 (Baird *et al.*, 1987); and 3) Violet Red Bile Agar (VRBA, Oxoid Inc., Nepean, ON) to enumerate *E. coli*. The suspension was dispersed on the MRSS and CFC plates by the spread-plate technique. Poured VRBA media under the conventional overlay method was used to plate the dilutions for the *E. coli* enumeration. The plates were incubated at room temperature for 3-5 days for MRSS, 48 hours for CFC and 36 hours for VRBA before colony forming units (CFU) were enumerated.

Treated meat samples were shipped back by plane to the Lacombe Research Centre and stored according to each treatment. Evaluation of day 0 and week 0 of the present experiment was conducted on the treated day at McGill University. Subsequent evaluations were done in the Lacombe Research Centre.

4.3.1 Statistical Analysis

Results of the experimental design were statistically analysed by the Statistical Analysis System (SAS) using the General Linear Model Procedure. A probability of (P) of < 0.05 was considered to be significantly different. The SAS output is compiled in a chart presented in Appendix A. Retail packaging statistical analysis are showed in Appendix A-1 to A-3 and vacuum packaging statistical analysis followed in the section of Appendix A4 toA6. Missing values were predicted using the GLM procedure but the SAS outputs of the predictions are not presented in this thesis.

V. RESULTS AND DISCUSSION

5.1 Preliminary Trials

The time of exposure and the power used on raw pieces of meat with our RF heating system needed to be evaluated at first to see the possible boundary of the treated product without inducing quality changes. To do so, pieces of raw beef were bought at the local super market and core samples similar to our experimental size were extracted from the pieces. No actual inoculation of bacteria was made to those samples and no microbiological analysis was conducted. The goal was to determine the critical treatment point between raw samples and "started to cook" samples. The result of this investigation was that under maximum power of our system (600 W), the longest time of exposure would be 60 s to keep good meat quality. This is why for preliminary trials the treatments provided were at maximum power and for a time of exposure of 45 s (RF1) and 60 s (RF2). Two treatment times were used to see if shorter exposure time would give satisfactory results compared to longer time exposure. The interest is that if the experiment gives promising results to be implemented in industry, shorter time of exposure would increase the profitability of the process.

Preliminary trials were done in October 1999 to verify the treatment time and power previously chosen and see its effect on bacteria. As mentioned in the material and methods section, the system set-up is located at McGill University. The RF treatments were applied to meat samples in the Montreal laboratory and half of each core was shipped back to the Lacombe Research Centre in Alberta for proper storage and analyses. The other half stayed in the McGill laboratory for storage and analyses. The reason for this action was to obtain duplicate sets of data for each core. Since the experiment was conducted with already duplicate samples for each treatment, we ended up with four replicates of the same treatment. For example, treatment 8A02 (RF2 treatment of 60s, 600 W; duplicate A; retail packaged sample of week 0, day 2) was cut in half (upper part for McGill, lower part for Lacombe Research Centre) to be analysed in both places. Two analyses of the same piece of meat with the same treatment were

obtained. For treatment 8B02, the same treatment is applied, as the treatment 8A02 except it is the duplicate B. The treatments and packaging methods are summarized in Table 5.1 where treatment 6 = positive control (inoculated pieces, no RF treatment), 7 = negative control (sterilized pieces, no RF treatment), 8 = RF1 (600 W, 45 s) and 9 = RF2 (600 W, 60 s).

Table 5.1: Summary of treatments for preliminary trials

RETAIL (7°C)									
STORAGE TIME		TREATMENT (DUPLICATE)							
WEEK	DAY	6(A)	6(B)	7(A)	7(B)	8(A)	8(B)	9(A)	9(B)
0	0								
0	2								
0	5								
0	7								
VACUUM (2°C)									
STORAGE TIME		TREATMENT (DUPLICATE)							
WEEK	DAY	6(A)	6(B)	7(A)	7(B)	8(A)	8(B)	9(A)	9(B)
0	0								
1	0								
2	0								
3	0								
4	0								
5	0								
6	0								

5.1.1 Microbiological Analysis

In order to follow the growth of each individual bacterium inoculated on the meat, three selective growing media were used. Cephaloridine Fucidin Cetrimide Agar (CFC) has been extensively used for enumeration of *Pseudomonas* spp from red meats and other foods. Violet Red Bile Agar (VRBA) is used to count and enumerate coliforms. Finally, deMan, Rogosa and Sharp Agar (MRS) is appropriate for cultivation of Lactic acid bacteria. Streptomycin sulfate was added to the medium to

inhibit growth of *Pseudomonas* D17 and *E. coli* which interfered with enumeration of the lactic acid bacterium on unamended MRS. Those three media were used to count *Pseudomonas* D17, *Escherichia coli* biotype 1, and *Carnobacterium* "845" respectively.

Graphs of the log number of bacteria over time in retail packaging are shown in Figures 5.1 to 5.6. Figure 5.1 corresponds to *Carnobacterium "845"* level over retail storage time period starting from day 0 to day 7 for McGill analysed half cores. Figure 5.2 presents the same core but stored and analysed at the Lacombe Research Centre (LRC). Differences of 1 log number are not considered to be important in Food Microbiology. Figure 5.1-5.4 do not show differences of many log numbers. The only major difference is in the numbers of *Pseudomonas* D17 at 5 and 7 days. RF treatments do not show a reduction in log numbers compared to the positive control samples. In fact, the level of bacteria seems to be higher when subjected to RF heating. Both treatments, as well as the positive control, show an increase in bacteria level until the end of the storage period. Figures 5.3 and 5.4 present the level of *E. coli* when stored under retail temperature and evaluated at McGill and LRC respectively. Again, no significant reduction is found for the RF treatments compared to the positive control, except for the day 7 of analysed in Alberta for RF2 samples where a large reduction is present. The large reduction in the numbers of *E. coli* at this time was explained by a rapid increase in the *Carnobacterium* population and the high pH of the cores. However, McGill results do not corroborate this reduction. Also, a reduction is noticed for all treatments over time probably due to competition among bacteria types. Figures 5.5 and 5.6 present the *Pseudomonas* level over time when stored under retail packaging for McGill and LRC analyses. The level of bacteria over time increased for all treatments. LRC results seem more acceptable and uniform than the McGill ones. No apparent log reductions of bacteria due to RF treatments are observed.

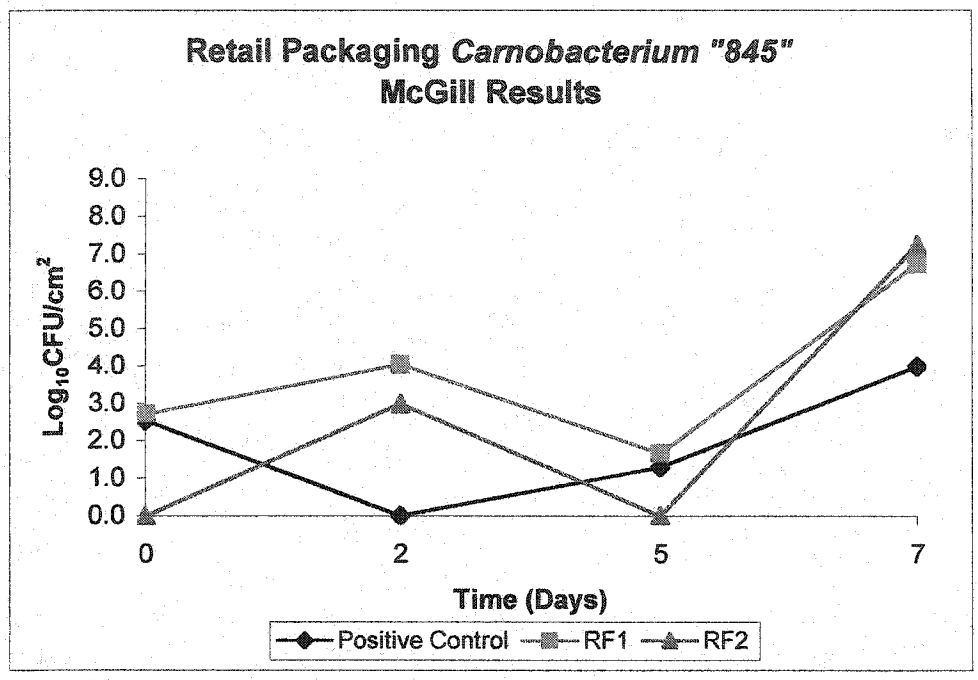


Figure 5.1: Log numbers of *Carnobacterium "845"* on meat samples (McGill results)

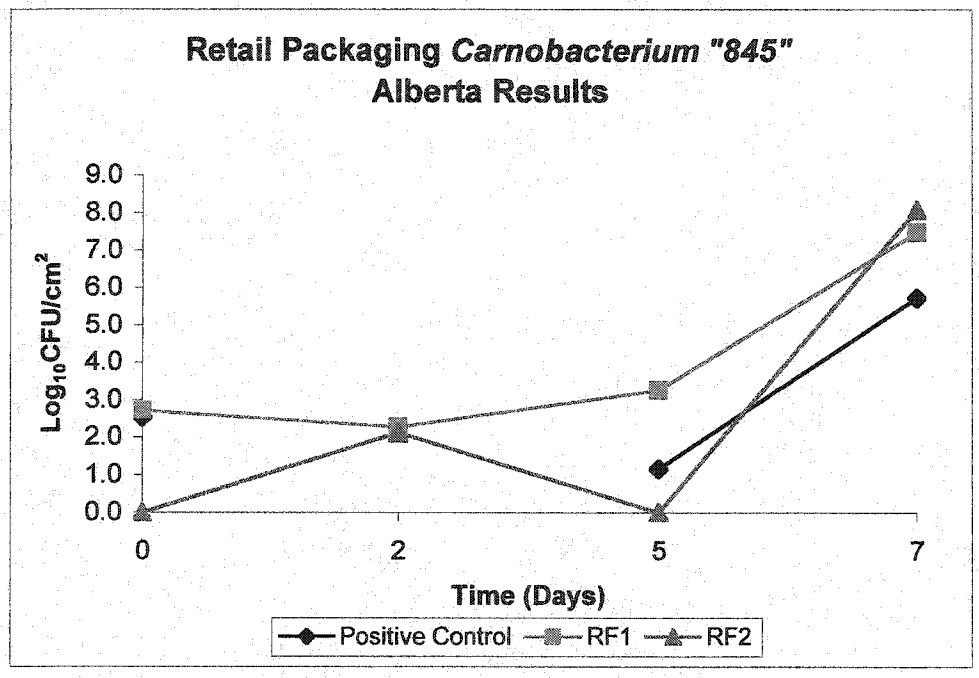


Figure 5.2: Log numbers of *Carnobacterium "845"* on meat samples (Alberta results)

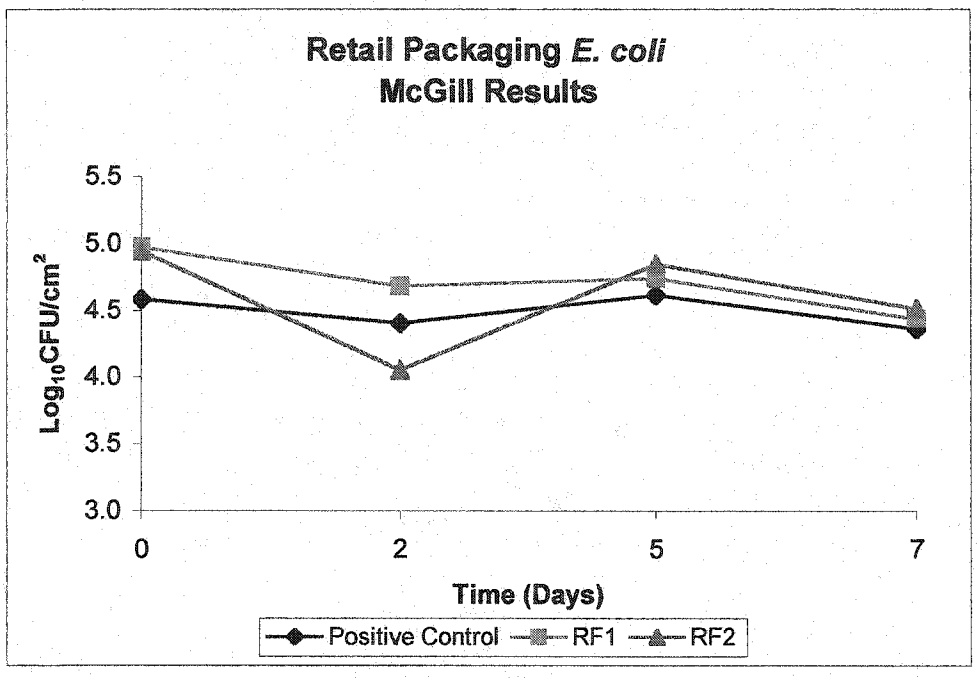


Figure 5.3: Log numbers of *E. coli* on meat samples (McGill results)

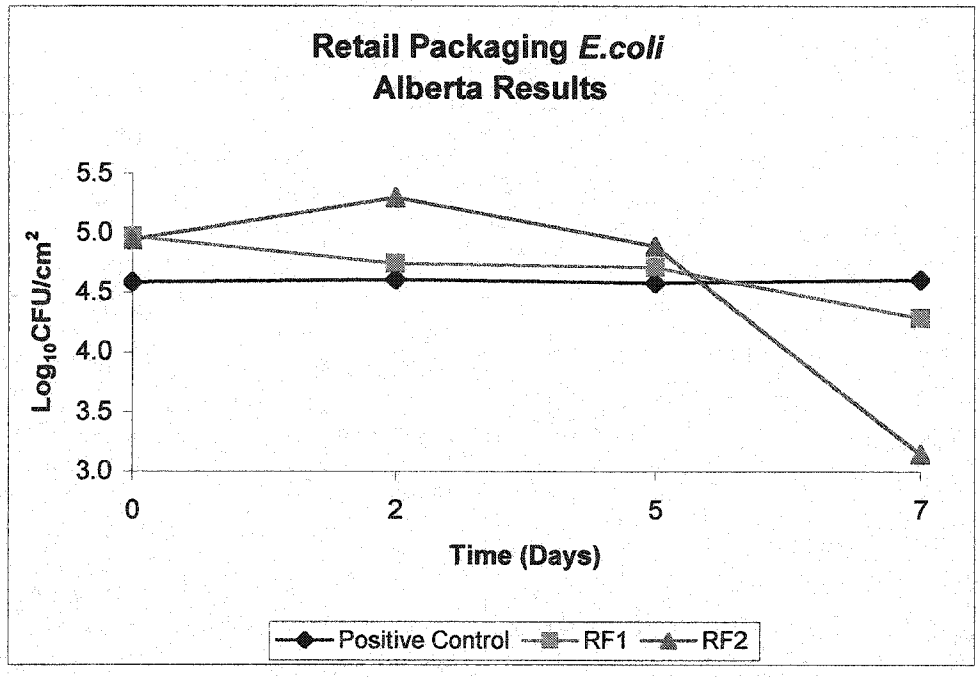


Figure 5.4: Log numbers of *E. coli* on meat samples (Alberta results)

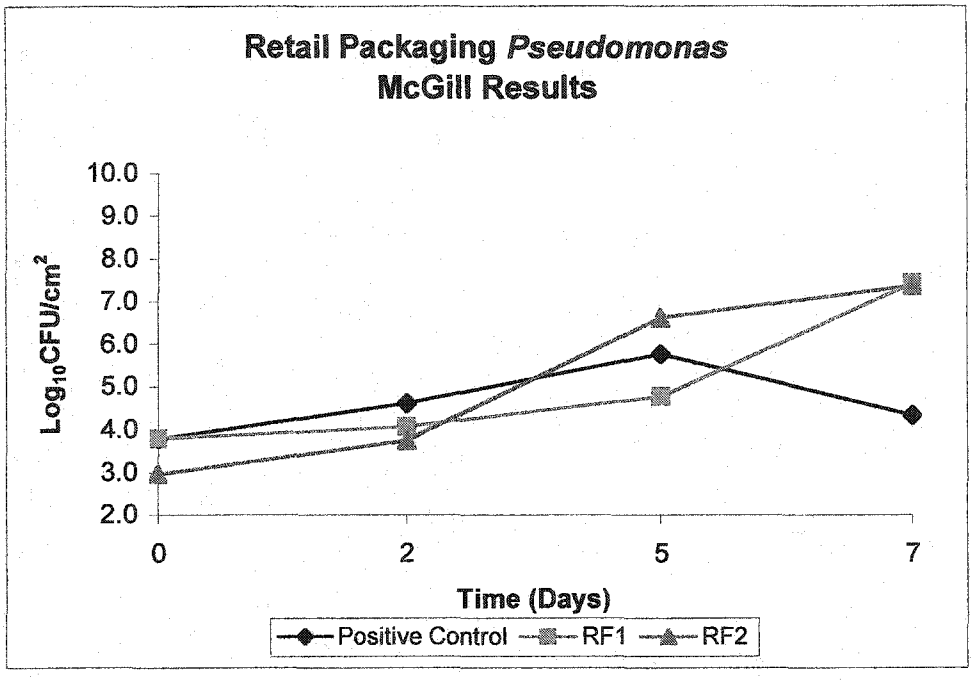


Figure 5.5: Log numbers of *Pseudomonas* on meat samples (McGill results)

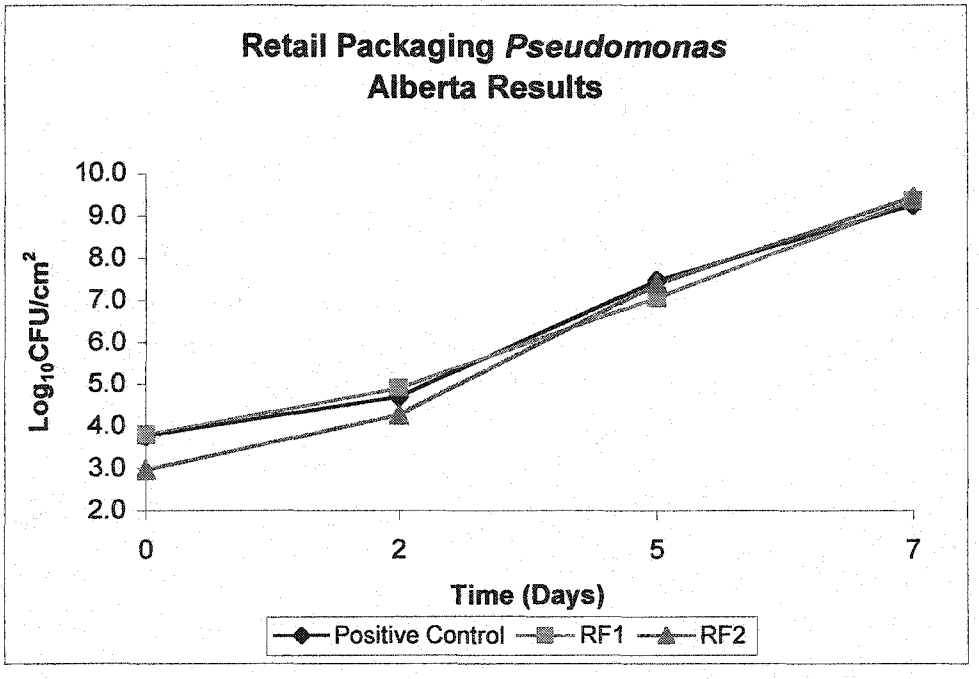


Figure 5.6: Log numbers of *Pseudomonas* on meat samples (Alberta results)

Graphs of the log number of bacteria over time in vacuum packaging are shown in Figures 5.7 to 5.12. In Figures 5.7 and 5.8, the level of *Carnobacterium "845"* increased over the storage period for McGill results and decreased for LRC. This could possibly be due to interference from the pseudomonads. At LRC we were able to incubate anaerobically thus inhibiting them. At McGill we were relying entirely on the streptomycin sulfate to keep the pseudomonads from growing. However, at both locations of analyses, the RF treatments showed no reduction compared to positive control, instead, higher log numbers are observed. The level of *E. coli* (Figures 5.9 and 5.10) in vacuum packaged environment over time stayed fairly constant for LRC results and showed a little decrease in log numbers for McGill results and this was evident for all treatments. No bacterial reductions due to RF treatments were noticed. Figures 5.11 and 5.12 present the level of *Pseudomonas* over time for both locations of analysis. Log numbers increased over time with no significant reduction due to RF treatments compared to positive control.

5.1.2 Measurement of pH

Figures 5.13 and 5.14 show the pH measurements on retail and vacuum packaged meat samples respectively for LRC analyses. McGill results are not shown here since the pH measuring instrument used was not accurate enough to consider the data obtained. Normal pH of muscle tissue is known to be between 5.5 and 5.8. In retail, the pH level for RF treatments increased to nearly 6.0 and 6.2 at day 7. High level of pH >6.0 usually refer to a dark, firm and dry piece of meat. In high pH meat the level of bacterial nutrients are lower which causes the tissue to spoil more rapidly than normal pH meat since amino acids are rapidly attacked (Borch, *et al.*, 1996). This is probably why the population of *Carnobacterium* had increased so rapidly in retail packaging. The pH of the vacuum packaged meat stayed fairly constant with little increase over the entire storage period for all treatments.

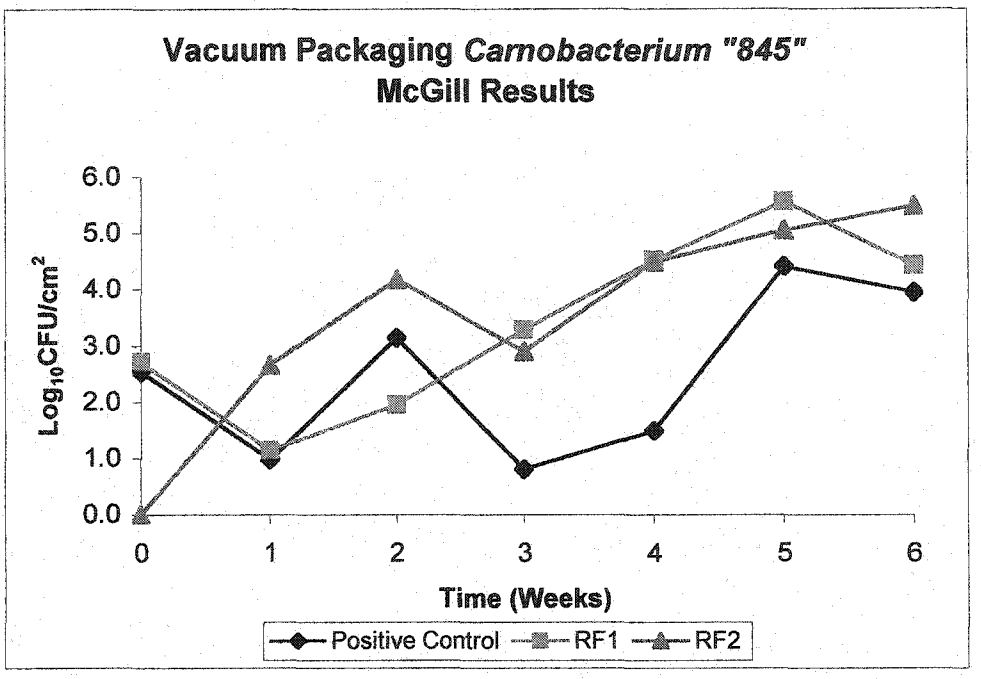


Figure 5.7: Log numbers of *Carnobacterium "845"* on meat samples (McGill results)

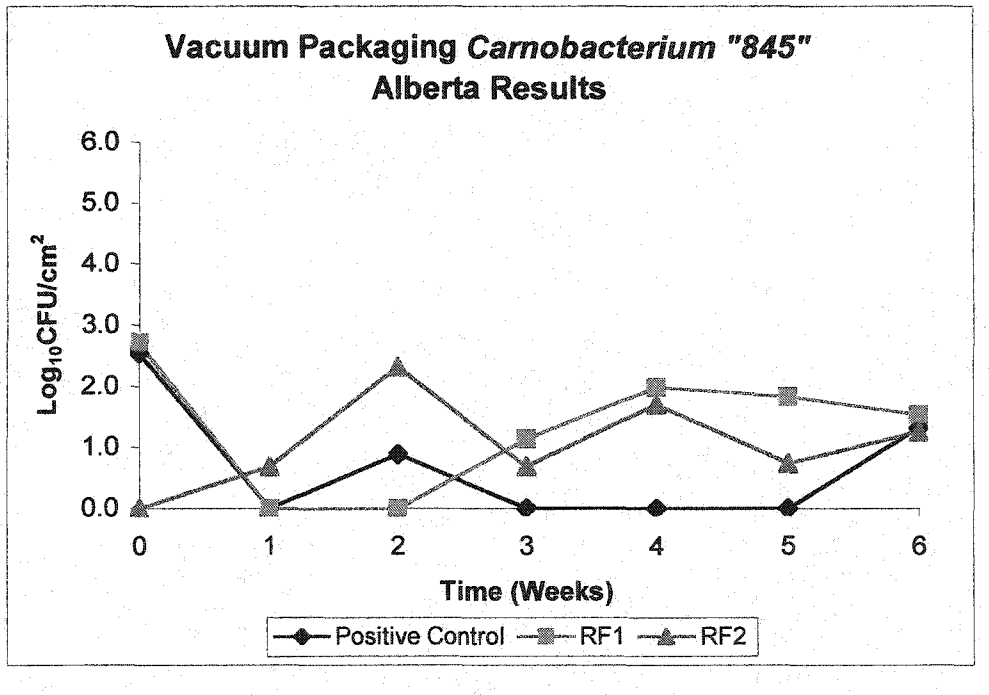


Figure 5.8: Log numbers of *Carnobacterium "845"* on meat samples (Alberta results)

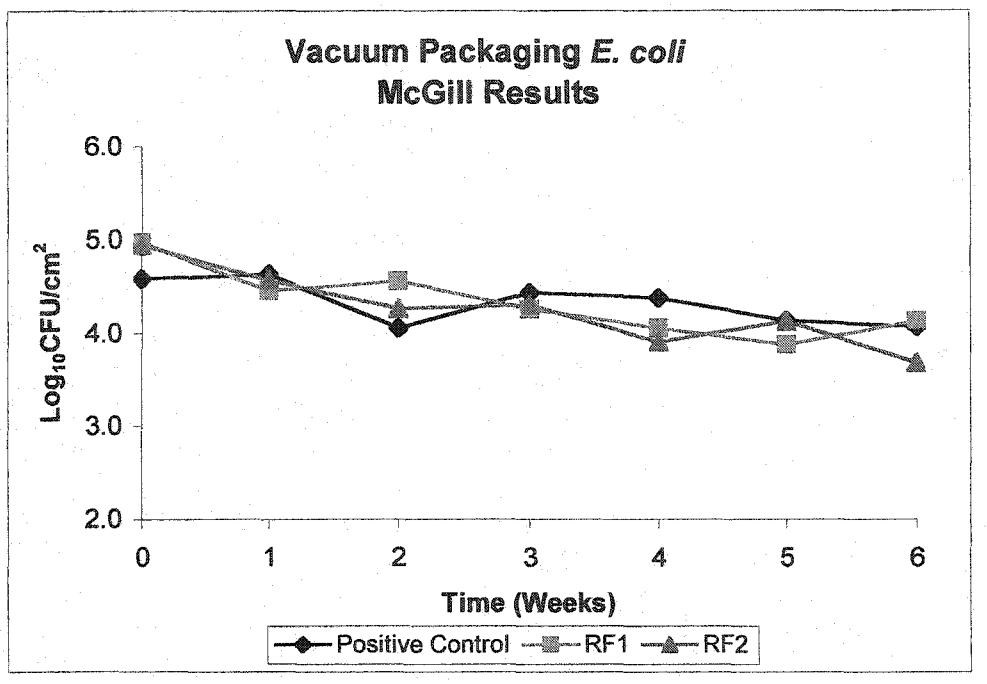


Figure 5.9: Log numbers of *E. coli* on meat samples (McGill results)

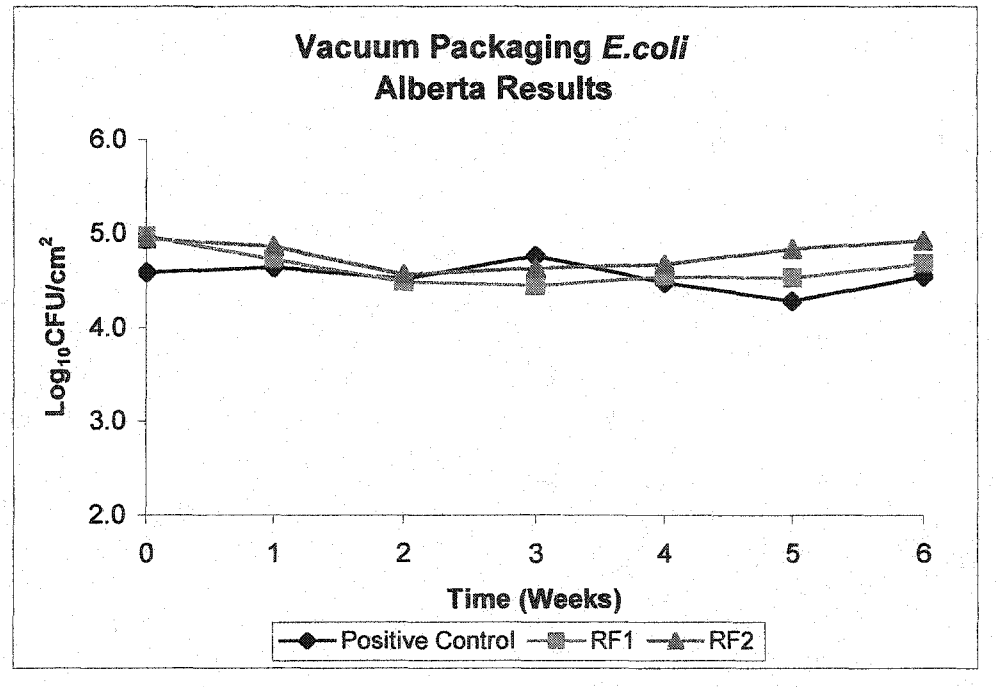


Figure 5.10: Log numbers of *E. coli* on meat samples (Alberta results)

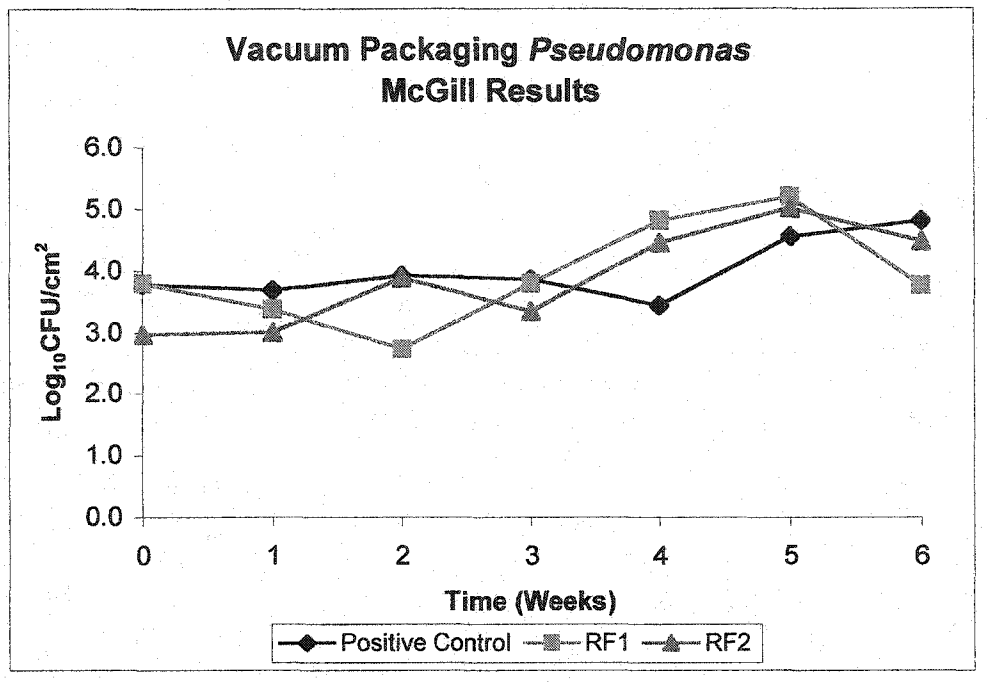


Figure 5.11: Log numbers of *Pseudomonas* on meat samples (McGill results)

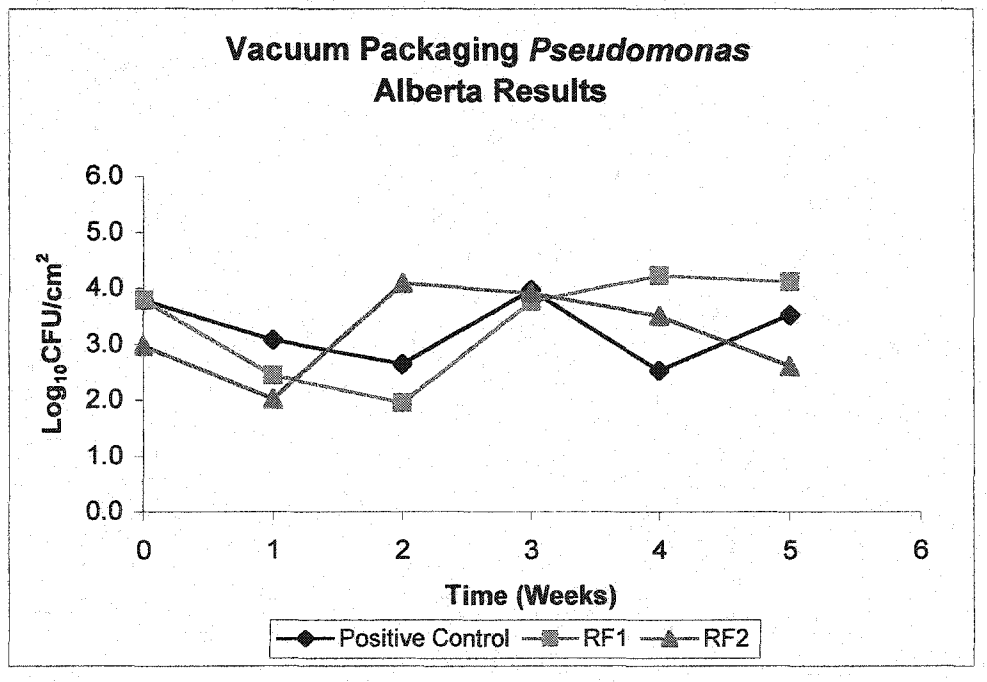


Figure 5.12: Log numbers of *Pseudomonas* on meat samples (Alberta results)

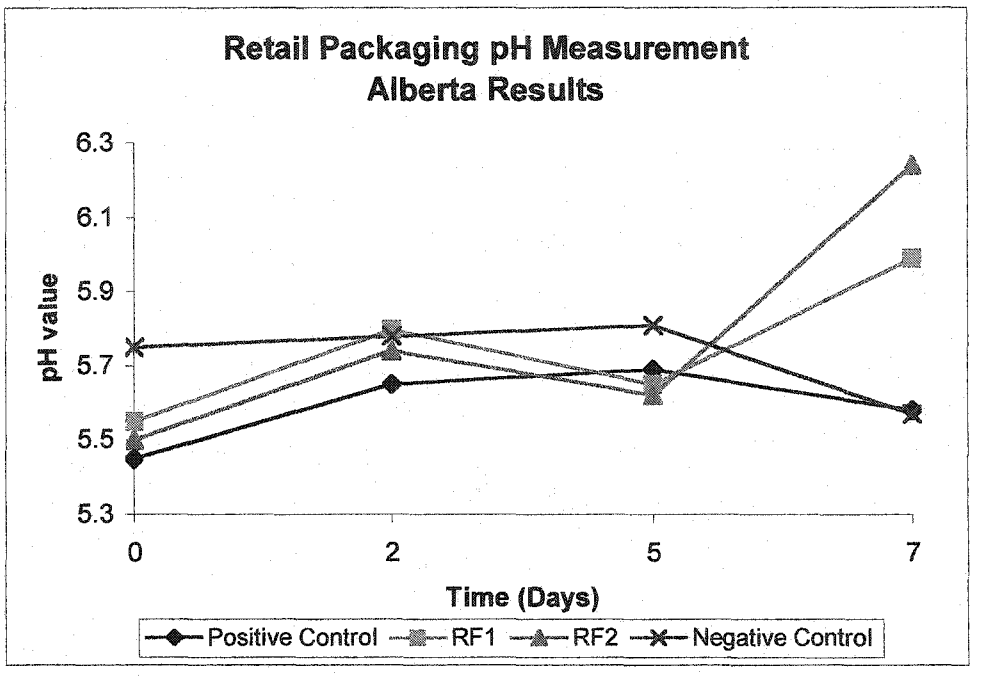


Figure 5.13: pH measurements on retail packed meat samples

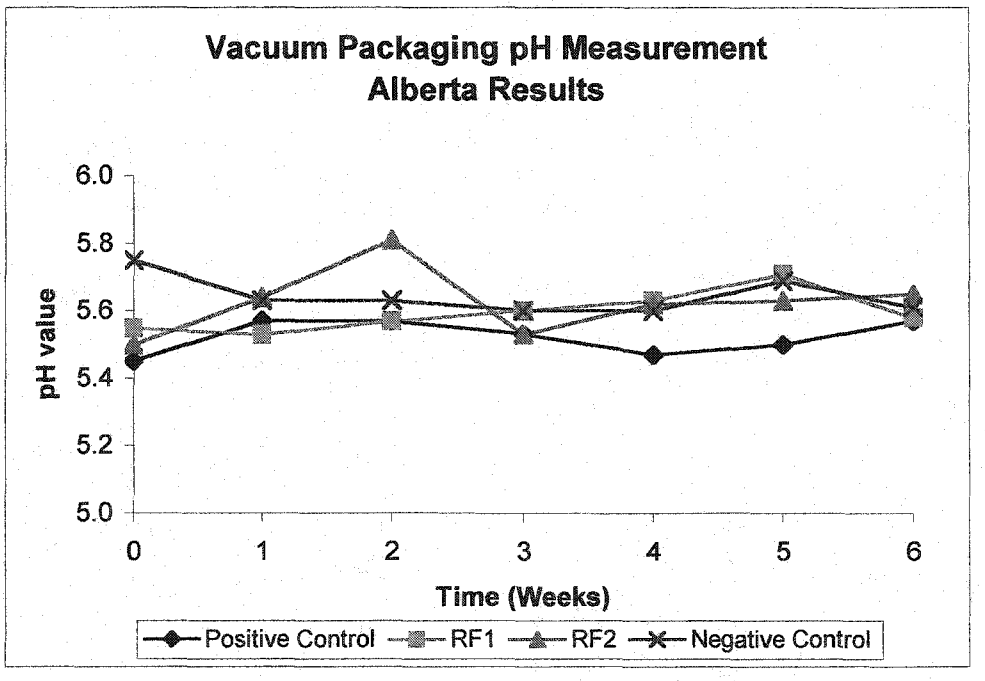


Figure 5.14: pH measurements on vacuum packed meat samples

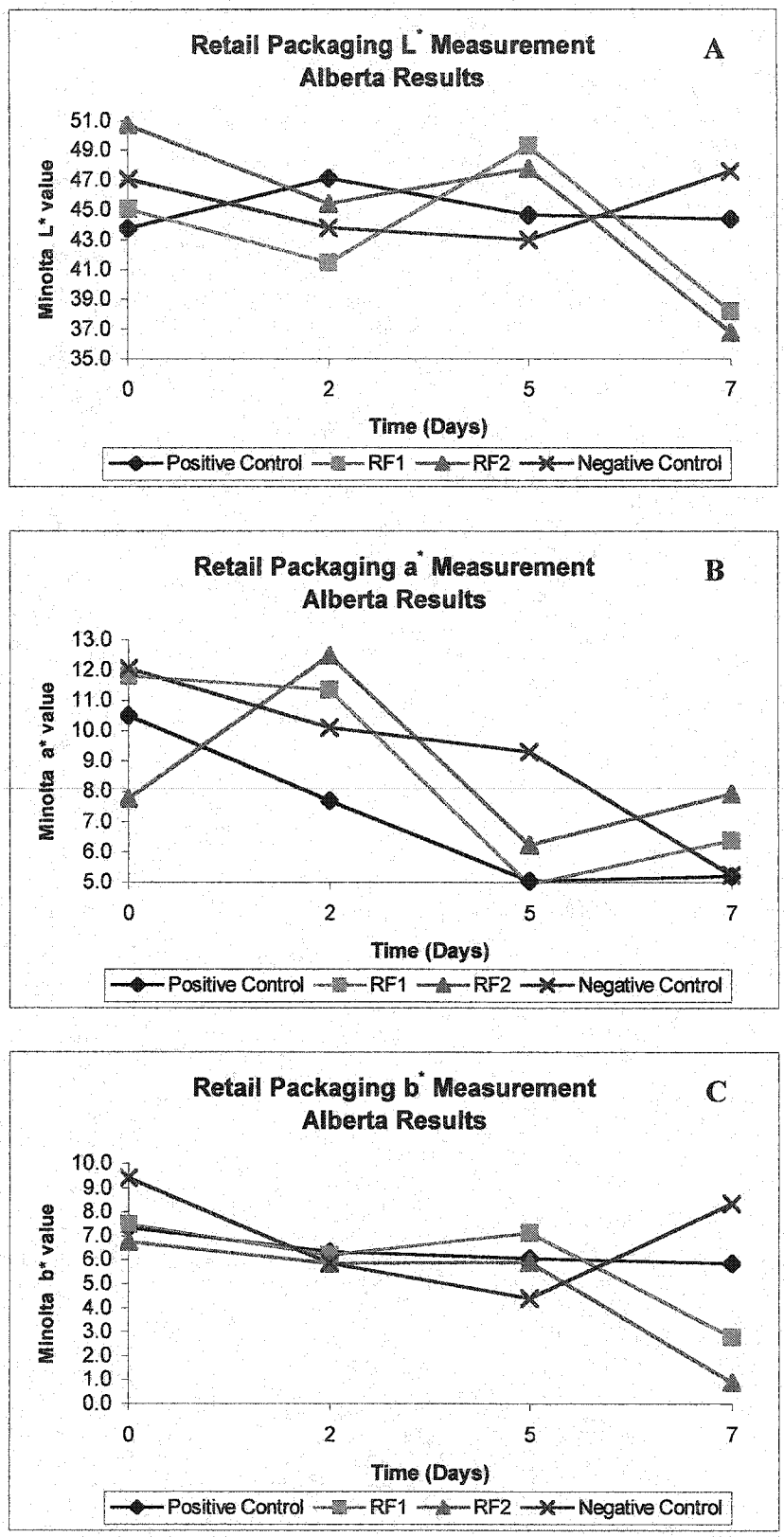
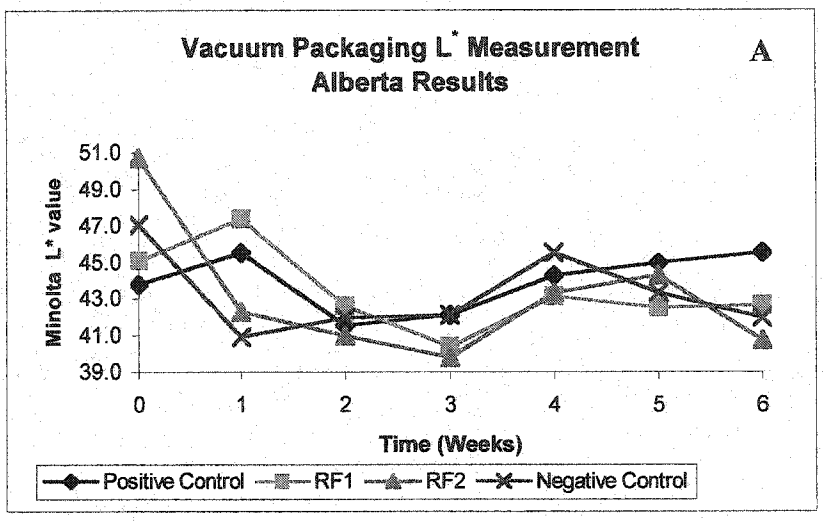
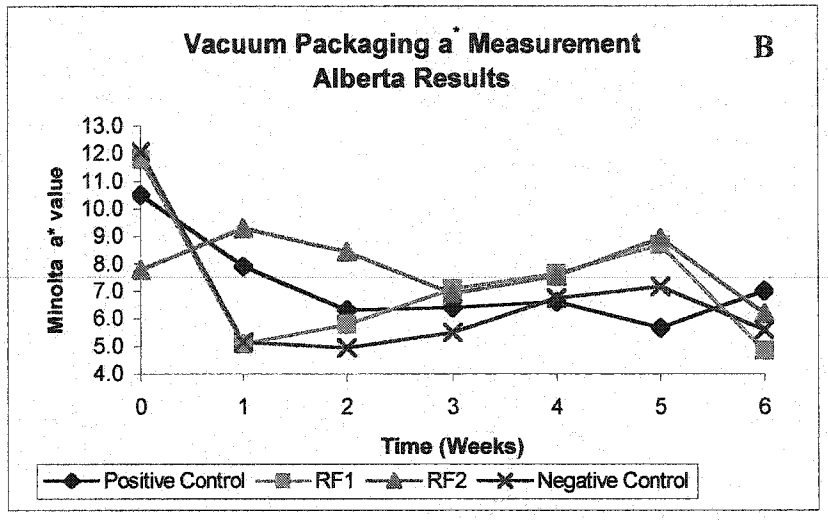


Figure 5.15: Colour measurement of retail packed meat samples A) L* value, B) a* value and C) b* value





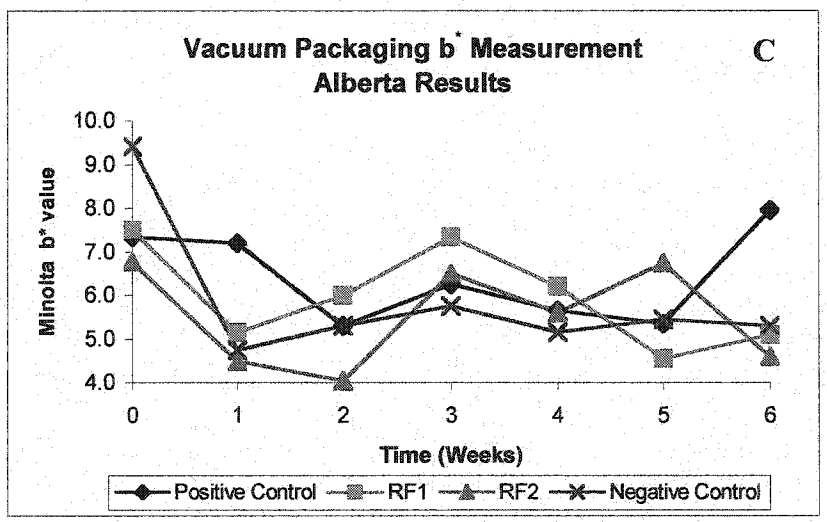


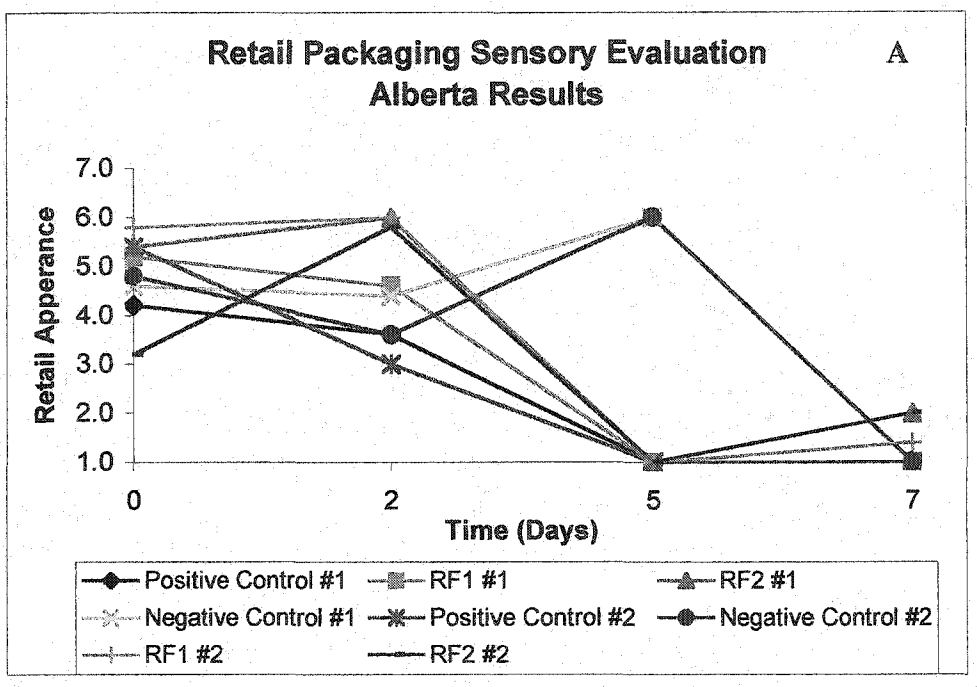
Figure 5.16: Colour measurement of vacuum packed meat samples A) L* value, B) a* value and C) b* value

5.1.3 Colour Measurement

Figure 5.15 (A, B, C) shows the changes in lightness (L*), redness (a*) and yellowness (b*) values of retail packed meat samples. The graphs shown are for LRC results only. Both L* and b* values for RF treatments after 7 days of storage show a significant reduction over controls. However, a* value increased compared to controls. Figure 5.16 (A, B, C) presents L*, a* and b* measurement for vacuum packed meat samples. All results seem to indicate a decrease in the colour measurements over storage time. Since colour is an important factor in consumer acceptability of fresh meat, any significant changes from the original state colour would negatively affect the product purchase decisions.

5.1.4 Sensory Evaluation

Trained panelists conducted the sensory evaluation before each analysis. For overall retail appearance, a rate of 1 is classified as extremely undesirable and 7 for extremely desirable. Figures 5.17-A and 5.18-A illustrate the average rating of 5 panelists for retail and vacuum packaged meat samples respectively. Only Alberta's results are shown here. For all treatments, the tendency is that at day 2 in retail packaging, the samples were still desirable. At day 5, the samples were rated undesirable except for the negative control ones. After 1 week in vacuum packaging, samples for all treatments were not desirable. Odour acceptability is presented for retail and vacuum packaging samples for Alberta's results in Figures 5.17-B and 5.18-B respectively. For treatments after 2 days the odour acceptability in retail packaging was rated neither acceptable nor unacceptable for controls and unacceptable for most RF treatments. In vacuum packaging, the results were not constant for weeks 1 and 2 fluctuating around the neither acceptable nor unacceptable category. Starting at week 3, the results showed unacceptable odour emanation from the meat samples.



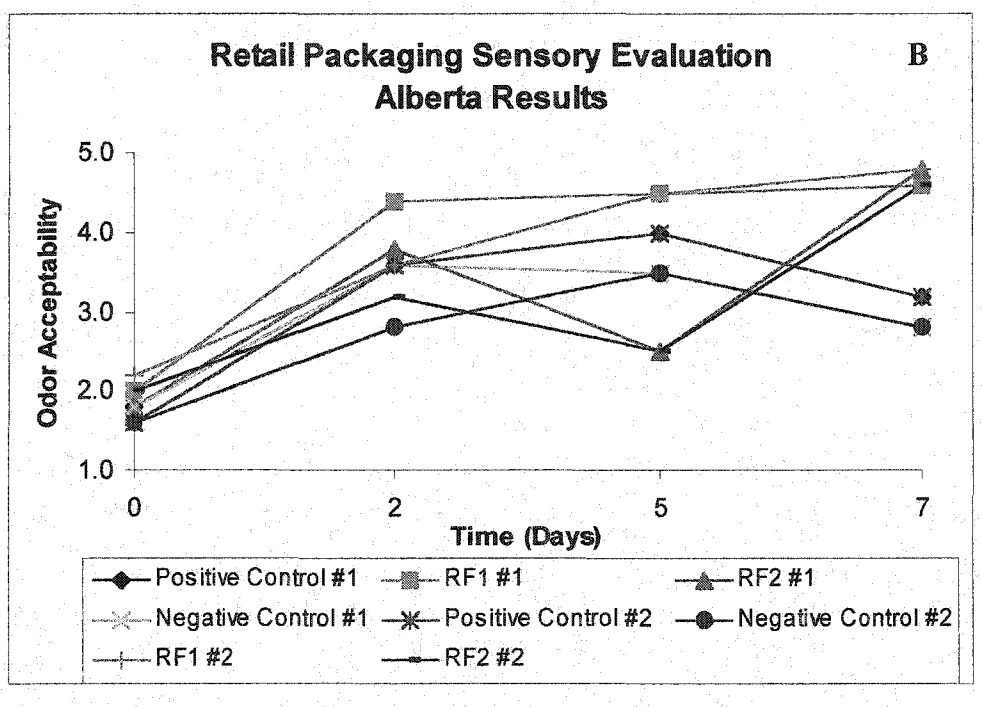
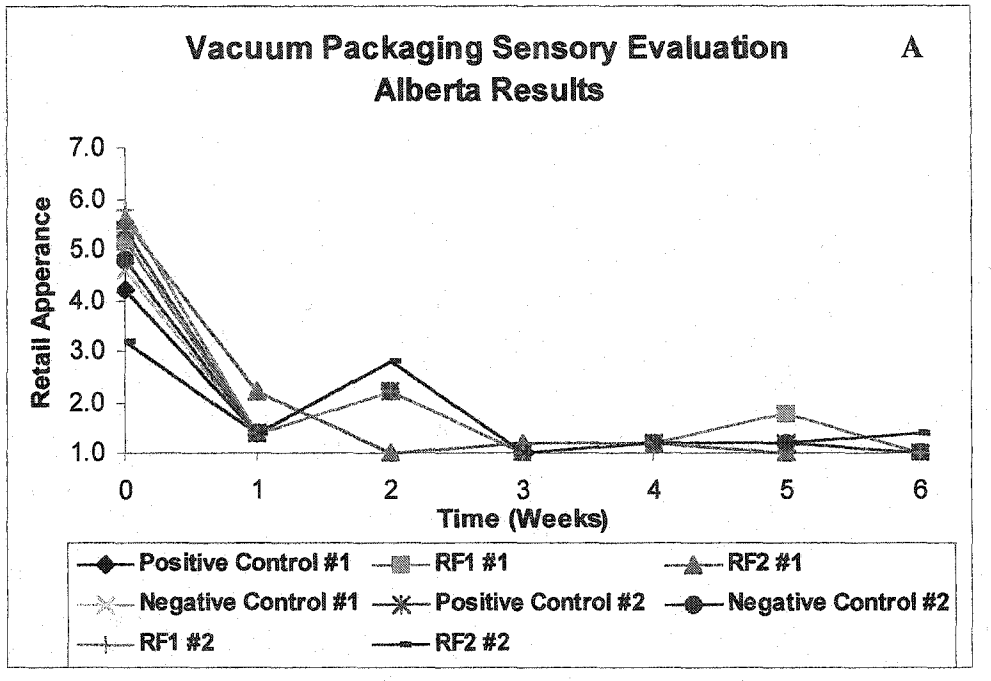


Figure 5.17: Sensory evaluation for retail packaged meat samples A) Retail appearance B) Odour acceptability



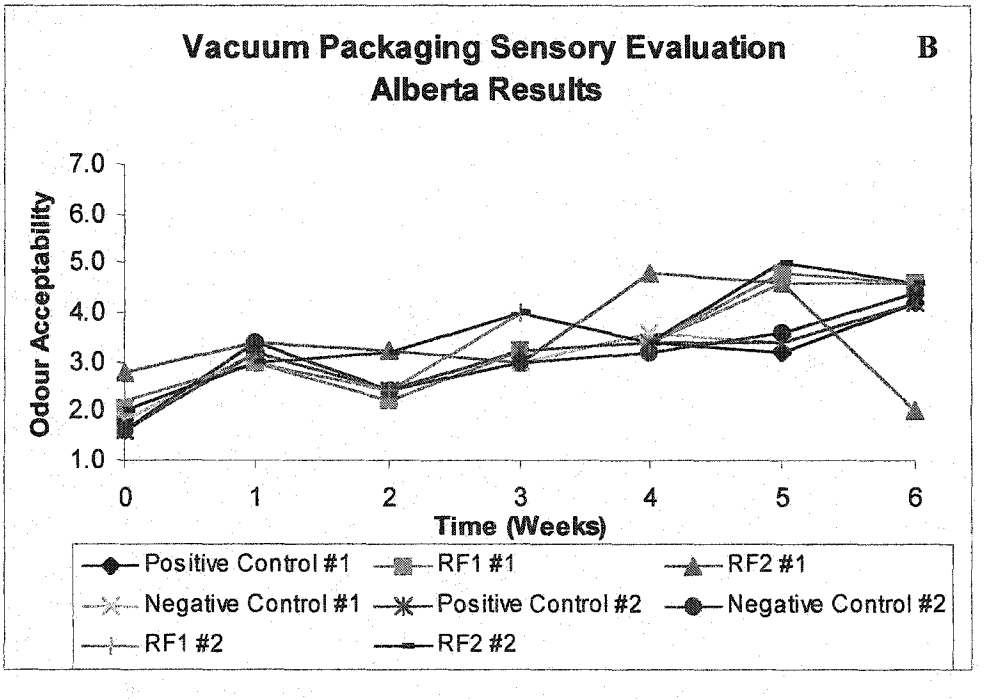


Figure 5.18: Sensory evaluation for vacuum packaged meat samples A) Retail appearance B) Odour acceptability

5.1.5 Surface Temperature

From all the samples treated, the average surface temperature recorded with the fiberoptic thermometer was ranging between 22°C and 26°C. However to achieve success, a heat based decontamination system needs to raise the surface temperature rapidly to a value (>70°) where pathogens are killed, and then rapidly reduce the temperature so that heat does not penetrate into the food and cause quality changes. Surfaces of RF treated samples were not brought to killing temperatures. Thus the quality attributes were not favourable as seen in the above sections.

5.2 Microwave Trials

Results obtained from the October trials showed us the inefficacity of the RF treatments applied with those particular times of exposure and power used. Microwave treatments were investigated to see if more bacterial kill was possible. The results of the best combinations obtained with those trials are presented in Figure 5.19. Only *Pseudomonas* D17 bacteria were used for those microwave trials and the samples were analysed just after being microwave treated. The results indicate that the samples reached higher surface temperatures (50-55°C) compared to RF treatments. However, the reduction obtained was not considerable (less than one log) and some discolouration or cooking signs were visible. No significant trend could be established and there was high standard deviations and limited repeatability between treatments and replicates.

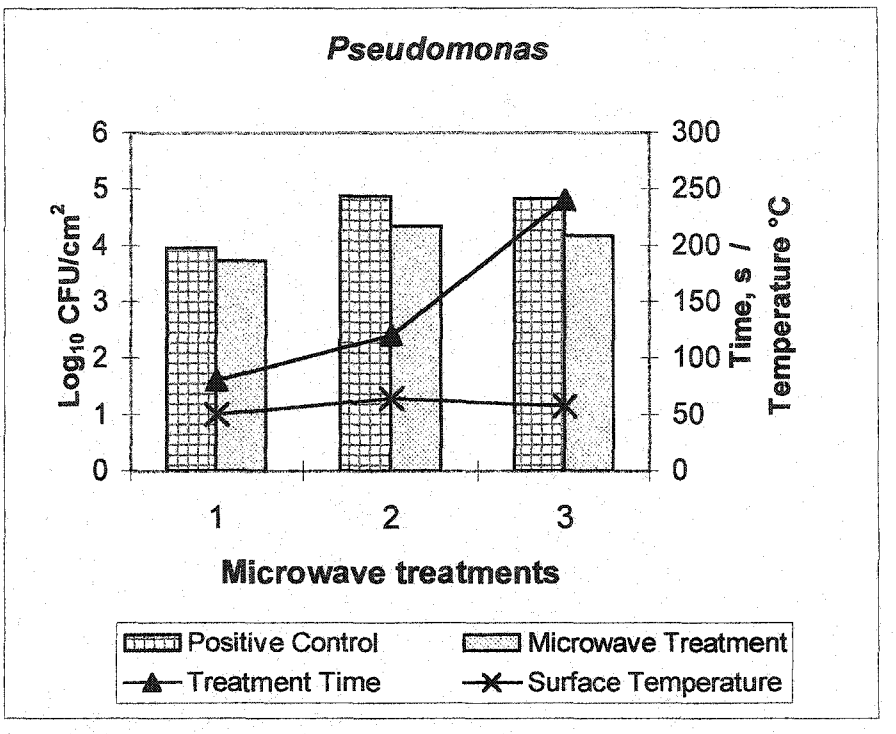


Figure 5.19: Average log numbers of *Pseudomonas* on meat samples after microwave treating with three best combinations of 30s pulsing at 25 Watts microwave incident power (0.7 W/g)

5.3 Experimental Design

In the period between the October trials and this experiment, more RF combinations were tested to find a better treatment. Also, antimicrobial solutions were introduced in the experiment since past research found that a combination of stressors on the bacterial cell (i.e. heat and an antimicrobial) could allow treatments which on their own would be sublethal to become lethal and possibly show synergistic effects. The RF treatment combinations chosen exposed the samples for a longer time period than in our preliminary trials. The present design consisted of exposing the samples for a total of 120 s and using 3 levels of power starting with the highest one (600 W). The results are graphically presented and are the average of the two replicates. Statistical analyses of the data were done and the comparative chart of the SAS outputs are presented in Appendix A-1 to A-3 for retail and in Appendix A-4 to A-5 for vacuum

packaged samples. Two sets of statistical analysis for each retail and vacuum packaged samples were performed. The first set of analysis included the starting status. The second set of statistical analysis did not include the starting status. The reason why such analysis was done is to note the difference between the two since the day and week 0 evaluations of the samples were done at McGill and later ones were done at the LRC.

5.3.1 Microbiological Analysis

The same kinds of media as of the preliminary trials were used to determine the population of bacteria on meat samples. However, new treatments were added and the RF treatments were modified. This experiment used two duplicates for each treatment and all the analyses were done at the LRC in Alberta except for the day 0 and week 0 which was performed at the McGill laboratory.

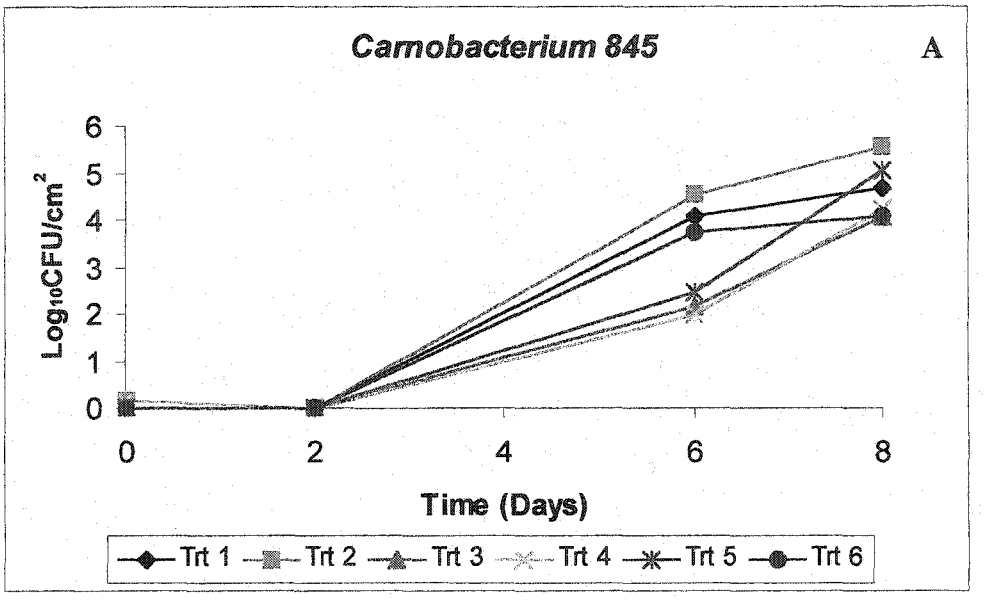
Figures 5.20 and 5.21 illustrate the bacterial growth of each microorganism under retail packaging. *Carnobacterium "845"* log bacterial numbers were lower at day 6 for treatments 3, 4 and 5 which are the treatments with the antimicrobial solution. The treatment of nisin-lysozyme alone (Trt 3) gives a lower log number than the combinations of nisin-lysozyme and RF treatments (Trt 4 and 5). However, the statistical analysis (Appendix A-1) shows that nisin-lysozyme alone and the combination of nisin-lysozyme and RF1 were not significantly different and obtained the lowest means in the treatment section. The SAS output revealed a treatment, day and treatment by day effect ($P<0.05$) for *Carnobacterium "845"* in retail packages. Day 0 and 2 are not significantly different in their means but differ from day 6 and 8. The tendency is to obtain an increase in the log number over time. On the graph of *E. coli* growth over time in retail packaging, there seems to be no difference between treatments just by looking at the graph. Although, the SAS output (Appendix A-1) indicated treatment and day effect but no combination of treatment by day effect ($P<0.05$), all treatments of nisin-lysozyme (Trt 3-4-5) and the RF2 treatment (Trt2) showed the lowest means. The tendency is to obtain a decrease in log numbers over time, which was also noted in the preliminary trials. This decrease is probably due to the increase in numbers of *Carnobacterium* causing competition for the nutrients and producing antimicrobial substances. Figure 5.21 illustrates the increased numbers of

Pseudomonas and shows no differences between the treatments (P<0.05) (Appendix A-1). Again, nisin-lysozyme treatments have lowered the bacterial numbers (lowest mean), but in this case, the small difference is only visible at Day 0. When analysed without the day 0, it is possible to observe that treatments 3-2-4 and 6 are not significantly different. Since in that category, treatment 6 (positive control) is showing the lowest mean, one can conclude that no treatments really affected the growth of bacteria. The population of microorganisms under vacuum packaging is shown in Figures 5.22 and 5.23. *Carnobacterium "845"* log bacterial numbers at week 1 for RF treatments (Trt 1 and 2) are higher than the positive control and all treatments of nisin-lysozyme or nisin-lysozyme /RF combinations (Trt 3, 4, and 5) illustrate lower counts of bacteria than the positive control. Starting at week 5, all treatments had reached the same level of bacteria on the meat surface. Statistically, there is a treatment, week and treatment by week effect (P<0.05) (Appendix A-4). *E. coli* population again had a tendency to decrease over time as in retail packaging. From week 4, all treatments revealed bacterial numbers higher than the positive control. RF treatments alone obtained the highest log numbers from week 0 to week 4. As observed in retail packaging, there is treatment and time effect but no treatment by week effect. Treatments of nisin-lysozyme, nisin-lysozyme /RF2 and the positive control (Trt 3-5 and 6) were not significantly different (Appendix A-4) from one another and showed the lowest means. All other treatments obtained higher count numbers than the positive control indicating that instead of killing the bacteria, the treatments encouraged them to grow. The graph representing the population of *Pseudomonas* D17 in vacuum packaging shows a small reduction at time 0 for antimicrobial treatments (Trt 3, 4, and 5). However, no differences between treatments are seen after time 0 (P<0.05) (Appendix A-4). At week 1, the bacterial log numbers went up to decrease slowly over time afterwards showing the week effect. The reduction of *Pseudomonas* D17 is probably due to the increase in *Carnobacterium* "845" population.

5.3.2 Measurement of pH

The variation of pH with treatment type over the storage period is represented for retail packed meat samples in Figure 5.24 and for vacuum packed meat samples at

Figure 5.25. In retail packaging, the pH level seems to increase over time with no apparent relationship due to treatment type. However, statistical analysis shows a treatment, day and treatment by day effect ($P<0.05$) (Appendix A-2). The graph of pH level for vacuum packaged meat samples shows more chaotic results. The pH increased from time 0 to week 2 to reach a peak at week 2 and then decreased at week 3 to stay fairly constant for the rest of the storage period. But again, as for retail packaged samples, the statistical analysis reflected a treatment, week and treatment by week effect (Appendix A-5). Variation in pH seems to have some relationship to treatment but the relationship is not clear.



Trt 1 = RF1 treatment: 600W – 30s, 400W – 30s, 200W – 60 s

Trt 2 = RF2 treatment: 600W – 30s, 400W – 30s, 100W – 60 s

Trt 3 = Antimicrobial mixture treatment

Trt 4 = Antimicrobial mixture treatment and RF1 treatment

Trt 5 = Antimicrobial mixture treatment and RF2 treatment

Trt 6 = Positive control

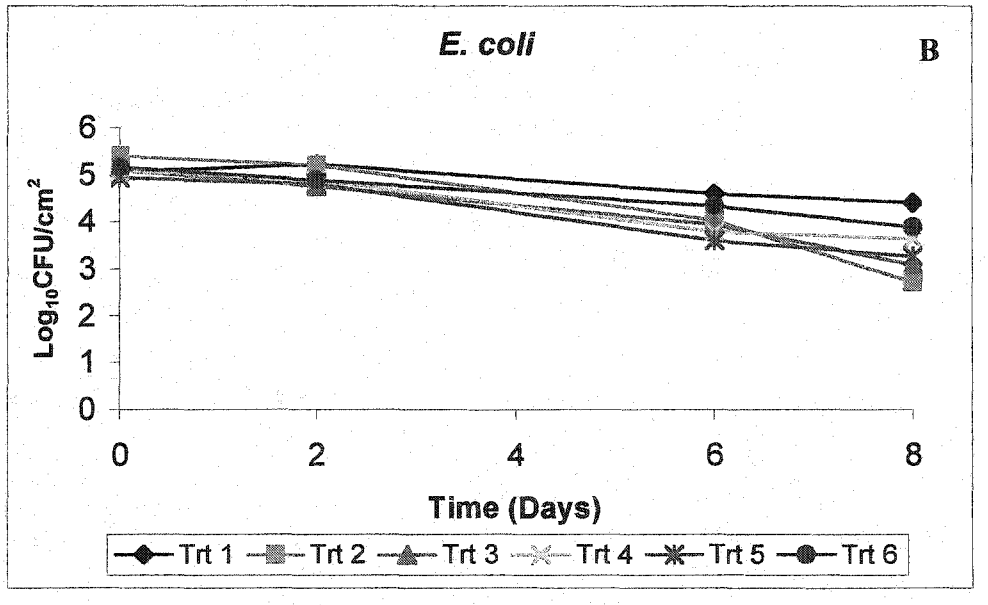


Figure 5.20: A) *Carnobacterium "845"* and B) *E. coli* population on retail packaged meat samples

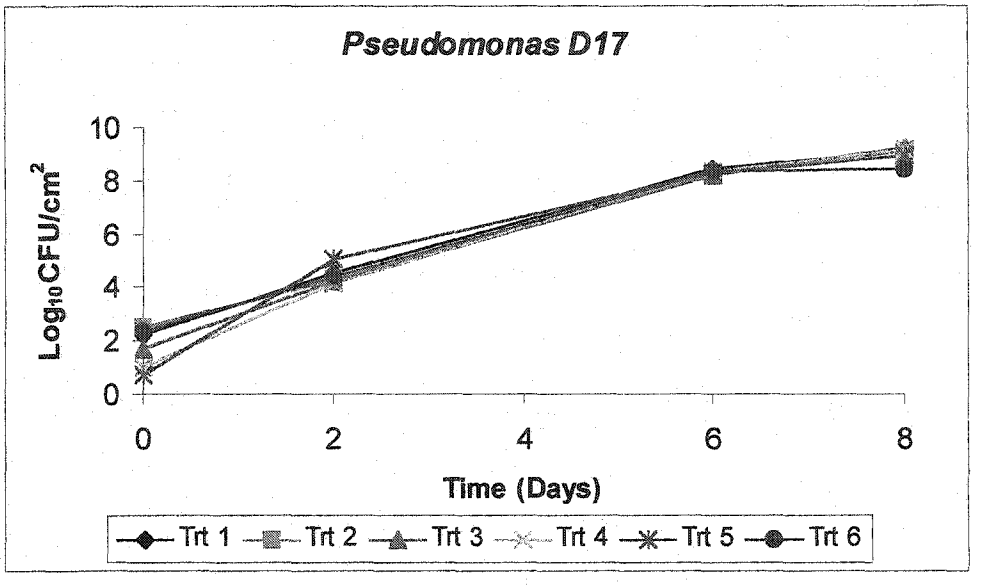


Figure 5.21: *Pseudomonas D17* population on retail packaged meat samples

Trt 1 = RF1 treatment: 600W – 30s, 400W – 30s, 200W – 60 s

Trt 2 = RF2 treatment: 600W – 30s, 400W – 30s, 100W – 60 s

Trt 3 = Antimicrobial mixture treatment

Trt 4 = Antimicrobial mixture treatment and RF1 treatment

Trt 5 = Antimicrobial mixture treatment and RF2 treatment

Trt 6 = Positive control

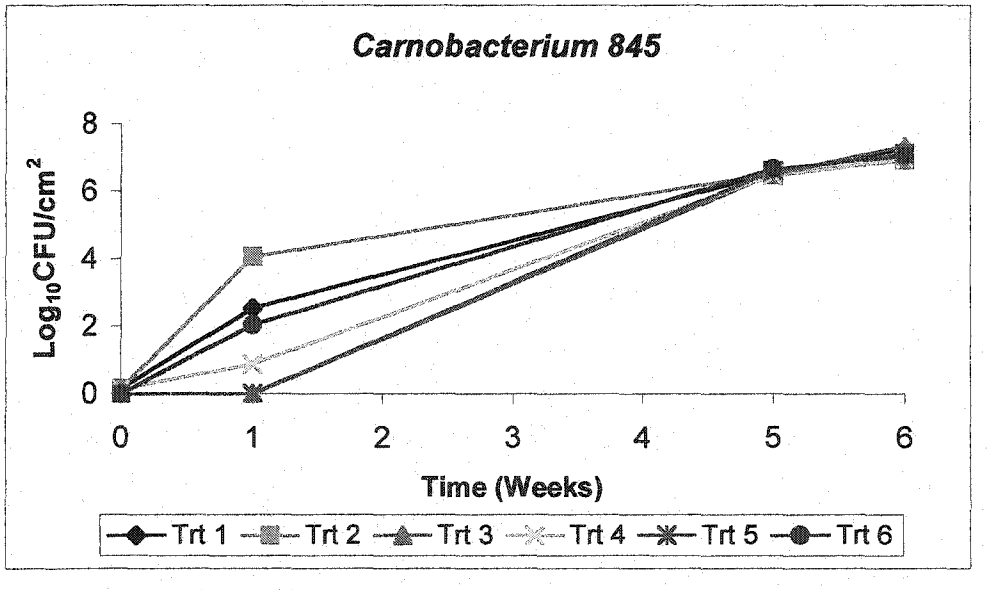
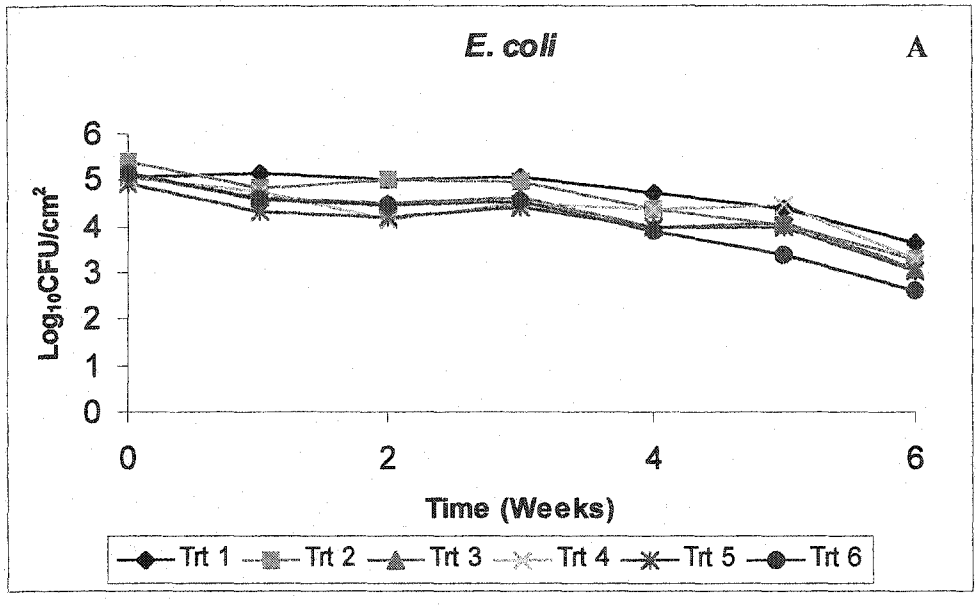


Figure 5.22: *Carnobacterium "845"* population on vacuum packaged meat samples



Trt 1 = RF1 treatment: 600W – 30s, 400W – 30s, 200W – 60 s

Trt 2 = RF2 treatment: 600W – 30s, 400W – 30s, 100W – 60 s

Trt 3 = Antimicrobial mixture treatment

Trt 4 = Antimicrobial mixture treatment and RF1 treatment

Trt 5 = Antimicrobial mixture treatment and RF2 treatment

Trt 6 = Positive control

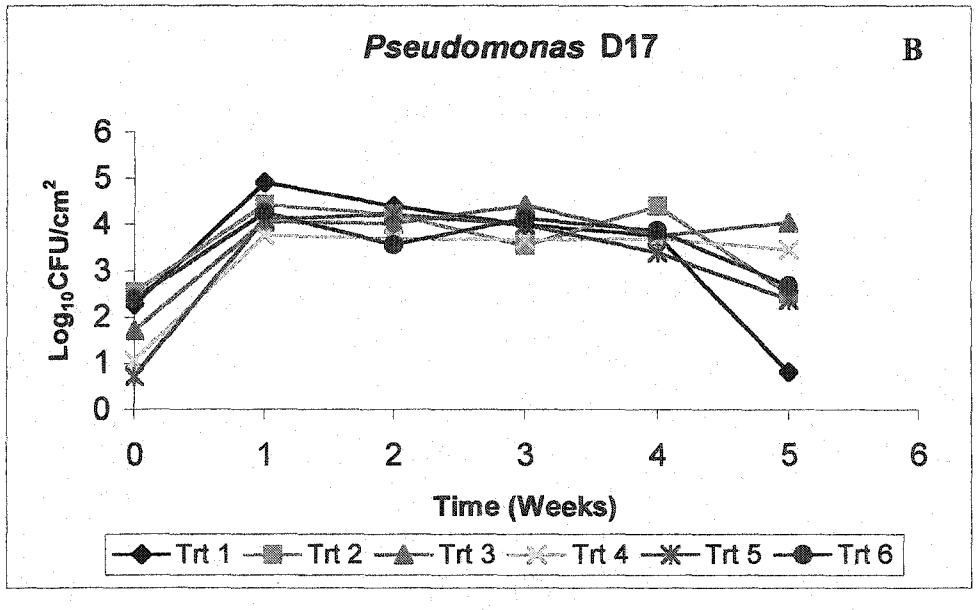


Figure 5.23: A) *E. coli* and B) *Pseudomonas D17* population on vacuum packaged meat samples

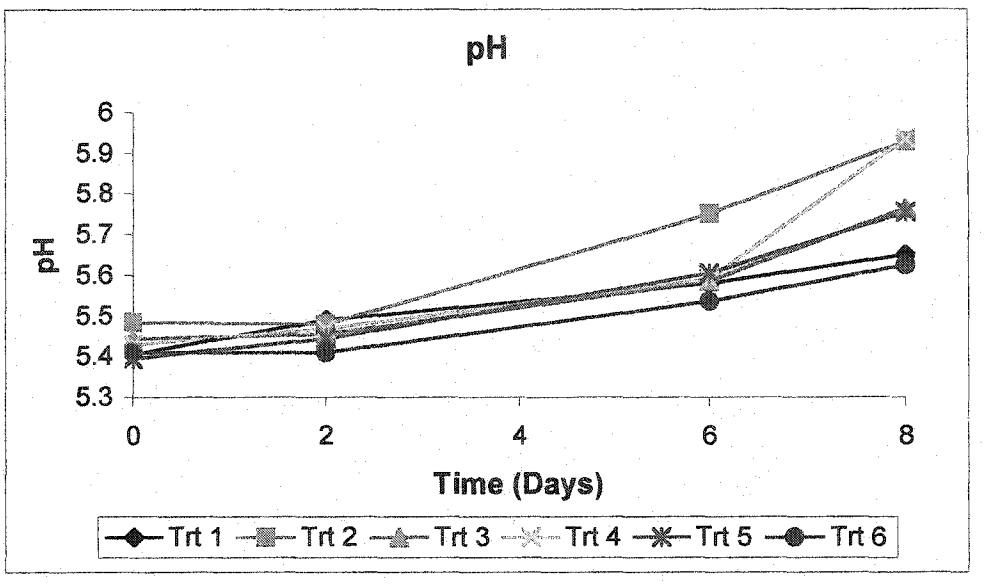


Figure 5.24: pH value of retail packed meat samples

Trt 1 = RF1 treatment: 600W – 30s, 400W – 30s, 200W – 60 s

Trt 2 = RF2 treatment: 600W – 30s, 400W – 30s, 100W – 60 s

Trt 3 = Antimicrobial mixture treatment

Trt 4 = Antimicrobial mixture treatment and RF1 treatment

Trt 5 = Antimicrobial mixture treatment and RF2 treatment

Trt 6 = Positive control

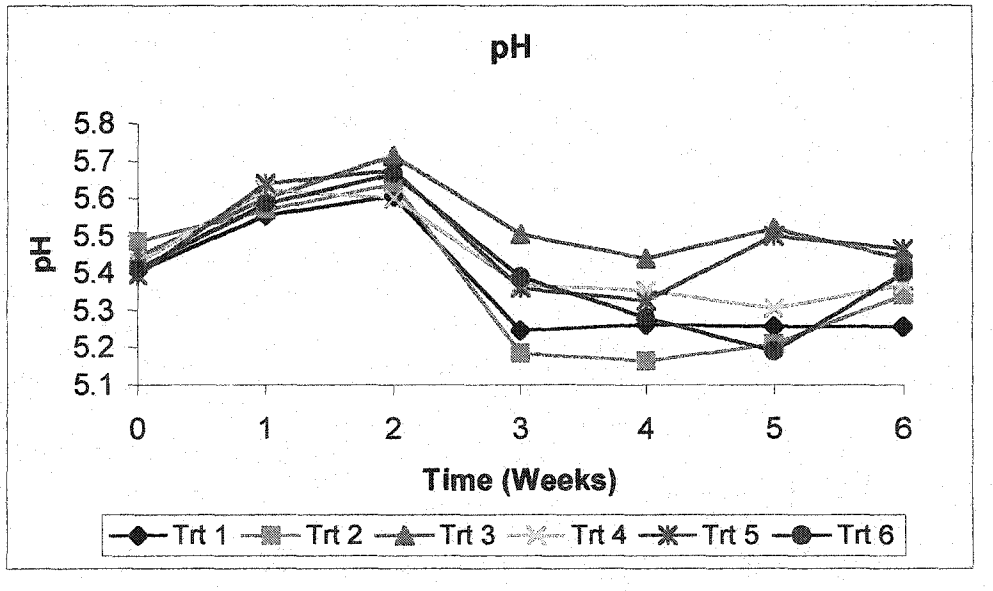


Figure 5.25: pH value of vacuum packed meat samples

5.3.3 Colour Measurement

Colour measurements of L*, a*, b* are presented graphically for all treatments and are the means of the two replicates. Statistical analyses are presented in Appendix A-2 and A-5 for retail and vacuum packaging respectively.

Retail packaged meat samples stored for 8 days at 7°C showed a peak in the L* value at day 6 (Figure 5.26-A). All the treatments seemed to react the same way by having a little decrease at day 2, a peak at day 6 and finally a decrease again at day 8. Day 6 is statistically different from days 0, 2 and 8 (Appendix A-2). All treatments reflected darker samples at the end of the storage period except for treatment 5, which became lighter and treatment 6, which stayed constant in colour. Darker or lighter meat colours are not really desirable if the difference is too extreme compared to control samples. Treatments 3, 2 and 4 are not significantly different from one another and possessed the lowest means indicating darker samples. SAS output indicated a treatment and a day effect on the samples colour ($P<0.05$) (Appendix A-2). But, there is no treatment by day effect on the meat ($P<0.05$). L* value for vacuum packaged meat samples kept at 2°C are shown in Figure 5.27-A. No differences between the treatment types are observed from the graphs except for treatment 6, which L* values are lower than all the other treatment indicating darker meat. However, there is a treatment and a time effect but no treatment by time effect ($P<0.05$) (Appendix A-5). Peaks in the values are observed at week 2 for treatments 1, 3 and 5 and at week 4 for treatments 2 and 6. Lighter samples at the end of the storage time for treatment 5 are obtained compared to week 0. Treatment 6 (positive control) gives relatively constant L* values for the storage period and generally all the treatments showed darker meat samples than the positive control samples. Treatment 4 (Nisin-lysozyme/RF1) seems to give darker samples as revealed in the SAS output (Appendix A-5). In general, for both retail and vacuum packaging methods, treatment 4 shows darker meat samples which is not desirable for customers.

The trend of retail a* value over the incubation period is to reach a peak at day 2 and then decrease at day 6 for all treatment types and the data are presented in Figure 5.26-B. Treatment 2 (RF2) and 4 (Nisin-lysozyme/RF1), however, did not peak at day 2 but decreased from day 0 to day 6. Treatment 2 did not fluctuate throughout the

storage period and the samples of this treatment got the highest a* value at the end of the experiment compared to other treatments. The a* value of treatment 4 samples increased a lot from day 6 to day 8. There is no real treatment effect found in the statistical analysis, but a time and treatment by time effect (Appendix A-2). The general tendency is to obtain lower a* value at the end of the storage period for retail packaged meat samples indicating a loss in the redness of the meat. Redness value (a*) of vacuum packaged meat samples (Figure 5.27-B) compared to retail packaged a* value are higher throughout the storage period. By the end of the storage period of the samples stored at 7°C, all a* values were below 10. For the samples stored at 2°C, only treatments 5 and 7 fell below 10. The lowest a* value observed for vacuum packaging was 7.35 compared to 6.03 for retail packaged samples. This comparison indicates that the redness value (a*) was more affected by warm storage temperature or by the retail type of packaging. Treatments RF1 and RF2 showed higher a* value than the positive control samples and Nisin-lysozyme alone treatment. Also, treatments 4, 5 and 7 gave lower values compared to positive control samples. By analysis of the statistical output (Appendix A-5), it was possible to observe a treatment, a week and a treatment by week effect when analysed with week 0. However, by removing the week 0 data, the day effect was gone. Either, the a* value is highly affected from week 0 to the other weeks or there is a difference caused by the different intruments used or by the possible difference in the light reflection of both laboratories.

Yellowness (b*) values of retail packaged meat samples are presented in Figure 5.26-C. The general trend was to obtain lower b* value for all treatments over the storage period which represented a significant time effect ($P<0.05$) (Appendix A-3). The SAS output shows also a significant treatment and treatment by day effect ($P<0.05$). The combination of nisin-lysozyme and RF2 treatments resulted in samples with higher b* value at the end of the incubation period than any other treatment at the same stage. Also, treatments 2 and 3 (RF2 and nisin-lysozyme alone respectively) obtained the lowest b* value at day 8 and were statistically different in their means compared to other treatments. A small peak was observed at day 2 for treatments 1, 3 and 6. Big fluctuations were obtained for the b* values of vacuum packaged meat samples as shown in Figure 5.27-C. Again, there was a significant (Appendix A-5)

treatment, week and treatment by week effect ($P<0.05$) as in retail packaging. In general, the trend was a decrease in the b* value from week 0, reaching the lowest peak at week 2 and an increase at week 3 to stay mainly constant up to week 6. The lowest value for all treatments was reached at week 2 (lowest mean). Treatments 4, 5 and 7 did not plunge as low as the other treatments at week 2. Treatment 1 and 3 showed, however, the highest mean suggesting yellowness of the meat samples compared to the others treatments. Except for the dip in the b* value of the vacuum packaged meat samples at week 2, the trends between retail and vacuum packaged samples were similar by showing a steady decrease in the b* value over the storage period. Lower b* value indicates less yellow colour reaching even the grey tint by getting closer to the 1.0 for b* value. Such colour is undesirable for customers. Treatments that were able to keep as much as possible a balance in the L*, a*, b* colour of the meat like at day 0 and week 0 throughout the storage period, would result in a high level of satisfaction for the customers.

Statistical analyses were done with time 0 and without time 0, to see if the different laboratory used could influence the results. The results of this action is compared in Appendix A where it is possible to conclude that in general there was no difference between the two except for the LSD results for treatment effect. Also, often in the graphs some peaks were observed at either day 2 or week 1 which were probably caused by the change in location for sample analyses. Not only the manipulators were different from one laboratory to the other but also the laboratory and the equipment itself. Different light could affect the L*a*b* recorded values. The incubators were keeping the temperature constant in the LRC, while in McGill laboratory the temperature was more variable. The environment was not properly ventilated at the McGill laboratory while the LRC was highly equipped for microbiology manipulations. The meat transportation by plane from one place to the other may have affected the products. Manipulation errors could also be involved.

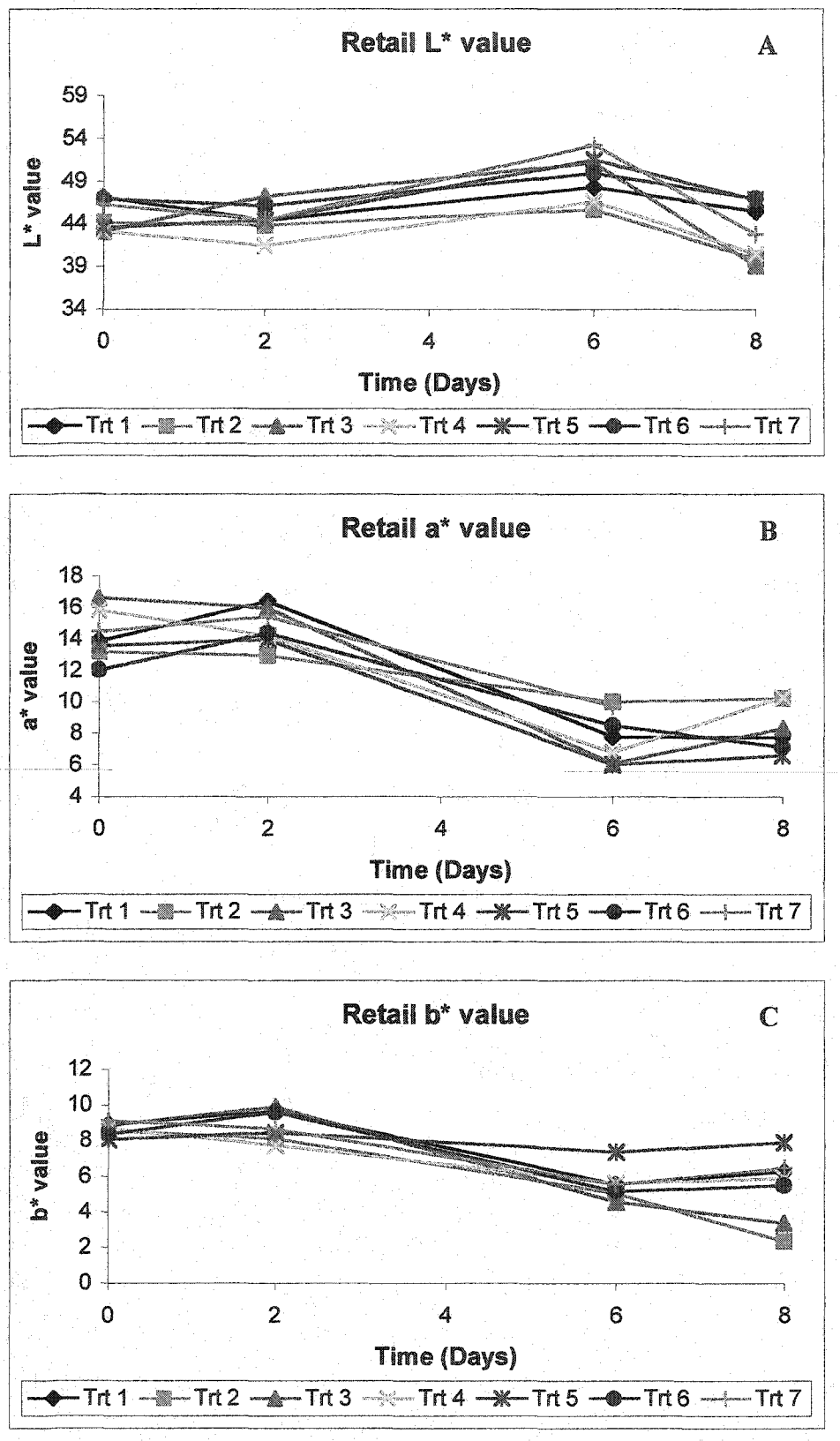


Figure 5.26: Colour measurement for retail packed meat samples over the storage period

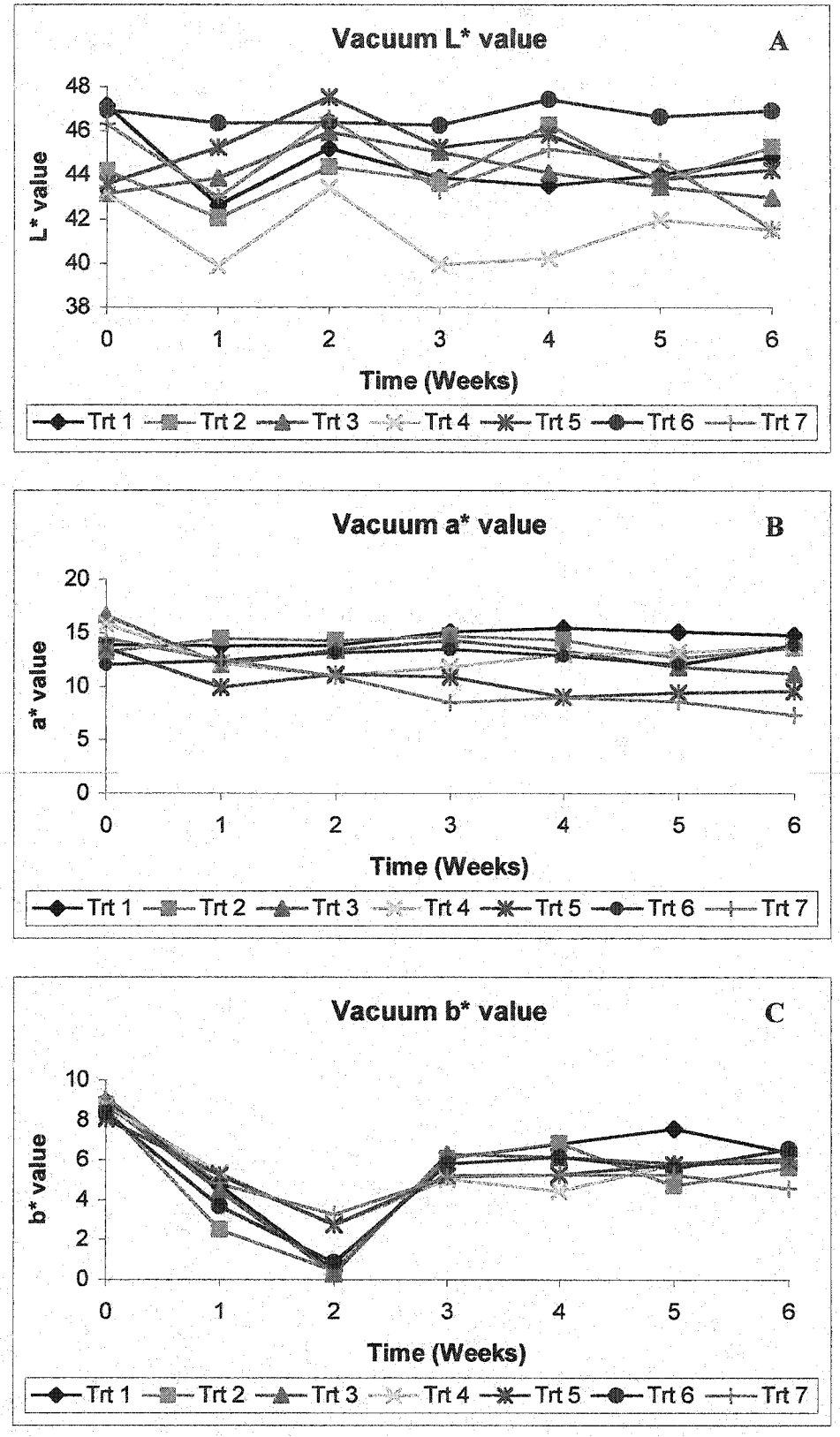


Figure 5.27: Colour measurement for vacuum packed meat samples over the storage period

5.3.4 Sensory Evaluation

Before any analysis, each sample was studied by a five-member panel to evaluate colour, discolouration, appearance, off-odour intensity and odour acceptability. The results are presented graphically in Figures 5.28 to 5.31 either for retail or vacuum packaging method for all treatment types over the storage period. The data presented are the means of the duplicate samples as well as the average of the sensory evaluation done by the five member panel. It has to be noted that the sensory evaluation at day 0 and week 0 was done at McGill and the subsequent evaluations were done at the LRC, which means that the panel members were different for the day-week 0 than the other days or weeks evaluated. The evaluation at the starting day may be different from one set of panelists to the other. Comparison of each incubation time to day-week 0 must thus be avoided since they were performed by two different groups. Statistical analyses were done with day-week 0 and without day-week 0 as presented in a comparative chart in Appendix A for better comparison.

For the retail packaged samples evaluated for colour, there are three treatments (Trt 1, 5 and 6), which reached a low peak at day 6 (Figure 5.28-A). At day 8, all the samples of all treatments were classified equally or with one point increase of the initial value they obtained at day 0. The only exception is the treatment 7 (negative control), which at day 8 reached the lowest point of the group indicating appearance of discolouration. Treatment 4 (Nisin-lysozyme/RF1) was evaluated as the darkest sample of all for the duration of storage. However, the samples at day 0 for this treatment had the highest colour evaluation indicating its dark colour. The SAS output indicated no significant treatment effect on the colour ($P<0.05$) (Appendix A-3). However, there is a day and treatment by day effect suggesting that the colour was affected by some treatment and by the storage period. Treatments 4, 2 and 3 were not significantly different from each other and were evaluated as dark samples compared to others. Day 6 was significantly different from the other days and gave lighter sample results. The same tendency was obtained for vacuum packaged samples (Figure 5.30-A). The treatment 4 got the highest colour evaluation compared to the other treatments for the entire incubation period. Also, treatment 7 obtained the lowest evaluation near the end of the storage period (week 5 and 6) as seen for retail packaged samples. All other

treatments demonstrated a constant colour evaluation among treatments and the samples were evaluated as darker than the treatment 6 (positive control). Treatments 6 and 7 were the lightest ones. There was significant difference among treatment, week and treatment by week ($P<0.05$) (Appendix A-6) for the vacuum packaged samples. Colour evaluation revealed a significant difference between week 0 and all the other weeks. Samples evaluated at week 6 were more discoloured and less desirable than the previous one.

Surface discolouration for retail and vacuum packaged samples are presented in Figure 5.28-B and Figure 5.30-B respectively. With the exception of the negative control treatment (Trt 7), all the treatments behaved the same way by showing a big difference in discolouration from day 2 to day 6 and presented a significant day effect ($P<0.05$) (Appendix A-3). Also, the SAS output showed a treatment effect and a treatment by day effect ($P<0.05$). Treatments 2 and 7 were not significantly different between each other and showed the least discolouration (lowest means) at day 6. The negative control samples were not showing a noticeable discolouration up to day 6 and showed even higher discolouration at day 8 than the other treatments. However, the statistical evaluation (Appendix A-3) confirmed that day 6 and 8 were not significantly different from each other instead, day 0 and 2 were different from each other. The trend for retail packaged samples showed higher surface discolouration over the storage period which is undesirable for customers. Evaluation of surface discolouration of vacuum packaged samples showed more inconsistent results than the samples in retail packaged. They also present treatment, week and treatment by week effect as in retail packages. However, the tendency was to obtain higher discolouration for treatments 4, 5 and 7 starting at week 2 while the others were still not showing significant discolouration. Starting from week 3, the negative control samples were more discoloured than all other treatments. Treatments 1, 2 and 6 were showing less discolouration over time than others. In general, all treatments (except treatment 5 and 4) were evaluated to be significantly different from each other (Appendix A-6). Weeks 6 and 5 show the highest surface discolouration. The surface discolouration of vacuum packaged samples tended to be less pronounced at the end of the storage period compared to retail packaging samples. Treatments 1 and 2 seem to show lower

discolouration for both retail and vacuum packaging samples. Further, it should be noted that the sample size was small (i.e. the core size) and that the subjective evaluation of colour attributes may have been difficult for the panelists. The treatments and storage effects may have resulted in inconsistencies within a core which would be very difficult to rate accurately.

The overall retail appearance was evaluated and is presented in Figures 5.28-C and 5.30-C for retail and vacuum packaged meat samples. The general trend is a decrease in the quality of the retail appearance over time. The majority of the treatments were evaluated as extremely undesirable at day 6 and only at day 8 for the negative control samples. At day 2, treatment 3 and 6 were rated more undesirable than all other treatments. There is a significant treatment, week and treatment by week effect ($P<0.05$) (Appendix A-3). In general, treatment 7 was evaluated as highly desirable compared to the other treatment samples. For the vacuum packaged samples, the behaviour was a little different. In general, treatments 1, 2 and 6 were giving better retail appearance over the storage period compared to the remaining treatments. Treatments 4, 5 and 7 were considered less desirable and significantly not different among one another (Appendix A-6). A peak is observed at week 2 for treatments 1, 2, 3 and 6. There is a significant treatment, week and treatment by week effect as in retail packaged samples ($P<0.05$). Retail appearance of vacuum packaged meat samples is observed to be more desirable than for the retail packaging samples.

Off-odour intensity evaluated in the sensory evaluation for retail packaged meat samples is illustrated in Figure 5.29-A. Statistical analysis shows treatment and day effect when evaluated with day 0 (Appendix A-3). However, without day 0, only day effect is shown to be significant ($P<0.05$). In general, treatments 6 and 5 were evaluated to produce the most off-odours while treatments 7 and 1 were rated to have less off odour. An average level of smell was evaluated to be given out by all other treatments but not much difference was noticeable. Day 8 was significantly different than the other days with the most undesirable off odour. However, as shown in Figure 5.31-A for vacuum packaged samples, the off-odour intensity developed with time, starting with more prevalent off-odour at week 3. No significant differences were noticeable among the treatments for off-odour intensity ($P<0.05$) (Appendix A-6).

However, there was a time effect and treatment by time effect when evaluated with week 0 and only time effect when evaluated without week 0. The time effect was predominant by the end of the storage period. Off odour intensity was becoming more prevalent for both retail and vacuum packaged samples with the storage time. Treatment effect was present in retail packaging but significantly different in vacuum packaging.

The general odour acceptability for retail stored samples is graphically shown in Figure 5.29-B. The data obtained is demonstrating a significant increase in the unacceptability of the product with time, as expected ($P<0.05$) (Appendix A-3). At day 2, more differences among the treatments odour acceptability were observed, having treatments 4 and 5 (Nisin-lysozyme/RF1; Nisin-lysozyme/RF2) being less acceptable than the other treatments including the positive control treatment (Trt 6). However, at day 6 and 8, all treatments were evaluated by the panelists and rated to be around the same acceptability level. Statistical analysis proved no significant treatment or treatment by day effect ($P<0.05$) (Appendix A-3). At day 8, the samples were still evaluated to be "Neither acceptable nor unacceptable" for most treatments. Figure 5.31-B illustrates the odour acceptability of vacuum packaged samples. As the storage period increased, the odour acceptability decreased; except for week 2 when most samples were evaluated to be more acceptable than week 1 or 3. In general, all treatments were evaluated as unacceptable by week 3. As for retail packaging, only time effect was significant ($P<0.05$) (Appendix A-3).

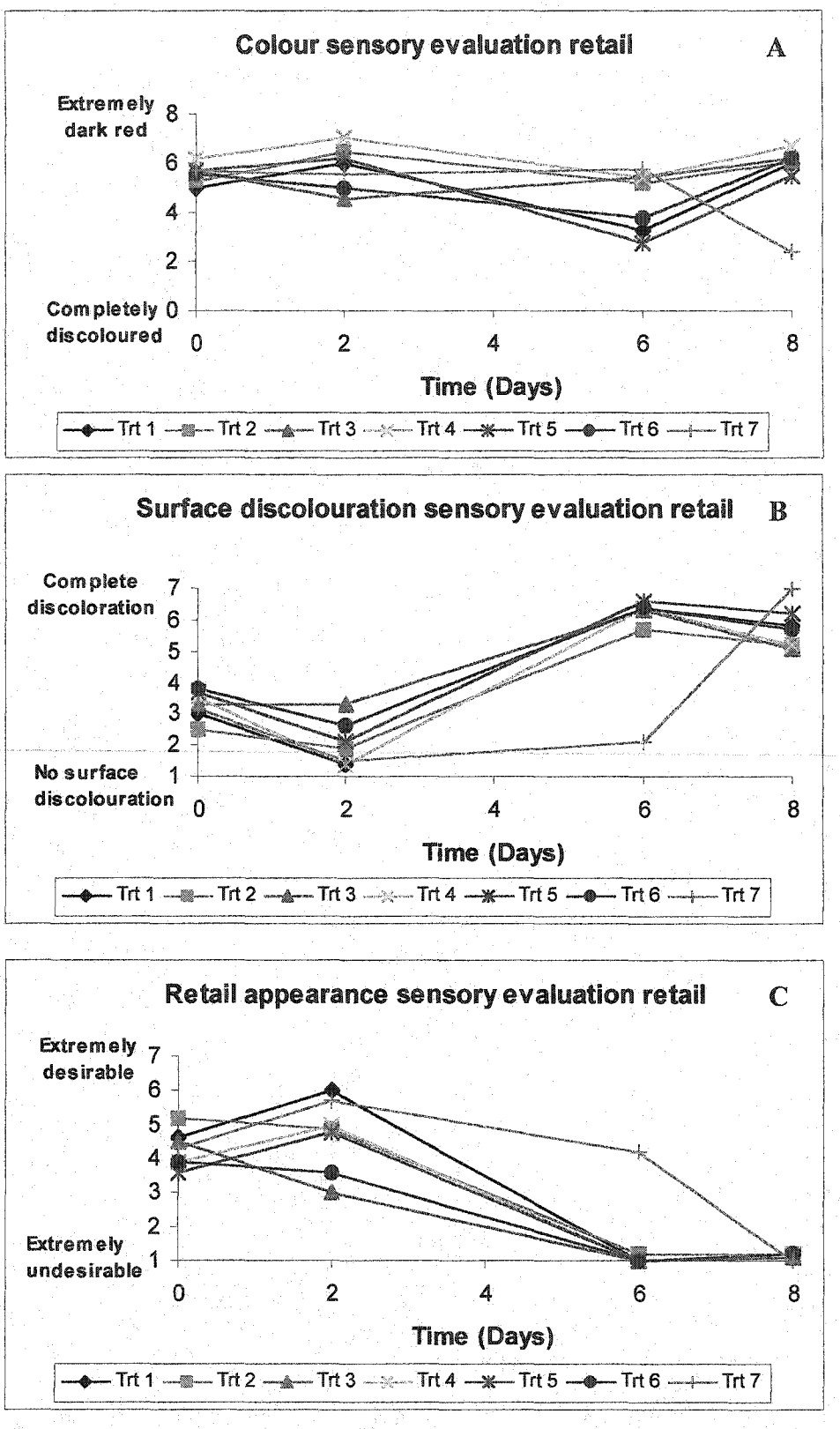


Figure 5.28: A) Colour, B) surface discolouration and C) retail appearance sensory evaluation for retail packed meat samples over the incubation period

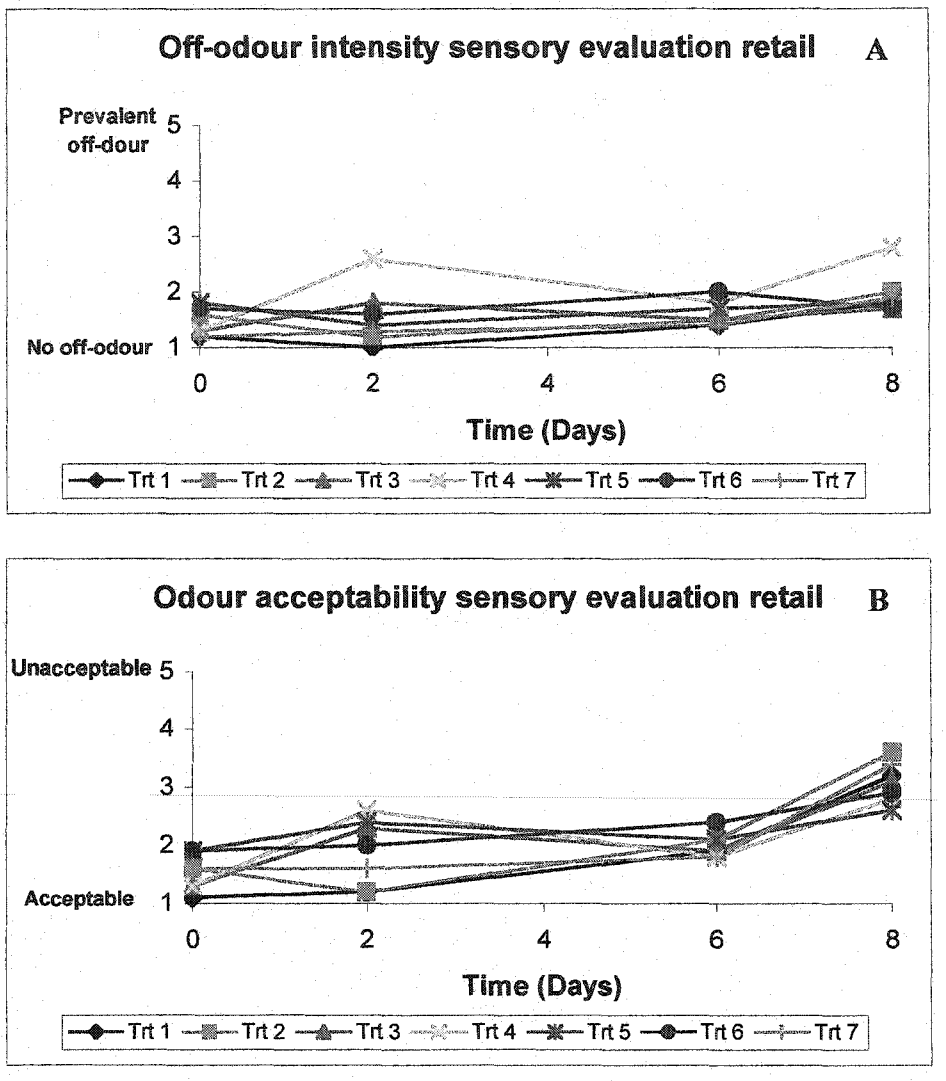


Figure 5.29: A) Off-odour intensity and B) odour acceptability sensory evaluation for retail packed meat samples over the incubation period

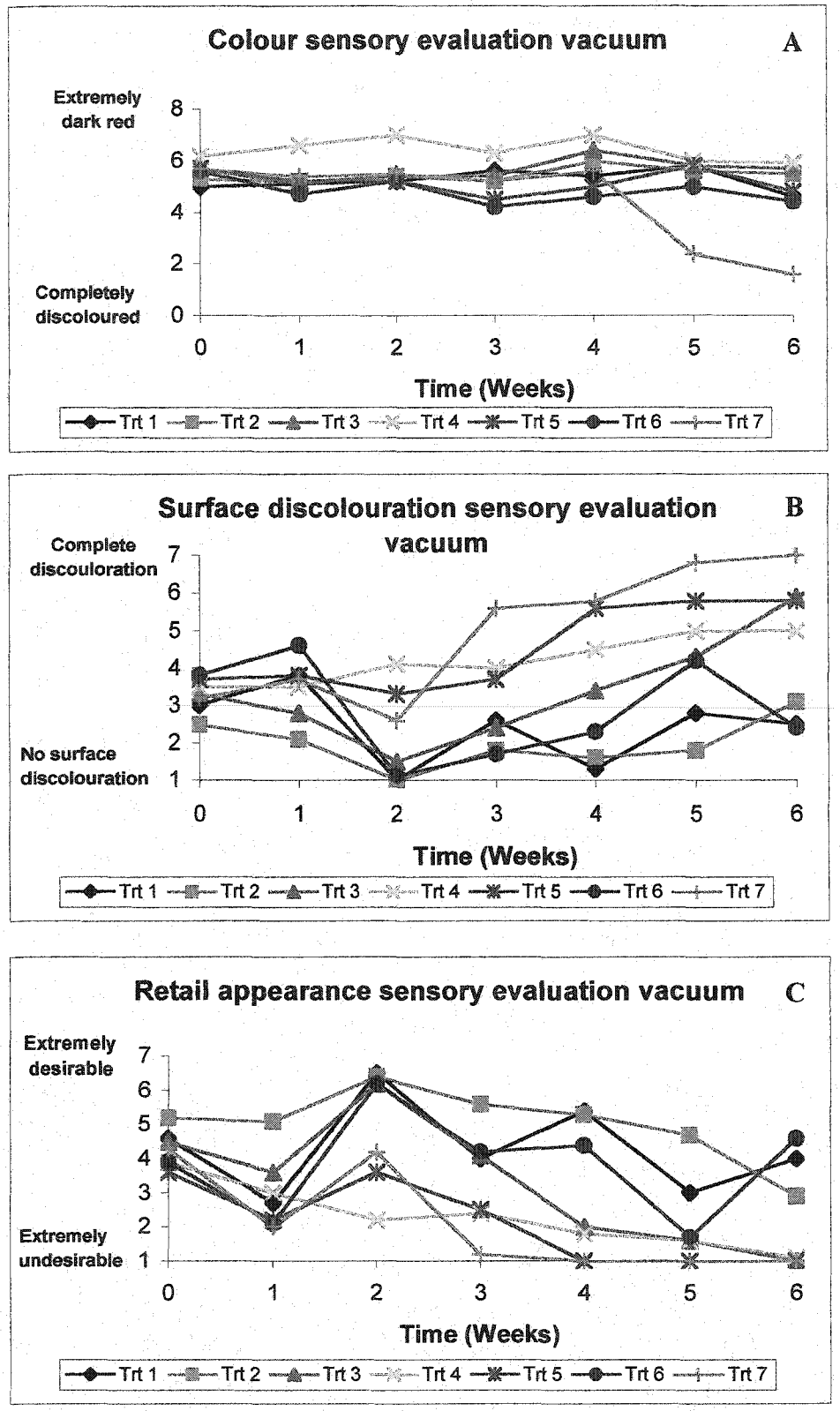


Figure 5.30: A) Colour, B) surface discolouration and C) retail apperance sensory evaluation for vacuum packed meat samples over the incubation period

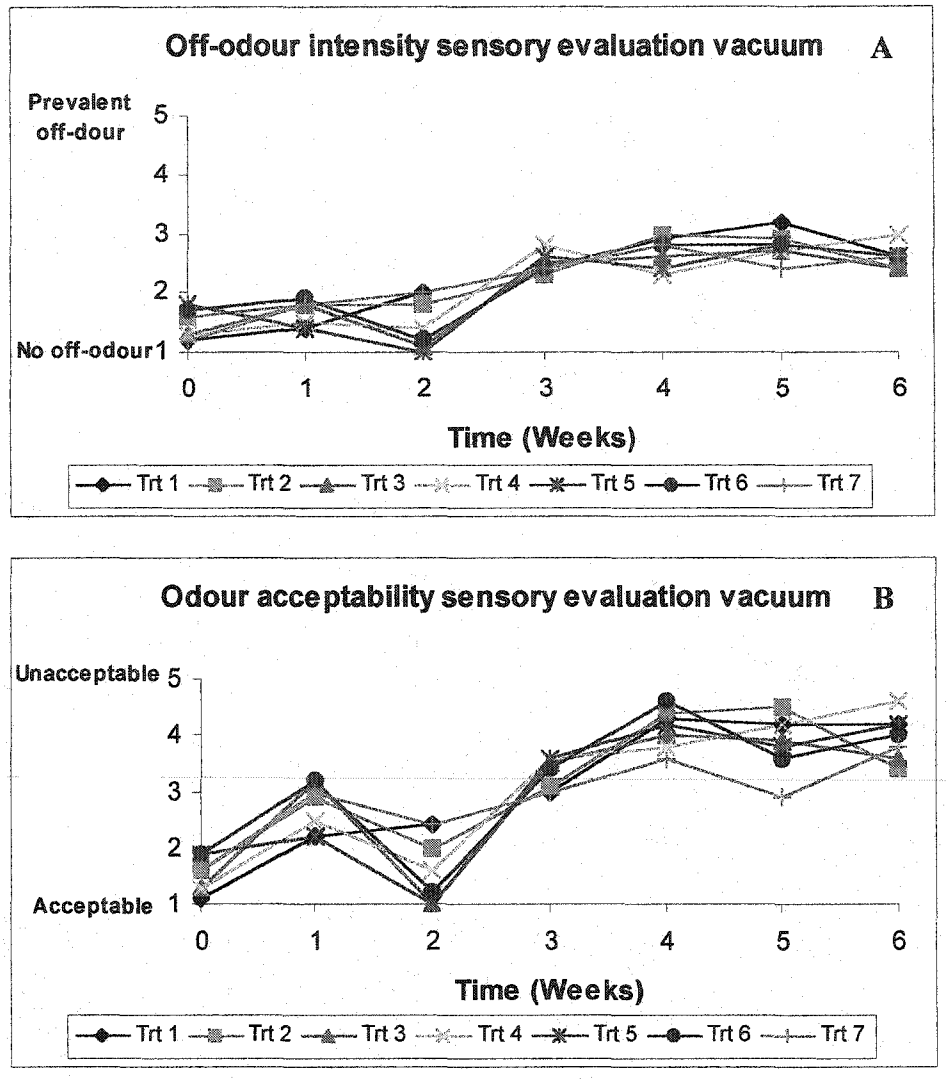


Figure 5.31: A) Off-odour intensity and B) odour acceptability sensory evaluation for retail packed meat samples over the incubation period

5.3.5 Surface Temperature

Although up to 600 W was generated by the RF-power source, for meat samples of 35-40 g, only approximately 1 to 2 W/g of material was actually absorbed by the material. Indeed, from equation 4, we can calculate the power absorbed by the material. Since there was an airgap of 0.5 cm at the top of the meat sample below the top electrode, there was considerable loss of energy through the system. This airgap was necessary to avoid arcing between the electrodes when the meat sample was in contact with the grounded electrode. This loss of energy dissipated in the matching box where the water cooling system was registering increases in temperature of up to 8°C.

The first few sample trials indicated that there was a high incidence of arcing in cases where there were fat pockets on the edges of the meat cores. Furthermore, there was high chance of scorching or browning of the edges of the meat samples. This can be explained with the schematic presented in Figure 5.32 representing the concentration of the electric field at the edges.

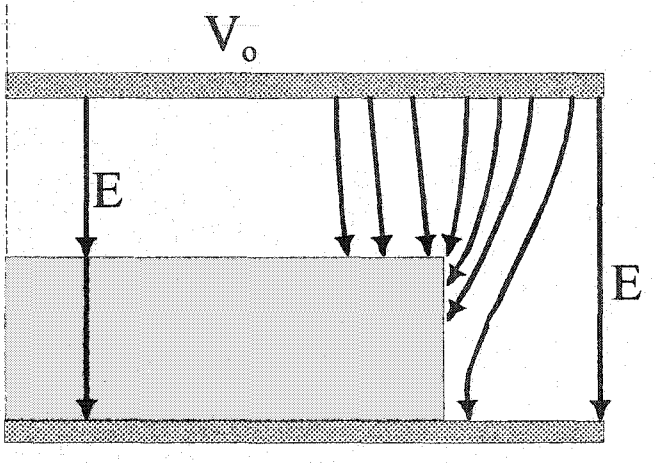


Figure 5.32: Sketch of the electric field concentrations at the corner of a cylindrical shaped material between parallel plates (Roussy and Pearce, 1995).

Experimental evidence has demonstrated that the electric field concentrates on the corner of the cylindrical material in a parallel plate configuration. This is because, at the corner there are conflicting boundary conditions that are satisfied by field concentration (Roussy and Pearce, 1995).

The temperature achievable at the surface of the meat samples, within a two minute RF treatment without protein denaturation, was around 30°C. Figure 5.33 presents the RF incident power for the RF1 treatment and the surface temperature attained on the meat core. As can be seen in Figure 5.33, the temperature was brought from refrigerated storage of 4-5°C up to 23°C in 2 minutes. For any longer treatment, protein denaturation was observed at the edges of the cores and cooking was occurring in the center of the meat samples.

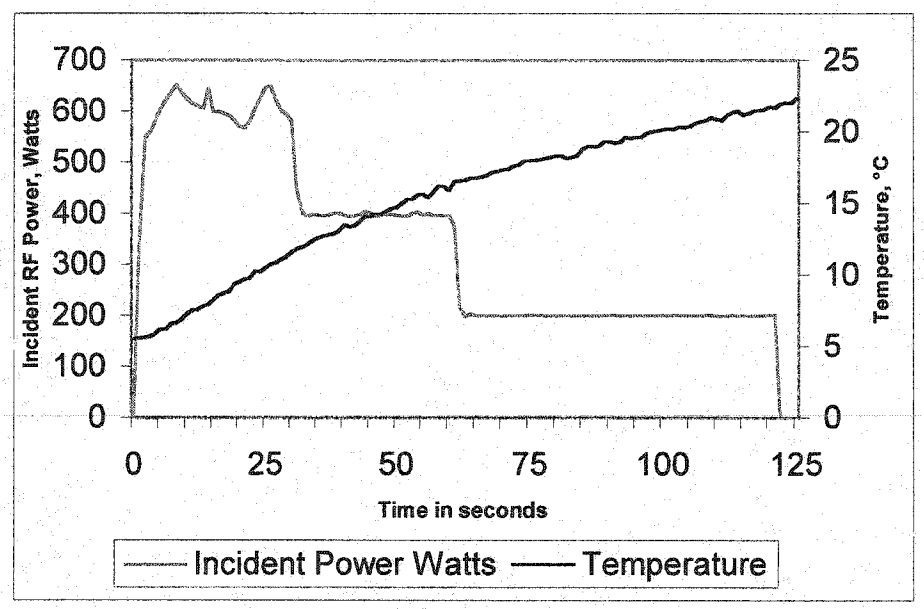


Figure 5.33: Evolution of the incident RF power in treatment RF1 and the temperature increase at the surface of the meat core

Evidently from Figure 5.33, we can see that the temperatures attained at the surface of the meat cores were inadequate to experience a reduction in the number of microorganisms. Therefore modified treatment would be needed to satisfy the reduction in the number of microorganisms.

VI. CONCLUSION

The present study focussed its energy in evaluating a recent pasteurization technology which in the past research showed inconsistent results. It is well known that consumers demand healthy and safe food products. In order to keep the product as fresh as possible, researchers have to develop techniques to alleviate the presence or the growth of microorganisms considered as the most important factor in deterioration of food products.

First of all, preliminary trials were completed in order to determine the right combination of time and power to apply on raw beef product while preventing protein denaturation. Maximum power (600W) was used for a period of 45 s (RF1) and 60 s (RF2). Experiments were performed using *Escherichia coli* biotype 1, *Pseudomonas* D17 and *Carnobacterium* "845". The cores were inoculated to introduce approximately log 3.5 cfu/cm^2 bacteria on the cores. Microbiological analysis for all three bacteria, for both retail and vacuum packaging revealed no reduction in the log numbers due to RF treatments compared to positive control samples. The level of *Carnobacterium "845"* and *Pseudomonas* D17 was even higher when subjected to RF heating. *Escherichia coli* log numbers showed a reduction for all treatments over time for both type of packaging and that was probably due to competition among bacteria types. The pH level was higher than normal over time in retail packaging and fairly constant for vacuum packaging. Colour measurement for both retail and vacuum packaged meat samples indicate a decrease over the storage time. Retail appearance was considered undesirable at day 5 for retail packaging and at week 1 for vacuum packaged samples. Odour acceptability for RF treatments was unacceptable at day 2 in retail and week 3 for vacuum packaged meat samples. Surface temperature on meat samples obtained while using RF technology was between 22°C and 26°C which was far from the >70° C required killing temperatures.

In general, the preliminary trials showed us the inefficacy of the treatment applied. Microwave technology was investigated in an attempt to obtain more positive

results. The temperature reached on the surface was higher compared to RF treatments but still no significant results were obtained with this technology.

The experimental design went on to include an antimicrobial solution. *Carnobacterium "845"* showed a treatment, time and treatment by time effect. *E. coli* seemed to decrease in number over time as observed in preliminary trials. This decrease was probably due to competition among bacteria types. *Pseudomonas* D17 did not reflect any treatment effect but was subjected to time and the combination of treatment by time effect. They were all significantly different at a level of $P<0.05$ and appeared on both packaging methods. In general, however, treatment with a combination of nisin and lysozyme showed a slight reduction of bacterial numbers but the reductions were too small to be considered practical. Measurement of pH revealed that there was an increase in pH level over time compared to the pH of control samples for retail packaged samples and fairly constant pH for vacuum packaged samples. For both packaging methods, the L* value indicated darker (lower L* value) samples for most treatments compared to the positive control, a* value was higher (darker) and the b* value stayed fairly constant. The sensory evaluation showed a negative effect of time on the samples. Samples became darker, more discoloured and emanating an off odour. No treatments resulted in a significant improvement compared to the untreated controls.

All analyses indicated a significant ($P<0.05$) (Appendix A) time effect which was expected. Treatment effects were not consistent nor predictable. However, most of the time the treatments reflected worst results than the control treatments ones showing the inefficacy of studied treatments. The only conclusion possible is to confirm that no treatments were really effective in preserving for a longer time period raw meat without altering its quality.

Further research investigating pasteurization of raw beef with RF technology should consider using higher power. Higher power may increase the surface temperature to a killing level while keeping the meat quality.

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APPENDIX A

SAS analysis comparative charts

APPENDIX A-1: Microbiological statistical analysis for Retail packaging samples

		Carnobacterium "845"		*E.coli*		*Pseudomonas*	
		With day 0	W/O day 0	With day 0	W/O day 0	With day 0	W/O day 0
	Trt effect	X	X	X	X	NS	NS
	Time effect	X	X	X	X	X	X
	Trt * Time effect	X	X	NS	NS	X	X
LSD test: Means with the same letter are not significantly different	A- Trt effect	2	2	1-6	1-6	1-2-6-3	5-1-3-2-4
	B	1	1	6-4-2-3	6-4-2-3	2-6-3-5	3-2-4-6
	C	6-5	6-5	4-2-3-5	4-2-3-5	6-3-5-4	
	D	4-3	3-4				
	E						
	A- Time effect	8	8	0-2	2	8	8
	B	6	6	6	6	6	6
	C	0-2	2	8	8	2	2
	D					0	
	E						

Legend:

X	Significant at a level of P< 0.05
NS	Not significant
Letters	T group
Numbers	Treatment number (treatment effect)
	Day (time effect)

Comprehensive example: *E.coli*; with day 0

There is a significant (P<0.05) treatment and time effect.
The treatment by time effect is not significant
The LSD test reveals no significant differences between treatment number 1 and 6 (LSD T group A)
However, there is a significant differences between LSD T group A (Treatments 1-6)
and LSD T Group B (Treatments 6-4-2-3) and LSD T Group C (Treatments 4-2-3-5)
The LSD test for time effect reveals no significant differences between day 0 and 2 (LSD T group A)
and a significant time effect between LSD T group A (Day 0-2) , group B (Day 6) and group C (Day 8)

APPENDIX A-2: Colour measurements and pH statistical analysis for Retail packaging samples

		L*		a*		b*		pH	
		With day 0	W/O day 0	With day 0	W/O day 0	With day 0	W/O day 0	With day 0	W/O day 0
	Trt effect	X	X	NS	NS	X	X	X	X
	Time effect	X	X	X	X	X	X	X	X
	Trt * Time effect	NS	NS	X	X	X	X	X	X
LSD test: Means with the same letter are not significantly different	A- Trt effect	6-7-5-1-3	6-5-7-1-3	4-3-2-7-1-6	2-1-7-4-3-6	5-1-7-6	5-1-7-6	2-4	2-4
	B	3-2-4	1-3-2-4	6-5	7-4-3-6-5	1-7-6-4-3	1-7-6-4	4-3-5	4-5-3
	C					3-2	7-6-4-3	3-5-1	5-3-1-6
	D						3-2	5-1-6	1-6-7
	E							1-6-7	
	A- Time effect	6	6	2-0	2	2-0	2	8	8
	B	0-2-8	2-8	8-6	8-6	6-8	6-8	6	6
	C							2-0	2
	D								
	E								

Legend:

X	Significant at a level of P< 0.05
NS	Not significant
Letters	T group
Numbers	Treatment number (treatment effect)
	Day (time effect)

APPENDIX A-3: Sensory evaluation statistical analysis for Retail packaging samples

		Colour		Discolouration		Retail Appearance	
		With day 0	W/O day 0	With day 0	W/O day 0	With day 0	W/O day 0
	Trt effect	NS	NS	X	X	X	X
	Time effect	X	X	X	X	X	X
	Trt * Time effect	X	X	X	X	X	X
LSD test: Means with the same letter are not significantly different	A- Trt effect	4-2-3	4-2-3-1	5-6-3-1	5-6-3-1	7	7
	B	2-3-6-1-5-7	2-3-1-6-5	6-3-1-4	1-4-2	1-2-4-5	1-2-4
	C		3-1-6-5-7	1-4-2	7	2-4-5	2-4-5
	D			2-7		4-5-6-3	5-6
	E						6-3
	A- Time effect	2-0-8	2-8	8-6	8-6	2	2
	B	6	6	0	2	0	6
	C			2		6-8	8
	D						
	E						

		Off Odour		Odour Acceptability	
		With day 0	W/O day 0	With day 0	W/O day 0
	Trt effect	X	NS	NS	NS
	Time effect	X	X	X	X
	Trt * Time effect	NS	NS	NS	NS
LSD test: Means with the same letter are not significantly different	A- Trt effect	6-5-3-2	6-3-5-2-7-4	6-5-3-4-2-7-1	3-6-4-5-2-7-1
	B	5-3-2-4-7	3-5-2-7-4-1		
	C	3-2-4-7-1			
	D				
	E				
	A- Time effect	8	8	8	8
	B	6-0-2	6-2	6-2	6-2
	C			0	
	D				
	E				

Legend:

X	Significant at a level of P< 0.05
NS	Not significant
Letters	T group
Numbers	Treatment number (treatment effect)
	Day (time effect)

APPENDIX A-4: Microbiological statistical analysis for Vacuum packaging samples

		Carnobacterium "845"		*E.coli*		*Pseudomonas*	
		With week 0	W/O week 0	With week 0	W/O week 0	With week 0	W/O week 0
	Trt effect	X	X	X	X	NS	NS
	Time effect	X	X	X	X	X	X
	Trt * Time effect	X	X	NS	NS	X	X
LSD test: Means with the same letter are not significantly different	A- Trt effect	2-1-6	2-1-6	1-2	1	3-2-6-1	3-2-6-4-5
	B	6-3	3-4-5	2-4	2-4	2-6-1-4	2-6-4-5-1
	C	3-4-5		4-3	4-3	6-1-4-5	
	D			3-5-6	3-5		
	E				5-6		
	F						
	A- Time effect	6	6	0	1-3-2	1-2-3	1-2-3
	B	5	5	1-3-2	4-5	2-3-4	2-3-4
	C	1	1	4-5	6	5	5
	D	2-0	2	6		0	
	E	0-3-4	3-4				

Legend:

X	Significant at a level of P< 0.05
NS	Not significant
Letters	T group
Numbers	Treatment number (treatment effect)
	Week (time effect)

APPENDIX A-5: Colour measurements and pH statistical analysis for Vacuum packaging samples

		L*		a*		b*		pH	
		With week 0	W/O week 0	With week 0	W/O week 0	With week 0	W/O week 0	With week 0	W/O week 0
	Trt effect	X	X	X	X	X	X	X	X
	Time effect	X	X	X	NS	X	X	X	X
	Trt * Time effect	NS	NS	X	X	X	X	X	X
LSD test: Means with the same letter are not significantly different	A- Trt effect	6	6	1-2	1-2	1-3	1-3-5	3	3-5
	B	5-1-2-7-3	5-2-3	2-3-4	6-4-3	3-5-4-6-7-2	3-5-6-4-7-2	5	4-6
	C	4	2-3-1-7	3-4-6	5-7			4-6	7-1-2
	D		4	5-7				2-1-7	
	E								
	F								
	A- Time effect	2-0-4	2-4	0	3-2-1-4-6-5	0	6-4-5-3	2-1	2-1
	B	0-4-5-3-6	4-5-3-6	3-2-1-4-6-5		6-4-5-3	1	0	6-3
	C	5-3-6-1	5-3-6-1			1	2	6-3	3-5
	D					2		3-5	5-4
	E							5-4	

Legend:

X	Significant at a level of P< 0.05
NS	Not significant
Letters	T group
Numbers	Treatment number (treatment effect)
	Week (time effect)

APPENDIX A-6: Sensory evaluation statistical analysis for Vacuum packaging samples

		Colour		Discolouration		Retail Appearance	
		With week 0	W/O week 0	With week 0	W/O week 0	With week 0	W/O week 0
	Trt effect	X	X	X	X	X	X
	Time effect	X	X	X	X	X	X
	Trt * Time effect	X	X	X	X	X	X
Means with the same letter are not significantly different	A- Trt effect	4	4	7	7	2	2
	B	3-2	3-2-1	5-4	5-4	1	1
	C	2-1-5	1-5	3	3	6	6
	D	6-7	6	6	6	3	3
	E		7	1	1	4-5-7	4-5-7
	F			2	2		
	A- Time effect	4-0-2	4-2	6-5	6-5	2	2
	B	0-2-1	2-1	4-1-0	4-1	0	3
	C	1-3-5	1-3-5	1-0-3	3	3	4-1
	D	6	6	2	2	4-1	6-5
	E					6-5	

		Off Odour		Odour Acceptability	
		With week 0	W/O week 0	With week 0	W/O week 0
	Trt effect	NS	NS	NS	NS
	Time effect	X	X	X	X
	Trt * Time effect	X	NS	NS	NS
Means with the same letter are not significantly different	A- Trt effect	2-1-6-7-4-5-3	1-2-7-4-6-3	2-6-4-1-5-3-7	1-2-4-6-3-5-7
	B		2-7-4-6-3-5		
	C				
	D				
	E				
	F				
	A- Time effect	5-4-6	5-4-6	4-6-5	4-6-5
	B	4-6-3	4-6-3	3	3
	C	1-2-0	1-2	1	1
	D			2-0	2
	E				

Legend:

X	Significant at a level of P< 0.05
NS	Not significant
Letters	T group
Numbers	Treatment # (treat. effect)
	Week (time effect)