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Aspects of the Microbiological Quality and Safety of Ready-to-Eat Foods in Sharjah Supermarket Stores

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By

Najla Ali Al-Mualla

Thesis

Submitted to

United Arab Emirates University

In Partial fulfillment of the Requirements

For the Degree of M.Sc. in Environmental Sciences

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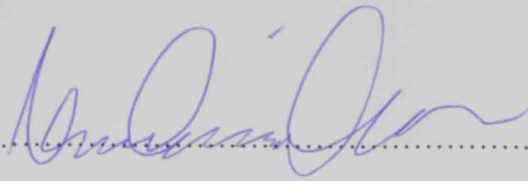
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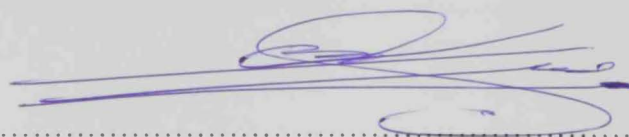
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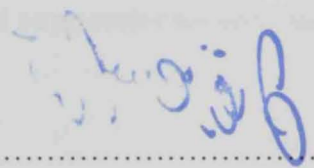
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Dedication

To the soul of my father

And supportive lovable mother

IV

Acknowledgments

ABSTRACT

Ready to eat foods covers a wide range of pre-processed food products that are readily eaten by consumers without further processing. Recently, there has been considerable increase in the demand of processed and convenience foods that are mildly pre-cooked, rapidly chilled, portioned and distributed for refrigeration prior to reheating and consumption by consumers. However, some of these foods such as, luncheon meals, ready to eat vegetables, fruit salad, spinach salad, and other deli-style meat and poultry products can be a harbor for pathogenic bacteria that are harmful to health when consumed by people especially with weak immune system.

Unfortunately, these foods quite often may suffer from temperature abuse of $10^{\circ}C$ or above for extended period of time giving chance to pathogenic bacteria to grow and survive to sufficient numbers that are harmful to human health. In spite of all the safety concern, ready to eat foods are very popular among a wide range of consumers. Over the last few years, these foods became popular in Sharjah grocery stores. The study evaluated the microbiological safety of some ready to eat foods sold in supermarket stores in Sharjah and the appropriate shelf-life for those products.

The results showed that the way of preparation of the food could be an indication of the quantity and quality of the presence of microorganisms as in tabbouleh, hommos, greek salad, and coleslaw (0.68, 0.50, 0.44 and 0.20 Log_{10} CFU/g) of *E. coli* and (3.57, 2.71, 2.76 and 2.52 log_{10} CFU/g) of total microorganisms. All ready to eat appetizers tested contained acceptable count of total bacteria except tabbouleh with the highest count. Twenty percent of all samples contained *E. coli* although the number can be as low as 3 log_{10} CFU/g. From the results of all products studied, it was recommended the shelf life of ready to eat appetizers should not exceed one day, according to UAE standards and it is recommended that Hazard Analysis Critical Control Point (HACCP) should be implemented in all locations studied.

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I. INTRODUCTION

1. Introduction and definition of ready to eat foods

Ready-to-eat (RTE) foods include various categories of food products and they can be divided in many different ways. According to the Codex Alimentarius Commission (An international commission of Food Agriculture Organization and World Health Organization) definition, RTE include any food (including beverages) that is normally consumed in its raw state, or any food handled, processed, mixed, cooked or otherwise prepared into a form in which it is normally consumed without further processing. RTE foods differ in different countries, according to local eating habits, availability and the integrity of the chill chain and regulations (CAC, 1999, FAO/ WHO, 2004).

Demand for RTE foods has led to an increase in the amount and selection of products available for the consumer. Some examples of RTE foods are luncheon meals, fresh cut vegetables, fruit salad, spinach salad, and other meals of meat and poultry products. The RTE appetizers prepared from vegetable may consist of trimmed, peeled, sliced, shredded, washed and disinfected vegetables. The vegetables may be combined with cooked vegetables, meats and pastas or salad dressing. The products are generally packaged and stored at refrigeration temperatures (Menlove, 2002). RTE products evaluated in this study were tabbouleh salad, greek salad, coleslaw salad and houmos.

1.1. tabbouleh salad

It is a Middle-Eastern salad dish. It consists of durum wheat, finely chopped parsley, mint, tomato, spring onion and lemon juice. Various seasonings added to it include black pepper, cinnamon and some other spices (Wikipedia Encyclopedia, 2008).

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1.2. Greek salad

Greek salad is a traditional Greek salad but is consumed in different places in the world. The salad consists of sliced or chopped lettuce, bell pepper, tomato, cucumber and red onion, seasoned with salt, black pepper, oregano and olive oil. Feta cheese, capers and olives are added at the end (Wikipedia Encyclopedia, 2008).

1.3. coleslaw salad

It is generally eaten as a side dish and it consists of shredded raw cabbage and carrots, mixed with mayonnaise which is made up of vegetable oil and egg yolks (Wikipedia Encyclopedia, 2008).

1.4. hommos

It is another Middle-Eastern dish made from mashed chick peas and sesame paste seasoned with salt, garlic, lemon and chopped green peppers, along with a little olive oil on the top. Different ratios of mashed chick peas and sesame paste are applied to suit individual tastes (Wikipedia Encyclopedia, 2008).

2. The microbiology quality and safety of RTE foods

The RTE foods contain several indigenous microflora of raw material from which they are prepared. Pathogens may form part of this microflora, posing a potential microbial problem. Also microbiological quality and safety of RTE foods are influenced by processing steps and storage conditions that may introduce some microorganisms.

2.1. Indigenous microflora

RTE foods prepared from vegetables harbor large and various populations of microorganisms, frequently percent at a rate of $10^5/g$-$10^7/g$ CFU (Gillian *et al.*, 1999). In general, vegetables contains eighty to nightly percent of bacteria are Gram-negative rods, predominantly *pseudomonas, Enterobacter* or *Erwinia* species (Manvell & Ackland, 1986; Brocklehurst, *et al.*, 1987; Nguyen-the & Prunier, 1989; Gary *et al.*, 1990; Magnuson *et al.*, 1990; Marchetti *et*

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al., 1992) Lactic acid bacteria have also been detected in mixed salads that are held at above 30°C temperatures (Manvell & Ackland, 1986).

2.2. Pathogens

RTE foods have a fairly acceptable track record in terms of food safety. However, foodborne pathogens may be present, and a number of outbreaks of food borne disease have been traced to RTE foods (Table 1). The predominant pathogens frequently associated with RTE foods are *Listeria monocytogenes*, *Salmonella* spp, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium botulinum*, *Aeromonas hydrophila*, *Yersinia enterocolitica* and *Campylocbacter jejuni*, and some of these pathogens were investigated in this study.

Microorganism	Product suspected	Place of infections	References
L. monocytogenes	-Shredded cabbage in coleslaw	Canada	Schlech *et al*.,1983
	-Raw vegetables in salads	US	Ho *et al*., 1986
	-Shredded cabbage in coleslaw	US	Solomon *et al*., 1990
Salmonella	Beansprouts	UK	O,Mahony *et al*.,1990
Enteroemorrhagicic E. coli	Salads of raw vegetables	Mexico	Nguyen-the & Carlin, 1994

Table 1.1: Examples of foodborne infections linked to the consumption of raw vegetables

2.2.1. *Listeria monocytogenes*

L. monocytogenes is a Gram-positive rod-shaped bacterium. This pathogen causes several diseases in human including meningitis, septicemia, still-births and abortions (ICMSF, 1996). The major concern with *L. monocytogenes* is its ability to grow at refrigeration temperatures 4°C; the minimum temperatures for growth are reported to be -0.4°C (Walker & Stringer, 1987). It is also a facultative anaerobe, capable to survive and grow under the low O_2 concentrations within modified atmosphere packages of RTE vegetable foods (Gillian *et al.*, 1999).

2.2.1.1. Sources and level of contamination

L. monocytogenes is considered ubiquitous in the environment, being isolated from soil, faeces, sewage, silage, manure, water, mud, hay, animal feeds, dust, birds, animals and human (Al-Ghaza & Al-Azawi, 1990; Nguyen & Carlin, 1994; Gunasena *et al.*, 1995). It is also associated with plant material including shrubs, wild grasses, corn, cereals and decaying vegetation (Welshimer & Donker-Voet, 1971). Since *L. monocytogenes* occurs widely in soil and agricultural environment in general, it is present naturally on many vegetables. Also, contamination of vegetables by *L. monocytogenes* may occur through agricultural practice, such as irrigation with polluted water or fertilization with contaminated manure (Geldreich & Bordner, 1971; Nguyen & Carlin, 1994).

L. monocytogenes has been also isolated from a wide range of whole vegetables such as cabbage, cucumber and lettuce (Steinbruegge *el al.*, 1988; Heisick *et al.*, 1989; McLauchin & Gilbert, 1990; De Simon *et al.*, 1992; Vahidy, 1992; Arumugaswamy *et al.*, 1994; Tang *et al.*, 1994; Gunasena *et al.*, 1995). Cases of recent occurrence of *L. monocytogenes* on fresh vegetables and RTE foods prepared from vegetables are summarized in Table 2; although none of these products were associated with outbreaks of food poisoning (Gillian *et al.*, 1999). *L. monocytogenes* has also been isolated from chilled RTE foods at concentration ranging from 0% to 44% (Petran *et. al.*, 1988; Fenlon *et. al.*, 1996, Sizmur & Walker, 1988; Beckers *et. al.*, 1989; McLauchlin & Gilbert, 1990; Velani & Roberts, 1991; Harvery & Gilmour, 1993; Arumugaswamy *et. al.*, 1994; MacGowan *et al.*, 1994; Doris & Seah, 1995).

Intact vegetables & Ready to eat appetizers	Number (and %) of positive samples	Country and observations	References
Beansprouts	6/7 (85%)	Malaysia	Arumugaswamy *et al.*,1994
Cabbage	6/18 (33%) 1/92 (1.1%)	Sri Lanka US	Gunasena *et al.*, 1995 Heisick *et al.*,1989
Cucumber	2/92 (2.2%) 1/15 (6.7%)	US Pakistan	Heisick *et al.*,1989 Vahidy, 1992
Lettuce	10/20 (50%) 1/28 (3.6%)	Sri Lanka Kuala Lumpur	Gunasena *et al.*, 1995 Tang et al., 1994
Salad vegetables	2/108 (1.8%)	UK	Velani & Roberts, 1991
Tomato	2/15 (13.3%)	Pakistan	Vahidy, 1992
Various vegetables	8/103 (7.8%)	Spain	De Simon *et al.*,1992
Cucumber slices	4/5 (80%)	Malaysia	Arumugaswamy et al.,1994
Coleslaw	2/92 (2.2%) 2/50 (4%) 3/38 (7.7%)	Canada Singapore UK	Schlech et al., 1983 Doris & Seah, 1995 MacGowan *et al.*,1994
Prepared mixed vegetables	8/42 (19%)	UK (contamination during processing	Velani & Roberts, 1991

		suspected;<200 g-l present)	
Fresh cut salad vegetables	11/25 (44%)	The Netherlands <10(2)g-1 present	Beckers *et al.*, 1989
Prepared vegetables	1/26 (3.8%)	UK	MacGowan et a*l.*,1994
Salad vegetables	4/16 (25%)	Northern Ireland	Harvey & Gilmour, 1993
Processed vegetables and salads and salads	(13%)	UK	McLauchlin & Gilber, 1990

Table 1.2: Occurrence of *L. monocytogenes* on fresh RTE foods

2.2.2. *Escherichia coli*

Coliform bacteria are the commonly-used bacterial indicator of sanitary quality of foods and water. They are defined as rod-shaped Gram-negative non-spore forming organisms that ferment lactose with the production of acid and gas when incubated at 35-37°C. Coliforms are abundant in the feces of warm-blooded animals, but can also be found in the aquatic environment, in soil and on vegetation. The typical genera include: *Citrobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, Serratia* and *Yersinia*. So *Escherichia coli (E. coli)*, a rod-shaped member of the coliform group, can be distinguished from most other coliforms by its ability to ferment lactose at 44°C, and by its growth and color reaction on certain types of culture media. Unlike the general coliform group, *E. coli* are almost exclusively of fecal origin and their presence is thus an effective confirmation of fecal contamination

Escherichia coli also a common inhabitant of the gastrointestinal tract of mammals. Gastroenteritis and hemorrhagic colitis are classical symptoms of which complications include thrombocytopenic purpura and hemolytic uraemic syndrome (Martin *et al.*, 1986).

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complication has been reported to lead to renal failure and death in 3-5% of juvenile cases (Karmali *et al.*, 1983; Griffin & Tauxe, 1991).

2.2.2.1. Sources and level of contamination

The principal reservoir of *E. coli* is believed to be the bovine gastrointestinal tract (Wells *et al.*, 1991; Doyle *et al*, 1997). Hence contamination of associated food products with faeces is a significant risk factor. Contamination and survival of the organism in natural water sources make these potential sources in the distribution of infection, particularly if untreated water is consumed directly or is used to wash uncooked foods (Gillian *et al.*, 1999).

2.2.3. *Salmonella*

Salmonella, a genus of the family enterobacteriaceae, are characterized as Gram-negative, rod-shaped bacteria. Some pathogenic species of *Salmonella* include *S. typhimurium, S. enteritidis, S. Heidelberg, S. saint-paul, and S. Montevideo*. Normal symptoms of gastroenteritis include diarrhea, nausea, abdominal pain, vomiting, mild fever and chills (ICMSF, 1996). *Salmonella* are mesophiles, with optimum temperatures for growth between 35-43 °C. The growth rate is substantially reduced at < 15 °C, while the growth of most *Salmonella* is prevented at <7 °C. *Salmonella* are facultative anaerobes, capable of survival in low O_2 atmospheres.

2.2.3.1. Sources and level of contamination

Salmonella are abundant in faecal material, sewage and sewage-polluted water; consequently they may contaminate soil and crops when they come into contact. Sewage sludge may contain high amounts of *Salmonella* and when used for agricultural practices, it can spread the bacterium. Once introduced into the environment, it can remain viable for months (ICMSF, 1996). This microorganism has been isolated from many mammals, poultry, cattle, birds, reptiles, fish, amphibians and insects. Food is the primary route of *Salmonella* to humans, especially foods from animal origin. *Salmonella* has been also isolated from several fresh intact foods (Doyle, 1990; Tauxe, 1991). In a study of whole foods sampled in the field or in retail outlets in Spain, 7.5% of the total samples were found to be contaminated with *Salmonella* spp.(Garcia-villanova Ruiz *et al.*, 1987). In a survey of food was conducted from

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shops in Italy, 68% of lettuce and 72% of fennel samples carried *Salmonella* spp. (Ercolani, 1976). *Salmonella* were also isolated from leafy vegetables (4%) and bean sprouts (20%) in Malaysia (Arumugaswamy *et al.*, 1994).

2.2.4. *Staphylococcus aureus*

Staphylococcus aureus is commonly associated with the human body, particularly moist areas such as the nose, throat, skin and arms pits. It produces large amounts of toxins, one of which causes the typical food poisoning of staphylococcal intoxication. Symptoms include rapid onset of vomiting (1-8 hours), sometimes accompanied by diarrhea, and this is usually self resolving within 1 day (Menlove, 2002). Growth of the organism needs to occur to high levels in the food before sufficient toxin is produced to cause illness. The toxin is very heat-resistant. Therefore, mild pasteurization temperatures of 70 °C that can destroy the organism cannot affect the toxin (Menlove, 2002).

2.2.4.1. Sources and level of contamination

Staphylococcus aureus can colonize the skin of humans and cause infection in cuts and sores, and it is also associated with boils. Personnel handling practices, raw materials, particularly meat, can also cause contamination by *Staphylococcus aureus*. The microorganisms cannot grow under chilled conditions (<10 °C) and are inhibited by pH values of <4. However, it can tolerate very low water activity (a_w) and produces enterotoxin at a_w 0.86 if other conditions are optimal (Menlove, 2002).

2.3. Outbreaks of foodborne disease associated with RTE foods prepared from vegetables

In England and Wales 4.4% of foodborne disease was associated with consumption of salad, fruit and vegetables (Mintel Market Intelligence Report, 2000). However, Listeriosis foodborne outbreak was the first to occur in the Maritime Provinces of Canada in 1981 and involved thirty four prenatal cases and 7 adult cases. It was found that the source of the outbreak was coleslaw where as *L. monocytogenes* was found in stored cabbage (Schlech *et*

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al., 1983). An outbreak of *L. monocytogenes* infection during the summer of 1979 involving 23 patients from eight Boston hospitals was reported by Ho *et al.*, (1986). This outbreak was attributed to the consumption of raw salad vegetables (celery, tomatoes, lettuce), but no attempt was made to isolate *L. monocytogenes* from the vegetables at the time of the outbreak. It was also reported that an elderly patient in a London hospital acquired Listeric septicemia after consuming contaminated lettuce (Fain, 1996).

The majority (32%) of food associated with *E.coli* 0157:H7 outbreak in the USA, between 1982 and 1994, was attributed to the consumption of ground beef. However, approximately 6% of the outbreaks were associated with the consumption of ready to eat foods (Doyle *et al.*, 1997). The large outbreak was associated with alfalfa sprouts (Como-Sabetti *et al.*, 1997). The origin of contamination in such cases was likely to result from the application of contaminated water or manure to the crops, coupled with inadequate washing procedures. Recently, at the end of September in 2005 there were 157 cases of *Escherichia coli* O157: H7 food poisoning from raw spinach in U.S.A. (US FDA, MD, USA, 2006).

Salmonella is one of the most frequently reported causes of foodborne outbreaks of gastroenteritis. A number of outbreaks of Salmonellosis have been linked to vegetables. An outbreak of Salmonellosis in U.K. in 1988 was associated with consumption of bean sprouts (attributed to *S. saint-paul* and *S. virchow* PT34; O'Mahony *et al.*, 1990). Epidemiological studies linked two multi-state outbreaks of Salmonellosis to raw tomatoes (Wood *et al.*, 1991; Hedlberg *et al.*, 1994).

2.4. Factors affecting the growth of pathogens on RTE foods

Raw vegetables may be contaminated with pathogens when they enter the processing stages (Nguyen & Carlin, 1994). The product itself and its source will often determine the numbers and types of microorganisms initially present. Analysis at different chains of processing showed that the end product is frequently less contaminated than the raw vegetable (King *et al.*, 1991). Because contamination with pathogens may occur during processing and distribution; scrupulous hygiene must be applied at all levels of processing.

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The development of food poisoning microorganisms on RTE foods depends on the properties of the microorganisms, intrinsic properties of the food itself and on the effects of processing, storage and packaging. Each produced item may go through a series of processing steps, including equipments, handling, slicing, contact, washing, packaging and storage. Each of these steps may affect microbial colonization, survival and growth (Gillian *et al.*, 1999). The more unit operations a product undergoes, the more its flora will reflect that of the environment in which it is produced (Gillian *et al.*, 1999).

2.4.1. Processing

Handling can damage fresh produce and it more suitable for growth and survival of spoilage and pathogenic microorganisms (Brackett, 1994). Damage of fresh produce can occur during harvesting, packaging, preparation, transportation and storage.

Contamination during processing may occur from lack of personnel hygiene or through product-to-machine impacts. Slicing and shredding destroy surface cell, bruise underlying layers and allow juices to leak from inner tissues on to equipment and on to fresh-cut products. Cutters and slicers can be potent sources of contamination because they usually provide inaccessible sites which harbor bacteria (Garg *et al.*, 1990). The presence of cut surfaces allows microbial infiltration and provides an increased surface area for contamination and growth (Brackett, 1994). Moisture and exudates on cut surfaces and on surfaces of utensils and equipments provide excellent media for rapid growth of microorganisms. Therefore, during the processes of slicing or shredding, there may be an increase in the number of bacteria present. Exposing foods to various types of cutting resulted in a six to seven -fold increase in microbial populations (Brackett, 1994). Garg *et al.* (1990) found that shredders used to prepare shredded lettuce and cabbage were major sources of contamination in a factory producing RTE foods (Gillian *et al.*, 1999).

L. monocytogenes may be present and can survive in the environment of many types of food processing plant, particularly in damp, moist conditions (Walker & Stringer, 1990; Farber & Peterkin, 1991). It has been recovered from the environment of processing operations used to

prepare RTE foods (Zhang & Farber, 1996). Slicing operation may increase contamination of RTE foods by *L. monocytogenes*. The pathogen was detected in 19% of prepared mixed vegetables examined, whereas only 1.8% of individual ingredients were contaminated (Velain & Roberts, 1991) The researchers concluded that substantial contamination by *L. monocytogenes* occurred during chopping, mixing and packaging. A case reported where *L. monocytogenes* was regularly found in shredded cabbage and on shredding machines in the processing line. Nguyen *et. al.*, (1996) reported the microbiological consequences of mixing cooked foods with raw vegetable salads and investigators observed that *L. monocytogenes* grew better at 6 and 9°C in a mixed salad (raw endive and sweet corn) than on raw endive alone.

Salmonella can initiate and multiply in the environment and equipment of a variety of food-processing facilities, potentially cross-contaminating raw foods during processing (ICMSF, 1996). However, producing RTE vegetables encounter less contamination chances than producing it with protein source.

2.4.2. Washing and antimicrobial dip treatments

An important step in the processing of RTE foods prepared from vegetables is thorough washing, often accompanied by dipping in an antimicrobial solution, to reduce microbiological load. Washing vegetables with water containing a free chlorine level of 100 mg L^{-1} has been found to reduce the number of bacteria on lettuce leaves by up to 100-fold (Adams *et al*., 1989). Dipping produce in ascorbic or citric acid solutions prior to packaging greatly reduced aerobic and anaerobic plate counts and these differences persisted during storage (McLachlan & Stark, 1985; O'Beirne & Ballantyne, 1987). Strict hygiene is critical during washing and disinfection in order to avoid contamination of produce. Chlorinated water from the washing bath on a line processing chicory salads was found to contain 10^3 bacteria ml^{-1} (Nguyen-the & Prunier, 1989). Washing produce in such washing bath may result in transfer of microorganisms, due to the re-use of wash water (Gillian, *et al*., 1999). Ultraviolet radiation may be useful in inactivating vegetative bacterial cells in wash water, thus avoiding cross-contamination (Gillian *et al*., 1999).

There are theoretical concerns regarding the safety of using antimicrobial dip for example, pathogens if present on raw vegetables, may not be fully eliminated by commercial disinfection procedures. On the other hand the influences of disinfection on the indigenous microflora may reduce or remove natural competitive organisms. As a result, disinfection may produce conditions which favor survival and growth of the pathogens (Gillian *et al.*, 1999).

The effects of sodium hypochlorite on *L. monocytogenes* have been widely studied *in vitro* and were shown to be very influence (Brackett, 1987; El-Kest & Marth, 1988; Tuncan, 1993). However, the elimination of *L. monocytogenes* from the surface of vegetables by disinfection is limited and unpredictable (Nguyen & Carlin, 1994). This ineffectiveness may be due to a number of factors; an aqueous hypochlorite solution may not wet the hydrophobic surface of the waxy cuticle or penetrate into the crevices, creases, and natural openings of the vegetables (Adams *et al.*, 1989). The most useful effect of chlorine may be in inactivating vegetative cells in washing water and on equipment during processing as part of a hazard analysis critical control points system, thereby avoiding build-up of bacteria and cross-contamination (Wilcox *et al.*, 1994).

Bracket (1987) examined the influence of chlorine on Brussel sprouts contaminated with *L. monocytogenes*, dried and immersed in a 200 mg L^{-1} chlorinated solution. The counts of *L. monocytogenes* were reduced approximately 100-fold, 10-fold more than those treated with water. In addition, *L. monocytogenes* was undetectable in the chlorinated solution or on Brussel sprouts subsequently immersed in the same chlorinated solution after inoculated samples. Zhang & Farber (1996) evaluated the influences of various disinfectants against *L. monocytogenes* on fresh-cut vegetables. The results for chlorine showed (200 ppm, 10 min), the maximum observed log_{10} reduction of *L. monocytogenes* at 4 °C and 22°C, respectively, were 1.3 and 1.7 for lettuce and 0.9 and 1.2 for cabbage. Thus, the bactericidal effect on *L. monocytogenes* was higher at 22 °C than at 4°C, and was higher on lettuce than on cabbage.
In relation to post-disinfection contamination, some researchers reported that disinfection of salad leaves or tomato slices with chlorine prior to inoculation with *L. monocytogenes* did not effect its subsequent growth during storage (Beuchat & Brackett, 1990; 1991).

By contrast, Carlin *et al.* (1996) reported that *L. monocytogenes* inoculated on disinfected (10% hydrogen peroxide) endive leaves grew better than on water-rinsed produce and gave higher counts after 7 days at 10 °C than on leaves rinsed with water. Similarly, Francis & O' Beirne (1997) reported that dipping shredded lettuce in a chlorine (100 ppm) or citric acid (1%) solution followed by storage at 8 °C resulted in significant increases in *Listeria* populations compared with untapped samples.

Influence of organic acid as disinfectants on the growth of *L. monocytogenes* on produce has received little attention. Lactic and acetic acid (1%) reduced numbers of *L. monocytogenes* on lettuce.

Salmonella are readily killed by disinfectants commonly used in the food industry (ICMSF, 1996). Zhuang *et al.* (1995) examined the efficacy of chlorine treatment on inactivation of *S. emonotevideo* on and in tomatoes. The populations of *S. emonotevide* on the surface and in the core tissues were substantially reduced by dipping tomatoes in solutions containing 60 or 110 ppm chlorine, respectively.

The efficacy of buffered sodium hypochlorite solution (Bionox) in controlling *S. enteritidis* contamination on vegetables (carrot, zucchini) was studied by Park *et al.*, (1991). The investigators found that the sanitizing solution was very effective in reducing *Salmonella* levels on vegetables (counts following 20 min exposure to the sanitizing solution were < 2 g^{-1}).

Beuchat & Ryu (1997) examined the efficacy of chlorine in removing *Salmonella* from alfalfa sprouts. Sprouts were dipped in solution containing 200, 500 or 2,000 ppm chlorine for 2 min. The pathogen was reduced by about 2 $\log_{10}$ CFU g^{-1} after treatment with 500 ppm chlorine compared to the control and to undetectable levels after treatment with 2000 ppm chlorine.

The effects of disinfection on the survival of *E.coli* or other pathogens on RTE foods were poorly understood. The tolerance of *E.coli* 0157:H7 to acidic pH is well recorded (Zhao *et. al.*, 1993; Conner & Kotrola, 1995), particularly with respect to organic acid sprays applied to beef surfaces (Brackett *et al.*, 1994). Survival of the organism for a period of 35 days when

held at 5 °C in mayonnaise based sauces (pH ~ 4.0) has been documented (Weagant *et al.*, 1994). Therefore, following cross-contamination, such foods may act as a transmission of infection, particularly in the context of salad bars (Gillian *et al.*, 1999).

2.4.3. Storage temperature

Storage temperature is probably the most important factor influencing the growth of microorganisms in RTE foods. Results obtained on shredded chicory salads (Nguyen-the & Carlin, 1994), carrot slices (Buick & Damoglou, 1987) and shredded lettuce (Bolin *et al.*, 1977; Beuchat & Brackett, 1990) found that growth of the mesophilic microflora was significantly reduced when storage temperature was decreased.

French regulations imposed 8 °C as a maximum storage temperature for RTE foods in 1987. This limit was lowered to 4 °C in 1988 (Gillian *et al.*, 1999). However, RTE foods are often distributed at higher temperatures. In the United Kingdom, according to the food hygiene regulations, retail packs of prepared salad vegetables should be maintained at temperatures below 8 °C. Guidelines for handling chilled foods, published by the UK Institute of Food Science and Technology (IFST, 1990), recommend that the storage temperature should range from 0 to 5 °C for prepared vegetables salads, noting that some foods may suffer damage if kept at the lower end of this temperature range (IFST, 1990).

Storage of RTE foods at adequate refrigeration temperatures limits the growth of pathogens that are psychrotrophic. Previous work has shown that *L.monocytogenes* survives or grows on a range of RTE foods such as ice-berg lettuce (Steinbruegge *et al.*, 1988; Beuchat & Brackett, 1990), shredded cabbage (Kallender *et al.*, 1991) and on a range of intact fresh vegetables at refrigeration temperatures (Berrang *et al.*, 1989). In addition, at low temperatures, reducing the storage temperature extends the lag phase and reduces the rate of growth (Beuchat & Brackeet, 1990; Carlin *et al.*, 1995). On the other hand, temperature abuses during storage markedly reduce the lag and generation time and permit rapid growth of *L .monocytogenes*.

Mesophilic pathogens are unable to grow when temperature control is adequate (4 °C or less). The minimum growth temperature of *Salmonella* was reported to be 5.2°C (ICMSF, 1996). Survival and slow growth in produce stored for extended period in chilled conditions (within the growth range for *Salmonella*) may be of concern. On a range of vegetables, *Salmonella* consistently was viable for more than 28 days at 2-4 °C (ICMSF, 1996). Populations of *S. hadar* on minimally processed cabbage were particularly similar to the initial levels after 10 days storage at 4 °C (Piagentini *et al.*, 1997). On samples stored at 12 and 20°C, *Salmonella* populations increased by 4 and 6 logs respectively after 10 days of storage (Piagentini *et al.*, 1997). Population of *S. Montevideo* on the surfaces of inoculated tomatoes stored at 10 °C not varies significant over 18 days. However, significant increases in population occurred within 7 days when tomatoes were stored at 20 °C (Zhuang *et al.*, 1995).

The mesophilic status of *E. coli* growth in RTE foods slows down under good refrigeration. Growth has been reported at 8 °C on minced beef (Weeratna & Doyle, 1991). The substantial amounts of nutrients provided through processing (shredding) of lettuce may help the growth of *E.coli* on such products (Abdul-Raouf *et al.*, 1993).

Besides its direct effect on microbial growth, storage temperature also determines respiration rate of the product and therefore changes in gaseous atmospheres within the package. This in turn may affect the behavior of pathogens. In general, inhibitory influence of modified atmosphere system increases as the storage temperature is lowered (Dixon & Kell, 1989).

2.4.4. Interactions between the indigenous microflora and pathogens

Interactions between pathogens and background microflora have been studied in meat and dairy products (Nguyen & Carlin, 1994), but there were lack of data on RTE foods.

The antagonistic properties of lactic acid bacteria have been reviewed by Piard & Desmazeaud (1992). Lactic acid bacteria pose antimicrobial effects due to one or more of the following mechanisms: lowering pH (Raccach & Baker, 1979); generating hydrogen peroxide (Price & Lee, 1970); competing for nutrients (Iandolo *et al.*, 1965); and probably by producing antimicrobial compounds, such as bacteriocins or antibiotics (Klaenhammer 1988; Harris *et al.*, 1989; Arihara *et al.*, 1993). Lactic acid bacteria are naturally present on vegetables usually

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at low numbers and they can reach high counts on some RTE foods, in particular products within modified atmosphere packages containing high concentrations of CO_2 (Carlin *et al.*, 1990). They may grow faster than the aerobic spoilage bacteria. Thus, the use of a particular gas atmosphere may select for proliferation of desired subpopulations, resulting in suppression of undesirable pathogens. Competitive effects of lactic acid bacteria may limit pathogen growth on RTE vegetable foods, but there is insufficient data available to prove this conclusively.

Pseudomonas are major components of the indigenous microflora of RTE vegetable foods (Nguyen & Carlin, 1994) and are capable of excreting cell wall degrading enzymes and thus releasing nutrients (Liao *et al.*, 1993). Since *Pseudomonas* are relatively sensitive to CO_2 (Enfors & Molin, 1980), their suppression can effect nutrient availability for other microorganisms. Marshall& Schmidt (1991) found that addition of *Pseudomonas* increased the multiplication of *L.monocytogenes*. These authors proposed that proteolytic *Pseudomonas* supplied free amino acids. There were no similar results for vegetables. Using an endive leaf medium, Bennik *et. al.* (1996) found that the addition of strains of fluorescent *Pseudomonas* very slightly reduced the final population density of *L.monocytogenes*. They reported that competition of the background microflora with *L.monocytogenes* resided mostly with enterobacteria and not with *Pseudomonas*. Francis & O'Beirne (1998) also found that competition of the background microflora of lettuce with *L. monocytogenes* resided primarily with *Enterobacter* species.

Little data has been published with regard to interactions between *E. coli* and other microorganisms. *E. coli* 0157:H7 is more competitive against spoilage microorganisms than *Salmonella* (Varnum & Evans, 1991) and, through its acid tolerance properties, is hypothetically more resistant to the acidic fermentation end-products of lactic acid populations.

Complex interaction between elements of the microflora may have significant influence on the growth and survival of pathogens (Gillian *et al.*, 1999). This need to be fully understood in order that novel mild preservation technologies can continue to be applied safely.

Shelf life of ready-to -eat foods

The shelf life of a food product can be defined as the length of time during which the product will remain microbiologically safe, will retain its sensory and physical characteristics and will comply with the declared nutritional data (Frances, 2006). The microbiological shelf life of ready meals can be highly variable depending on the individual product formulation but, in general, there are two main criteria that must be considered in establishing the shelf life. First, the potential for growth of those pathogenic bacteria that may be present in the product; second, the potential of spoilage microorganisms. Shelf life will be influenced by the initial microbiological load of food, as the more microorganisms are present at the time of storage, the more rapid the deterioration of the product. So, the rate of growth of spoilage organisms is the most important factor influencing shelf life unless the food has undergone a commercial

Sterilization process or has a water activity so low it will not permit microbial growth (Frances, 2006). However, the safe shelf life can only be set by understanding the microbial pathogens that may be present and assessing how they may grow in the RTE foods, given the normal product formulation and the expected storage temperature (Menlove, 2002). Mathematical models that predict the growth of different foodborne microorganisms are available and can be used to estimate the growth of food-poisoning bacteria under variable conditions of pH, water activity, temperature and gaseous environment (Menlove, 2002). Such models can help in identifying potentially unsafe shelf lives and assist in predicting the formulation changes, e.g. pH reduction, that should be made to achieve a safe shelf life or, indeed, what restrictions in shelf life that could be applied to ensure safety (Menlove, 2002). Mathematical models need to be used carefully by professional individuals and, in many cases, these models should be supplemented with challenge testing of individual products in experimental facilities to determine more accurately the growth potential of key pathogens in the food (Menlove, 2002). So, chilled food which can support the growth of pathogens for example storage trial would normally be held at 5°C, but the shelf life trial would be held at temperatures above this, in order to assess food safety under conditions of temperature abuse (Frances, 2006).

In the majority of RTE foods, the predominant microbial pathogens known to be capable of growing in the final product stored under good chill conditions are *L. monocytogenes* and *C. botulinum* (psychotropic strains). Although some strains of *B. cereus* can grow and produce toxin at 4 °C, outbreak of foodborne illness implicating this organism has resulted almost exclusively from temperature abuse of the foods, and it is not considered a major hazard in relation to the growth in most RTE foods that have short shelf life (8-14 days) and where storage temperatures are generally <5 °C with short periods up to 8 °C (Menlove, 2002). Products in which the organism may need more attention include those containing raw ingredients where it may occur in higher numbers, such as highly spiced meals, rice or pasta dishes and dairy based products (Menlove, 2002). In such cases, control is usually done by regular monitoring of the raw ingredients for the organism to ensure that levels are under effective control. In addition, it may also be of concern to those products where the shelf life is extensive, for under vacuum product where greater potential exists for growth owing to the longer life. Under such conditions, the control options include processing to destroy the organism, formulating to stop rapid growth or strict maintenance of low temperatures (<4 °C).

Products that are cooked in hermetically sealed containers to 90 °C for 10 minutes or equivalent are normally considered to be safe with regard to *C. botulinum* and *L. monocytogenes* and, although some strains of *B. cereus* may survive the heat treatment, as detailed above, such products should achieve safe shelf life of several weeks, need to be substantiated for *B. cereus* in long-shelf-life products. In these *cases*, *L. monocytogenes* may be destroyed if the temperature passed 70 °C for 2 minutes but sporeforming organisms such as *C. botulinum* may remain as a hazard (Menlove, 2002).

However, storage of the same product at higher temperatures, e.g. 8 °C, can significantly accelerate the speed of growth. In many cases, the shelf life of products in this category is restricted by food-spoilage bacteria, including thermotolerant species such as some lactic acid bacteria, clostridia and even sporeforming yeasts and molds (Menlove, 2002). Indeed, for the previous category of RTE foods heated to 90 °C for 10 minutes, it must be remembered that any contaminations left in the product have very little microbial competition as the process

eliminates most other microorganisms (Menlove, 2002). Therefore, growth of contaminating or surviving spoilage and pathogenic microorganisms can often be unchallenged. Nevertheless, these products, because they exclude the presence of many post-process contaminants, can achieve fairly long shelf life at 5 °C or below 10-14 days (Menlove, 2002).

For products that are cold-mixed or filled at temperatures below 70 °C *L. monocytogenes*, *C. botulinum* and *B. cereus* may all be present in the finished product and need to be controlled (Menlove, 2002). Under such conditions, the shelf life is usually restricted to <10 days at 5°C or below unless individual product parameters are shown to restrict the growth of the contaminating pathogens using mathematical models or challenge tests in experimental facilities (Menlove, 2002). It should also be remembered that such products will allow the survival of any other contaminating pathogens such as *S. aureus*, *Salmonella*, etc., that may have been introduced from the raw ingredients or through handling or processing (Menlove, 2002). Such organisms will not generally grow at low temperatures, for example <8 °C, but refrigeration will not effect on their survival. As far

as the potential for foodborne pathogens, concerned RTE foods that are not hot-filled carry a fairly high loading of general microflora from the component ingredients, equipment and general handling (Menlove, 2002). Yeasts and molds, enterobacteriaceae, lactic acid bacteria and *Pseudomonas* predominate, although sporeforming microorganisms can be high in some products, particularly highly spiced meals. The ingredients, formulation and storage conditions play an important role in the nature of the spoilage that the product succumbs to (Menlove, 2002). Neutrally, food can be spoiled by *Pseudomonas*, with the production of objectionable off-odors apparent at time intervals dependent on the temperature of storage and, to some degree, by the strength of flavor of the product (Menlove, 2002).

Products with large amounts of fermentable carbohydrates, such as sucrose and lactose, often succumb to more visual spoilage caused by the growth and gas production by yeasts and sometimes Enterobacteriaceae (Menlove, 2002). Both of these organisms grow quickly and can produce plentiful quantities of gas, causing blowing of the pack. Low-pH products restrict the growth of *Pseudomonas* and Enterobacteriaceae (Menlove, 2002). Spoilage tends to occur

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as a result of the growth of yeasts, although a high proportion of spoilage in these and many other ready meals is caused by mold. Mold spoilage can develop fm single spores and, although growth is relatively slow in the low concentrations of oxygen available and at low temperatures, they can scavenge low oxygen levels sufficient to from visible colonies on the food such colony being readily visible to the eye and most often greeted by product rejection (Menlove, 2002). Although less frequently, RTE foods can be spoiled by lactic acid bacteria growth in longer-shelf life products with the production of acid and sickly sweet taints. In many spoiled products, lactic acid bacteria and other organisms, particularly *Pseudomonas*, can produce visible slime on products, but microbial levels will usually be exceptionally high ($>10^8$/g) at the point where this occurs (Menlove, 2002).

Microbiological quality is best determined by means of simple storage experiments with determination of microbial growth during the life which is more dependent on storage time than on storage temperature (Riva *et al.*, 2001). Although it is possible to make some judgment regarding shelf life using microbial counts, it is best assessed in conjunction with organoleptic assessment of the product (Menlove, 2002). Shelf life determination should be done under conditions that simulate those experienced by the product. Ideally, this should be done using production samples, packed and stored under similar conditions intended for the final product. Product should be stored at temperatures simulating those experienced through manufacture, distribution, retail and with the consumed (Menlove, 2002). Although never likely to be absolutely accurate, storage at 5-8 °C gives a good reflection of the durability of the RTE appetizers under normal conditions (Menlove, 2002). Others have found it equally suitable to store product under regimes of different temperatures, such as 4-6 °C for periods from manufacture and through distribution, 4-6 °C for retail storage and 8-10 °C for customer storage, with a short period at ambient temperature (2-4 h) to simulate consumed usage after purchase. It is not possible to get a perfect shelf life test that simulates all conditions, but using the above strategies has proved to be adequate (Menlove, 2002).

Microorganisms to be examined should include all those expected to be present or capable of causing spoilage in the final products. This usually includes a basic aerobic plate count, together with yeast and mold, Enterobacteriaceae, *S. aureus*, *E. coli*, *B. cereus*, *Salmonella* and

Listeria (Menlove, 2002). Examinations may also be supplemented with *C. perfringens* for highly spiced and meat-containing products, where its presence may occasionally be expected. High levels of aerobic plate count, particularly at the start of life, should be investigated to determine the nature of the organisms present (Menlove, 2002).

Shelf life can be extended by storage under modified atmospheres using carbon dioxide and nitrogen or by the addition of preservative factors (Menlove, 2002). Increased salt concentrations restrict the growth of many spoilage bacteria, as will the presence of additional organic acids, for example: acetic, lactic, citric, although these are most effective at decreased pH (<5.5). Preservatives such as benzoate and sorbates can restrict the growth of molds and achieve longer open life, although it should be noticed that molds are best controlled by effective hygiene and environmental cleaning programs in the filling area (William & Dennis, 1988).

II. OBJECTIVES

2. OBJECTIVES

The objectives of this study were:

1. To evaluate and assess the prevalence of the most common pathogenic microorganisms (*Staphylococcus*, *E. coli*, *Salmonella* and *Lister. monocytogenes*) ready to eat foods (tabbouleh, houmos, Greek salad and coleslaw).

2. To evaluate the effect of storage temperature and time on the survival and growth of the isolated and identified pathogenic microorganisms from the RTE foods.

3. To evaluate the shelf-life of the prepared ready-to-eat foods at different storage temperatures.

III. Materials and Methods

3. Materials and Methods

1. Location of the experiments

Sharjah Municipality Food Laboratory, Sharjah

Description of experimental procedures:

1) Ready-to-eat foods Category:

tabbouleh

houmos

Greek Salad

coleslaw

2) List of the microbiological methods:

1. Standard plate count
2. *E. coli* Enumeration, isolation and identification
3. *Staphylococcus* enumeration, isolation and identification
4. *Salmonella* enumeration, isolation and identification
5. *Listeria monocytogenes* enumeration, isolation and identification

2. Materials

2.1. Ready to eat foods samples

2.1.1. Selection of RTE foods:

The Ready-to-eat foods used and analyzed for this study were tabbouleh salad, Greek , coleslaw salad and houmos.

2.1.2. Ready-to-eat foods sampling and locations

The RTE foods were collected from three different branches of the Supermarket Stores in Sharjah. These Supermarket Stores represent the majority of ready-to eat foods on sale in Sharjah Supermarket Stores. The number of samples of each product was 30 which replicate three times and the period of sampling was one and half month which started from 10 of June 2007 to 19 of July 2007 (Appendices VII.1).

2.2. Media and reagents

All media and reagents required for the enumeration and identification of total bacterial count, *Staphyloccocus aureus*, *E. coli*, *Salmonella*, and *Listeria monocytogenes* were prepared and stored as manufacture requirements (Appendices VII.2)

Type of microorganisms	Media and Sublimates used	Biochemical reagents and strips
Total bacterial count	- Standard Plate Count Agar (SPCA), (CM0463, Oxoid). - Ringer Solution 1/4 strength: (BR-52 tablets, Oxoid).	-

Escherichia coli	h: (BR-52 tablets, Oxoid). - MacConkey Broth Purple: (CM 5a Oxoid) - Brilliant Green Bile Broth 2% BGB: (CM31 Oxoid) - Tryptone Water: (CM 87 Oxoid)	- Indole Reagent: (Kovacs) - Rapid ID 32E (REF 32 700)
Staphyloccocus aureus	- Baird Parker agar base: (CM 275 Oxoid) - Egg yolk Tellurite Emulsion: (SR 54 Oxide)	- Staphylase test kit: (DR 595 Oxoid) - ID 32 STAPH (REF 32 500)
Salmonella spp	- Buffered Peptone Water (BPW): (CM 509, Oxoid) - Vassiliadis Soya Peptone broth (RVS): (CM866 Oxoid) - Muller-Kauffmann tetrathionate novolicon broth (MKTTn broth): (CM-1048, Oxoid) - Xylose Lysine Desoxycholate Medium (XLD): (CM 469, Oxoid)	- Rapid ID 32E (REF 30242) - Specific Antisera: (polyvalent O and /or polyvalent H)

	- Brilliant Green Agar (Modified): (Code CM 329, Oxoid) - Violet Red Bile Agar: (Code: CM 107 Oxoid)	
Listeria monocytogenes	- Primary enrichment (UVM), SR014E - Secondary enrichment (UVM∏), SR0143E - Oxford agar (CM0856), SR140 - PALCAM agar (CM0877), SR0150B	- Api Listeria (REF 10 300) - Catalase reaction

Table III.3: microbiology media and reagents

2.3. Other materials

All required microbiology materials such as petri dishes, pipettes, glassware sterile, incubators, pH meter (model Kent EIL 7045/46), refrigerators, water bath and incubators calibrated for the shelf life study were used from Sharjah Municipality Food Control Laboratory.

3. Methods

3.1. Sample collection

The RTE foods were freshly prepared inside the kitchen within the supermarkets and displayed on sale in refrigerated counters. Appropriate amounts of each RTE foods packed in their original containers (plastic plates with cover) were collected then transferred to the Sharjah Municipality Food Control Laboratory in sterile cooler bags padded with ice blocks. At the laboratory, the samples were aseptically divided into sterile stomacher bags and stored at three different temperatures (5, 25 and 40 °C). For 5 °C, the samples were analyzed at 0 h, 1, 2, 4 and 6 days, where as 25 and 40 °C samples were analyzed at 1, 2, 4 and 6 days. To check the quality of the samples, total bacterial count test was used, for hygiene testing *Escherichia coli* detection was done; finally for detecting microbial pathogens: *Salmonella* spp, *Staphyloccocus aureus* and *Listeria monocytogenes* detection were used. At the end, pH of all products was measured after microbial experiment to avoid cross contamination, and the below flow chart shows the procedure of the sampling (Figure III 1).

Three locations

tabbouleh   Greek salad   coleslaw   houmos

Sampling under aseptic technique

5 °C   25 °C   40 °C

0 h   1 day   2 days   4 days   6 days

Microbiology tests

Aerobic Plate Count

Escherichia coli

Staphyloccocus aureus ────────► pH measured

Salmonella spp

Listeria monocytogenes

pH measured at the end of the shelf life
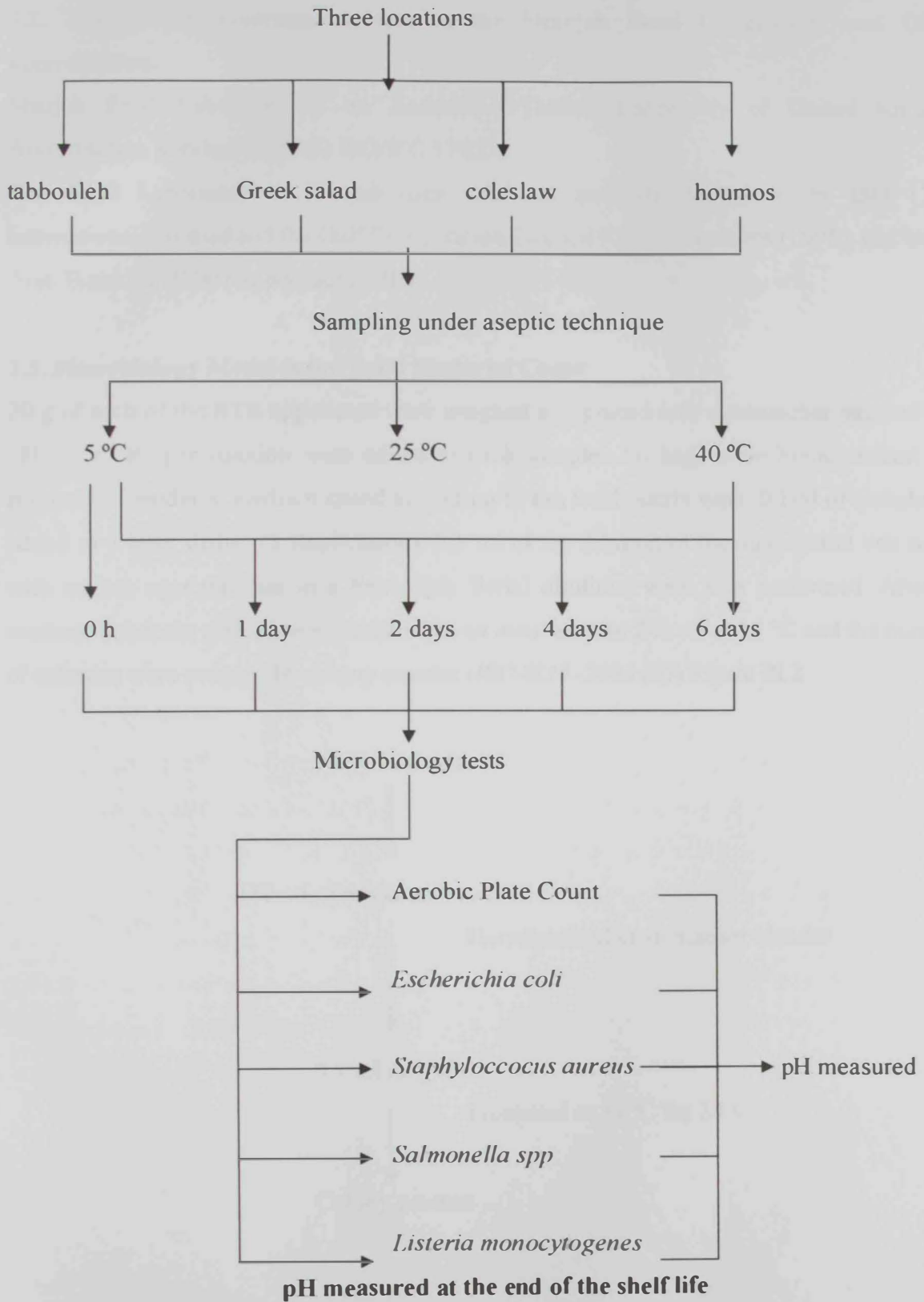
Figure III.1: the procedure of the sampling.

3.2. The quality assurance system in the Sharjah Food Laboratory and UKAS accreditation.

Sharjah Food Laboratory is an Accredited Testing Laboratory of United Kingdom Accreditation Services (UKAS) ISO/IEC 17025.

The Food Laboratory in Sharjah uses validated methods Accredited by ISO 17025 International standard and the Gulf Co-operation Council (GCC) Standards (1998), and United Arab Emirates, 2000 (Appendices VII.3).

3.3. Microbiology Methods for Total Bacterial Count

20 g of each of the RTE appetizers were weighed and placed into a stomacher bag and then 180 ml of Ringer solution were added to each sample. All bags were homogenized with stomacher blender at medium speed according to the food matrix type. 0.1 ml of sample was added to 3 Petri dishes (3 Replication). 0.1 ml of the dilution of the suspension was mixed with molten agar medium in a Petri dish. Serial dilutions were also performed. After the medium dried, the plates were incubated in an incubator for 24h ±2 at 37 °C and the numbers of colonies were counted by colony counter (*ISO 4833-2003 (E)*) Figure III.2.

20 g of sample

↓

180 ml of ringer solution

Homogenized in stomacher blander

↓

0.1 ml in SPCA

Incubated at 37 °C for 24 h

↓

Colony counter
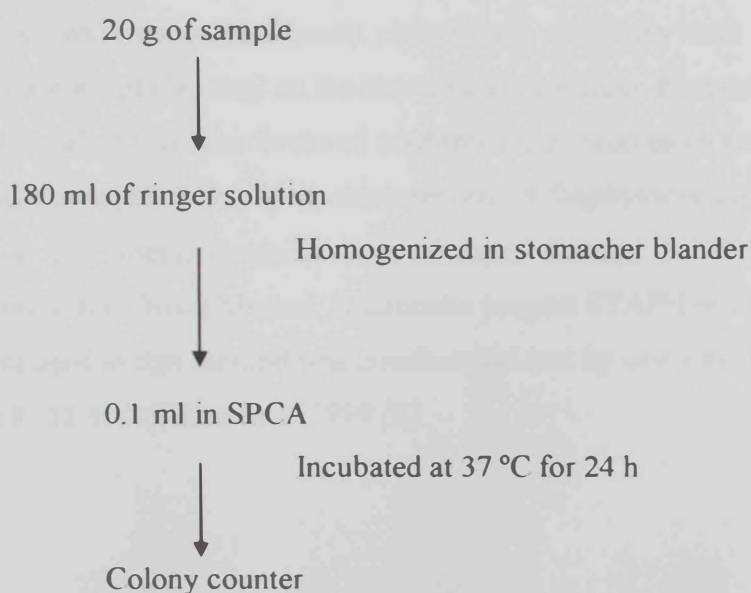
Figure III.2: Microbiology methods for total bacterial count

3.4. Microbiological Methods for isolation of *Staphylococcus aureus*

20 g of sample

180 ml of ringer solution

Homogenized in stomacher blander

0.1 ml in BPA

Incubated at 37 °C for 24 hr or 48 hr

Confirmation

(Staphylase test, Biochemical test)
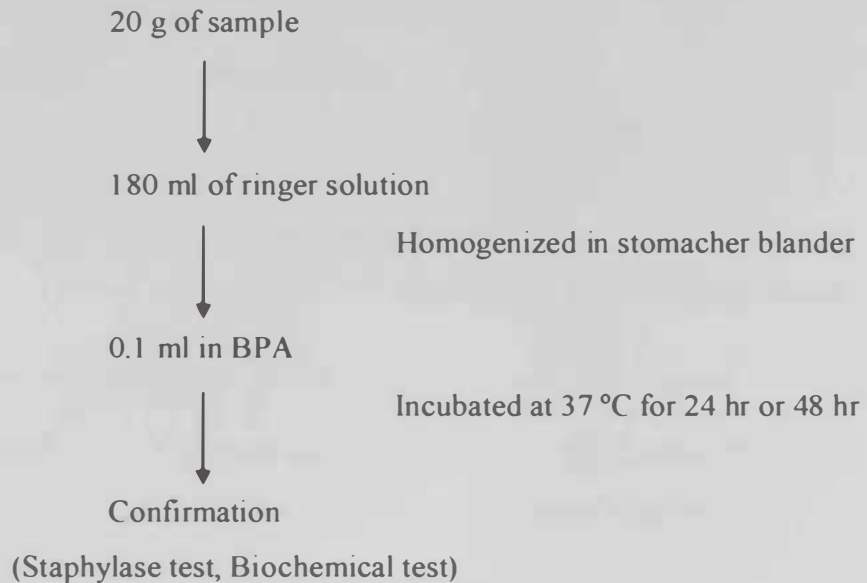
Figure III.3: Microbiology method of *Staphylococcus aureus*

The number of colonies were recorded of each plate (black and shiny with narrow white margins surrounded by a zone of clearing) on the Baird-Parker medium. Five colonies of each dilution were selected (or all if less than five) and confirmed the colonies by used Staphylase test which was generally accepted identifying characteristic of *Staphylococcus aureus* is the ability to produce free and bound coagulated (or clumped factor), the test method was followed the instruction in the Oxoid Manual, Diagnostic reagent STAPH test Kit DR 595A. Other confirmation test used in this method was Biochemical test by using the ID 32 STAPH from Bio Merieux, REF. 32 500 *(ISO6888-1:1999 (E)*.

3.5. Detection and enumeration of Coliforms / Faecal Coliforms in foods by most probably number (MPN).

20 g of sample

↓

180 ml of ringer solution

Homogenized in stomacher blender

↓

| 1 ml in 3* 10 ml of MacConky broth | 0.01 ml in 3 * 10 ml of MacConky broth | 0.001 ml in 3* 10 ml of MacConky broth l |

↓

Incubated at 37 °C for 24 h

Identification

↓

BGB   BGB   TW

↓

Incubated at 37 °C for 24 h

Incubated at 44 °C for 24 h
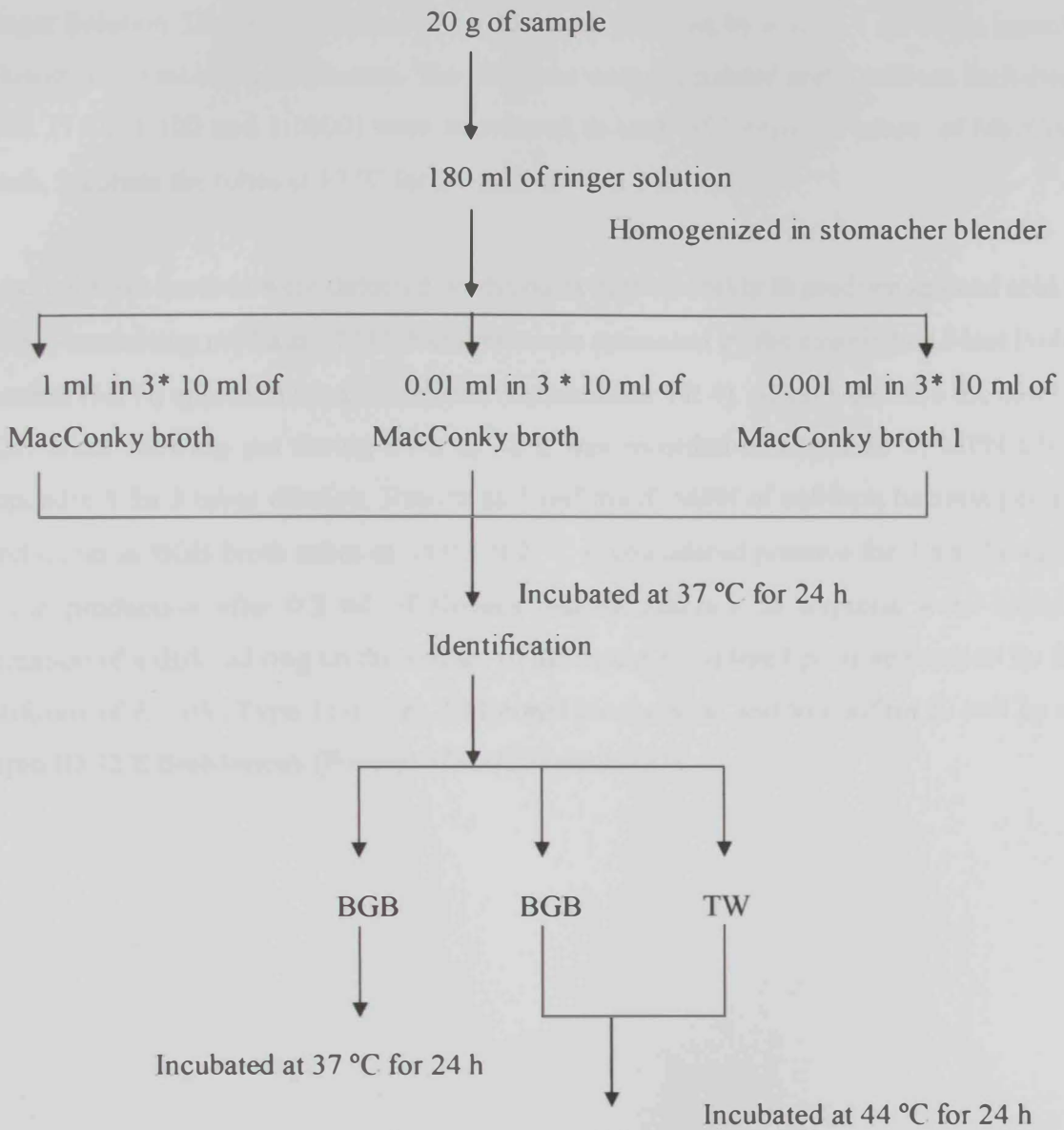
Confirmation

((Gas production, Kovace Reagent (Dark red ring, Gram staining), Biochemical test))

Figure III.4: Microbiology method of Coliforms / Faecal Coliforms

Samples were homogenized at a 1:10 dilution level. Decimal dilutions of 1:100 were prepared by adding 1 ml of the homogenated 1 ml of the dilution 1:10 of the liquid food , to 9 ml of Ringer Solution. Decimal dilutions of 1:1000 were prepared by adding 1 ml of the inoculated diluents into 9 ml of blank diluents. The dilutions were inoculated and 1 ml from each dilution level (1:10, 1:100 and 1:1000) were transferred to each of 3 separate tubes of MacConkey Broth. Incubate the tubes at 37 °C for 24 ± 2h or 48 ± 2 h.

Total coliform bacteria were detected on the basis of their ability to produce gas and acid from lactose-containing media at 37 °C. Numbers were estimated by the established Most Probable Number (MPN) approach used successive (Appendices VII.4). At each dilution the number of BGB tubes showing gas during 24 h or 48 h was recorded and referred to MPN tables in Appendix 3 for 3 tubes dilution. Results as "confirmed" MPN of coliform bacteria per g. Gas production in BGB broth tubes at 44.0 ± 0.2 °C is considered positive for Faecal coliforms. Indole production after 0.2 ml of Kovacs reagent addition to tryptone water tubes and formation of a dark red ring on the surface of the liquid considered positive reaction for faecal coliforms of *E. coli* (Type 1) origin. Additional biochemical test to confirm *E. coli* by using Rapid ID 32 E BioMerieux (France). (*ISO7251-2005 (E)*).

3.6. Detection of *Salmonella* spp. in foods

25 g of sample

↓

225 ml of BPW

Homogenized in stomacher blander
Incubated at 37 °C for 20 h

1 ml in 10 ml of MKTTn Broth   0.1 ml in 10 ml of RVS broth.

Incubated at 37 °C for 24 h   Incubated at 41.5 °C for 24 h

3 *M*m in XLD   3 *M*m in BGA   3 *M*m in XLD   3 *M*m in BGA

Incubated at 37 °C for 24 h

Confirmation

(Gram staining, Biochemical test, Serological test)
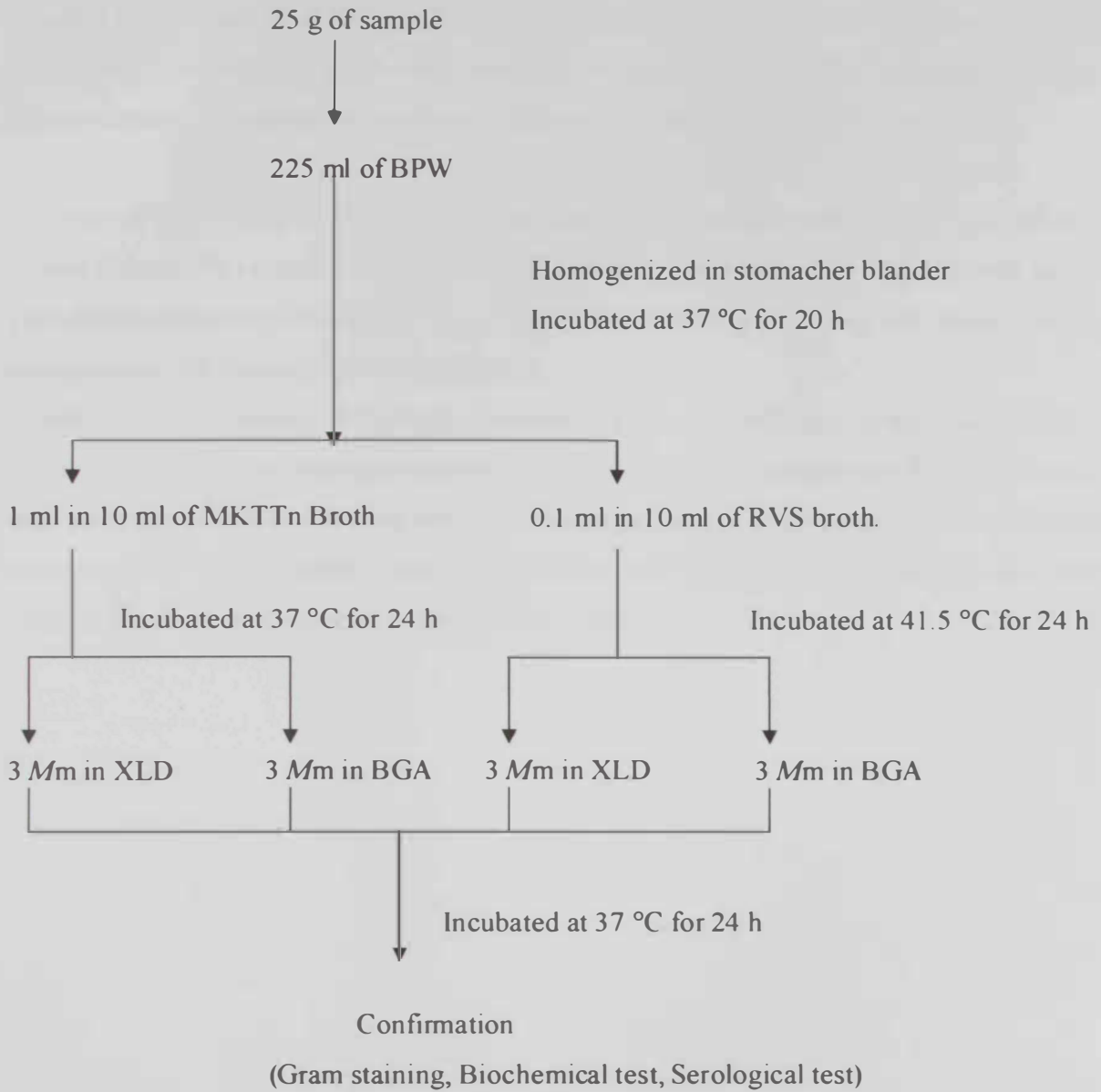
Figure III.5: Microbiology method of *Salmonella* spp

Selection and growth of suspect colonies

Figure 5 shown method of *Salmonella* spp after incubation 24 h, the BGA plate was examined for *Salmonella* colonies which were colorless to pink, surrounded by bright red medium; lactose-fermenting organisms (coliforms) form yellow-green colored colonies.

Colonies of *Salmonella* on XLD plates were append pink with or without black centers. 1- 5 typical *Salmonella* - suspect colonies were selected and purified by streaking onto pre-poured petri dishes containing SPCA medium and then incubated for 24 ± 3 h at 37 °C. Gram Staining was done and the Oxidase test was performed.

Confirmation of suspect *Salmonella* colonies from Standard Plate Count Agar (SPCA) medium carried out by serological test by used polyvalent O and polyvalent H antisera and by performed the identification used biochemical Rapid ID 32 E, BioMerieux (France). Results were reported as *Salmonella* Detected / Not Detected in 25g food after confirmation with biochemical & serological tests (*ISO6579-2002 (E)*).

3.7. Detection of *Lsiteria monocytogenes*

25 g of sample

↓

225 ml of Primary enrichment media

Homogenized in stomacher blender
Incubated at 30 °C for 24 h

0.1 ml in Oxford agar plate   0.1 ml in PALCAM agar

Incubated at 37 °C for 48 h

Confirmation

(Gram staining, Rapid ID 32 E, Serological test)

Or

Added 0.1 ml in secondary enrichment media

Incubated at 37 °C for 24 h

0.1 ml in Oxford agar plate   0.1 ml in PALCAM agar

Incubated at 37 °C for 24 h

Confirmation

(Gram staining, Catalase reaction, Biochemical test)

Figure III.6: Microbiology method of *Lsiteria monocytogenes*

Figure III.6 shown the method of *L. monocytogenes* and typical colonies of *Listeria* spp. grow on Oxford agar for 24 h were small grayish colonies surrounded by black halos and after 48 h colonies become darker and increased in size was incased. Otherwise, typical colonies of *Listeria* spp. grow on PALCAM agar for 24 h were small or very small grayish green and sometimes with black centers and black halos.

The gram stain was preformed on a colony isolated from Total Plate Count, *Listeria* spp were revealed as Gram-positive slim, short rods. In the Catalase reaction gas bubbles indicated a positive reaction and then interpretation of biochemical test by used api Listeria 10 300. (*ISO11290-1:1996 (E)*).

3.8. Measurement of pH

The pH was measured by using a pH meter (Kent EIL 7045/46) fitted with standard combination glass electrode. The pH meter was calibrated before determination by freshly prepared buffer solutions of pH 7.0 and pH 4.0. The diluted samples of salads were poured in a beaker and taken the reading of instrument.

4. Statistical analysis and software

Data mean, average, Standard deviation, Standard error and graphs were analyzed using Excel program.

IV. RESULTS AND DISCUSSIONS

4. Results & Discussions

1. Results

A total of 120 ready to eat foods samples were collected from 3 different locations of Sharjah supermarkets to evaluate the prevalence of the most common pathogen microorganisms (*Staphylococcus*, *E. coli*, *Salmonella* spp and *Listeria monocytogenes*) among the RTE foods.

1.1. Aerobic bacteria and prevalence of pathogenic microorganisms in ready-to-eat foods

High count of total aerobic bacteria was obtained and the results of series of biochemical tests in this study revealed that *E. coli*, were low in number; while, *Salmonella* species, *Listeria monocytogenes* and *Staphylococcus aureus* were absent in all products

The total aerobic plate count is shown in (Figure IV.7). The initial total plate count of ready to eat foods were significantly different ($p<0.05$). Coleslaw and hommos had significantly lower colonies 4.8 log colon forming unit (CFU/g) and 4.9 log CFU/g, than tabbouleh and Greek salad that had 5.08 log CFU/g and 5.09 log CFU/g respectively. Coleslaw and hommos were not significantly different from each other.

For *E.coli*, coleslaw had significantly lower numbers as per log MPN of 0.20/g than Greek salad, hommos and tabbouleh. The numbers of bacteria per gram for Greek salad, hommos and tabbouleh were as per log (MPN/g) of 0.44, 0.50 and 0.68 respectively. However, there was no significant difference between hommos and Greek salad. The *E.* coli count is shown in Figure IV.8.
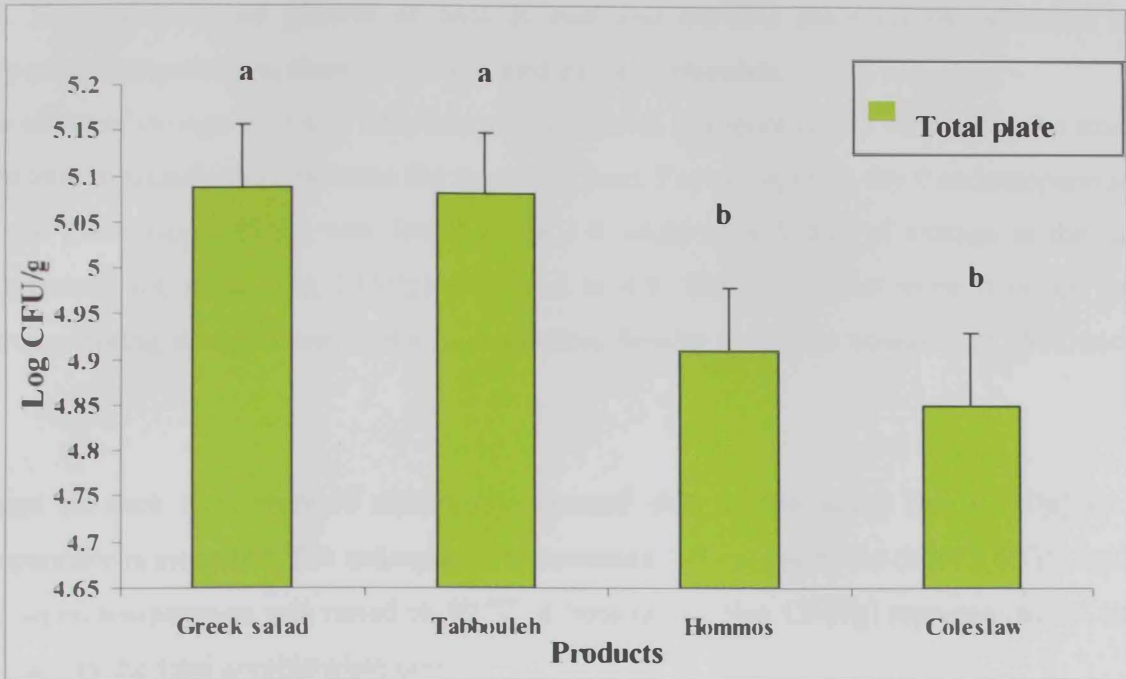
Figure IV.7: Microbial loads in ready to eat foods (columns with similar letters are not significantly different)
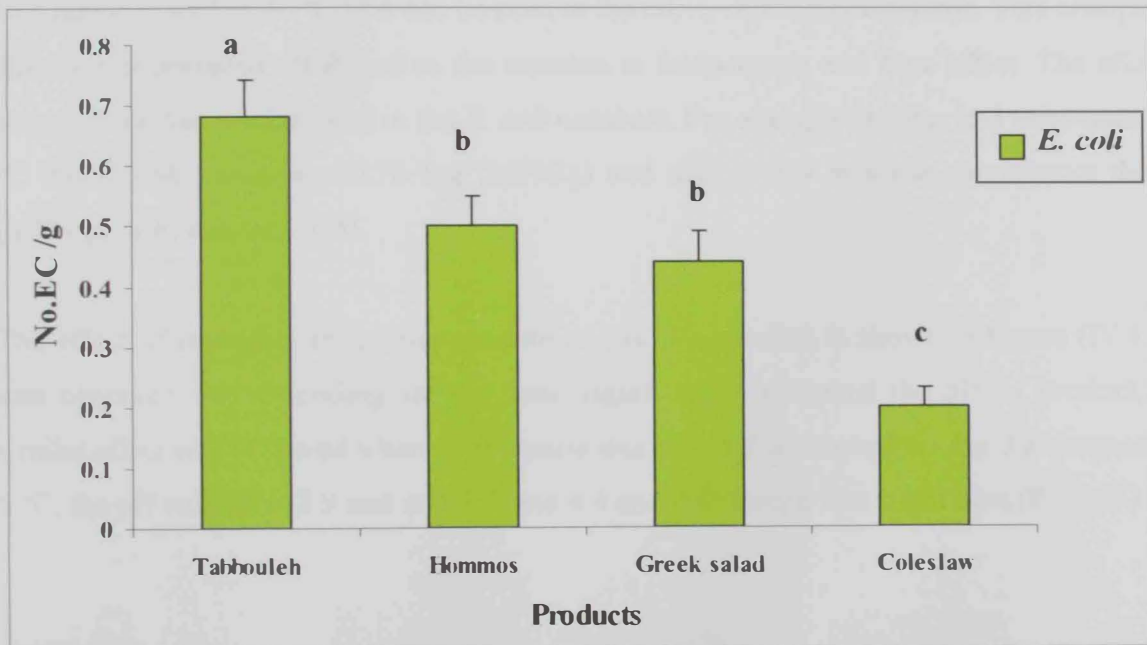
Figure IV.8: Prevalence of E. coli in ready to eat foods (columns with similar letters are not significantly different)

1.2. The survival and growth of both *E. coli* and aerobic plate microorganisms with respect to temperature, time of storage and pH in tabbouleh.

The effect of storage time and temperatures are shown in Figure (IV.9). Extending the storage time tend to significantly increase the microbial load. For example, at day 0 and temperature 5 °C the mean (log CFU/g) was found to be 3.8 while after 6 day of storage at the same temperature the mean (log CFU/g) increased to 4.9. This represents more than ten times increase during storage times under investigation. Similar trend was observed at 25 °C and 40 °C.

It can be seen that, there is remarkable upward shift in the mean (log CFU/g) as the temperature is increased. For example, at temperature 5 °C at day 6, the (log CFU/G)/ was 4.6 and when temperature was raised to 40 °C, it became 6.1 (log CFU/g) representing 15 times increase in the total aerobic plate count.

The effect of storage time and temperature on *E. coli* growth is given in Figure (IV.10). Unlike the case in total plate count, *E. coli* count had significantly decreased when the temperature was raised from 5 to 40 °C as it can be seen in the curve shifting downwards. This unexpected decrease is primarily attributed to the increase in temperature and time effect. The effect of storage time has similar trend in the *E. coli* numbers. For example at 0 day and temperature 40 °C the *E. coli* count was 0.78 log (MPN/g) and after 6 day at same temperature the log (MPN/g) of *E.* coli was 0.20.

The effect of storage time and temperature on pH of tabbouleh is shown in Figure (IV.11). It was observed that extending storage time significantly increased the pH of product, and similar effect was observed when temperature was raised. For example at day 0 at temperature 5 °C, the pH value was 3.9 and at day 6 was 4.4 and the change was significant (P< 0.05).

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Figure IV.9: The effect of temperature and time of storage on the aerobic plate count in tabbouleh
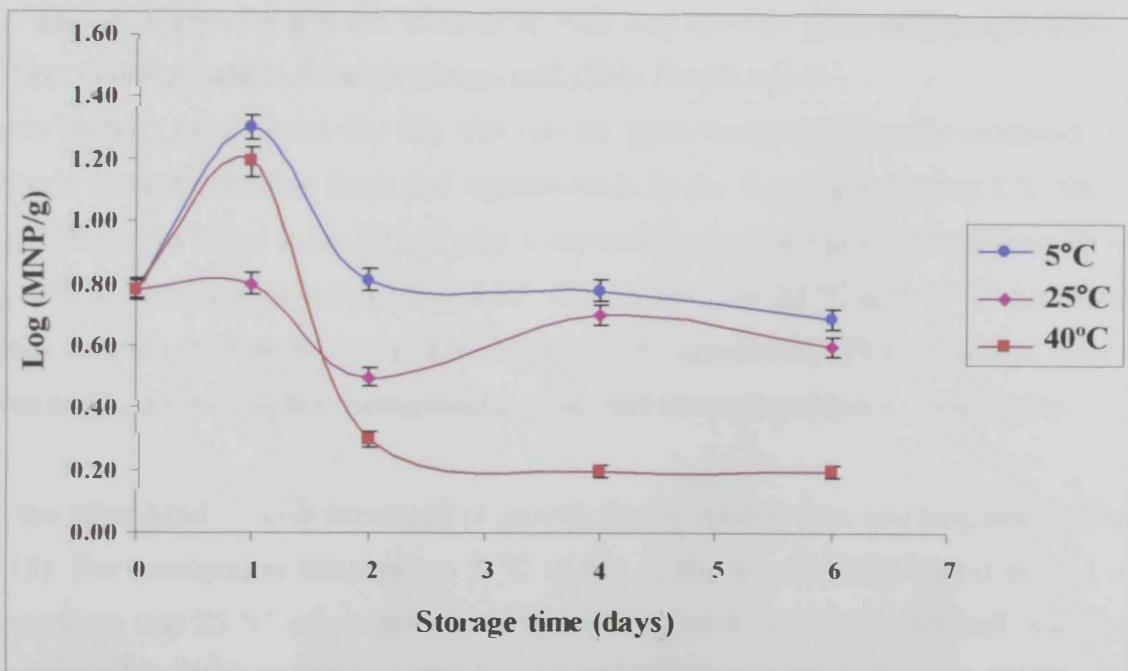
Figure IV.10: The effect of temperature and time of storage on the growth and survival of *E. coli* in tabbouleh

Figure IV.11: The effect of temperature and time of storage on pH of tabbouleh.

1.3. The survival and growth of both *E. coli* and aerobic plate microorganisms with respect to temperature, time of storage and pH in Greek salad.
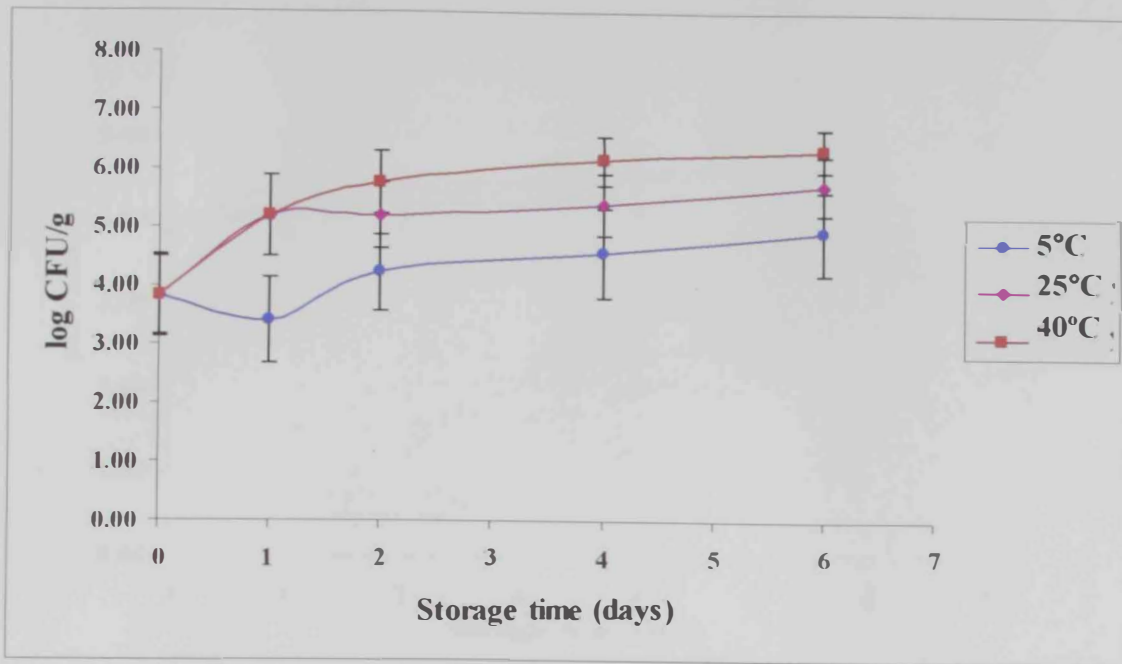
It can be seen from Figure (IV.12), that aerobic plate count significantly increased during different storage condition (time and temperature). At day 0 and temperature 5 °C the mean (log CFU/g) was found to be 3.8 and after 6 days of storage at the same temperature the mean log colony forming unit increased to 4.07. Also in day 6 at 25 °C and 40 °C the mean log colony forming units were 5.80 and 6.20 log CFU/g, respectively. This represents more than seven and four times higher during storage times and temperature under investigation.

On the other hand, *E. coli* decreased in growth during storage time and temperature (Figure IV.13). For example, at temperature 5 °C at day 1, the log (MPN/g) was 0.40 and when temperature was 25 °C it becomes 0.75 log (MPN/g) at 6 day the number of bacteria was decreased (from 0.40 to 0.20 Log (MPN/g) at 5 °C and from 0.75 to 0.45 log (MPN/g) at 25 °C), and similar trend was observed at temperature 40 °C.

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The last figure is Figure IV.14 shows the pH of Greek salad. It was slightly increased of pH of Greek salad taken at the different storage conditions. For example, at 0 day and temperature 5 °C, the pH values was 4.80 and at day 6 was 5.16 and the change was significant (P<0.05).

Figure IV.12: The effect of temperature and time of storage on the count of aerobic plate count in Greek salad
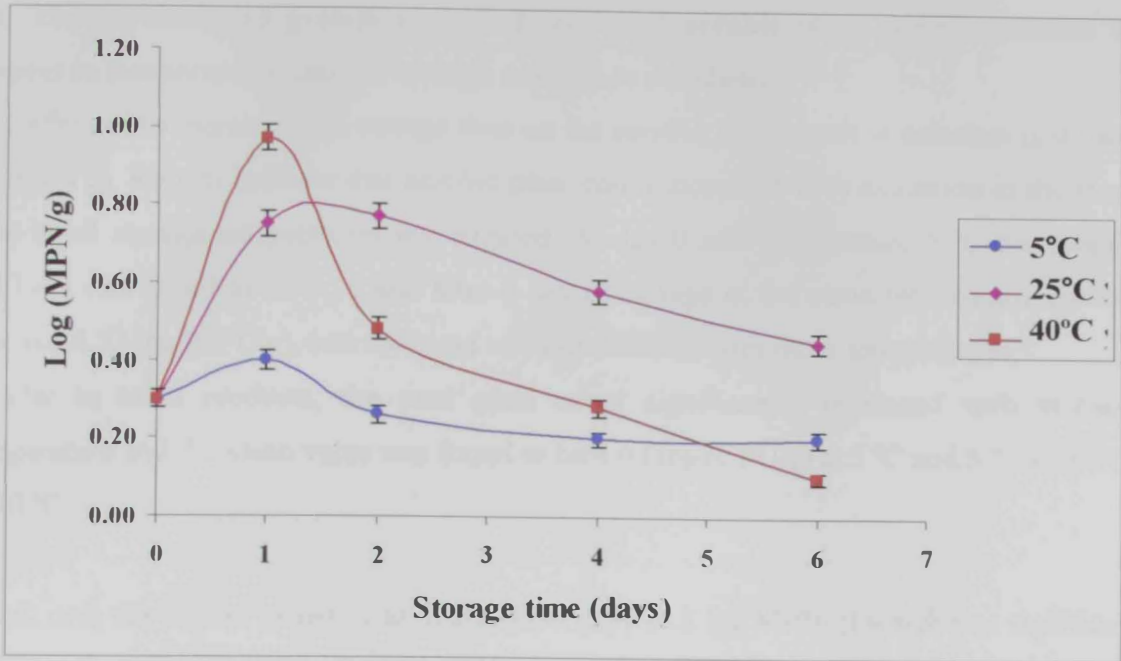
Figure IV.13:. The effect of temperature and time of storage on the growth and survival of *E. coli* in Greek salad
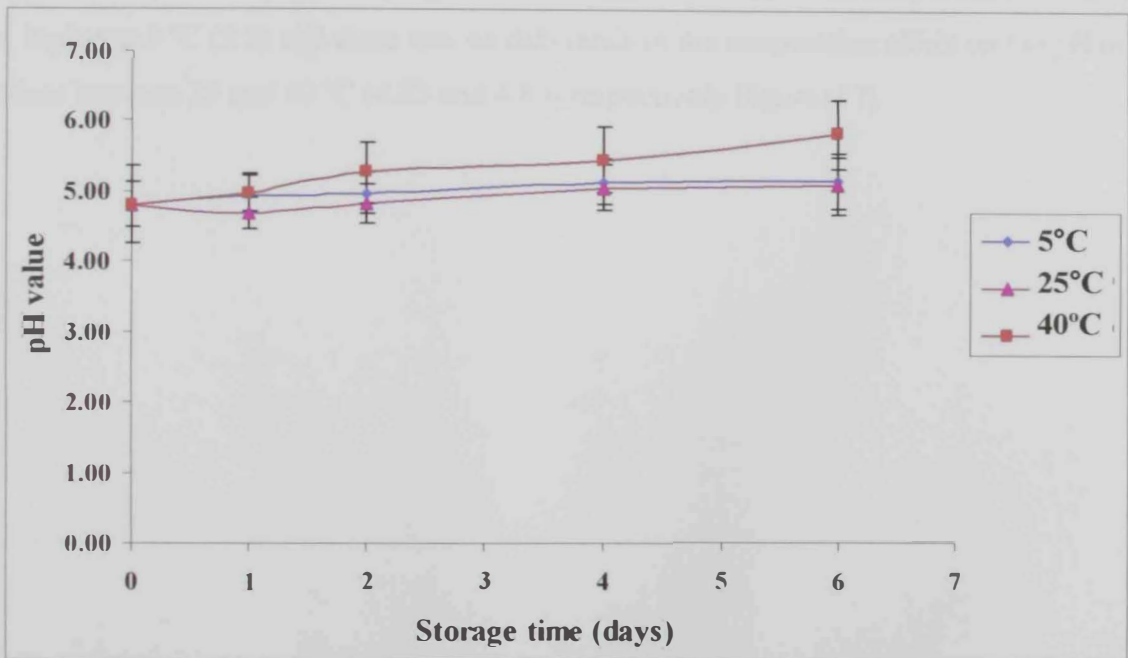
Figure IV.14: The effect of temperature and time of storage on pH of Greek salad.

1.4. The survival and growth of both *E. coli* and aerobic plate microorganisms with respect to temperature, time of storage and pH in coleslaw.

The effect of temperature and storage time on the aerobic plate count in coleslaw is shown in (Figure 15). Results indicate that aerobic plate count increased with extension in the storage time at all storage temperatures investigated. At day 0 and temperature 5 °C the mean log (CFU/g) was found to be 3.16 and after 6 day of storage at the same temperature the value reached 4.73 log (CFU/g), and the trend was not different with other temperatures.

Similar to other products, the total plate count significantly increased with increasing temperature and the mean value was found to be 4.04 log (CFU/g) at 5 °C and 5.7 log (CFU/g) at 40 °C

For *E. coil*, the highest growth was observed in day1 (0.2 log MPN/g) which was significantly different from day (0.12 log (MPN/g). There was no significant difference between day 1, 2 and 4.in the growth of *E. coli*. With respect to temperature the effect was not significant, but the trend indicates that higher temperature has killing effect on *E. coli* (Figure 16).

The pH of the product (coleslaw) registered slight difference between temperatures. The value was higher at 5 °C (5.2) and there was no difference in the temperature effect on the pH of the product between 25 and 40 °C (4.85 and 4.89) respectively Figure (17).
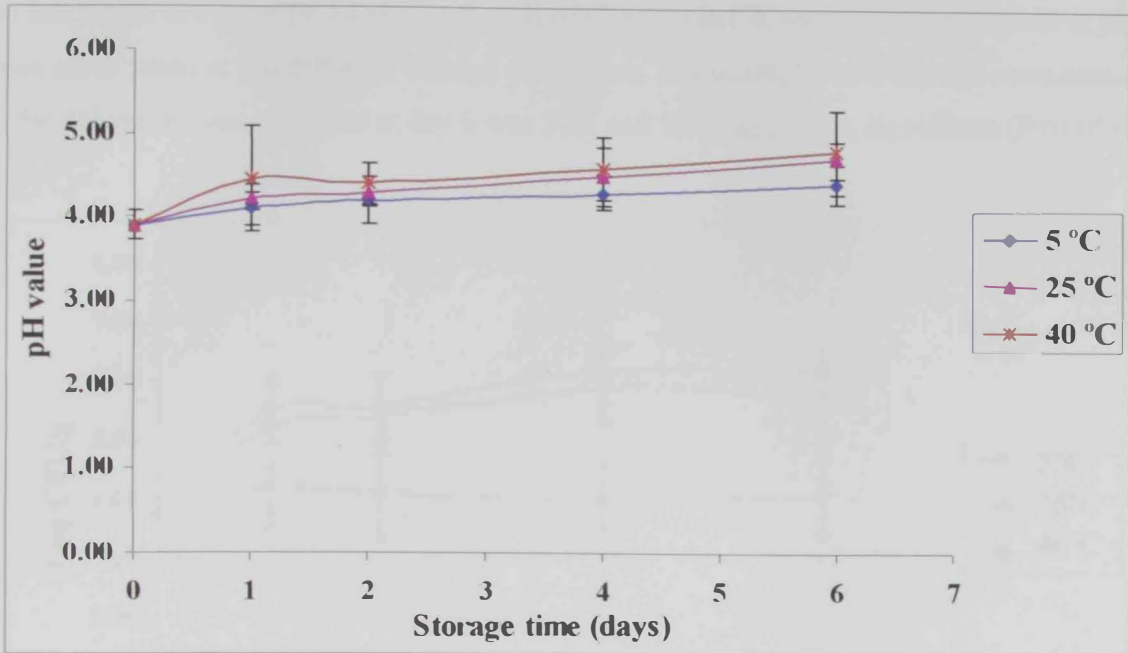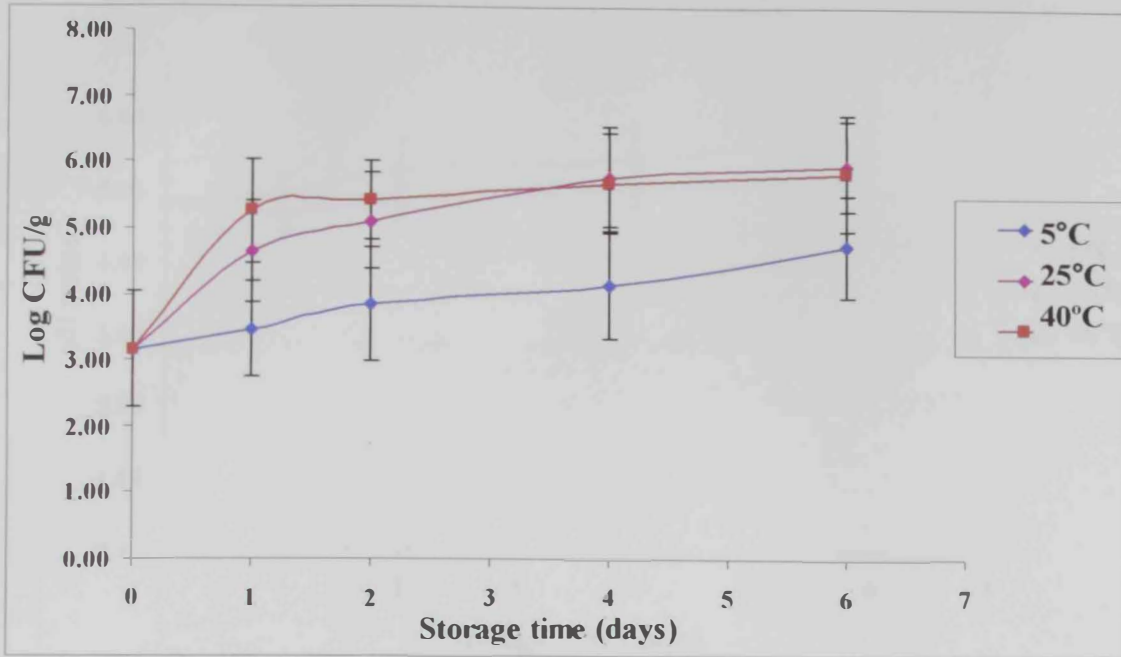
Figure IV.15: The effect of temperature and time of storage on the count of aerobic plate count in coleslaw
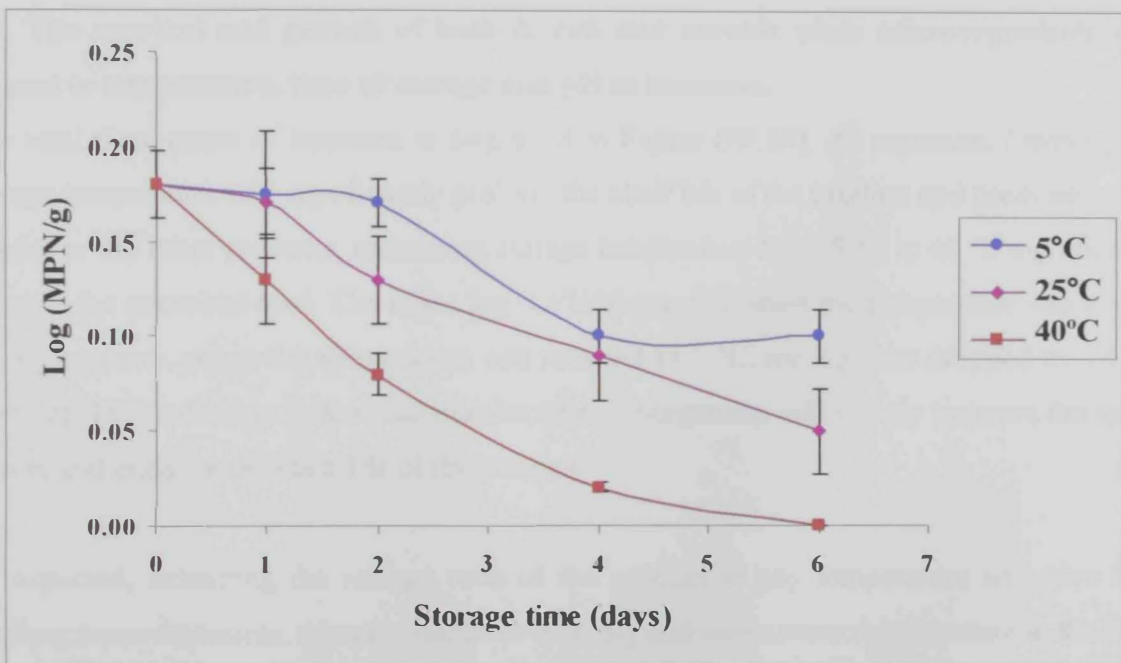
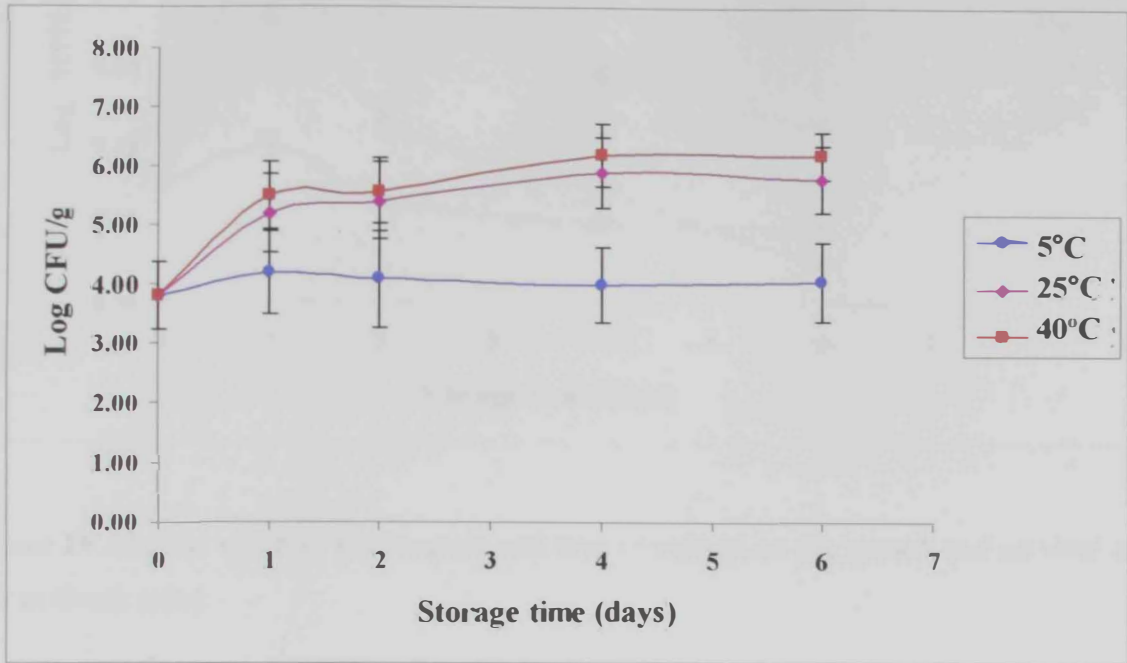**Figure IV.16:** The effect of temperature and time of storage on the count of *E. coli* in coleslaw
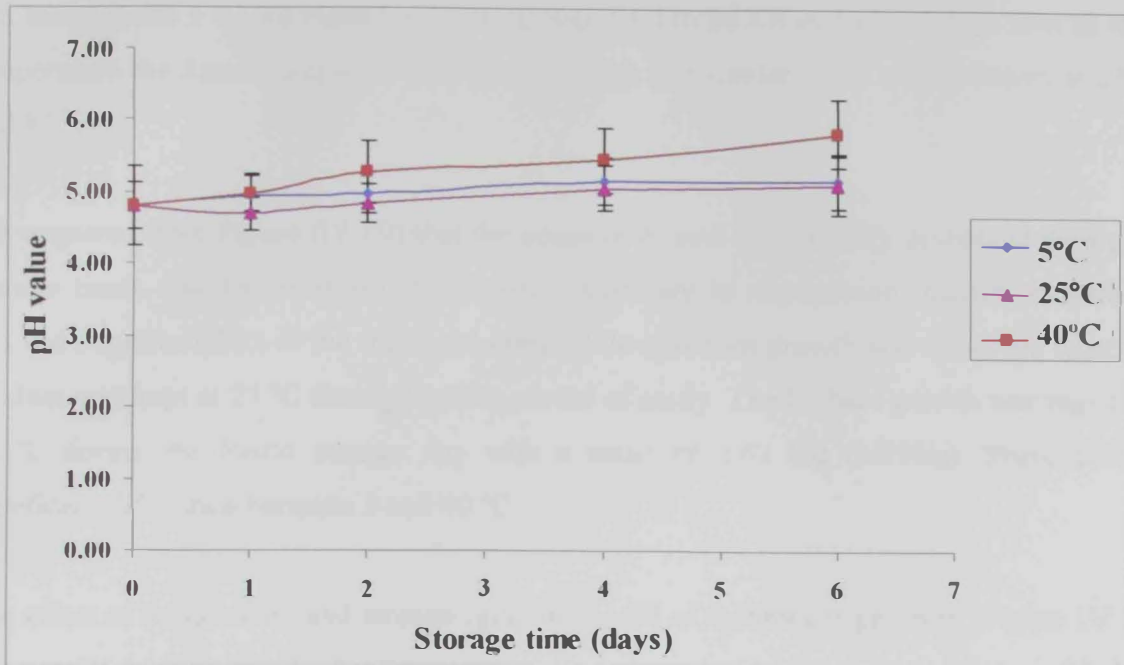
Figure IV.17: The effect of temperature and time of storage on pH of coleslaw

1.5. The survival and growth of both *E. coli* and aerobic plate microorganisms with respect to temperature, time of storage and pH in hommos.

The total plate count of hommos is displayed in Figure (IV.18). As expected, lowering the storage temperature will significantly prolong the shelf life of the product and promote safety. Similar to the other products, increasing storage temperature from 5 °C to 40 °C significantly increase the microbial load. The mean log (CFU/g) was 5.3 when the temperature was kept at 40 °C, however, when the temperature was reduced to 5 °C, the number dropped to 4.4 log (CFU/g). This reduction in the total number of microorganism will greatly improve the safety quality and enhance the shelf life of the product.

As expected, extending the storage time of the product at any temperature will give food spoiling microorganisms, a better chance to multiply and cause more deterioration in the food. This negative effect can be easily observed when the microbial population of the fresh product is compared with the load six days after at all investigated temperatures. For example at day 0

and temperature 5 °C the mean log (CFU/g) was fond to be 4.8 and after 6 days later at same temperature the figure jumped to 6.01 log (CFU/g) and similar trend was observed at 25 °C and 40 °C.

It is apparent from Figure (IV.19) that the count of *E. coli* significantly decreased during the storage times and temperatures investigated. Contrary to expectation, higher temperature showed negative effect in the mean colonies while optimum growth was observed when the product was kept at 25 °C throughout the period of study. The highest growth was register at 25 °C during the fourth storage day with a mean of 0.67 log (MPN/g). There was no significant difference between 5 and 40 °C.

The effect of temperature and storage time on the pH of hommos is given in (Figure IV 20). The results suggest that higher temperature may promote certain reactions that are likely to lower the acidity of the food which may result into negative consequences in the safety and stability of the food. There was no significant difference between 5 and 25 °C at ($P<0.05$)
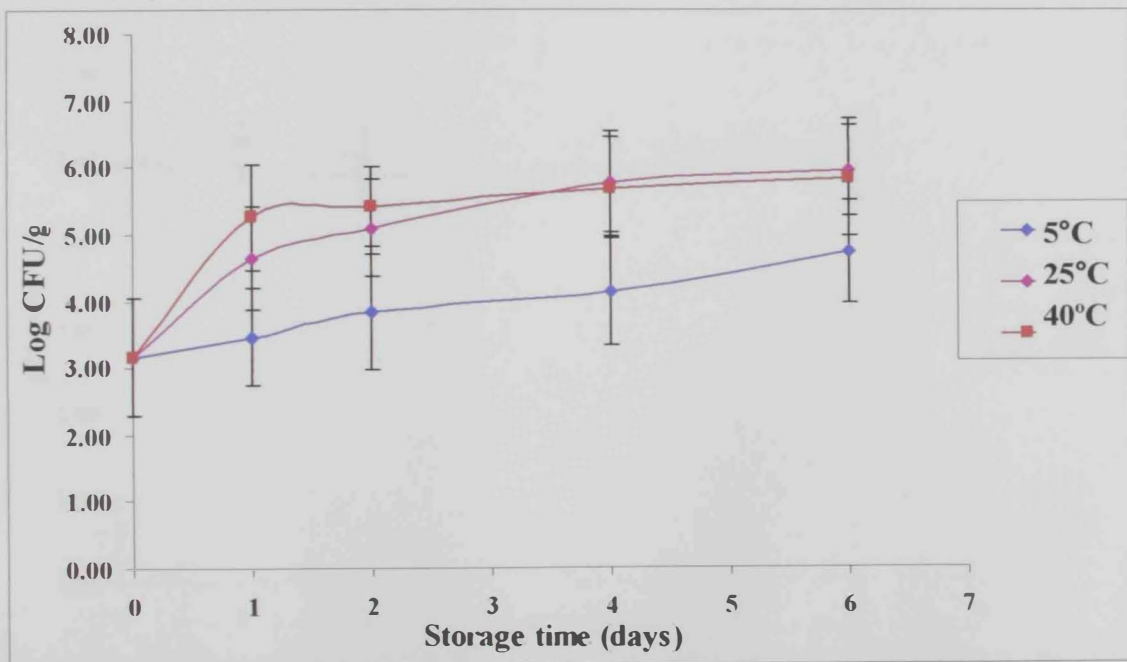
Figure IV.18: The effect of temperature and time of storage on the count of aerobic plate count in hommos
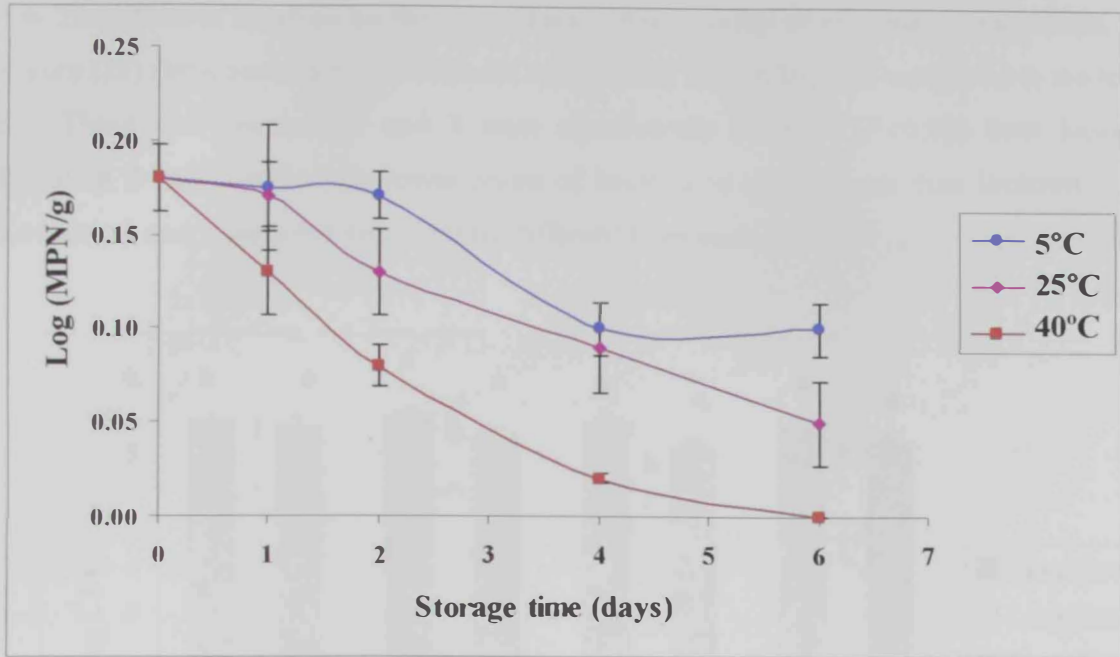
Figure IV.19: The effect of temperature and time of storage on the count of *E. coli* on hommos
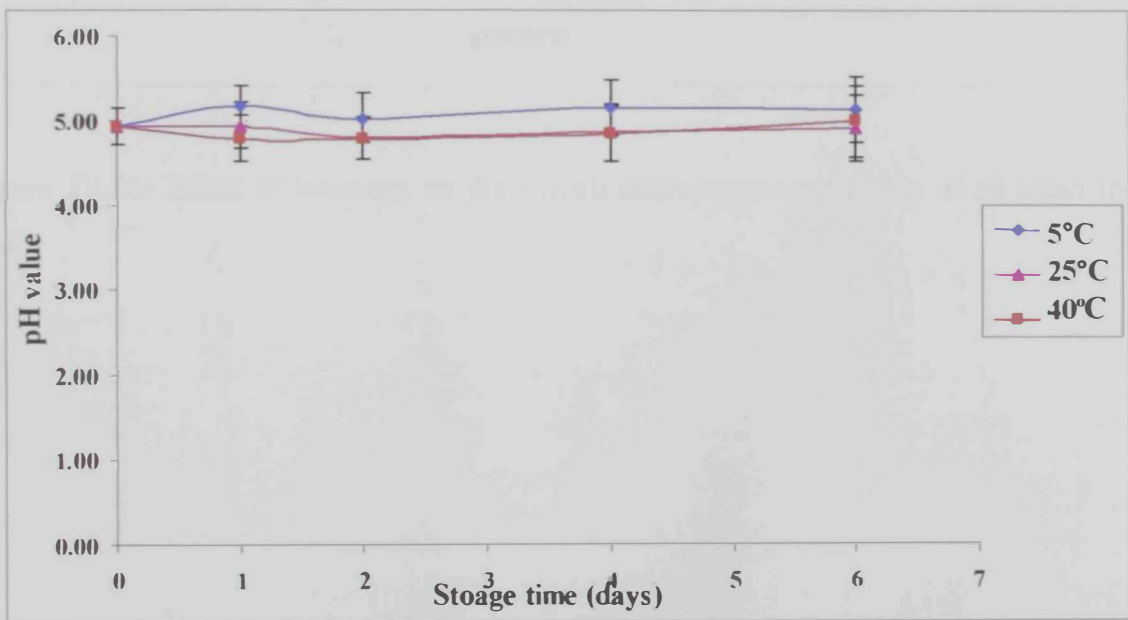
Figure IV.20: The effect of temperature and time of storage on pH of hommos

1.6. The effect of location on the overall microbial quality of all ready to eat foods.

Figure (21) demonstrates that locations (1 and 3) had lack in hygiene compared to the location (2). These two locations 1 and 3 were significantly different (P<0.05) from location 2. Location 2 had significantly lower count of bacteria in all products than location 1 and 3. Location 1 and 3 were not significantly different from each other.
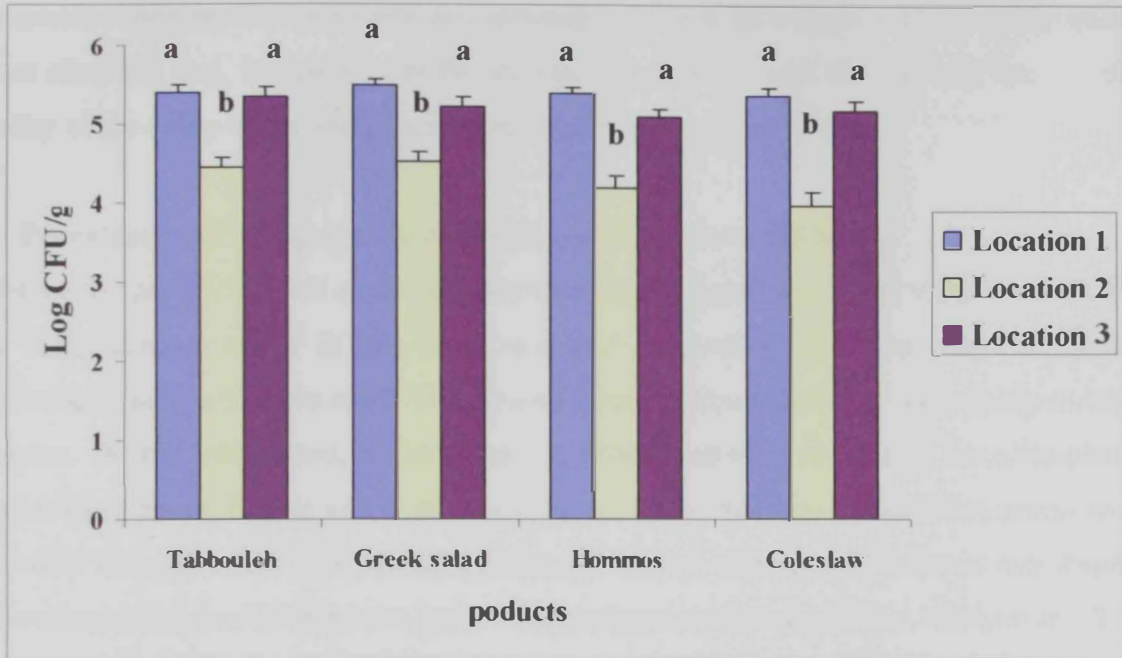
Figure IV.21: Effect of locations on the overall microorganisms quality of all ready to eat foods

2. Discussion

As it was mentioned previously, the UAE standards for read-to-eat foods allows a "one day" shelf-life and the products are to be kept refrigerated under 5 °C; but during the sampling, the products indicated 4 days shelf-life (best before the manufacturing date) supposedly refrigerated at 5 °C in open-counter. However, there is no indicator of the temperature in refrigerator counter. Consequently this practice may lead to a high number of the bacteria count after one day, irrespective of the storage temperature, and this affected the microbial quality of the ready-to-eat foods samples evaluated during this study.

2.1. Prevalence of Pathogenic microorganisms in ready-to-eat foods

Salmonella species, *Listeria monocytogenes* and *Staphylococcus aureus* were not detected in any RTE samples tested in this study and this observation was also reported by other researchers such as Soriano *et al.*, (2000) who observed the absence of *Salmonella* in lettuce samples. On the other hand, Meldrum *et al.*, (2006) reported high level contamination of aerobic plate count, *E. coli*, and *S. aureus* which could be due to poor sanitation practices *and B. cereus* in ready to eat food but no detection of *Salmonella* spp and *Listeria* spp; there are other reasons that could inhibit the growth of pathogenic microorganisms. Rauha et al., (2000) reported that in nature there are large numbers of different types of antimicrobial compounds that play an important role in the natural defense of all kind of living organisms. The possible presence of antimicrobial compounds like allicins – released during the cutting of the onion which is one of the ingredients in certain ready-to-eat foods could inhibit the growth of pathogenic microorganisms (Cowan, 1999). Also, Yucel and Karapinar, (2005), found that vinegar and the lemon juice containing acetic and citric acids which are naturally used as flavoring and acidifying liquids for vegetables salads could be considered as an alternative disinfectant which removes or at least reduces pathogens causing no health risk to consumers. Gillian (1999) reported, that fewer than 4 °C mesophilic pathogens are unable to multiply, also the minimum growth temperature of *Salmonella* was 2 °C as reported by ICMSF (1996). We did not measure any antimicrobial activities during this study; however, the above published papers could be one of the reasons for not detecting the most common outbreak pathogenic microorganisms in the ready-to-eat salads sampled during this study.

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2.2. Tabbouleh

In this study, the counts of *E. coli* were high. tabbouleh salads are made of leafy vegetables and herbs particularly parsley. Johnston *et al*., (2005) found that those vegetables contain high level of aerobic plate count and *E. coli*.

This study showed that, during the storage at different temperatures and during the shelf life, the counts of *E. coli* decreased. Naimi *et al*., (2003) reported similar results and found that holding of large quantities of parsley at room temperature increased the risk of sporadic low level of *E. coli*. This study showed that the *E. coli* counts under low temperature decreased over time which could be an indication of survival and adaptation to the storage conditions. This had been proven by Menlove (2002) and Seo *et al*., (1999) where *E. coli* decreased in number when the produce was stored at 4-5 °C with a pH less than 4.4.

The pH of tabbouleh salads slightly increased from 3.9 to 4.8 during the storage irrespective of the storage temperature. Prescott et al., (2002) found some of microorganisms made their environment more alkaline by generating ammonia though amino acid degradation.

2.3. Greek salad

In this product, the total bacteria count at 25 and 40 °C increased significantly during the shelf life. The count at 5 °C was steady during the storage. Petram (2005) reported, that the aerobic plate count for leafy vegetables and salads could be high but low for fruit vegetables and salad such as tomatoes, peppers and aubergines due to the cross contamination. Many species of microorganisms such as bacteria, yeast and mold can grow between optimal temperatures (20-30 °C); and in general the count of microorganisms is stable at 5 °C as observed by Prescott *et al*., (2002). This could be an explanation of the results observed in this study for the survival of aerobic plate count in Greek salad.

The growth of *E. coli* in this study decreased when stored at low temperatures, this was in agreement with the findings of Skandamis & Fand Nychas, (2000), where the pH, the storage temperature and some essential oils reduced the count of *E. coli* at different storage temperature (0, 5, 15 °C) and pH adjusted to 4, 4.5 or 5. Seo & Frank, (1990) reported that the survival and growth of pathogens (*E. coli*) on lettuce decreased in number when the produce was stored at 4-5 °C. In addition, Fang *et al*., (2001) reported, *E. coli* was high at 18 °C in

ready to eat food samples. Moreover, Prescott *et al.*, (2002), documented that *E. coli* species could produce bacteriocin at high temperature 40 °C which will eventually inhibit the growth of Enterobacters.

2.4. Coleslaw

In coleslaw, the total bacteria count increased during the storage time and temperature. Coleslaw salads are made of made shredded cabbage and mayonnaise. Man & Jones (1994) reported that the complexity and rate of growth of the associative microflora found in mayonnaise based salads were related to the concentration of the various organic acids in the dressings and its pH. These particular conditions in mayonnaise base salads such as coleslaw would affect the decreased of *E. coli* (Brocklehurst *et al.*, 1987). These authors also observed the absence of *E. coli* and *Staphylococcus aureus* in coleslaw at 7 °C of storage temperatures and a pH range of 4.6 to 5.1 but the presence of *Pseudomonas* spp, Enterobacteriaceae and yeast. This study showed a relatively stable pH of coleslaw salads between 4.9 and 5.1 irrespective of the temperature of storage. In general, vegetable contains a wide range of gram-positive and Gram-negative bacteria such as Enterobacteriaceae and *Streotococceae*, *Pseudomonas* spp, lactic acid bacteria and yeast (Manvell & Ackland,1986; Brockehurst, 1987). Gary *et al.*, (1990) found that these microorganisms were derived mainly from the naturally associated microflora of the plant tissues, in addition to the processing equipment (shredders and slicers) that could give rise to the subsequent contamination of processed vegetables.

2.5. Hommos

In the hommos salads, the bacteria count was the lowest count when compared to other RTE; however, the count increased was significantly over time particularly when the products were stored at 25 and 40 °C, but *E. coli* significantly deceased at all of the storage temperatures. In a study done by Gohil *et al.*, (1996) fecal coliforms were isolated from 22 samples of hommos. Klaenhammer (1988); Harris *et al*, (1989) and Arihara (1993), found that, some microflora in vegetables produced antimicrobial compounds such as antibiotics and bacteriocins that could inhibit the survival of certain microorganisms. Another factor that could exercise a bactericidal effect was the presence of olive oil and citric acid in hommos

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according to Gohil *et al.*, (1996), where in their study without olive oil, the bacteria count of the stored hommos showed a slight increase in number at 4 °C over a period of 3 days incubation but at 10 °C the count remained stable, whereas at 20 °C and 40 °C the count declined significantly after one and half day.

The hommos samples in this study experienced dryness when the temperature and storage time increased; this latter situation caused a decline in *E. coli* numbers. This observation supported the results of Menlove (2002) who noted that *E. coli* growth was inhibited by fairly low a_w < 0.95 in ready to eat foods. Also, Robinson (2002) showed that, *E. coli* can grow between a_w 0.97 to 0.99 at 22.5 °C in foods. On the other hand, McEldowney & Fletcher (1988) and Hirai (1991) showed that dryness exerted a significant bactericidal effect.

2.6. The effect of locations on the overall microorganism's quality and safety of all salad products.

Location 1 and 3 were poor in hygiene compared to the location 2 due to huge load of bacteria count in the finished products, averaging 5.5 Log CFU/g for location 1 and 3 and 4.5 Log CFU/g for location 2. These three locations used the same raw ingredients to prepare the salads. During this study, poor manufacturing practices were observed when preparing the RTE salads. Kaneko *et al*, (1999) conducted a study on the bacterial contamination in the environment of food factories processing ready-to-eat fresh vegetables and found heavy contamination of aerobic plate count isolated from interior surfaces of equipment of washing, slicing, dehydrating, and blending. It was also reported that the surface of blades for slicing and the floor surfaces of operation rooms, harbored various type of microorganisms which could compromise the safety of the products; also *E. coli* strains were detected in the surface of the operation room floors and employees gloves. In this study, all locations prepared the salads in house. The fact that all kitchen surfaces and processing areas might exhibit some degree of bacterial contamination illustrates the potential risk of bacteria being transferred to food, and two items that are consistently contaminated are the working surfaces, the chopping boards and knives that might be used for slicing vegetables (Komirose *et al.*, 1972, Bloomfield and Scott, 2003)

V. Conclusion

5. Conclusion

The aims of this study were to evaluate and to assess the prevalence of the most common pathogen microorganisms (*Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Staphylococcus aureus*) among selected RTE foods based on the effect of temperature and time of storage. The growth and survival of total bacteria were also assessed during the shelf life study at different temperatures of storage. In addition the locations of the preparation of the RTE foods were also evaluated in relation to the hygiene and source of the contamination of the RTE foods. In this study, we found that when the temperatures increase, the total plate count increases for all the products studied. Prolonging the storage time increased the total plate count for all the products studies. Also the pH increases within the limit of pathogenic microorganism's growth rate.

Salmonella, *Listeria monocytogenes* and *Staphylococcus aureus* were not detected in any of the products studied. However, *E. coli* counts decreased in all products studied during the storage periods at different temperatures (5, 25 and 40 °C).

There is strong relationship between the factors studied were temperature, time, and location and the growth of both total bacteria and *E. coli*.

The results showed that the way of preparation of the food items and processing could give you an indication of quantity and safety of the microorganisms present in tabbouleh, hommos, Greek salad and coleslaw. In fact, in this study, there were some observations that some fridges were not operating at cold temperature to keep food safe. Also, most of the employees who worked at the supermarket stores have no qualification in food handling and preparation. From the microbiological results of all products studied, and after one day of storage the high count of total bacteria and high count of *E. coli* exceeded the maximum acceptable standards. Consequently the shelf life of ready to eat foods should not exceed one day, according to UAE General Secretariat of Municipalities (No.8/2003). Based on the data from the study and the food preparation handling at the three locations in Sharjah Supermarket, it is recommended that HACCP should be implemented in all locations studied.

VI. REFERENCES

6. REFERENCES

AbdulRaouf, U. M., Beuchat, L. R. & Ammar, M. S. (1993). Survival and growth of *E. coli* 0157: H7 on salad vegetables. *Applied and Environmental Microbiology*, **59**, 1999-2006.

Adams, M. R., Hartley, A. D. & Cox, L. J. (1989). Factors affecting the efficacy of washing procedures used in the production of prepared salads. *Food Microbiology*, **6**, 69-77.

Al-Ghaza;I, M. R. & Al-Azawi, S. K. (1990). *Listeria monocytogens* contamination of crops grown on soil treated with sewage sludge cake. *Journal of Applied Bacteriology*, **69**, 642-647.

Arihara, K., Cassens, R. G. & Luchansky, J. B. (1993). Characterization of bacteriocins from *Enterococcus Faecium* with activity against *Listeria monocytogenes*. *International Journal of Food Microbiology*, **19**, 123-134.

Arumugaswamy, R. K., Rusul Rahamat Ali, G. & Nadzriah Bte Abd. Hamid, S. (1994). Prevalence of Listeria monocytogens in food in Malaysia. International Journal of Food Microbiology, **23**, 117-121.

Beckers, H. J., In't Veld, P. H., Soentoro, P. S. S. & Delfgou-van Ash, E. H. M. (1989). The occurrence of *Listeria* in foods. In: *Foodborne Listeiosis, Proceedings of a Symposium on September 7*, 1988. 83-. 97 Wiesbaden, Hamburg, FRG.

Bennik, M. H. J., Peppelenbos, H. W., Nguyen-the, C., Carlin, F., Smid, E. J. & Gorris, L. G. M. (1996). Microbiology of minimally processed, modified atmosphere packaged chicory endive. *Postharvest Biology and Technology*, **9**, 209-221.

Berrang, M. E., Brackett, R. E. & Beuchett, L. R. (1989). Growth of *Listeria monocytogenes* on fresh vegetables stored under controlled atmosphere. *Journal of Food Protection*, **52**, 702-705.

Beuchat, L. R. & Brackett, R. E. (1990). Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. *Journal of Food Science*, **55**, 755-758.

Beuchat, L. R. & Brackett, R. E. (1991). Behaviour of Listeria monocytogenes inoculated into raw tomatoes and processed tomato products. *Applied and Environmental Microbiology*, **57**, 1367-1371.

Beuchat, I. R. & Ryu, J. H. (1997). Produce handling and processing practices. *Emerging Infectious Diseases*, **3**, 459-465. Centers for Disease Control.

Bloomfied, S. F. (2003). Home hygiene: A risk approach. International *Journal Hygiene Environmental Health*, **206**, 1-8.

Bolin, H. R., Stafford, A. E., King, A. D. Jr & Huxsol, C. C. (1977). Factors affecting the storage stability of shredded lettuce. *Journal of Food Science*, **42**, 1319-1321.

Brackett, R. E. (1994). Microbiological spoilage and pathogens in minimally processed refrigerated fruits and vegetables. In: *Minimally Processed Refrigerated fruits and vegetables* (edited by Wiley, R. C.). New York: Chapman & Hall. **pp**: 29-312

Brackett, R. E. (1987). Antimicrobial effect of chlorine *on listeria Monocytogenes*. *Journal of Food Protection*, **50**, 999-1003.

Bracket, R. E, Hao, Y. y & Doyle, M. p. (1994). Ineffectiveness of hot acid sprays to decontaminate E. coli 0157: H7 on beef. *Journal of Food Protection*, **57**, 198-203.

Brocklehurst, T. F., Zaman-wong, C. M. & Lund, B. M. (1987). A note on the microbiology of retail packs of prepared salad vegetables. *Journal of Applied Bacteriology*, **63**, 409-415.

Buick, R. K. & Damoglou, A. P. (1987). The effect of vacuum packaging on the microbial spoilage and shelf-life of 'ready-to-use' sliced carrots. *Journal of the Science of Food and Agriculture*, **38**, 167-175.

CAC. (1999). Revised regional guidelines for the design of control measures for street-vended foods in Africa. CAC/GL-22 –Rev. 1 (1999).

Carlin, F., Nguyen-the, C. & Abreu da Silva, A. (1995). Factors affecting the growth of *Listeria monocytogenes* on minimally processed fresh endive. *Journal of Applied Bacteriology*, **78**. 636-646.

Carlin, F., Nguyen-the, C., Chambroy, Y. & Reich, M. (1990). Effects of controlled atmospheres on microbial spoilage, electrolyte leakage and sugar content on fresh, 'ready-to-use' grated carrots. *International Journal of Food Science and Technology*, **25**, 110-119.

Carlin, F., Nguyen-the, C. & Morris, C. E. (1996). The influence of the background microflora on the fate of Listeria monocytogenes on minimally processed fresh broad leaved endive (Cichorium endivia var. *latifolia*). *Journal of Food Protection*, **59**, 698-703.

Coleslaw. (no date). [Online]. Available: http://en.wikipedia.org/wiki/Coleslaw. [2008, June 20].

Como-Sabetti, K., Reagan, S., Allaire., Parrott, C. M., Simonds, S., Hrabowy, S., Ritter, B. *et al.* (1997). Outbreaks of *E. coli* 0157: H7 associated with eating alfalfa sprouts – Michigan and Virginia, June-July 1997. *Morbidity and Mortality Weekly Report*, **46**, 741 – 744.

Conner, D. E. & Kotrola, J. S. (1995). Growth and survival of *E.coli* 0157: H7 under acidic conditions. *Applied and Environmental Microbiology*, **61**, 382-385.

63

Cowan, M., M.(1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, **12**(4), 564-582.

De Simon, M., Tarrago, C. & Ferrer, M. D. (1992). Incidence of *Listeria monocytogens* in fresh foods in Barcelona (Spain). *International journal of Food Microbiology*, **16**, 153-156.

Dixon, N. M. & Kell, D. B. (1989). The inhibition by CO_2 of the growth and metabolism of micro-organisms: a review. *Journal of Applied Bacteriology*, **67**, 109-136.

Doris, L. K. Ng & Seah, H. L. (1995). Isolation and identification of *Listeria monocytogenes* from a range of foods in Singapore. *Food Control*, **6**, 171-173.

Doyle, M. P. (1990). Fruit and vegetable safety- microbiological considerations. *Hortscience*, **25**, 1478-1481.

Doyle, M. P., Zhao, T., Meng, J. & Zhao, S. (1997). 0157: H7. In: *Food Microbiology – Fundamentals and Frontiers* (edited by M. P. Doyle, L. R. Beuchat & T. J. Montville). Washington DC: American Society of Microbiology Press. **pp**:171-191.

El-Kest, S. E. & Marth, E. H. (1988a). Temperature, pH, and strain of pathogen as factors affecting inactivation of *Listeria monocytogenes by chlorine*. *Journal of Food Protection*, **51**, 622-625.

El-Kest, S. E. & Marth, E. H. (1988b). inactivation of *Listeria monocytogenes by chlorine*. *Journal of Food Protection*, **51**, 520-530.

Enfors, S. O. & Molin, G. (1980). Effect of high concentrations of carbon dioxide on growth rate of *Pseudomonas fragi, Bacillus cereus* and *Streptocoous cremoris*. *Journal of applied Bacteriology*, **48**, 409-416.

Ercolani, G. L. (1976). Bacteriological quality assessment of fresh markted lettuce and fennel. *Applied and Environmental Microbiology*, **31**, 847-852.

Fain, A. R. (1996). A review of the microbiological safety of fresh salads. *Dairy, Food and Environmental Sanitation*, **16**, 146-149.

Fang, T. J., Que-King W., Chia-Wei., Min-Ju. H, Tzu-Hui W. (2001). Microbiological of 18 °C ready-to-eat food products sold in Taiwan. International Journal of Food Microbiology, **80**, 241-250.

FAO/WHO. (2004). Risk assessment of *Listeria monocytogens* in ready-to-eatfoods: Guidelines. *FAO/WHO Microbiological Risk Assessment Series*, No. 4. **pp**:1.

Farber, J. M. & Peterkin, P. I. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiology Reviews*, **55**, 476-511.

Fenlon, D. R., Wilson, J. & Donachie, W. (1996). The incidence and level *of Listeria monocytogens* contamination of food sources at primary production and initial processing. *Journal of Applied Bacteriology*, **81**, 641-650.

Francis, G. A. & O´Beirne, D. (1997). Effects of gas atmosphere, antimicrobial dip and temperature on the fate of *L. innocua and L. monocytogenes* on minimally processed lettuce. *International Journal of Food Science and Technology*, **32**, 141-151.

Francis, G. A. & O´Beirne, D. (1998). Effects of the indigenous microflora of minimally processed lettuce on the survival and growth of L. Monocytogenes. *International Journal of Food Science and Technology*, **33**, 477-488.

Frances, P. (2006). A practical look at shelf-life evaluation. International Food Hygiene, **16**, 7-23.

Garcia-Villanova Ruiz, B., Galvez Vargas, R. & Garcia-Villanova, R. (1987). Contamination on Fresh vegetables during cultivation and marketing. *International Journal of Food Microbiology*, **4**, 285-291.

Gary, N., Churey, J. J. & Splittstoesser, D. F. (1990). Effect of processing conditions on the microflora pf fresh-cut vegetables. *Journal of Food Protection*, **53**, 701-703.

Geldreich, E. E. & Bordner, R. H. (1971). Feacal contamination of fruit and vegetables during cultivation and processing for market: a review. *Journal of Food Technology*, **34**, 184-195.

Gelderich, E. E. & Bordner, R. H. (1971). Fecal contamination of fruits and vegetables during cultivation and processing for market: a review. Journal of Food Technology, **34**, 184-195.

Gillian A. F., Thomas. C & O'Berine. (1999). The microbiological safety of minimally processed vegetables. *International Journal of Food Science and Technology*, **3**, 1-22.

Gohil, V.S. (1996). Growth and survival of *Listeria monocytogens* in two traditional foods from the United Arab Emirates. Food Microbiology, **13**, 159-164.

Greek salad. (no date). [Online]. Available: http://en.wikipedia.org/wiki/Greek_salad. [2008, June 20].

Griffin, P. M. & Tauxe, R. V. (1991). The epidemiology of infections caused by *E. coli 0157: H7*, other enterohaemorrhagic *E. coli*, and the associated haemolytic syndrome. *Epidemiological Reviews*, **13**, 60-98.

Guidelines for the handling of chilled foods (1990). 2nd ed. ISBN 0905367073. London: Institute of Food Science and Technology.

Gulf Co-operation Council Standards. (1998). Microbiological Criteria for Food Stuffs.

Gunasena, D.K., Kodikare, C. P., Ganepola, K & Widanapathirana, S. (1995). Occurrence of Listeria monocytogens in food in Sir Lanka. Journal of the National Science Council of Sir Lankla, **23**, 107-114.

Harris, L. J., Daeschel, M. A., Stiles, M. E. & Klaenhammer, T. R. (1989). Antimicrobial activity of lactic acid bacteria against *Listeria monocytogene. Journal of food Protection*, **52**, 384-387.

Harvey, J. & Gilmour, A. (1993). Occurrence and characteristics of *Listeria* in foods produced in Northern Ireland. *International Journal of Food Microbiology*, **19**, 193-205.

Hedberg, C. W., MacDonald, K. L. & Olsterholm, M. T. (1994). Changing epidemiology of food-borne disease: a Minnesota perspective. *Clinical Infectious Disease*, **18**, 671-682.

Heisick, J. E., Wagner, D. E., Nierman, M.L. & Peeler, J. T. (1989). *listeria* spp. Found on fresh market produce. *Applied and Environmental Microbiology*, **55**, 1925-27.

Hirai, Y. (1991). Survival of bacteria under dry condition; from a viewpoint of nosocomial infection. *Journal of Hospital Infection*, **19**, 191-200.

Ho, J. L., Shands, K. N., Freidland, G., Eckind, P. & Fraser, D. W. (1986). An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. *Archives Internal Medicine*, **146**, 520-524.

Hummus. (no date). [Online]. Available: http://en.wikipedia.org/wiki/Homous. [2008, June 20].

Iandolo, J. J., Clark, C. W., Bluhm, L. & Ordal, Z. J. (1965). Repression of *Staphylococcus aureus* in associative culture. *Applied Microbiology*, **13**, 646-649.

67

Institute of Food Science & Technology IFST (1990) Shelf Life of Food - Guidelines for its Determination and prediction. Institute of Food Science & Technology (UK), London.

International Commission on Microbiological Specifications for Foods ICMSF (1996). In : Microbiological Specifications of Food Pathogens (edited by T. A. Roberts, A. C. Baird-Parker & R. B. Tompkin). London: Blackie Academic & Professional.

International Standard ISO 11290-1:1996(E) (1996). Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of the *Listeria monoytogenes* – Part 1: Detection method. ISO copyright office: Switzerland.

International Standard ISO 4833:2003(E) (2003). Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms – Colony – count technique at 30 °C. ISO copyright office: Switzerland.

International Standard ISO 6579:2002(E) (2002). Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Salmonella* spp. ISO copyright office: Switzerland.

International Standard ISO 6888-1:1999(E) (1999). Microbiology of food and animal feeding stuffs - Horizontal method for enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium. ISO copyright office: Switzerland.

International Standard ISO 7251:2005(E) (2005). Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of presumptive *Escherichia coli* – Most probable number technique. ISO copyright office: Switzerland.

Johnston, L. M., Jaykis, L. A., Moll, D., Martinez, M. C., Anciso, J., Mora, B., Moe, C. L. (2005). A field study of the microbiological quality of fresh produce. Journal of Food Protection, **68**(9): 1840-7.

Kallender, K. D., Hitchins, A. D., Lancette, G. A., Schmieg, J. A., Garcia, G. R., Solomon, H. M. & Sofos, J. N. (1991). Fate of *Listeria monocytogenes* in shredded cabbage stored at 5 and 25 °C under a modified atmosphere. *Journal of Food Protection*, **54**, 302-304.

Kaneko, K., Hayashidani, H., Takahashi, K., Shiraki, Y., Imawongpranee, S., Ogawa, M. (1999). Bacterial contamination in the environment of food factories processing rady to eat fresh vegetables. *A service of the National Library of Medicine and the National Institutes of Health*, **62**, 800-4

Karmali, M. A., Steele, B. T., Petric, M. & Lim, C. (1983). Sporadic cases of haemolytic uremic syndrome associated with faecal cytotoxin-producing *E. coli*. *Lancet*, I, 619-620.

King, A.D. Jr, Magnuson, J. A., Torok, T. & Goodman, N. (1991). Microflora and storage quality of partially precessed lettuce. *Journal of Food Science*, **56**, 459-461.

Klaenhammer, T. R. (1988). Bacteriocins of lactic acid bacteria. *Biochimie*, **70**, 337-349.

Kominos, S., D. Copeland, C., E. Grosiak, B, Postic, B. (1972). Introduction of *Pseusomonas aeruginosa* into a hospital via vegetable. *Journal of Applied Microbiology*, **24**(4), 567-570.

Liao. C. H., McCallus, D. E. & Wells, J. M. (1993). Calcium-dependent pectate lyase production in the soft-rotting bacterium *Pseudomonas fluorescens*. *Phytopathology*, **83**, 813-818.

MacGowan, A. P., Bowker, K., McLauchlin, J., Bennett, P. M. & Reeves, D. S. (1994). The occurrence and seasonal changes in the isolation of Listeria spp. In shop bought food stuffs, human faeces, sewage and soil from urban sources. International Journal of Food Microbiology, **21**, 325-335.

Magnuson, J. A., King, A. D. Jr & Torok, T. (1990). Microflora of partially processed lettuce. Applied and Environmental Microbiology, 56, 3851-3854.

Man, C. D. & Jones. A. A. (1994). Shelf life evaluation of food. Blackie Academic and Professional, an imprint of Chapman & Hall, Wester Cleddens.106-114.

Manvell, P.M & Ackland, M.R. (1986). Rapid detection of microbial growth in vegetable salads at chill and abuse temperatyres. *Food Microbiology*, **3**, 59-65.

Marchetti, R., Casadei, M. a. & Guerzoni, M. E. (1992). Microbial population dynamics in ready-to-use vegetable salads. *Italian Journal of Food Science*, **4**, 97-108.

Marshall, D. L. & Schmidt, R. H. (1991). Physiological evaluation of stimulated growth *of Listeria monocytogenes* by *Pseudomonas* species in milk. *Canadian Journal of Microbiology*, **37**, 594-599.

Martin, M. L., Shipman, D., Potter, M. E., Wachsmuth, J. G., Wells, K., Hedberg, K., Tauxe, R. V. et al. (1986). Isolation of *E. coli* 0157: H7 from dairy cattle associated with two cases of haemolytic uremic syndrome. *Lancet*, ii, 1043.

McElodowney, S., Fletcher, M. (1988). Effect of temperature and humidity on survival of bacteria attached to dry solid surfaces. *Letters in Applied Microbiology*, **7**, 83-86.

McLachlan, A. & Strak, R. (1985). Modified atmosphere packaging of selected prepared vegetables. *Campden Food Preservation Research Aeeociation Technical Memorandum no.* bv412ev. Chipping Campden, UK. **pp**78-92

McLauchlin, J. & Gillert, R. J. (1990). *Listeria* in foods. Report from the PHLS Committee on Listeria and listeriosis. *PHLS Microbiology Digest*, **7**, 57-55.

Menlove, A. (2002). Ready Meal Technology. England: Leatherhead Publishing. **pp**192-228

Mintel Market Intelligence Report, (2000). Fresh Fruit and Vegetables.

Mohamed, I. (2002). Microbioloical guideline for some traditional Arabic food. Department of Nutrition & Food industry Agriculture College, Jourdan Univiersity.(Arabic).

Naimi, T. S., Wicklund. J. H, et al., & Hedberg C. W. (2003). Concurrent Outbreaks of Shiglla sonnei and Enterotoxigenic Escherichia Coli Infection Associatd with Parsley: Implication for Surveillance and Control of Foodborne Illness. Journal of food protection, 66, 535-541.

Nguyen-the, C. & Carlin, F. (1994). The microbiology of minimally processed fresh fruits and vegetables. Critical Reviews in Food Science and Nutrition, 34, 371-401.

Nguyen-the, C., Halna-d,. u-fretay, B. & Abreu da Silva, A. (1996). The microbiology of mixed salad containing raw and cooked ingredient without dressing. *International Journal of Food Science and Technology*, 41, 481-487.

Nguyen-the, C. & Prunier, J. P. (1989). Involvement of pseudomonads in the deterioration of 'ready-to-use' salads. *International Journal of Food Science and Trchnology*, 24, 47-58.

O'Beirne, D. & Ballantyne, A. (1987). Some effects of modified atmosphere packaging and vacuum packaging in combination with antioxidants on quality and storage life of chilled potato strips. International Journal of Food Science and Technology, 22, 515-523.

O'Mahony, M., Cowden, J., Smyth, B., Lynch, D., Hall, M., Rowe, B., Teare, E. L., Tettmar, R. E., Rampling, A. M. & Coles., M. (1990). An outbreak of *Salmonella saintpaul* infection associated with beansprouts, *Epidemiological Infections*, 104, 229-235.

Park, D. L., Rua, S. M. Jr & Acker, R. F. (1991). Application of a new hypochlorite sanitizer for reducing bacterial contamination on foods. *Journal of Food Protection*, 54, 960-965.

Petran, R. L., Zottola, E. A. & Gravani, R. B. (1988). Incidence of *Listeria monocytogens* in market samples of fresh and frozen vegetables. *Journal of Food Science*, **53**, 1238-1240.

Petram, W. (2005). Managing Risks in vegetable and salad production. *International Food Hygiene*, 16, 3.

Piagentini, A. M., Pirovani, M. E., Guemes, D. R., Di pentima, J. H. & Tessi, M. A. (1997). Survival and growth of *Salmonella hadar* on minimally processed cabbage as influenced by storage abuse conditions. *Journal of Food Science*, **62**, 616-618.

Price, R. J. & Lee, J. S. (1970). Inhibition of *Pesudomonas* species by hydrogen peroxide producing lactobacilli. *Journal of Milk Food Technology*, **33**, 13-18.

Piard, J. C. & Deesmazeaud, M. (1992). Inhibiting factors produced by lactic acid bacteria. 2. Bacteriocins and other antimicrobial substances. *Lait*, **72**, 113-142.

Prescott L M, Harley J P and Klein D A. (2002). Microbiology.McGraw-Hill Companies, Inc., New York.113-125.

Raccach, M. & Baker, R. C. (1979). The effect of lactic acid bacteria on some properties of mechanically deboned poultry meat. *Poultry Science*, **58**, 144-147.

Rauha, J. P., Remes, M., Heinonen, M., Hopia, A., Kahkonen, M., Kujala, T., Pihlaja, K., Vuorela, H. and Vuorela, P. (2000). Antimicrobial effect of Finnish plant extracts containing flavonoids and other phenolic compounds, *International Journal of Food Microbiology*, **56**, 3-12.

Riva, M. Franzetti, A. & Galli, A. (2001). Effect of storage temperature on microbiological quality and shelf-life of ready to use salads. *Journal of Annals of Microbiology*, **51**, 39-52.

Robinson, R, K., Batt, CA. & Patell, P, D. (2002). Encyclopedia of Food Microbiology. Academic Press., London, **pp:** 542-544.

Saddik, M. F., El-Sherbeeny, M. R. & Bryan, F. L. (1985). Microbiological profiles of Egyptian raw vegetables and salads. *Journal of Food Protection*, **48**, 883-886.

Schlech, W. F., Lavigne, P. M., Bortolussi, R. A., Allen, A. C., Haldane, E. V., Wort, A. J., Hightower, A. W. et al. (1983). Epidemic Listeriosis-evidence for transmission by food. New England Journal of Medicine, **308**, 203-206.

Seo, K.H., &. Frank. (1999). Attacchment of Escherichia Coli 0157 :H7 to Lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scaning Laser microscopy. J. Food Protection **62**, 3-9

Sizmur, K. & Walker, C. W. (1988). *Listeria* in prepacked salads. Lancet, i, 1167.

Skandamis, P, N., & Nychas, G, E. (2000). Development and Evaluation of a Model Predicting the survival of Escherichia coli 0157: H7 NCTC 12900 in Homemade Eggplant salad at various temperatures, pH and oregano. *Applied and Environment Microbiology*, **66**, 1646-1653.

Steinbruegge, E. G., Maxcy, R. B. & Liewen, M. B. (1988). Fate of *L. monocytogens* on ready to serve lettuce. *Journal of Food Protection*, **51**, 596-599.

Solomon, H. M., Kautter, D.A., Lilly, T. & Rhodehamel, E. J. (1990). Outgrowth of *Cl. Boutulinum* in shredded cabbage at rome temperature under modified atmosphere. *Journal of Food Protection*, **53**, 831-833.

Soriano, J. M., Rico, J, C., Manes, J. (2000). Assessment of the microbiological quality and wash treatments of lettuce served in University restaurants. International *Journal of Food Microbiology*, **58**, 123-128.

Sutherland, J. P, Varnam, A, and Evans, M. (1986). A colour Atlas of Food Quality Control. Netherlands: Wolfe Publishing Ltd. London, pp: 172-174.

Tabbouleh (no date). [Online]. Available: http://en.wikipedia.org/wiki/Tabbouleh. [2008, June 20].

Tang, M. Y., Cheong, Y. M. & Zainuldin, T. (1994). Incidence of Listeria spp. In vegetables in Kuala Lumpur. *Medical Journal of Malaysia*, **49**, 217-222.

Tauxe, R. V. (1991). Salmonrlla: a postmodern pathogen. *Journal of Food Protection*, **54**, 563-568.

Tessi, MA., Aringoli, EE., Vincenzini, AZ., Sabbag, NG., Costa, CS, Garcia CC., Zannier MS., Silva, ER., Moguilevsky, MA. (2002). Microbiological quality anf safety of ready to eat cooked foods from a centralized school kitchen in Argentina. *A service of the National Library of Medicine and the National Institutes of Health*, **65**, 636-42.

Tuncan, E. U. (1993). Effect of cold temperature on germicida efficacy of quaternary ammonium compound, iodophor and chlorine on *Listeria*. *Journal of Food Protection*, **56**, 1029-1033.

UAE standard (2000). Microbiological Criteria for Food Stuffs. General Secretariat of Municipalities. UAE.

US FDA, MD, USA (2006).USA: *Esherichia coli* O157: H7 outbreak. Expert Review of Anti-infective Therapy. [online]. Available: http://www.future-drugs.com/doi/abs/10.1586/14787210.4.5.723. [2007, january 14].

Vahidy, R. (1992). Isolation of *Lsteria monocytogens* from fresh fruits and vegetables. *Hortscience*, **27**, 628.

74

Velani, S. & Roberts, D. (1991). *Listeria monocytogenes* and other *Listeria* spp. In prepacked mixed salads and individual salad ingredients. *PHLS Microbiology Digest*, **8**, 21-22.

Varnum, A. H. & Evans, M. G. (1991). *Foodborne Pathogens – an Illustrate Text*. England: Wolfe publishing.

Walker, S. J. & Stringer, M. F. (1990). Microbiology of chilled foods. In: *Chilled Foods: the State of the Art* (edited by T. R. Gormley). pp. 269-304. Elsevier.

Walker, S. J. & Stringer, M. F. (1987). Growth of *Listeria monocytoges* and *Aeromonas hydrophila at chill temperatures. Campden Food Preservation Research Association Technical Memorandum* no. CFPRA: Chipping Campden, UK. 462.

Weagant, S. T., Bryant, J. L. & Bark, D. H. (1994). Survival of *E.coli* 0157: H7 in mayonnaise-based sauces at room and refrigerated temperatures. *Journal of Food Protection*, **57**, 629-631.

Weeratna, R. D. & Doyle, M. P. (1991). Detection and production of verotoxin 1 of *E.coli* 0157: H7 in food. *Journal of Applied and Environmental Microbiology*, **57**, 2951-2955.

Wells, J. G., Shipman, /l. D., Greene, K. D., Sowers, E. G., Green, J. H., Cameron, D. N., Downes, F. P. (1991). Isolation of *E. coli* 0157: H7 and other Shiga-like toxin producing E. coli from dairy cattle. *Journal of Clinical Microbiology*, **29**, 985-989.

Welshimer, H. J. & Donker-Voet, J. (1971). *Listeria monocytogens in nature. Applied Microbiology*, **21**, 516-519.

Wilcox, F., Tobback, P. & Hendrickx, M. (1994). Microbial safety assurance of minimally processed vegetables by implementation of the Hazard Analysis Critical Control Point system. *Acta Aliment*, **23**, 221-238.

William, C., F. & Dennis, C., W. (1988). Food Microbiology. McGraw-Hill Book Co. Sangapore. 203-205

Wood, R. C., Hedberg, C, & White, K. (1991). A multistate outbreak *of Salmonella javana* infactions associated with raw tomatoes. In: *CDC Epidemic intelligence Service 40th Annual Conference*. Abstracts Atlanta Centers for Disease Control. 69

Yucel, S, I., Mehmet, K. (2005).Effectiveness oh household natural sanitizers in the elimination of *Salmonella typhimurium* on rocket (Eruca sativa Miller) and spring onion (Allium cepa L.). *International Journal of Food Microbiology*, **98**, 319-323.

Zhang, S. & Farber, J. M. (1996). The effects of various disinfectants *against Listeria monocytogenes* on fresh-cut vegetables. *Food Microbiology*, **13**, 311-321.

Zhao, T., Doyle, M. P. & Besser, R. E. (1993). Fate of enterohaemorrhagic *E. coli* 0157: H7 in apple cider with and without preservatives, *Applied and Environmental Microbiology*, **59**, 2526-2530.

Zhuang, R.-Y., Beuchat, L. R. & Angulo, F. J. (1995). Fate of *Salmonella montevideo* on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied and Environmental Microbiology*, **61**, 2127-2131.

VII. APPENDICES

7. Appendices

Appendices I

1. Preparation of diluents

.Quarter-Strength Ringer's solution tablets (RS, BP 52):

Composition	g/l
Sodium chloride	2.25
Pottasium chloride	0.105
Calcium chloride 6H2O	0.12
Sodium bicarbonate	0.05
Final pH of 7.0	

One tablet of ingredients for Ringer's solution was dissolved in 500 ml of distilled water, and 9 ml aliquots of the mixture were dispensed into screw-capped bottles. The solution was sterilized by autoclaving at 121 °C for 15 minutes.

2. Preparation of media

. Standard Plate Count Agar (SPCA, CM 463):

Composition	g/l
Yeast extract	2.5
Pancreatic digest of casein	5.0
Glucose	1.0
Agar	15.0
Final pH of 7.0 ± 0.2	

An amount of SPCA (23.5 g) was added to 1 litter of distilled water, boiled, distributed into bottles and sterilized by autoclaving at 121°C for 15 minutes.

. Baird Parker Agar Base (BPAB, CM 275):

Composition	g/l
Tryptone	10.0
'Lab-Lemco' powder	5.0
Yeast extract	1.0
Sodium pyruvate	10.0
Glycine	12.0
Lithium choride	5.0
Agar	20.0
Final pH of 6.8 ± 0.2	

An amount of BPAB (63 g) was added to 1 litter of distilled water, boiled, distributed into tubes or flasks and sterilized by autoclaving at 121°C ± 1°C for 15 minutes.

The medium was then cooled to 50°C and, at this temperature, aseptically added 50 ml of Egg Yolk Tellurite Emulsion SR54, and mixed well before pouring.

. Macconkey Broth (purple) (MB, CM 5a, powder):

Composition	g/l
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Bromocresol purple	0.01
Final pH of 7.4	

An amount Macconkey Broth powder (23.5 g) was added to 1 litter of distilled water, distributed into containers fitted with fermentation (Durham) tubes and sterilized by autoclaving at 121°C for 15 minutes.

. **Brilliant Green Bile (2%) Broth (CM 31):**

Composition	g/l
Peptone	10.0
Lactose	10.0
Ox bile (purified)	20.0
Brilliant green	0.0133
Final pH of 7.4	

Dissolved (40.0 g) in 1 litter of distilled water, mixed well, distributed into containers fitted with fermentation (Durham) tubes and sterilized by autoclaving at 121°C for 15 minutes.

. **Tryptone Water (CM 87):**

Composition	g/l
Trypton	10.0
Sodium chloride	5.0
Final pH of 7.5	

An amount Tryptone Water (15 g) was added to 1 litter of distilled water, distributed into final and sterilized by autoclaving at 121°C for 15 minutes.

. Buffered Peotone Water (BPW) (CM 509):

Composition	g/l
Peptone	10.0
Sodium chloride	5.0
Disodium phosphate	3.5
Potassium dihydrogen phosphate	1.5
Final pH of 7.2	

An amount BPW (20 g) was added to 1 litter of distilled water, distributed into final containers and sterilized by autoclaving at 121°C for 15 minutes.

. Rappaport Vassiliadis Soya (RVS) peptone broth (CM 866):

Composition	g/l
Soya peptone	4.5
Sodium chloride	7.2
Potassium dihydrogen phosphate	1.26
Di-potassium hydrogen phosphate	0.18
Magnesium chloride (anhydrous)	13.58
Malachite green	0.036
Final pH of 5.2	

An amount RVS (26.75 g) was added to 1 litter of distilled water, heated, distributed 10 ml into tubes and sterilized by autoclaving at 121°C for 15 minutes.

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. **Muller-Kauffmann Teterthionate Broth Base (MKTBB, CM 343):**

Composition	g/l
Tryptone	7.0
Soya peptone	2.3
Sodium chloride	2.3
Calcium carbonate	25.0
Sodium thiosulphate	40.0
Ox bile	4.75
Final pH of 6.8 ± 0.2	

An amount of MKTBB (82 g) was added to 1 litter of distilled water, boiled, The medium was then cooled to 45°C and, at this temperature, aseptically added 19 ml of iodine solution and 9.5 ml of a 0.1% brilliant green solution, and mixed well before filled out into sterile tubes.

Ioden Solution	g/l
Iodine	20
Potassium iodide	25
Distilled water to	100

. XLD medium (CM 469):

Composition	g/l
Yeast extract	3.0
L-Lysine	5.0
Xylose	3.75
Lactose	7.5
Sucrose	7.5
Sodium desoxycholate	1.0
Sodium chloride	5.0
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	12.5.75
Final pH of 7.4	

An amount of XLD (53 g) was added to 1 litter of distilled water, heated, the medium was then cooled to 45°C and, at this temperature and poured into plates.

. Brilliant Green Agar (BGA, CM 329):

Composition	g/l
'Lab-Lemco' powder	5.0
Peptone	10.0
Yeast extract	3.0
Disodium hydrogen phosphate	1.0
Sodium dihydrogen phosphate	0.6
Lactose	10.0
Sucrose	10.0
Phenol red	0.09
Brilliant green	0.0047
Agar	12.0
Final pH of 6.9	

An amount of BGA (53 g) was added to 1 litter of distilled water, heated to boil, the medium was then cooled to 50°C and, at this temperature and poured into plates.

. Violet Red Bile Glucose Agar (VRBGA, CM 485):

Composition	g/l
Yeast extract	3.0
Peptone	70
Sodium chloride	5.0
Bile salt No.3	1.5
Glucose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.0
Final pH of 7.4	

An amount of VRBGA (38.5 g) was added to 1 litter of distilled water, boiled, the medium was then mixed well and poured into tubes.

. Listeria Selective Agar (Oxford formulation) (LSA, CM 856):

Composition	g/l
Columbia Blood Agar Base	39.0
Aesculin	1.0
Ferric ammonium citrate	5.0
Lithium chloride	15.0
Final pH of 7.0	

. **Listeria Selective Supplement (Oxford formulation) (LSS, SR 140):**

Composition	mg
Cycloheximide	200.0
Colistin sulphate	10.0
Acriflavine	2.5
Cefotetan	1.0
Fosfomycin	5.0

Suspended (27.75 g) of the LSA CM 856 in 500 ml distilled water, boiled, sterilized by autoclaving at 121°C for 15 minutes, the medium was then cooled to 50 °C and, at this temperature, added the contents of one vial of LSS SR 140 which reconstituted with 5 ml of ethanol/sterile distilled water (1:1), mixed well then poured into sterile petri dishes.

. **Listeria Enrichment Broth Base (UVM), (CM 863):**

Composition	g/l
Proteose peptone	5.0
Tryptone	5.0
'Lab-Lemco' powder	5.0
Yeast Extract	5.0
Sodium chloride	20.0
Disodium hydrogen phosphate	12.0
Potassium dihydrogen phosphate	1.35
Aesculin	1.0
Final pH of 7.4	

. Listeria Primary Selective Enrichment Supplement (UVM 1), (SR 142):

Composition	mg
Nalidixic acid	10.0
Acrifla vine hydrochloride	12.5

Suspended (27.2 g) of the UVM CM 863 in 500 ml distilled water, sterilized by autoclaving at 121°C for 15 minutes, the medium was then cooled to 50°C and, at this temperature, added the contents of one vial of UVM 1 SR 142 which reconstituted with 2 ml of sterile distilled water, mixed well then poured into sterile containers.

. Listeria Secondary Selective Enrichment Mediun (UVM II), (CM 863):

Composition	g/l
Proteose peptone	5.0
Tryptone	5.0
'Lab-Lemco' powder	5.0
Yeast Extract	5.0
Sodium chloride	20.0
Disodium hydrogen phosphate	12.0
Potassium dihydrogen phosphate	1.35
Aesculin	1.0
Final pH of 7.4	

. Listeria Secondary Selective Enrichment Supplement (UVM II), (SR 143):

Composition	mg
Nalidixic acid	10.0
Acrifla vine hydrochloride	12.5

Suspended (27.2 g) of the UVM CM 863 in 500 ml distilled water, sterilized by autoclaving at 121°C for 15 minutes, the medium was then cooled to 50°C and, at this temperature, added the contents of one vial of UVM 1 SR 143 which reconstituted with 2 ml of sterile distilled water, mixed well then poured into sterile containers.

. Palcam Agar Base (CM 877):

Composition	g/l
Columbia blood agar base	39.0
Yeast extract	3.0
Glucose	0.5
Aesculin	0.8
Ferric ammonium citrate	0.5
Mannitol	10.0
Phenol red	0.08
Lithium chloride	15.0
Final pH of 7.2	

. Palcam Selective Supplement (SR 150):

Composition	mg
Polymixin B	5.0
Acrifla vine hydrochloride	2.5
Ceftazidime	10.0

Suspended (34.5 g) of the Palcam Agare CM 877 in 500 ml distilled water, boiled, sterilized by autoclaving at 121 °C for 15 minutes, the medium was then cooled to 50 °C and, at this temperature, added the contents of one vial of SR 150 which reconstituted with 2 ml of sterile distilled water, mixed well then poured into sterile petri dishes.

Appendices 2: Collecting Samples schedule form Sharjah Supermarket Stores

Location:

The samples were collected from 3 different supermarket stores in Sharjah

Samples Schedule

Locations	Date Sunday-Thursday	Products	Weight
1	10/06/07 To 21/06/07	tabbouleh salad greek salad coleslaw salad houmos	790g 790g 790g 790g
2	24/06/07 To 05/07/07	tabbouleh salad greek salad coleslaw salad houmos	790g 790g 790g 790g
3	08/07/07 To 19/07/07	tabbouleh salad greek salad coleslaw salad houmos	790g 790g 790g 790g

Appendices 3: Microbiology Criteria for Foodstuffs (Read-to-Eat foods)

GULF STANDARD   **GS 1016/1998**

6. Tomato products, Salad, Vinegar and spices

Item	Microorganisms	Limit per ml or gram			
		n	c	m	M
Tomato Ketchup, Tomato juice, tomato paste, tomato puree, tomato sauce and tomato products	Shall pass commercial sterility test. Incubation at $25 - 30\,°C$ for 10 days	No signs of microbial growth, no swells or seam defective on packages are found during incubation			
Coleslaw (cabbage)	Aerobic plate count	5	1	10^5	10^6
	Staphylococci	5	1	10^2	10^4
	Escherichia coli	5	2	10	10^6
	Listeria monocytogenes	5	0	0	-
Salad of raw vegetable	Aerobic plate count	5	1	10	10^2
	Salmonella	5	0	0	-
Mayonnaise, mustard, salad sauce and other sauces dfawedv dvz	Aerobic plate count	5	1	10^3	10^5
	Coliforms	5	1	10	10^2
	Yeasts and moulds	5	2	20	10^2
	Salmonella	5	0	0	-
Vinegar	Aerobic plate count	5	1	30	10^2
Spices	*Staphylococci*	5	1	10^2	10^4
	Salmonella	5	0	0	-
	Yeasts and moulds	5	2	10^2	10^4
	Fecal coliform	5	2	10	10^3

Definitions

Sampling plan

A laboratory plan defining the number of product unit's ¨n¨ that should be examined and acceptance or rejection levels and tolerance values. It comprises the following:

n = Number of sample units to be examined.

m = Value or level of microbiological criterion to be met in the food product.

c = The maximum number of sample units allowed to have a microbiological criterion value greater than m and below the value of M.

M = The maximum criterion value that should not achieved or exceeded any of n units.

Sample unit = A sample from the food product examined as one unit from ¨n¨. It is either a single or a part of a package or a mixed compound of the product.

Appendices 4: UAE standard of Most Probable Number

Most probable number (MPN) of bacteria; three tubes at each dilution.

Number of positive tubes at each dilution level.

Confidence limits

10^{-1} dilution	10^{-2} dilution	10^{-3} dilution	MPN Per g	99 % Lower	Upper	95 % Lower	Upper
0	0	0	<3	-	-	-	- (D)
0	1	0	3	<1	23	<1	17
1	0	0	4	<1	28	1	21
1	0	1	7	1	35	2	27
1	1	0	7	1	36	2	28
1	2	0	11	2	44	4	35
2	0	0	9	1	50	2	38
2	0	1	14	3	62	5	48
2	1	0	15	3	65	5	50
2	1	1	20	5	77	8	61
2	2	0	21	5	80	8	63
3	0	0	23	4	177	7	129
3	0	1	40	10	230	10	180
3	1	0	40	10	290	20	210
3	1	1	70	20	370	20	280
3	2	0	90	20	520	30	390
3	2	1	150	30	660	50	510
3	2	2	210	50	820	80	640
3	3	0	200	<100	1900	100	1400
3	3	1	500	100	3200	200	2400
3	3	2	1100	200	6400	300	4800
3	3	3	>1100	–	–	–	– (C)

Calculated from data of de Man (1975)

A - At each dilution level, inoculate 1 ml into each of three tubes of media.

B - To calculate MPN from dilutions greater than those shown, multiply the MPN by the appropriate factor of 10, 100, 1000, etc. For example, if tubes selected come from 10^{-2} 10^{-3}, and 10^{-4}, dilutions multiply by 10; if from 10^{-3}, 10^{-4}, and 10^{-5} dilutions, multiply by 100.

C- Adapted from ICMSF (1978)

D - Practical Food Microbiology (1995), 2nd Ed., Edited by D.Roberts, W.Hooper and M.Greenwood.

F - Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed, Edited by Card Vanderzant, Don F.

الملخص

الأغذية الجاهزة للأكل تغطي مدى واسع من منتجات الأغذية الشبه محضرة والتي تأكل مباشرة دون الحاجة إلى التحضير المسبق. في السنوات الأخيرة زاد الطلب عل هذا النوع من الأغذية، و هي تعتبر من الوجبات السريعة التي تطبخ ثم تبرد سريعا ثم تقسم وتوزع سريعا في المبردات قبل إعادة تسخينها و استخدامها من قبل المستهلكين. بعض الأغذية الجاهزة للأكل و على سبيل المثال: سلطات الخضروات ، الفواكه و السبانخ،منتجات اللحوم و الدواجن تعتبر بيئة مناسبة لنمو الميكروبات الضارة المسببة .

تعتبر هذه الأغذية غير أمنة و خاصة عند تخزينها عند درجة حرارة 10° م لأنها تتيح الفرصة لنمو و تكاثر الميكروبات الضارة بصحة الإنسان.

لذلك هذه الدراسة مدى سلامة الأغذية الجاهزة للأكل من الناحية الميكروبية و من ضمن هذه الأغذية: التبولة، السلطة اليونانية، سلطة الملفوف و الحمص، و المباعة في الشارقة ، كما تحدد فترة صلاحيتها.

من أهم نتائج هذه الدراسة، أن طرق إعداد و تحضير هذه الأغذية الجاهزة للأكل مؤشر عن مدى جودة وسلامة الأغذية من ناحية وجود الميكروبية. و من أهم التوصيات التي خرجت منه هذه الدراسة: فترة صلاحية الأغذية الجاهزة للأكل أن لا يتعدى اليوم الواحد كذلك يجب على المؤسسة تطبيق نظام تحليل نقاط المناطق الحرجة.

جامعة الامارات العربية المتحدة

عمادة الدراسات العليا

برنامج ماجستير علوم البيئة

عنوان الرسالة

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رسالة مقدمة من الطالبة

نجلاء علي المعلا

إلى جامعة الأمارات العربية المتحدة

إستكمالا لمتطلبات الحصول على درجة الماجستير في علوم البيئة

2008/2009