READY-TO-EAT MEALS

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Ready-to-eat (RTE) is defined as the status of the food being ready for immediate consumption at the point of sale or serve. It could be served as raw or cooked, hot or chilled, and can be consumed without further heat-treatment including reheating (Anon., 2001). RTE vegetables and salads, also called minimally-processed products, are raw products that must preserve as much as possible the nutritional, sensorial and microbial qualities of fresh products. A very wide range of vegetables are used, both cut and whole. Even during refrigerated storage, the fresh fruits and vegetables are characterized by active metabolism. Brecht (1995) indicated that some of the factors affecting the intensity of wounding are species, variety, maturity index, temperature, oxygen (O_2) and carbon dioxide (CO_2) concentrations and water vapor pressure. In general the problems with whole fruits and vegetables are rapid quality deterioration of the fruit and enzymatic browning, moisture loss, weight loss, shriveling, flaccidity (due to water loss), fungal attack, loss of flavor, decay and textural changes, physiological disorders, physical injuries and limited shelf life (Ben-Yehoshua, 1966; El Ghaouth *et al.*,1992b; Lerdthanangkul and Krochta, 1996; Miller *et al.*, 1983; Motlagh and Quantick, 1998; Taşdelen and Bayındırlı, 1998; Zhang and Quantick, 1997).

The production process of RTE salad is generally very simple: the vegetables are selected, washed, cut, dried and packaged in sealed pouches or in plastic trays wrapped with an extensible high permeability polymeric film. The lack of heat treatment does not ensure microbial destruction, while physical damages, determined by cutting, increase the metabolic activity of the plant tissues bringing about oxidative reactions, dehydration, senescence and ethylene production. Consequently, the products show modifications of sensorial properties such as necrosis, browning, loss of texture, and the discharge of cellular liquids favours microbial proliferation faster than that in fresh unprocessed vegetables. An improvement of shelf-life can be obtained with special cares during processing and packaging and along the chain trade, using good quality raw products and a control of relative humidity and temperature so as to reduce microbial hazards and physical damages. The stability of these products depends on many factors, but temperature is certainly the most important parameter especially during the marketing process, when the control of this parameter is more difficult. Under the same thermal conditions shelf-life can vary according to the type of vegetables, the production process, the packing and the quality of raw materials used (Riva *et al.*, 2001). In the Table 1 there is shown the Q_{10} values for some vegetables. Q_{10} is a unit less quantity. It is the factor by which the rate increases when the temperature is raised by ten degrees. For many biological processes, particularly those that involve large-scale protein conformational changes, Q_{10} values are greater than two.

Table 1. Parameters for commercial shelf-life of different ready-to-eat vegetables.

Vegetables	Q_{10} value	Stability time at 5 °C (days)
Cut cicorino	4.51	6.5
Lettuce	5.16	6.7
Carrots	2.66	4.5

Q_{10} – temperature coefficient

There are number of studies about the improving quality of RTE lettuce. Recent emerging technologies to reduce the initial microbial load and browning during storage of minimally processed lettuce were the use of ozone, electrolyzed water, ultrasound, irradiation, as well as warm water treatment, warm water treatment in combination with chlorine in combination with hydrogen peroxide or with irradiation. Most studies on minimally processed lettuce were performed on laboratory scale or have dealt with samples obtained at the retail level. Up to now, only few studies on the effect of different washing treatments have been conducted on pilot plant or industrial scale Therefore, detailed process relevant data are scarce. According to the microbial criteria recommended by the German Society for Hygiene and Microbiology (DGHM, 2002), the load of aerobic mesophilic microorganisms on mixed, packaged salads at the consumer level should not exceed 7.7 log (5×10^7) colony forming units per gram (cfu/g). Additionally, the use-by date should not exceed 6 days, and the direction for use should advise to keep the product below 6 °C (Baura *et al*., 2004).

It is also well known that microbial safety has main importance for RTE salads. This type of food can be dangerous to consumers' health if safety measures are not strictly complied with at the time of preparation. In these cases, contamination can be caused by animal manure and contaminated irrigation water. Several authors make reference to the presence of bacteria *(E. coli, Listeria monocytogenes)* and illness outbreak have been associated to the consumption of raw vegetables such as cabbage, lettuce, tomato, etc. (Pellicer *et al*., 2002). *Stafylococcus aureus* is detected on fresh produce and RTE salads, most likely because of contamination by food handlers (Beuchat, 1996). *L. monocytogenes* has been detected on lettuce between 1–2% of incidence. (Heisick *et al*., 1989).

Although various studies deal with the growth of *L. monocytogenes* on lettuce, no growth data on RTE lettuce under MAP initially flushed with a mixture of CO_2, O_2 and N_2 gases are available. Studying the behaviour of *L. monocytogenes* on RTE lettuce (and in general, on RTE vegetables) becomes highly relevant, as it does not require decontamination practices such as washing at retail, foodservice or consumer stage, which could reduce a potential contamination of the microorganism (Carrasco, *et al*., 2008). To minimize the risk of infection or intoxication associated with raw fruits and vegetables, potential sources of contamination from the environment to the table should be identified and specific measures and interventions to prevent and/or minimize the risk of contamination should be considered and correctly implemented. Where the possibility of contamination cannot be excluded, the application of the most effective decontamination processes should be considered. Application of good hygienic practices during production, transport and processing, combined with the Hazard Analysis Critical Control Point (HACCP) system, will certainly minimize the contamination of fruits and vegetables and reduce the risk of illness associated with these foods. However, food handlers and consumers also need to observe good hygienic practice during processing and preparation of these foods for consumption including treatments for reducing the number of pathogens. The simple practice of washing raw fruits and vegetables in hot water or water containing detergent or permanganate salts removes a portion of the pathogenic and spoilage microorganisms that may be present, but studies showing the efficacy of these treatments are few. Even washing fruits and vegetables in potable water, then again washing or rinsing in potable water would aid in removing microorganisms. Additional 10-fold to 100-fold reductions can sometimes be achieved by treatment with disinfectants. Viruses and protozoan cysts on fruits and vegetables generally exhibit higher resistance to disinfectants than do bacteria or fungi. However, relative resistance varies greatly with the type and pH of disinfectant, contact time, temperature and the chemical and physical properties of the fruit or vegetable surface. Little is known about the efficacy of disinfectants in relation to the roughness of fruit and vegetable surfaces, although higher amounts of cuticle material may protect against embedding of cells, thereby increasing the need for exposure to chemical treatments. Several types of treatment are known to be partially effective in removing disease-causing organisms from the surface of whole and cut raw fruits and vegetables or from contact surfaces during handling. Perhaps with the exception of irradiation, none of these treatments can be relied upon to totally disinfect raw produce, at least when administered at levels that will not cause deterioration in sensory quality. Even irradiation may not be completely effective in killing viruses on fruits and vegetables. Rather, these treatments should be considered as methods of disinfection, causing reductions in populations of microorganisms but not always yielding fruits and vegetables free of pathogens.

Each type of disinfectant has its own efficacy in killing microbial cells. Effectiveness depends on the nature of the cells as well as the characteristics of fruit and vegetable tissues and juices. Some types of disinfectants are appropriate for use in direct contact washes, while others are suitable only for equipment or containers used to process, store or transport fruits and vegetables. The mechanism of action of many disinfectants on microbial cells and the influence of factors associated with plant materials is poorly understood. The legal use of various treatments differs from country to country. Available information on the effect of a number of disinfecting agents on *L. monocytogenes* was summarized in the review published by the WHO in 1998 (WHO, 1998) *L. monocytogenes* was stated to be generally more resistant to disinfectants than *Salmonella*, pathogenic *Escherichia coli* and *Shigella* (Beuchat, 1998).

At 200 ppm chlorine reduces the count of *L. monocytogenes* on brussel sprouts, shredded lettuce and cabbage by about 1–2 $\log_{10}$ units. However, simply dipping inoculated sprouts in sterile water reduced *L. monocytogenes* on sprouts by 1 $\log_{10}$ unit. The action of chlorine appears to occur during the first 30 seconds of exposure, so longer periods did not affect the reduction. However, the effectiveness of chlorine is increased if the temperature of the treatment solution is higher than the temperature of the fruit or vegetable (Beuchat, 1998).

On the other hand hurdle technology has been around for many years already as a concept for the production of safe, stable, nutritious, tasty and economical foods. It advocates the intelligent use of combinations of different preservation factors (hurdles) in order to achieve multi-target, mild preservation effects. The spoilage and poisoning of foods by microorganisms is a problem that is not yet under adequate control despite the range of preservation techniques available (*e.g.* freezing, blanching, pasteurizing, canning, drying). Because food manufacturers increasingly rely on mild preservation techniques (*e.g.* refrigeration, modified atmosphere packaging, biopreservation) in order to meet the consumer demand for fresh-like foods, this problem could even increase. According to the hurdle technology concept the desired safety and durability of fresh-like foods may be obtained by using combinations of mild preservation techniques. Hurdle technology (or combined processes) advocates the deliberate combination of existing and novel preservation techniques in order to establish a series of preservative factors that no microorganism present should be able to overcome. These hurdles may be temperature, water activity (aw), pH, redox potential, preservatives, etc. It requires a certain amount of effort from a microorganism to overcome each hurdle. The higher a hurdle, the greater this effort is (*i.e.* the larger the number of organisms needed to overcome it). Some hurdles, like pasteurization, can be high for a large number of different types of microorganisms, whereas others, like salt content, have a less strong effect or the effect is limited in the range of types of microorganisms that it affects (Gorris, 1995).

The purposes of this group work are to establish a RTE lettuce salad producing factory and to describe the needs for safe production. RTE lettuce salad consisting of fresh cut lettuce, naturally fermented olive, RTE tomato cubes, and salad sauce (vinegar-olive oil) was chosen as product and needs were described in this study.

HACCP TEAM

The HACCP team should have a competent and experienced team leader. HACCP team selection should be done by the team leader. HACCP team should be multi-disciplinary and consists of different specialists – quality manager, technical manager, warehouse manager, production manager, logistics manager and maybe of some additional specialists. It means that the members of the team have wide range and specific knowledge of HACCP, production processes, food safety and hazards, machinery, storage, transport etc (Table 2). The team should not be larger than 6 people. When the team has assembled all the members should participate in the

HACCP training to understand of HACCP, critical control points (CCP), prerequisite programs (PRP), hazards etc. Company's senior management should demonstrate commitment and support to the HACCP team (Dillon & Griffith, 1997; Anon., 2008, www.codexalimentarius.net).

Table 2. HACCP team members and their competences.

Team member	Field of competence
Quality manager	Product description, product contamination and hazards, hygiene, cleaning and disinfection, product inspection and laboratory tests
Production manager	Production processes, product design and development, pest control, garbage and waste
Technical manager	Machinery, maintenance, hygienic design, calibration
Logistics manager	Dispatch, picking, transport
Warehouse manager	Raw material storage and specifications, control of raw material

HACCP DOCUMENTATION

Documented HACCP procedures should be assembled. Efficient and accurate documentation provides the manufacturer a confidence that product is safe and helps HACCP auditors to do their work. All the prerequisite programs (PRP-s) should be documented: factory layout with machinery, movement of personnel, raw material, garbage, product; cleaning and sanitation procedures; garbage plan; pest control procedures; hygiene rules; plan of product inspection and laboratory test; storage and transportation procedures, maintenance, product flow diagram and product description, raw material description, complaints, training procedures. All the identified and relevant hazards, critical control points and critical limits, preventive and corrective actions should be documented. Monitoring system consists of records about the control of process, critical control points and prerequisite programs. Verification process and procedures and all the results of HACCP audit should be documented. Company should record all the consumer complaints and corrective actions (Dillon and Griffith, 1997, www.codexalimentarius.net).

PRODUCT DESCRIPTION

RTE lettuce salad including 2 pieces of naturally fermented black olive, RTE canned tomato cubes and pre-packed olive oil-vinegar sauce product for airplane passengers was chosen for this study. The detailed description for product and production is given below. Potential hazards in raw materials and in the process are given in Table 3.

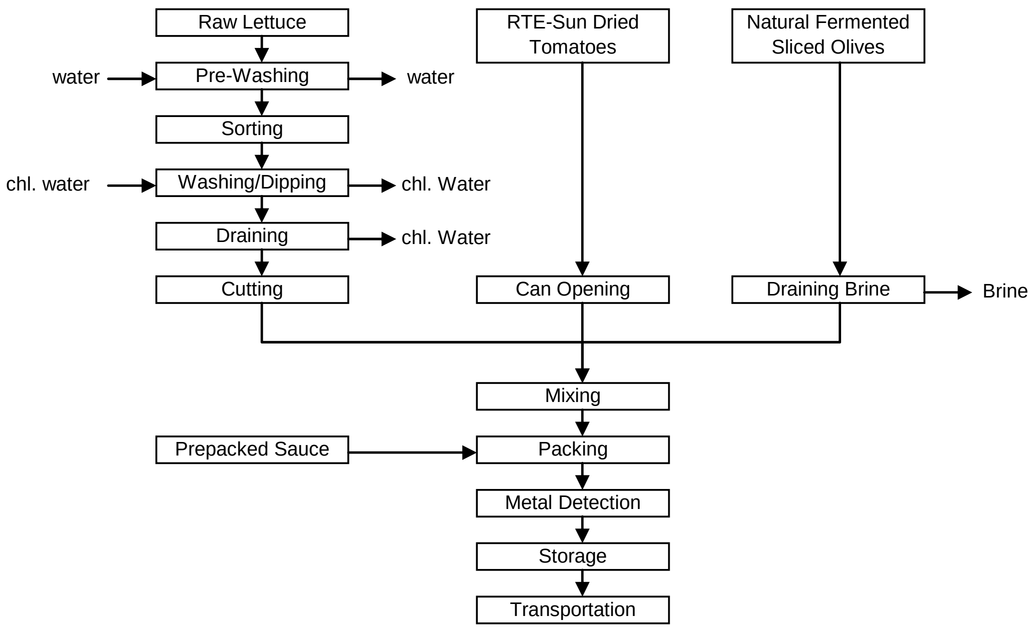

Figure 1. Flow chart of RTE lettuce salad production.

Table 3. Potential hazards.

Material / process	Biological hazard	Chemical hazard	Physical hazard
Raw material	Bacteria Yeast Mould Pests	Pesticides	Soil & stones Pieces of packaging Not hermetically sealed dressing packaging
Packaging materials	Bacteria		Broken packaging materials
Process	Microbes by cross contamination & employees	Wrong conc. of dipping solution Cleaning agents and disinfectants	Wrong temperature Pieces of equipment Materials from employees Pieces of packaging materials

Raw material

Main ingredient in salad is lettuce. It should be fresh, not physically damaged. Short shelf-life and strict control of refrigeration (it should be +2 – +5 C°) limit the growth of pathogenic and spoilage microorganisms (Francis, Thomas & O'Beirne, 1999). Sources of microorganisms are soil (*L. monocytogenes, Cl. botulinum* etc), water e.g. *A. hydrophila, Salmonella,* faeces e.g. *E. coli, Salmonella, L. monocytogenes, Cl. botulinum* etc) (Francis, 1999). Because of contaminated irrigation water and poor hygiene practices parasites like *Giardia lamblia, Entamoeba histolytica* and *Ascaris* spp can be present in vegetables (Francis, 1999). Lettuce should be bought as often as possible, temperatures should be strictly controlled. Quality of lettuce should be checked before taking it to production. During fermentation of olives lactic and acetic acid are produced which increase the acidity level of the brine and lower its pH. Low pH in combination of high salt content is reducing the risk of overgrowth of harmful microbes. Polyphenols in olives provide some degree of protection against microorganisms. Olives should be produced by GHP. (Zervakis, 2005). Naturally fermented black olives should be stored at temperatures under +15 C°, kept from contamination.

Process and Zoning

In production it is needed to follow: 1) Temperature in rooms – if it is too high, the growth of microorganisms will increase; 2) Producing time – as there are raw materials with short shelf life, it is necessary to wash, cut, mix and pack as quickly as possible; 3) Personal hygiene of employees – if hygiene is poor, product may be contaminated with big quantities of extra microorganisms. If personnel is not following the rules and wears jewelers, artificial fingernails etc in production, don't cover hair correctly, those can drop into the product. Also washing and disinfesting hands and wearing sanitary clothes must be strictly followed. Personnel should be well trained about HACCP and their work; 4) Cleanliness of machines – shredding and mixing destroy surface cells, bruise under laying layers and allow juices to leak from inner tissues on to equipment and on to fresh-cut products. Moisture and exudates on cut surfaces and on surfaces of utensils and equipment provide excellent media for rapid growth of microorganisms. (Francis, 1999); 5) Clean water used in washing, dipping – unclean water can cause extra contamination. Ultraviolet radiation may be useful in inactivating vegetative bacterial cells in wash water (Francis, 1999); 6) Cleanliness of the rooms – if not well cleaned, product can be contaminated by extra microorganisms, pests, rodents and their excrements. Cleaning solutions should be with correct concentrations and rinsed off well & 7) Pest control – if system is not working, there is a risk of contamination with pests, rodents and their excrements.

HACCP plan for RTE lettuce salad is given in Table 4. RTE tomatoes, naturally fermented sliced olives and salad sauce are purchased according to the specifications which are set by the quality manager. Raw lettuce is purchased from a supplier who guarantied that there are no herbicides and pesticides used in the product. They are organically grown lettuces. The processing steps for the RTE lettuce salad is shown in Figure 1. Washing/dipping step is considered as critical control point since if the microbial hazards cannot be inhibited in this step, the product could cause a severe risk to the consumers. The metal detection process is considered as the second critical control point in this process. The temperature in packaging and storage areas need to be controlled an hourly basis and corrected if a deviation occurs. The distribution of the products needs to be performed under cold-chain to inhibit product spoilage.

Before workers enter the production area they need to wash and disinfect their hands. The disinfection of the hands will open the door to the production area (leaving one person through), thereby preventing workers without disinfected hands entering the production area. The toilets are placed outside the production area to prevent workers who went to the toilet to continue work without washing their hands. Suppliers are only allowed to bring supplies to the storage room, and the shipping agent is only allowed to take the end product from the storage room. Both the suppliers and shipping agent are not allowed to enter the production area to prevent contamination.

Training Activities Needed for Staff

Every food manufacturer has to make sure that their staff has sufficient knowledge about personal hygiene and food handling. It is important to have regular food hygiene training to ensure the adequate level of competence. According to EU regulation 852/2004 food handler has to ensure that: 1) food handlers have had enough training in food hygiene to perform required working tasks & 2) trainees themselves have had adequate training in HACCP and food hygiene.

Table 4. HAACCP Plan for RTE lettuce salad (modified from Hentges, 2003).

Process Step/ CCP	Potential Hazard	Critical Limits	Monitoring				Corrective Action(s)	Recording Keeping	Verification
			What	How	Frequency	Who			
Washing / dipping	*L. monocytogenes, E. coli* O157:H7, *Salmonella S.aureus*	Potable water @ pH 7.0	pH	pH meter	before processing; 2 times / shift	QA[a], test kits/ meters evaluated by QA regularly	Preprocessing batch adjustment; manually adjust water	Recording charts monitored and procedures reviewed	Random sampling; QA[a] audit; HACCP plan validated & review annually
		Potable water. ~1 ppm free residual Cl* for min 30 s	Free chlorine	Test kit/ automated	Continuous		Hold product from last correct reading for rewashing; record incident in deviation log	Continuous strip chart	
Metal detection	Metal pieces	3.5-mm stainless steel **	Metal	Sample run through detector	Hourly	Line operator	Keep correctly calibrated product and rerun it; record incident and product status in deviation log; identify source of metal and investigate line; add to maintenance program	Calibrated metal detector records by QA every shift; records monitored by QA every shift	Random sampling for metal analysis; QA audit; HACCP plan validated every year
Storage[#]	Microbial	+7 °C	Temp.	Thermometer	Continuous	QA personnel of the storage rooms	Mechanical regulation of cooling equipment. Continue with cooling until the required temperature is achieved.	Recording sheets of temperature control at storage rooms by QA in every shift	Random temp. control of the final products with calibrated thermometers. Regular data analysis

[a] QA: quality assurance personnel

* or other appropriate concentration of approved antimicrobial solution for wash water.

** or according to manufacturer's guidelines or customer specifications.

[#] Previously storage room temperatures were the part of prerequisite programs in present food enterprise. According to HACCP team decision the Control Points related with storage rooms temperature control were determined to Critical Control Points because of frequent not compliances registered in this area.

The quality manager has to organize food hygiene training that personnel would understand the importance of the means to anticipate the contamination of food products. Staff should be trained so that they can handle food according to good hygiene practice and has knowledge of the HACCP procedures at least in their working area. This requirement applies also to those working part time and staff dealing with working equipment. In spite of training, quality manager has to check regularly the level of understanding and following of the food hygiene principles. It is advisable to have information about food hygiene at their working place (e.g. on the corridor wall) but not directly in the production area. A new staff member should not work before he or she has passed the hygiene training and testing. Afterwards training frequency should be at least once in every two years but in some cases more often. For example if changes in technology or working procedures are made it would always include some additional training.

A plan of the food hygiene training is put together by the quality manager. The purpose, frequency and scope are determined in the plan. According to the plan quality manager organizes food training and measures and tests knowledge in food hygiene. If there are some deficiency or if food hygiene principles are not followed correctly, quality manager will increase the extent or/and frequency of the training. All training and testing should be documented. To ensure a high quality production process staff should, besides having good competence in food hygiene, be able to work together as a team. Also they should be on top of new trends in salad producing technology. Twice a year staff should have training in food technology were new trends of salad making are studied. At least once a year staff including the management will go out to travel to evolve their team working abilities and clarify their goals. As a result we will have an excellent, hard working and innovative team of professionals.

HACCP VERIFICATION

HACCP plan should be reviewed at least once a year. Review of HACCP plan by the team is needed when changing some raw material, recipe, production conditions, equipment, packaging, distribution conditions, consumer use etc. For the verification processes a procedure and audit checklist is needed. Competent auditors should carry out the audits and they should be independent from the audited field. During HACCP audit critical control points (CCPs), hazard analysis, prerequisite programs, documentation and records, non-conformance and corrective action should be assessed. The auditor should focus to the food safety and critical control points, but the full production should be viewed (Dillon and Griffith, 1997; www.codexalimentarius.net, Anon., 2008).

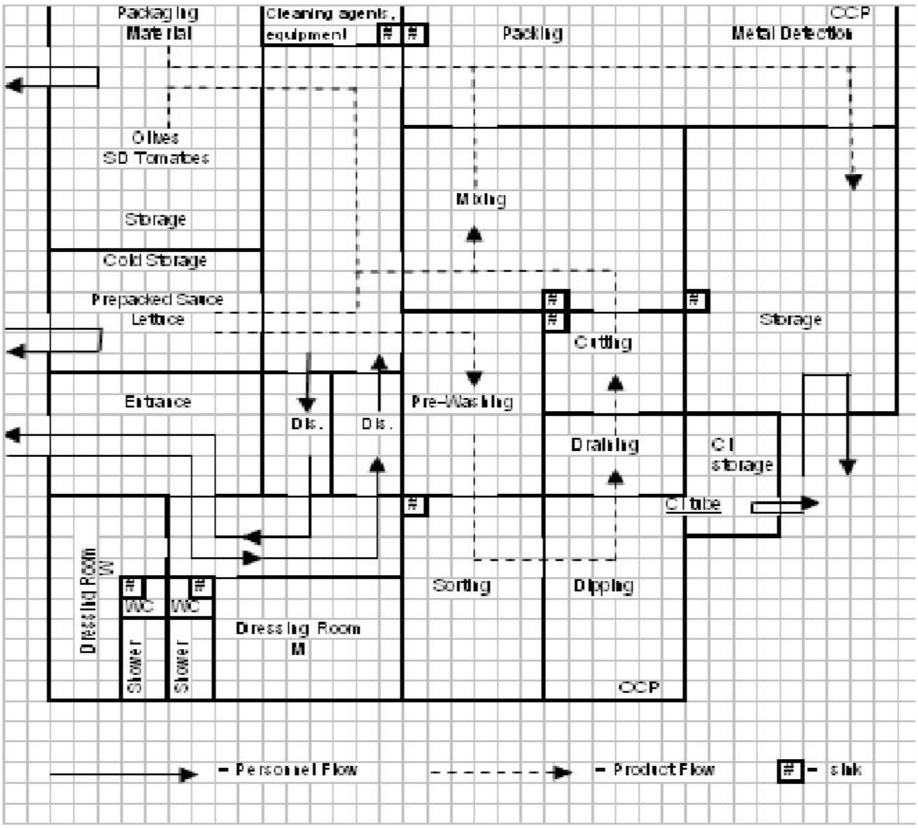

Figure 2. Floor plan for RTE lettuce salad factory.

RESEARCH NEEDS FOR TO IMPROVE FOOD QUALITY

There has been rapidly growing interest produce to ready to eat or ready to use foods and to prolong the shelf life and improve the quality. Specifically for RTE lettuce salad production one of the most important step is to trainee of farmers and stuff. Hygiene and microbial knowledge can be improving product safety. Cleaning of raw material and machinery are also important this kind of production. According to researches there are number of different disinfectants, detergents and cleaning agents could be use in production for to improve hygienic conditions. According to the hurdle technology concept, the desired safety and durability of fresh-like foods may be obtained by using combinations of mild preservation techniques. Hurdle technology (or combined processes) advocates the deliberate combination of existing and novel preservation techniques in order to establish a series of preservative factors that no microorganism present should be able to overcome. These hurdles may be temperature, water activity (aw), pH, redox potential, preservatives, etc.

„There are theoretical concerns regarding the safety of the use of antimicrobial dips. For example, pathogens if present on raw vegetables may not be fully eliminated by disinfection procedures, while at the same time the effects of disinfection on the indigenous microflora may be to reduce or remove natural competitive organisms. As a result, disinfection may produce conditions which favor survival/growth of the pathogen (Francis, 1999). Chlorine can be replaced with organic acids as lactic and acetic acid. To remove soil and reduce the number and growth of surface microorganisms, fresh produce is washed in potable water and cooled as quickly as possible after harvest. For storage is used modified-atmosphere packaging (MAP), perishable products are packaged in atmospheric gas compositions different than that of air (Larson *et al*., 1997) MAP extents the self life of minimally processed fruits and vegetables by suppressing the growth of aerobic spoilage microorganisms, reducing the rate of oxidation and enzymatic degradation and reducing the loss of water (Austin *et al*., 1998). Optimal oxygen (at least 2%) and carbon dioxide (no more than 20%) concentrations are generally maintained within the package to prevent anaerobic respiration, which accelerates senescence accompanied by development of off flavors and microbial growth (Watada *et al*., 1996). Refrigerated storage (5 °C) is a primary method used to suppress microbial growth and ensure safety of fresh cut produce. Psyhrotrophic pathogens such as *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Yersinia enterocolitica*, however, can survive and may reproduce at temperatures below 7 °C (Callister and Agger, 1989). Bacteria can survive and grow in the pH range of 4.0 to 9.0; however, specific genera need a narrower pH range, with pathogenic bacteria being the most fastidious. The optimum growth pH of many pathogens is within the pH range of vegetables (pH 4.2–7.3).

In addition TTI (Time Temperature Indicators) may be used and improve to monitoring existing of chilled condition after production. Furthermore, research can be done for to build new biosensors for *L. monocytogenes* and salmonella. Research can also performed to using natural antimicrobials such as lysoyme, edible coatings and films incorporated antimicrobials and intelligent packaging materials.

CURRENT AND FUTURE IMPLICATIONS

Although MAP, acidity, antimicrobials and surface disinfection practices are methods frequently used to inhibit pathogen growth, maintaining refrigeration and decreasing storage time before consumption are the most efficient ways to ensure the safety of fresh-cut produce (Nguyen-the and Carlin, 1994). Opportunities for marketing fresh cut salads will increase as consumers continue to demand freshness and convenience. But, pathogenic microorganisms are capable of growing on fresh-cut salads subjected to packaging and distribution practices common to the produce industry. To ensure the safety of salads, the further exploration is needed to identify safe, effective and affordable alternatives to chlorine for surface disinfection. Several non-thermal physical techniques, such as oscillating magnetic fields, high-intensity pulsed light, ultrasonics and hydrostatic pressure, are being developed that may offer alternative treatments for improving the quality and safety of minimally processed salads (Hentges, 2003).

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