COMBINING LASERS WITH EMERGING TECHNOLOGIES FOR MINIMAL PROCESSING

by

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ABSTRACT

Conservative estimates place the economic cost of food poisoning at hundreds of millions of pounds per annum. Recent market expansion of minimally processed fresh fruit and vegetables has resulted from the consumer's desire of convenience foods that have been peeled, cut, shredded or even washed. These produce, however, are likely to be contaminated. Such produce has a natural high microbiological burden. Minimally processing the food results in surface damage that is likely to favour bacterial adhesion. The cutting process leads to cellular damage, which coupled with increased adhesion and leakage of intracellular material, can lead to increased growth rate of spoilage organisms or pathogens. Minimally processed fruit and vegetables are often washed in water or water containing chemicals. The wash water can help distribute bacteria into the damaged sites on the processed fruit or vegetables. The use of chlorine has been associated with the production of carcinogenic and mutagenic substances and consequently there are fears over its use and potential accumulation within the body. Many government policies are encouraging people to eat more fresh fruit and vegetables. It is important therefore, that processors use the best practice available to supply fruit and vegetables with the minimum risk to consumers through digestion of pathogens and with a long a shelf life as possible to aid distribution channels, minimise waste and increase profits for processors.

This research was seeking to identify novel ways to reduce the levels of contamination on carrots and potatoes and extend their shelf-life. Technology was developed to exploit different parts of the electromagnetic spectrum to kill bacteria. The systems included a CO_2 laser, conventional UV lamps, and microwave irradiation. As an adjunct to these processes, H_2O_2 treatments and combination treatments were used. These systems were combined to kill bacteria on different substrates, fruit and vegetables. Comparison of shelf life tests and optimum treatment processes were also done. On carrots and potatoes the investigation of vitamin A and C levels, respectively, also indicated that their nutritious quality had not been degraded with any of the treatments.

Using ozone as a bactericide and bioluminescence as a measurement of viability or metabolic activity, a real time monitoring system was developed to measure surface decontamination. The *Escherichia coli* strain DH5α PT7-3 with its plasmid encoded with ampicillin resistance contained the lux ABCDE genes from Xenorhadus luminescence, were used as the reference organism. Results showed that the bioluminescence responded

instantaneously to treatment. Bioluminescence measurements were recorded in decibel, after 15 min of ozone treatment the bioluminescence was reduced by 4 dB and *E. coli* (lux) were inactivated.

To investigate the response of various Gram-positive and Gram-negative organisms to ozone treatment, *Campylobacter jejuni, Bacillus cereus, Escherichia coli, Listeria monocytogenes, Salmonella typhimurium* and *Staphylococcus aureus* were inoculated on plates and treated with ozone. The treatment achieved about 2 – 2.5 D-value reduction on plates. The same treatment was less effective when the experiments were repeated on contaminated chicken and salmon samples. A log reduction range between 0.37 - 1.5 was achieved.

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NOMENCLATURE

°C	-	degrees Centigrade
σ	-	absorption cross-section for ozone at 254.3 nm
A_v	-	Avagodro's number
B. cereus	-	*Bacillus cereus*
N(t)	-	total number of molecules per cm^3 as a function of time
C. jejuni	-	*Campylobacter jejuni*
C_O	-	The concentration of ozone gm/cm^{-3}
CO_2	-	carbon dioxide
cfu	-	colony forming unit
DNA	-	deoxyribonucleic acid
E. coli	-	*Escherichia coli*
H_2O_2	-	hydrogen peroxide
HACCP	-	Hazard analysis critical control points
I_o	-	irradiance with no ozone present
$I(t)$	-	irradiance as a function of time
jcm^{-2}	-	joules per square centimeters
L. monocytogenes	-	*Listeria monocytogenes*
LAF	-	Laminar air flow
M	-	molar mass
N	-	number of molecules
TVC	-	total viable counts
V	-	volume
ml	-	millilitre
msec	-	millisec
µsec	-	microsec
µl	-	microlitre
SEM	-	scanning electron microscope
RBDS	-	real time bioluminescence detection system
S. aureus	-	*Staphylococcus aureus*
S. typhimurium	-	*Salmonella typhimurium*

UV	-	ultraviolet
W	-	Watts
wt	-	weight

CHAPTER 1 Introduction to sterilization

1.1 Spoilage organisms and pathogens

Microorganisms are found almost everywhere and are capable of growth under a wide range of conditions. However, different types differ widely in nutritional and extrinsic growth requirements. Bacterial and other microorganisms are made up of proteins, polysaccharides, lipids, nucleic acids and other molecules and macromolecules [1]. To maintain viability and to grow these organisms must take in the major essential elements like hydrogen, oxygen, carbon, nitrogen, phosphorus and sulphur. These and other essential elements can be found naturally in foods and, by their very nature, such foods are highly nutritious and metabolizable. The organisms feed on the food substrate and produce by-products that result in spoilage. According to L. Gram *et al* in 2002 [2] of all foods produced globally, losses due to microbial food spoilage occurring during post-harvesting was estimated to be as high as 25%. Consumer demands and commercial interests in the food processing and agricultural industries have prompted the increase in research of emerging and new technologies for minimal processing and prolonging the shelf life of food-stuffs.

Organisms that have the potential to produce metabolites that are associated with spoilage of a particular food product can be characterised as spoilage organisms. Their metabolic activities can result e.g. in an off-odours, changes in colour or formation of slime [3]. However, food spoilage can also be gauged by the level of the consumer's acceptance of the food product, even if it is clinically safe for consumption. The factors that render food spoiled are often very subjective.

The growth of spoilage organisms in food, in a given set of environmental conditions, depends on the intrinsic properties of the food substrate. For example, vegetables with a moderately acidic pH encourage the growth of soft-rot producing bacteria such as *Erwinia carotovora* and *Pseudomonas*. Where low pH products like beer enable high microbial water activity, which encourages the growth of very high acid tolerant bacteria such as *Pecinatus* and especially *Megasphaera* in higher alcohol content beverages. This causes turbidity and off-flavours [4]. In other produce different spoilage organisms may

dominate. Generally *Enterobacteriaceae* [5] are found in meat and in fish, *Pseudomonas* [6] is one of the main spoilage organisms. Their natural function is to break down proteins of all kind. So, they grow on meat, fish, shell fish, milk products and any substance containing proteins.

Table 1.1 Some bacteria related to foodborne illness

Bacteria	Important reservoir / carrier	Transmission			Multiplication in food	Examples of some incriminated foods
		Water	Food	Person to Person		
Bacillus cereus	Soil	-	+	-	+	Cooked rice, cooked meats, Vegetables, starchy puddings
Campylobacter jejuni	Chickens, dogs, cats, cattle, pig, wild birds	+	+	+	-	Raw milk, poultry
Escherichia coli :						
Enterotoxigenic	Man	+	+	+	+	Salads, raw vegetables
Enteropathogenic	Man	+	+	+	+	Milk
Enteroinvasive	Man	+	+	+	+	Cheese
Enterohaemorrhgic	Cattle, poultry	+	+	+	+	Raw milk, cheese
Listeria monocytogenes		+	+	0	+	Soft cheeses, milk coleslaw, pate,
Salmonella typhi	Man	+	+	+/-	+	Dairy produce, meat products, shellfish, vegetable salads
Salmonella (non-typhi)	Man & animals	+/-	+	+/-	+	Meat, poultry, eggs, dairy produce
Staphylococcus aureus (enterotoxins)	Man	-	+	-	+	Ham, poultry, egg salads, cream filled bakery produce, ice-cream

+ Yes - No +/- Rare 0 No information

Table modified from [7]

1.2 Organisms causing foodborne diseases

Microorganisms that can cause disease are termed pathogens. Quoting form the World Health Organisation, "foodborne disease has been defined as a disease of an infectious or toxic nature caused by, or thought to be caused by, the consumption of food and water" [7]. It has been estimated that microbial contamination of food was 100 000 times greater than the risk from pesticide contamination. Some common pathogenic organisms that are found in our daily produce like meat, poultry, fish and vegetables are *Bacillus cereus,*

Campylobacter jejuni, Escherichia coli, Listeria monocytogenes Salmonella typhi and Staphylococcus aureus. **Table 1.1** shows the various types of bacteria and their common means of transmitting foodborne illnesses in food produce.

1.2.1 *Bacillus cereus*

Bacillus cereus is a bacterium that is common in the natural environment and in a variety of foods. They are Gram-positive, anaerobic, spore-forming and rod shaped. The bacterium can grow between the range of 8 to 55°C and optimally around 28-35°C. *B. cereus* spores are heat resistant, and can survive a temperature of 95°C in phosphate buffer for up to 36 min [8]. This characteristic poses a problem for the food industry, as it is not always possible to apply enough heat during food processing to kill spores [9]. For example, improper disinfection and insufficient heating of rice-containing dishes is a common source of *B. cereus* food poisoning [10]. The high survivability of *B. cereus* spores in dehydrated conditions also means that they are difficult to control even with preservation processes like drying. Their dispersal is so widespread that it is almost impossible to prevent the organism from contaminating certain foods.

B. cereus is frequently isolated from various kinds of contaminated raw and processed food products such as rice, spices, milk, dairy products, vegetables, meat, farinaceous foods, desserts and cakes [11]. Although, there were significant *B. cereus* food poisoning outbreaks in Norway in 1995 [12] such incidents are much less common than illnesses caused by *Campylobacter jejuni, Salmonella,* or *Staphylococcus aureus. B. cereus* causes two types of foodborne illness. A diarrhoea type of illness, caused by strains of *B. cereus* that produce a diarrhoea genic toxin, and a vomiting (emetic) type of illness caused by strains that produce emetic toxins [11].

1.2.2 *Campylobacter jejuni*

Campylobacter jejuni is an organism that is largely responsible for the majority of foodborne infections in the UK and other countries (see e.g. in **Figure 1.1** for U.S. data). Over the years, sporadic outbreaks of *Campylobacter* infection have been reported all over the globe: Japan [13], Indonesia [14], Germany [15], Britain [16], United States [17] and others [18]. *Campylobacter* are non-spore-forming, oxidase-positive, Gram-negative rods. The bacteria grow optimally at a temperature of 42°C under atmospheric conditions that are microaerophilic, with 5 – 10% oxygen and 3 – 4% carbon dioxide concentrations [19].

They survive poorly at room temperature and do not grow below 28°C, but they do survive through several weeks of chilled or frozen storage. *C. jejuni* is commonly present in the gastrointestinal tract of healthy cats, dogs, [20] cattle, or birds, which usually have higher body temperatures then humans [21]. Raw chicken is thought to be a major source of *C. jejuni* foodborne illnesses. Cross-contamination of food or utensils with raw chicken has been a factor in many of the reported outbreaks. Occasionally, they can be isolated from contaminated streams, lakes, ponds and even greywater [22]. These fastidious organisms can be easily destroyed by cooking. *Campylobacter jejuni* outbreaks are usually due to improper food handling or eating of raw or under-cooked chicken, or via contact with pets or farm animals [16]. Enteropathogenic *Campylobacter* causes an acute enterocolitis resulting in severe abdominal pain, fever and diarrhoea [8]. Campylobacter infection may be linked to Guillain-Barré Syndrome, a rare, but serious, form of neuromuscular paralysis causing limb weakness [23].

1.2.3 *Escherichia coli*

Escherichia coli is common species of bacterium that can be found in the intestinal tract of almost every human and warm-blooded animal. It is Gram-negative, non-sporing and belongs to the *Enterobacteriaceae* family. *E. coli* are typically mesophiles. They grow from 7 - 10°C, and up to 50°C with an optimum temperature around 37°C [8]. Most strains of *E. coli* are harmless, however, other strains like O157:H7 are capable of causing foodborne illness and death.

Virulent strains of *E. coli* can be categorised into Enterotoxigenic, Enteroinvasive, Enteropathogenic and Enterohaemorrhagic. Enterotoxigenic *E. coli*, or ETEC, is the name given to the group of *E. coli* that produce toxins which stimulate the lining of the intestines, causing them to secrete excessive fluid, thus producing diarrhoea. Enteroinvasive *E. coli* (EIEC) produce an illness known as bacillary dysentery, similar to that caused by *Shigella* [8]. Enterohaemorrhagic *E. coli* (EHEC) is a particularly dangerous type, and considered to be the common cause of haemorrhagic colitis (HC) [24]. Haemorrhagic *E. coli* O157:H7 is one of the most frequently isolated *E. coli* from humans. Major outbreaks were reported in the Western, United States in 1992-1993 [25;26] and Japan in 1996 [27]. One cause of such outbreaks has been the consumption of undercooked ground beef [28;29] which maybe widely distributed through 'fast food' restaurant chains [25]. These strains are associated with other foods such as salami, raw

milk, pasteurized milk, yogurt, lettuce, radish, sprouts and also the handling of potatoes reported in the United Kingdom [30]. Also there was a big outbreak in Scotland in December 1996 killing 21 people reported by Hugh P. [31]

1.2.4 *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive anaerobic, non-sporeforming organism. The coccoid to rod shape cells grow over a wide range of temperatures from 0 - 42°C, with the optimum temperature between 30-35°C [8]. It is able to grow below 5°C but at a reduced rate. *L. monocytogenes* is highly pathogenic and causes Listeriosis. Listeriosis causes immunocompromise and may lead to meningitis, septicaemia and even abortion [32;33]. This disease is a major public health concern because of the severe, non-enteric nature of the disease and high fatality rate. Outbreaks where high fatalities were reported involved 279 cases with 85 deaths [32].

The widespread distributions of *L. monocytogenes* during various phases of food production are accredited to the bacterium's ability to grow at temperatures as low as 5°C and with minimal nutrient requirements which permits multiplication during refrigeration. Food-associated outbreaks have been due to refrigerated food consumed without prior or adequate cooking. *L. monocytogenes* has been associated with foods such as raw milk, cheeses, ice cream, raw vegetables [34;35], raw meats [36], and raw and smoked fish [37;38].

1.2.5 *Salmonella*

Salmonellae are Gram-negative non-sporeforming rods belongs to the *Enterobacteriaceae* family, which primarily inhabits the gastrointestinal tract. They grow between 5-7°C and optimally at 37°C. They cause Salmonellosis disease which may develop into acute intestinal distress with sudden onset of headache, fever, abdominal pain, diarrhoea, nausea, and sometimes vomiting. More invasive strains of *Salmonella* like the *S. typhi*, can escape the confines of the intestine and be disseminated by the blood to other organs after multiplication [8]. Numerous types of *Salmonella* cause disease in both wild, domestic animals and humans [39]. While there is much variation in the relative prevalence of different types of *Salmonella* species, *Salmonella enteritidis* and *Salmonella typhimurium* are the two most commonly reported [39]. A multidrug-resistant strain of *Salmonella typhimurium*, called Definitive Type 104 (DT104), first emerged in England and Wales in

1984. These DT104 isolates posed a significant threat because they were resistant to several antibiotics normally used to treat people with *Salmonella* infections including ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline [8].

Salmonella outbreaks are commonly caused by insufficient cooking, cross-contamination through direct contact or indirectly via kitchen utensils, and long periods of standing food that encourages their growth. Other types of *Salmonella* related foodborne illness are often caused by drinking unpasteurised milk, eating poultry products such as eggs [40], seafood [41] or vegetables [42].

1.2.6 *Staphylococcus aureus*

Staphylococcus aureus are Gram-positive coccus, anaerobes with spherical to ovoid cells. *S. aureus* belong to the mesophile group capable of growth between the temperature of 7-48°C and optimum growth occurs at 37°C. It is commonly heat resistant and salt tolerant. It grows readily in media containing 5 - 7% NaCl but some strains can grow in media with 20% NaCl [8].

Globally, food poisoning related to staphylococcal intoxication is very common [43]. *S. aureus* food poisoning cases are generally the result of ingestion of pre-formed toxins produced by growth of the bacterium in foods [44]. *S. aureus* intoxication has a relatively short incubation period, typically 2 – 4 hrs [45;46]. The principal habitat of staphylococci is the skin, skin glands, and the mucous membranes of warm blooded animals [8]. It is frequently part of the normal human microflora [46].

In 1999, a large outbreak of food poisoning due to enterotoxigenic strains of *Staphylococcus* was reported in Brazil in cheese and raw milk. Among 30 *S. aureus* outbreaks in Japan, the bacterium was isolated from a large variety of other produce including sandwiches, soy, sweet bean paste, rice dumplings, rice and also cooking tools [47]. Ham has been described as the vehicle most frequently associated with *S. aureus* food poisoning, but other foods such as chicken, pork, beef, meat dishes or salads have also been commonly implicated [48]. Outbreaks of this nature are largely due to unhygienic treatment of food often associated with highly manually-handled food [49].

1.3 Hygiene in the food industry

Conservative estimates of food poisoning costs place it at millions of pounds per annum. In reality many estimates are too low. The emergence of convenience foods and in particular fruit and vegetable processing and packaging has seen significant growth in recent years and has presented new potential problems. Conventional wash processes for fruits and vegetables use chlorine. Chlorine, however is being phased out due to concerns over its carcinogenic nature. There is, therefore, considerable effort going towards finding replacement technologies and processes for microbial decontamination of foodstuffs. Numerous methods and processes are being developed that are either chemically-free, utilise different chemical combinations or even plant extracts, or a combination of these technologies and approaches. An important issue other than reduction of microbial bioburden is the effect of the treatment on the shelf-life of the produce and produce quality. Any extension that can be made to the shelf-life may reduce wastage, leading to increased profits for the grower, packer, handler or processor.

1.3.1 Routes to food contamination

In recent years, there has been considerable attention given to problems of contamination within the food chain. Recent outbreaks of food poisoning have been attributable to a number of causes such as improper handling or cross-contamination. To reduce risk of outbreaks, European legislation requires food handlers to be supervised or trained in food hygiene matters. Some studies suggest that this has increased knowledge of food safety but not created improved food handling behaviour [50;51]. Howes also suggested that 97% of outbreaks were due to improper handling by the food handlers [50]. More recently, Clayton *et al* [52], have studied the beliefs of food handlers and their practices. They concluded that the training should be on a risk-based approach, and that training alone did not result in a behavioural changes. Additionally, it was clear that adequate resources and a supportive management culture were necessary to implement good hygiene practice.

The impact of reporting outbreaks to the public can have a dramatic effect on their risk assessment and beliefs. Miles and Frewer [53] assessed the increase in public concern over food safety and in particular studied the impact of BSE, GM food, high fat diets, pesticide residue and *Salmonella* food poisoning (eggs). Most concerns were dependent

on the issue. For *Salmonella* poisoning, most concerns to the consumer were over health and risk of death. Interestingly, consumers felt safest when they were preparing there own food but at most risk when eating out. It was suggested that the risk assessors should discuss what is being done to deal with a particular outbreak as well as the scientific facts.

A prime example of improper handling and cross-contamination is when contaminated, uncooked food is placed either in direct or indirect contact with cooked foods or those eaten raw. This can happen by the food handlers or by the consumers themselves. An analysis of the domestic risk was made by Griffith *et al* [54]. Over 95% of consumers (108 samples) when following a number of recipes "failed to implement one or more basic hygiene practices due to lack of knowledge or failure to implement known food safety procedures." If eaten, these meals would have constituted a food poisoning risk. Only 4.6% used appropriate food safety measures. 3.7% posed a high level of risk.

There are numerous examples of food poisoning throughout the world, some of which have already been cited. In North America, in 1996 and 1997, there were cluster outbreaks of cyclosporiases that were associated with eating fresh fruit and vegetables. [55]. In England and Wales, Aeromonas *spp.* are found in over 500 cases of gastrointestinal infection each year. Isolates were identified in 19 of 25 salad samples that were assessed by Mattick *et al* [56]. *Salmonella typhimurium* was isolated in 372 cases between 1 August and 13 September 2000, in England and Wales [57]. In these cases the age-range of infection was from 1 month to 94 years old. The sources were thought to be lettuce, tomatoes and cucumber in baguettes, burgers or kebabs that were purchased from takeaways.

Between 1992 and 2000, 1518 outbreaks of infectious intestinal diseases (IID) were reported to the Public Health Laboratory Service (PHLS) [58], affecting 3438 people, 69 of whom were admitted to hospital and one person died. 5.5% of these (83) were due to consumers eating salad vegetables or fruit. *Salmonellae* (41%) and Norwalk-like virus were the most frequently reported pathogens. 67.5% of the outbreaks were traced to commercial catering premises. Three outbreaks were due to lettuce contaminated with *S. Typhimurium* DT 204b, which accounted for 501 cases.

A study of 370 samples of lettuce, meat and Spanish potato omelette were taken from

Spanish restaurants to assess the incidence of a range of microorganisms [59]. It was found that *Escherichia coli* and enterococci had the highest incidence on lettuce and *Staphylococcus aureus* was found most frequently on meat. A sample of 320 vegetarian foods purchased in Taiwan by Fang *et al* [60], showed that vacuum packed vegetarian foods had the lowest incidence of microorganisms

A review by the PHLS Communicable Disease Surveillance Centre (CDSC) [61], found that 60 cases out of 1408 (4.3%) of food poisoning cases reported between 1992 and 1999 in England and Wales were due to contamination of ready to eat fruit and vegetables. Additionally, it was reported that viruses account for 6% of foodborne infection and 5% of waterborne infection causing gastrointestinal illness. Of concern was that viruses could be recovered from inoculated samples of fruit and vegetables after prolonged storage at temperatures of 4 and 8°C. The viruses survived longer than the shelf-life of the produce. Washing gave between 0.5 and 2.5 log reduction of the contaminating organisms.

A study by Wheeler *et al* [62] suggested that, it is likely that figures reported for food poisoning are underestimated but in general outbreaks constitutes less than 10% of laboratory reports of *Salmonella* and less the 1% of reports of campylobacter.

1.3.2 HACCP

It is clear that contamination of foods with potential spoilage and pathogenic organisms is frequent. Appropriate steps have to be taken to minimise risk at every stage from farm to fork. This includes planting, harvesting, processing, packaging, transportation and selling.

Handlers, processors or packers need to identify critical control points of likely contamination through Hazard Analysis Critical Control Points [63] and then put in place measures, to ensure that contamination does not occur and microbiological burdens are kept within norms. HACCP also considers the design of the processing plant. Measures should also be taken to monitor contamination levels with appropriate normal and emergency decontamination protocols in place. Such measures may include downtime to clean equipment at the end of each day. Whilst it is realised that such measures can prevent microbial burdens rising too significantly and reducing the likelihood of food poisoning outbreaks, clearly there is room for improvement to reduce microbial levels .

HACCP provides a basis for ensuring that best practice is always done. It is important that this is an ongoing process, with data fed back to develop and maintain the HACCP systems [64]. However, the US meat and poultry industry stress the importance of minimizing the level of pathogens [65].

Direct contamination occurs when raw or contaminated food or utensils come into contact with the food and indirect contact is when the contaminated food is used on a chopping board or cut with a knife, and these implements are then used to prepare the food which is to be eaten raw. Most people associate contamination and cross-contamination with meat. Chicken is a prime example where people, in general, do exercise proper care and attention. They would separate raw and cooked meat in the fridge and be careful with cooking utensils, making sure that different chopping boards are used or that they are washed in hot water with detergent. People are less careful when it comes to preparing fruit and vegetables. If someone had a steak and salad and was subsequently ill, they are more likely to attribute their illness to a contaminated steak than a contaminated salad. In reality, it is more likely to be the salad that has caused the food-borne illness than the cooked steak. Public perception is a major issue when it comes to preventing contamination and food poisoning. The producer or food manufacturer can only use the best practice available. Once produce is sold, the consumer has a responsibility to ensure that the produce is not cross-contaminated.

There are legal issues for the supplier of food if a person becomes infected. Tests can be done on remaining suspected food to identify the source of the outbreak. However, the reliability of the tests is an important issue, as discussed by Anklem [66]. It then becomes important that the food producer can trace the source of the food sold to a particular customer or retail outlet. This is done for two reasons:

Firstly, it helps identify the source of any infection, which can then be eradicated, and other possible related products can be called back or public warnings given about concerns over food quality. Example of this occur when the food has been found to contain broken glass or chemical contaminants, where product recalls are given. In some cases, such as the Coca-Cola incident in Belgium in June 1999, there was a major outbreak of dioxin contamination reported. There was, however, no physiological evidence and it was concluded by Nemery [67] that it was mass sociogenic illness. This demonstrates the

importance of public risk assessment and how incorrect handling can lead to other social problems. Appropriate reporting is necessary.

Secondly, it helps protect the producer. It may be that the source of infection was due to poor consumer hygiene. In which case there is no likelihood of the producer being sued if this can be proven. The producer, therefore, needs to be able to defend their position which is best done through adopting the best available practice. Even with HACCP, however, improvements can still be made in the microbiological quality of restaurant meals [68].

With increasing litigation, it is ever increasingly likely that producers will be taken to court over cross-contamination and food foodborne illnesses due to poor consumer handling because contaminated produce has been supplied. Furthermore, an even more significant risk for producers is for those companies that supply food that is eaten raw, allowing possible litigation for direct contamination of consumers. This is a serious issue that can affect industries profitability. As markets continue to grow, cases of outbreaks must be controlled.

Buzby and Frenzen [69] investigated the US liability system for food poisoning. They quoted there was an estimated 6.5 to 33 million foodborne illnesses in the US per year and up to 9000 deaths; costing the US economy $6.6 to $37.1bn annually. The authors assessed whether there was strong incentive to produce safer food. They concluded that the product liability provided a weak incentive for US firms to produce safer food, and there were weak incentives to pursue litigation because expected monetary compensation was in general, low. 0.01% of all foodborne illnesses are litigated in the US. Only about 56% of claims are compensated by firms. Arguments were made that governments must drive increased food safety as there was no incentive for companies to operate above minimum levels.

One of the most significant growth industries in recent years has been the sale of minimally-processed raw foods such as carrots, salad stuff and vegetables. These may be eaten raw and therefore the producer has a legal responsibility to use the best practice available to ensure their produce is fit for human consumption. The produce is generally processed in someway, perhaps washed, cut, or peeled, and then packaged. Recycling of

water is becoming more common as water charges are increasing but there are special considerations needed when applying HACCP to water reuse [70], which itself may be used to clean fruit or vegetables.

Minimally-processed fruit or vegetables are a convenience food as the consumer does not have to cut or peel the produce, and very often they do not bother to wash the produce either. How the food is produced, how it is washed and cleaned and made ready for human consumption is therefore a very important issue in relation to the consumer's health and welfare and, also, the welfare of the supplying industry.

1.3.3 Planting and harvesting

Before the consumer can buy produce, it has to be harvested and made ready for market. Procedures adopted post-harvest are dependent on the produce and its market place. Ultimately these procedures have a significant impact on the bioburden and determining the product's shelf-life. In general, most decontamination technologies such as washing, chlorination or other chemical procedures only produce 1 or 2 log reductions in the total viable counts. It is essential therefore, to reduce the risk of contamination at every stage of from harvest to when the produce is market ready.

The practice of spreading manure onto farm land to act as a fertilizer can produce surface contamination of produce as pathogens can survive in soil for many months. Contamination of soil, however, can also lead to internalisation of bacteria in the produce [71]. This places even greater emphasis on the quality of the soil prior to planting of any crop and the need to control pathogens in soil. Contamination by faecal matter, animal waste, poor water quality or recent flooding may lead to excessive soil contamination [72].

Detailed by Mayberry for carrot and potato [73] production, the cost of minimising risk of contamination has to be weighed against the cost of production of crops. During harvesting, the produce must not become damaged. This can lead to possible sites of infection, more waste as the produce does not meet basic quality requirements and a reduced shelf-life. Overall, this will result in reduced profit margin for growers. The time of harvesting is critical to ensure high quality produce. Shelf-life can be dominated by the maturity of the crop. If the produce is too young, it may not ripen sufficiently from harvest. If the product is too old then the produce may not have the firmness to have

sufficient shelf-life to even reach market. Additionally for potatoes, the glycoalkaloid content of the potato can be affected by the harvest date [74]. The farmer may have to consider other important practicalities such as the costs of production, planting techniques and yield [75;76], that directly effect the profitability of their business.

1.3.4 Post harvest treatment of fruit and vegetables

Once harvested, the produce may be stored or prepared immediately for market. Storage conditions and practices are vitally important to maintain high quality product during the time of storage. The major reason for storage is satisfying timely market demand to maximize revenues. If produce can be stored until the produce is out of the local growing season, then the produce can be sold at premium rates. The grower will be able to compete with products grown in other countries that suffer the additional burden of transportation costs. The extra potential profit from the premium rate has to be assessed against the cost of storage incurred to control critical environmental factors such as temperature and humidity, which for example, is essential for potato storage [77].

It is important that for processed foods, or those minimally produced, that the additional process does not contribute to the overall microbiological bioburden or significantly reduce its shelf-life. According to Garcia- Gimeno, the shelf life of ready-to-eat salads is typically three days and the recommended storage temperature is 5°C [78]. Whilst packers, food handlers, and transporters have controlled chilled rooms and vehicles, there is continued reliance on the consumer to follow advice with proper storage conditions and temperatures to ensure safe food consumption within the recommended shelf-life. Johnson [79] led an investigation into food safety practices amongst the elderly living at home, and found that the majority do not meet recommended safety standards. Temperature measurements from 645 fridges found that 70% (451) were too warm with temperatures from 6 to 13°C and only one was too cold, at a temperature of -2°C. The warmer temperatures were insufficiently cold to prevent the growth of pathogens. 557 people stated that they kept their fridge stocked with food, indicating that the food was stored for long periods of time without rapid turnover or use.

Carrots are sometimes eaten raw and may be minimally processed by washing, cutting or shredding. There are significant industrial concerns regarding microbial burdens from washing fruit and vegetables and the subsequent effect on the shelf life of the produce.

Additionally, the minimal process itself may adversely affect the shelf-life of the product by damaging plant cells, leading to faster deterioration.

It has been common practice to wash produce in chlorine. Recently, there has been reported carcinogenic and mutagenic properties of chlorine by products, such as trihalomethanes, chloroform and chlorophenols [80]. This has led to development of other treatments and chlorine is being phased out in some countries and not pursued at all in others, but the situation remains unclear.

Some foodstuff, such as potatoes are cooked by the consumer, and consequently, there are fewer fears over their natural bioburden. However, the potato industry is concerned about an outbreak being attributed to a contaminated potato via cross-contamination and the effect that would have on their industry. Processing of potatoes leads to products that are either cooked by the processor or the consumer, but again microbiological levels need to be below allowed limits once the product is available to the market. Guidelines to acceptable limit was published in September 2000 by the Public Health Laboratory Service and is shown **Table 1.2** and **Table 1.3** below.

Table 1.2 Guidelines for the microbiological quality of various ready-to-eat foods

Food Category	Criterion	Microbiological quality (CFU per gram unless stated)			
		Satisfactory	Acceptable	Unsatisfactory	Unacceptable potentially hazardous
	Anerobic colony				
1	count 30 °C/48	$< 10^3$	$10^3 < 10^3$	$\geq 10^4$	N/A
2		$< 10^4$	$10^3 < 10^4$	$\geq 10^5$	N/A
3		$< 10^5$	$10^3 < 10^5$	$> 10^6$	N/A
4		$< 10^6$	$10^3 < 10^6$	$\geq 10^7$	N/A
5		N/A	N/A	N/A	N/A
	Indicator organisms				
1-5	*Enterobacteriaceae*	<100	100-$<10^4$	$> 10^4$	N/A
1-5	*E. coli* (total)	<20	20-<100	$\geq$ 100	N/A
1-5	Listeria spp (total)	<20		$\geq$ 100	N/A
	Pathogens				
1-5	*Salmonella* spp	Nil in 25g			Detected in 25g
1-5	Campylobacter spp	Nil in 25g			Detected in 25g
1-5	*E. coli* 0157 & other VTEC	Nil in 25g			Detected in 25g
1-5	*V. cholerae*	Nil in 25g			Detected in 25g
1-5	*V. parahaemolyticus*	<20	20-<100	100-$<10^4$	$\geq 10^3$
1-5	*L. monocytogenes*	<20	20-<100	N/A	$\geq$ 100
1-5	*S. aureus*	<20	20-<100	100-$<10^4$	$\geq 10^4$
1-5	*C. perfringens*	<20	20-<100	100-$<10^4$	$\geq 10^4$
1-5	*B. cereus* and other pathogenic *Bacillus spp*	$<10^3$	10^3-$<10^4$	10^4-$<10^5$	$\geq 10^5$

Table modified from [81]

Table 1.3 Colony count categories for different types of ready-to-eat foods

Food Group	Product	Category
Meat	Beefburgers	1
	Brawn	4
	Faggots	2
	Ham - - raw	5
	Kebabs	2
	Meat meals	2
	Meat pies	1
	Meat, sliced (cooked ham)	4
	Meat, sliced (beef, haslet, port poultry)	3
	Pork pies	1
	Poultry (unsliced)	2
	Salami and fermented meat products	5
	Sausages (British)	2
	Sausages (smoked)	5
	Sausage roll	1
	Scotch egg	1
	Tripe and other offal	4
Seafood	Crustaceans (crab, lobster, prawns)	3
	Herring/roll mop and other raw pickeled fish	1
	Other fish (cooked)	3
	Seafood meals	3
	Molluscs and other shellfish (cooked)	4
	Smoked fish	4
	Taramasalata	4
Vegetable	Coleslaw	3
	Fruit and vegetables (dried)	3
	Fruit and vegetables (fresh)	5
	Prepared mixed salads and crudites	4
	Rice	3
	Vegetables and vegetable meals (cooked)	2

Table modified from [81]

In recent years, the convenience of minimally-processed foods has led to significant market penetration of prepared salads and vegetables. Such produce is attractive to restaurants, fast food outlets and retail markets. This market appeal, and the inherent problems of contamination of these foods, has led to considerable scientific work investigating the quality of fresh-cut produce [82]. This work has led to an understanding of the factors that can contribute to reduced quality. Controlling these factors reduces deteriorating effects and prolongs the shelf-life. Examples of these factors are appropriate harvest time, low temperature, relative humidity and appropriate gas mixture for packaging such as CO_2 and N_2 rich environments [83].

Minimal processing, however, can make the produce more susceptible to discolouration and infection because of damaged cells and tissue around the cut and the possibility of contamination into the cut region. This leads to generation of an uneven topology where bacteria will adhere more easily, and may be more difficult to remove from the substrate. Additionally, any decontamination treatment is likely to be less effective in regions where the topology is non-uniform or highly irregular. This leads to the need for sharp cutting tools that produces an even a cut surface as possible. This adds additional hygiene cost to minimal processing as the cutting tools need to be kept sharp or replaced at frequent intervals, this introduces down time into the production operation.

Additionally, exposed flesh can lead to discolouration of some produce because of enzymatic activity. Whilst this is not a significant effect for carrots, it can lead to rapid deterioration of some fruits and vegetables such as potatoes [84] apples or celery. Carrots can become dry and white in colour at regions where they have been cut, leading to an aged appearance and lacking consumer appeal. This is due to dehydration of damaged cells [85]. It was found that dehydration of carrots, and the whitening, was reduced by treating them with calcium and a high relative humidity [86].

Of fresh produce available from markets, approximately 40% is sensitive to chilling. In general, however, slight chilling injury is preferable to injury caused by higher temperature and natural deterioration.

1.3.5 Post harvest technology

There has been substantial effort by the scientific community to investigate the factors that contribute towards the shelf life of fruit and vegetables [78;82;83]. These processes have utilised chemical washing with chlorine and optimisation of the environmental factors such as pH or temperature [87]. However, because of public concern over the use of chemicals and the undesirable effect these may have in cumulative quantities in the human body, there have been attempts to produce new techniques or procedures that are chemical-free or use chemicals that are less harmful. Additionally, the efficacy of chemical washes or different sanitizers has been assessed [88]. Chlorine dioxide, ozonated water and thyme oil were used to decontaminate *Escherichia coli* O157:H7 inoculated onto lettuce. They concluded that the inoculation method, incubation time, population size and number of washes had a significant impact on the efficacy of the sanitizer. Chlorine dioxide was used by Han *et al* [89] in gaseous form to assess its ability to kill *E. coli* and its effect on attachment of the microorganism to pepper surfaces that were either damaged or undamaged [89]. It was found that ClO_2 was more effective than water washing alone. Water washing on damaged surfaces achieved a 1.5 log reduction in viable bacteria numbers, whereas 2.44 log reductions was achieved on undamaged surfaces. When ClO_2 gas was used, it produced 3 or 6 log reduction on surface injured pepper surfaces with chemical concentrations of 0.62 and 1.24 mgl^{-1} ClO_2 respectively. With washing, it is important that the wash water is clean and free of contamination. Additionally, if ice is used during the refrigeration process, as is common in the fishing industry, then its microbiological quality plays an important role in maintaining the overall quality of the produce. Commercially available ice was found to be contaminated with coliforms, *E. coli*, *Salmonella*, *Shigella*, *Yersinia*, *Vibrio cholerae* and *Aeromonas* spp. [90].

Steam treatment of winter carrots was used by Afek [91] to reduce the bioburden on organically-grown carrots. A 3 second exposure to steam resulted in only 2% of carrots decaying as opposed to 23% in the control group. The carrots were kept at 0.5°C for 60 days and a week on the shelf. There was a different decay rate for spring carrots because *Erwina carotovora* was present on the produce during this time of the year.

Inhibition of browning of potatoes was investigated by Kaaber *et al* [92] after they had been peeled (minimally processed). Sulphite is the normal method to inhibit browning,

but Kaaber used CO_2 and N_2 gas in the immersion water to remove oxygen. The storage temperature was 4°C. Interestingly, CO_2 completely inhibited browning whereas there was no effect with N_2. It was thought that this was due to the low pH in the CO_2-treated water. Quite clearly, the bacterial survival rate on any produce is dependent on the pH of the substrate medium and the organism, and the pH can affect enzymatic activity. Indeed, it was suggested by Braun *et al* [93] that enzyme activity produced by spoilage–causing bacteria could be used as a measure of the shelf-life estimation.

1.3.6 Emerging decontamination technologies and processes

There are many different emerging technologies that have been assessed for decontamination applications, including decontamination of fruit and vegetables. Some of these technologies have been developed for other applications or markets and their suitability for decontaminating or extending the shelf-life of fresh produce is being evaluated. It is clear, however, that there is no one technology that can effectively decontaminate everything. This is often phrased as there being no golden bullet. This is particularly true for organic produce where there is a need to balance the decontamination or shelf-life extension treatment with its effect on the substrate medium. As with food poisoning itself, public perception of risk and the form of treatment used is crucial. One example here is the use of gamma irradiation. This has been available for some time since its use for sterilization within the medical industry. Its use on food however, has in the past been resisted by public opinion. A recent review by Hunter [94] has suggested that this attitude may be changing, with consumers becoming more willing to purchase irradiated food.

More recently, there have been investigations into combining gamma irradiation with more conventional processes. For example, the shelf life of beef products after gamma-ray and microwave treatment was investigated by Aziz *et al* [95]. They found that the shelf-life was enhanced with microwave treatments at lower levels. Steam and gamma radiation was combined to treat mango pulp stored at refrigerated temperatures [96]. The addition of steaming the pulp for 12 min before irradiation (1-2 kGy) increased the shelf-life from 90 days (gamma-rays only) to 270 days. This suggests that gamma irradiation alone may not be sufficient to substantially increase the shelf life, at least at the levels used in this investigation. Of course, investigators have also studied the effect of gamma irradiation directly. Aziz and Moussa [97] examined the effect of irradiation on

mycotoxin-producing mould in fruit. Mycotoxins production in fruit decreased with increasing exposure to irradiation and above 5 kGy there were no mycotoxins produced.

Some of the aspects of emerging technologies were addressed by Butz and Tauscher [98]. They considered: ohmic heating, high electric field pulses, UV light, light pulses, oscillating magnetic fields, ultrasound, and high pressure processing. Ohmic heating utilises the conductive electrical resistance within a conductive liquid or solid material in food to generate heat at a controlled energy input to reduce thermal damage to food, while generating enough heat to cause cell membrane rupture killing the bacteria. As for high electric field pulsed treatment, bacteria are inactivated because of the electrical breakdown where electroporation is generated by high voltage pulses between two electrodes. UV technologies use UV light in the germicidal wavelength range between 200 – 280 nm. The absorption of the UV light causes cross-linking between neighbouring pyrimidine nucleoside bases in the same DNA strand, preventing DNA transcription and replication necessary for multiplying and eventually leading to cell death [99]. In light pulse technology, intense and short duration pulses of broad-spectrum "white light" were used instead of just the UV spectrum. The spectrum of light for pulsed light treatment includes wavelengths in the ultraviolet (UV) to the near infrared region. At the UV-rich wavelength it causes photochemical changes while at visual and infrared wavelengths, photothermal changes take place resulting in antimicrobial effects. Preservation of foods with oscillating magnetic fields (OMF) involves magnetic fields oscillating between 5 to 500 kHz and they could be applied to food sealed in a plastic bags. According to [100] theories explaining the mechanisms of microbial inactivation were that OMF could loosen the bonds between ions and proteins that are vital to cell metabolism and inactivation may be the result in the breakdown of covalent bonds in the DNA molecule. Ultrasound treatment technology uses energy generated by sound waves of 2 kHz or more. They cause intracellular cavitations by disrupting cellular structure and cellular functional components, and cell lysis with micro-mechanical shocks [98] to achieve inactivation. Another emerging technology used in the food industry utilizes high pressure treatment of up to 800 MPa on liquid and solid foods. During high pressure processing biological membranes breakdown through cellular mass transfer that denaturizes enzymes and proteins killing the organisms [98]. Karaman and Erkmen report that in high pressure systems inactivating of *E. coli* in broth [101], at pressures of 25 to 100 atmospheres, the inactivation rate increased with increasing pressure, temperature and exposure time. In

recent years, great effort has focussed on high pressure processing, with industrial applications generally centred on high value products such as orange juice. An analysis of the changes in the functional properties of vegetables created by high pressure treatment [102] concluded that there was no loss in the nutrientionel benefits of the vegetables (carrots, tomatoes and broccoli) but there were changes in the physico-chemical properties such as water retention, reduced extractability, or a higher glucose retardation index. Other developments include the use of CO_2 gas where it has been used in a dissolved state against Gram-negative bacteria, with a view for modified atmosphere packaging (MAP) [103].

It is seen that with such a diverse range of produce that needs treatment and such a large number of potential treatments and physical variables, it is clear that there is a range of optimum treatment variables. This is made significantly more complicated by the enormous number of variables associated with each part of the process, particularly with emerging technologies. Some of these are quite complex, such as pulsed electric field, or pulsed light. This complexity is increased further when it is considered that there are still the variables associated with the growth of the produce, the harvesting technology and handling, post harvest treatment, environmental conditions at each stage, and the environmental parameters associated with the treatment condition such as temperature and pH.

1.4 Ozonation

Ozone is an unstable gas, toxic at high concentrations and a strong oxidiser. It is very reactive and tends to attack many chemicals of biological importance compared to other oxidisers such as oxygen and carbon dioxide [104]. Ozone is commonly used for disinfection of drinking water [105-108]. Driven by consumer demand, the food industry is currently in search of innovative processing technologies of prepared ready-to-eat products containing fruits and vegetables. Ozone is powerful sanitizer and has been reported to kill *E. coli* 600-3000 times faster then chlorine [109]. Not only that, the ozonation process has the advantage of not leaving harmful residuals after treatment [104] as the ozone decays. With the approval of regulatory agencies and the acceptance of consumers, food industries are now looking into direct ozone applications on food [110].

Over the years there have been various hypotheses of the mechanism of ozone inactivation. According to Christensen and Scott in 1963 [111;112], ozone attacks the microbial cell envelope or membrane by altering unsaturated double bond of the lipids in these structures. The ozone alters the permeability of the membrane and ultimately results in leakage of the cell constituents. Others have reported that the high reactivity of ozone with numerous organic compounds acts as a general protoplasmic oxidant [113]. Damage to DNA leading to the inactivation of *E. coli* was also reported to be another mechanism of ozone inactivation of *E .coli* [114]. Most recently, inactivation was reputed to be due to single-strand breaks in DNA [115].

1.5 Bioluminescence

In an ideal world, a real time detection process for microbial viability would enable the effect of all the system and physical variables to be assessed through instantaneous measurement of the changes in the microbial contamination level.

Conventional microbiological methods are based on detection, enumeration, and presumptive identification from visible growth on various media followed by final identification counting procedures and subsequent biochemical tests. These take at least 24 hrs and sometimes even 2–3 days depending on the microorganisms [116;117]. With the bioluminescence assay, the detection time is reduced to 1 hr and has a detection limit of 10^4 cells per grams [118]. On food where plate counts are impractical, bioluminescence assays can be used for monitoring bacterial survival where both the food matrix and the microflora may alter survival characteristics. Some examples of bioluminescence applications on food were reported by Gordon [118] where sausages, mortadella, German salami, Italian salami and bierwurst were overlaid with bioluminescent bacteria to determine bacteria distribution.

1.6 Current work

Some of the current work was based on an EU contract [119]. Minimal processing with combined emerging unit operations - laser, UV, microwave and chemical - for microbial killing and improved food quality.

The project objective was to build a novel test rig combining laser, UV, microwave and chemical treatment and to reduce wastage of fruit and vegetables by increasing their shelf life and increasing their safety by reducing levels of microbial contamination. The shelf life of tomatoes, carrots and potatoes after treatment with individual and combination systems was determined.

Eight partners from five member states were involved with this work. Rofin Sinar (Germany) provided the laser source. The microwave system was fabricated by Medexpo (Netherlands) and the UV and chemical systems were built by Glasgow University, including the author, and Medexpo. A detailed design and fabrication of an optimized UV system was done by the author. Integration of the subsystems was done by Glasgow University and the author. Country Crest (Ireland) provided a roller table that was typical of those used in industry; this facilitated the movement of the carrots and potatoes through the treatment systems.

After the EU contract was completed, further work was done by the author investigating the response of *Campylobacter jejuni, Bacillus cereus, Escherichia coli Salmonella typhimurium,* and *Staphylococcus aureus* to ozone treatment. This work led on naturally from the EU contract where ozone can be generated from UV lamps.

The work on ozonation led to the fabrication of a rapid, real-time monitoring system to identify the effects of the decontamination treatment on fish and chicken.

Chapter 2 describes the systems that were used in the work. This includes the laser, microwave, UV and chemical system. The integration of the subsystems into the decontamination system is described. This is detailed from the subsystem level to the way in which the produce was moved through the treatment areas on the roller table.

Chapter 3 describes the protocols that were developed to test the efficacy of the subsystems and the combined treatments. The individual treatments included laser, microwave, and UV. The results of using these different processes individually and combined on the microbial burden of artificially inoculated carrot and potato segments is detailed. For completeness, the results from the individual chemical treatments are

reported in the final report [120].

Chapter 4 gives the protocols and results of the effect of the individual treatments and combination on the shelf life of whole carrots, potatoes and tomatoes. In general the produce was rolled through the treatment area, bagged and stored under different conditions. The treatments included the laser, UV, microwave and H_2O_2. The combination treatments included UV and H_2O_2.

A bioluminescence real time monitoring system of the decontamination process of ozone is reported in Chapter 5. The system is described along with some experimental results of treating plates lawned with *E. coli* (lux) and in suspension. These results were compared with those from using a luminometer or a sensitive CCD camera. An ozone monitoring system was built to determine the level of theozone treatment.

Chapter 6 describes the decontamination efficiency against *Campylobacter jejuni, Bacillus cereus, Escherichia coli, Listeria monocytogenes, Salmonella typhimurium* or *Staphylococcus aureus* inoculated onto plates, chicken or salmon samples.

The conclusions are given in Chapter 7 along with proposals for future work.

Appendix I details the design of the UV lamp system to extend the shelf-life of the carrots and potatoes. Appendix II describes how the ozone concentration was calculated.

1.7 Figures

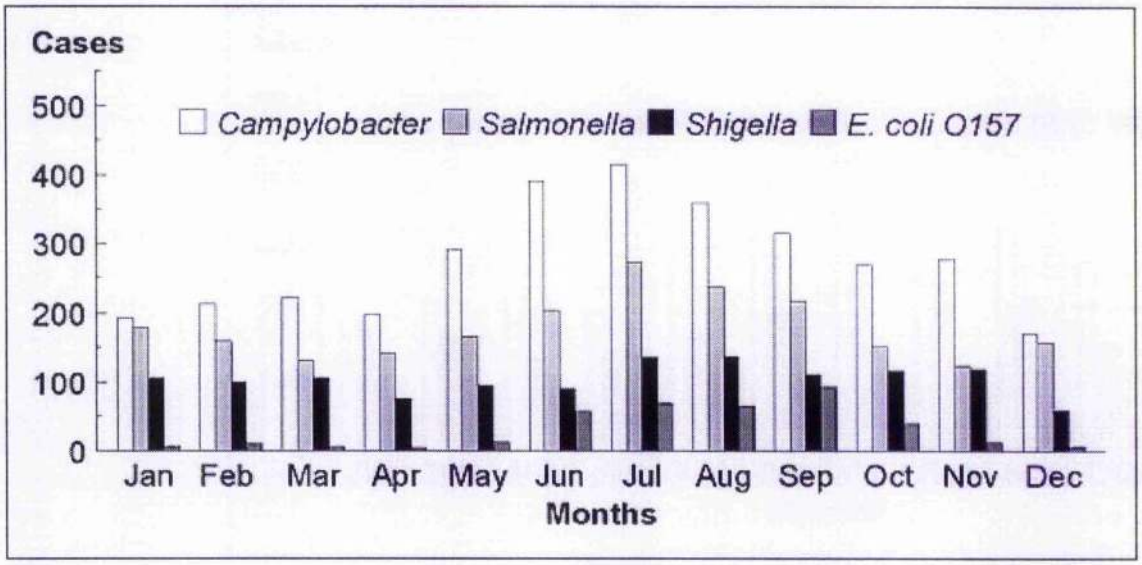


Figure 1.1 Cases of Campylobacter and other foodborne infection by month of specimen collection; Centres for Disease Control and Prevention/U.S Department of Agriculture/Food and Drug Administration Collaborating Sites Foodborne Disease Active Surveillance Network, 1996. [18]

CHAPTER 2 Decontamination system

2.1 Introduction

In order to perform an investigation into minimal processing with individual and combined emerging unit operations using laser, UV, microwave and chemical a test-rig was fabricated. The system comprised a 2 kW CO_2 laser, germicidal ultraviolet lamps, a 1 kW microwave, a chemical wash system and an industrial conveyor. The individual and system components are described below.

2.2 CO_2 laser

The switch mode power supply, **Figure 2.1**, provided high voltage to the laser discharge through the cathodes and anodes that were inserted into the laser gas. The switch mode power supplies (SMPS) provided the high voltage for the gas discharge sections. The laser output power was controlled by the discharge current control settings. The SMPS (2 off) were located in the upper section of the laser and each consisted of 4 units with transistorised switching and control and HV sections. The HV sections were located in an oil filled aluminium unit to prevent arcing. The SMPS structure can be seen in **Figure 2.2**.

A control console, containing an operator panel and switch gear, was assembled as a separate unit and connected via cables to the power supply, **Figure 2.3**

The laser discharge system was located in the centre section of the laser, **Figure 2.4**. This consisted of a kinematically mounted, temperature stabilised resonator. The resonator optics were mounted on end plates which were aligned with each other by means of four resonator tubes made of an alloy with a low thermal expansion coefficient (invar). The resonator end plate can be seen in **Figure 2.5**. The resonator was folded once using two fold mirrors. This is a patented arrangement with one of the fold mirrors aligned at 45 degrees to the horizontal which defines the polarisation of the output beam. In this way, only one quarter wave retarding optic was required to convert the beam into circular polarisation. For safety reasons the optical components were earthed. The mirror holders of the rear mirror and output coupler can be seen in **Figure 2.6** and **Figure 2.7**, respectively.

The discharge tubes, as well as the anode and cathode mounts were recessed into a moulded support made of special warp-resistant resin composite. A water-cooled mechanical shutter was located in the path of the laser beam in front of the output coupler and could be closed to prevent the laser beam from passing to the safety enclosure.

The shutter was always closed when the laser was running in standby mode and if a fault occurred. The laser beam was diverted onto an absorber by the shutter mirror and the heat from the beam was transferred into the cooling water.

A sensor located behind the rear mirror continuously monitored the power from the laser. This mirror allowed a fraction of a percent of the intra-cavity power to escape onto the power sensor and the reading from the sensor was shown on the display. The laser power was adjustable from the console panel.

The laser cooling system was built to standard design. The cooling system was located in the lower portion of the laser and consisted of a Rootes blower, heat exchangers and vacuum pump, plus associated pipe work. **Figure 2.8** shows the position of these components and the direction of the flow of fluids through the laser system. The laser gas was circulated via the Rootes blower through two heat exchangers, which cooled the gas by indirect contact with the chilled water provided by an external chiller. The laser gas pressure was maintained below atmospheric by the vacuum pump. The hot gas from the laser discharge was cooled in one heat exchanger, accelerated by the blower, cooled by a second heat exchanger and passed back into the discharge. A fresh gas mix of helium, nitrogen and carbon dioxide was fed into the laser discharge at pre-set intervals and the used gas was expelled to atmosphere. The pressure inside the discharge was maintained by the vacuum pump.

The laser was assembled in sections at Rofin's manufacturing facility (York Way, Willerby, Kingston upon Hull, U.K. HU10 6HD), each section being tested and approved before it was mated with an adjoining section. The discharge (centre) section was located on top of the cooling section (lower portion) and the power supply section (upper section) installed on the top. A control console, containing the operator panel and switch gear, was assembled as a separate unit and connected via cables to the power supply. **Figure 2.9** shows a picture of the assembled laser.

The laser was tested in special safety compartments to ensure protection from high voltage and high power laser beams. The individual units were tested before the laser was finally assembled and then the complete laser was tested when fully assembled. Interlocks were tested. The laser power, pulse performance and controls were tested by firing the laser into Perspex sheet to produce beam profiles, **Figure 2.10**; this is a standard laser testing procedure. Certificates were generated, detailing the results of the tests.

Once the subsystems were installed, the laser was commissioned. The laser was connected to the control unit and to the 3 phase electrical supply. The 3 laser gases were connected. The cooling water from the external chiller was connected to the laser. The safe and correct operation of the external parameters was verified, these included: electrical voltage, water flow and temperature, and gas flow and pressures. Once these were confirmed correct, the laser was started and the power and mode of the laser beam checked. **Figure 2.11** shows a burn print of the laser beam on thermally-sensitive paper. The alignment of the beam was checked via a He-Ne laser located in the CO_2 laser.

The safe operation of the laser required installation of an appropriate chiller, power supply, a safe operating environment, and a gas supply. These methods are detailed below.

2.2.1 Chiller

The chiller cooled the water to a set temperature, and worked against the thermal load of the laser system. The chiller, Kaw 330 (**Figure 2.12**), was purchased from Total Process Cooling Ltd (West Midlands, UK) to cool the laser system at 55 litres min^{-1} and the controller at 5 litres min^{-1}. The chiller provided sufficient cooling capacity for the laser at its maximum water pressure supply of 6 bars, the laser required 18 kW of power to be dissipated. The water temperature had to be maintained to 20 ± 0.5 C° to ensure safe operation of the laser. The chiller was a stand-alone unit housed in a steel cabinet. The Kaw 330 was also chosen as it was equipped with a crankcase heater that was necessary to pre-heat the lubrication oil inside the chiller's compressor and to avoid cold starting the laser. An amount of 5 litres of pure ethylene glycol was added to the water coolant, which acted as an anti-freeze reagent. The chiller required 7 kW of power to function, which was supplied from a three-phase supply. Connections from the chiller were made to the power supply and the laser controller.

2.2.2 Power Supply

A three-phase power source for the laser system was provided. From the technical specifications, the laser controller required a 400 V (±6-10 %) 50/60 Hz, 3 phase main power supply with the connected load standing at 20.5 and 22.5 kVA for nominal and maximum output respectively. Given that the maximum current consumption was 33 A, a 63 A 3 phase beaker was necessary to accommodate any initial current surge. The controller unit also required a 3 phase 415 V, 50 Hz supply.

A switch and fuse system was installed that ran along the bus-bar chamber on the top of the built-wall and into the laser and controller unit. Circuit safety inter-locks were mounted on the wall and the experimental enclosure to provide the necessary safety switches during system operation.

2.2.3 Safe operating environment (wall and connections)

The laser was housed outside of the safety enclosure where the roller table (conveyor) was positioned. However, a safe operating environment had to be provided for the laser, and a wall was fabricated to separate the laser controller, the chiller and the power supply. Overhead conduits were used to route the electricity connections, and the water connections were mounted on the wall. A pressure relief valve was installed to prevent any over pressure in the chiller, which may damage the pump motor. A steel structure was fabricated around the safety enclosure. On and off valves for the cooling system were fitted to ensure greater controllability of the water flow rate. Varidos lasercool GLS (Central chemical services Ltd, Garrison House, Garrison St, Birmingham B9 4BN), an organic corrosion inhibitor, was added to prevent corrosion of the cooling water circuit. This also served as a bacteriostatic agent and a hardness stabiliser.

2.2.4 Gases

The gases needed for operation of the laser were fitted; a filtered compressed air line was supplied for purging dust from the system. Copper tubing was used to transport the gases to the laser unit, except for the interface between the copper and the inlet/outlet connectors where High Density Polyethylene (HDPE) tubing was used. The laser gases were fed to the laser separately with each gas pressure regulated between 4 and 7 bar. An automated balanced pressure regulator was present within the laser unit itself, which further

monitored the pressure and flow of the gases.

2.2.5 Laser beam delivery system

Design constraints were imposed on the optical system because of the constraints of the mechanical system and the way in which the produce had to be moved through the system to avoid bruising. The beam was scanned in a conventional manner using a moving mirror.

The parabolic mirror was 14 cm in diameter, and was mounted in a water cooled enclosure, **Figure 2.13**; this prevented any thermal runaway or heating effects which may have distorted the reflected beam and damaged the mirror.

The mirror mount was attached to a stepper motor that was interfaced with the motor driver and linked to the computer. A "Delphi V" computer software code was written (Mr. Ian Peden, Department of Mechanical Engineering, University of Glasgow) to provide the scanning motion. The path of the laser beam was limited to that solid angle subtended by the length of one roller. The movable steel rods which held the scanning assembly were attached to the roof of the welded steel frame.

An aluminium tube was positioned between the laser exit window and the safety enclosure. Provision was made to add a purge gas or filtered compressed air into this tube so that the laser and beam delivery optics could be kept free of contamination via the positive pressure. The alignment of the laser beam, the scanning optics and the roller were optimized. This was achieved by taking a series of burn prints on thermally sensitive paper placed on the roller.

A He-Ne laser was incorporated in the laser system to allow alignment of the CO_2 laser beam; which is invisible to the naked eye. Once aligned, the CO_2 laser beam diameter was measured on the conveyor belt as 12 cm.

Figure 2.14 shows the scanning mirror system mounted above the roller table and **Figure 2.15** and **Figure 2.16** show burn prints of the laser beam on thermally sensitive paper. In **Figure 2.15** the beam was stationary and the laser was operating in the TEM_{01} mode.

2.3 Microwave system

An industrial microwave oven with the specification given in **Table 2.1** was fabricated (Medexpo, Netherlands). This custom-made microwave system included the power supply, electronic controls, cavity, magnetron and safety features

Table 2.1 Specification of variable power microwave module

Power rating, infinitely variable	100-1000 watts
Frequency	2450 MHz
Complete with timer and quick release door Wired for 230 VAC, single phase, 50 Hz	

2.3.1 Safety features of the supplied system

The magnetron system and safety features were designed to be similar to a domestic microwave so that the operators were completely safe. The power output was infinitely variable and a special safety latch door and electronics were designed. The magnetron was only operational when the door was closed. If the door was opened at any time the magnetron was automatically turned off.

Figure 2.17 shows a picture of the microwave system and entry hatch. Further detailed experiments on the effect of microwaves on extending the shelf life of the produce were done. The internal dimensions of the microwave cavity were 30 x 30 x 20 cm^3. This design was critical so that a standing wave pattern was established within the microwave cavity and the impedance of the load was matched to the magnetrons.

The microwave power supply was integrated into the main chassis, adjacent to the cavity and connected to the magnetron terminals, control panel and cooling fan. The safety trips were tested. The microwave output power was measured with a microwave power meter and measured and calibrated as a function of the switch setting. It was ensured that the reading on the microwave system agreed with the meter reading by trimming resistors as appropriate.

2.4 UV enclosure subsystem

An UV decontamination system comprising a bank of germicidal UV mercury lamps (254 nm) was fabricated in-house. **Figure 2.18** shows an exploded view of a UV system that was originally designed **Figure 2.19**. The ballast and electronic controls were placed above the lamps that were mounted in a grid fashion underneath. They were designed to slide easily in and out of the assembly so that any of the lamps could be easily replaced in case of their failure. The UV light was contained in the enclosure by placing plastic curtains over the inlet and outlet of the system. The width of the system was optimized to fit a standard roller table. Additionally, lugs were placed on the side support so that the system could be secured to the table. **Figure 2.19** shows the original design with the lid partially closed. The lid was interlocked to the UV lamps so that if it was opened it would trip the lamp supply and turn the lamps off. The lid was slightly curved to allow the installation of new components into the assembly as required.

In practice, this original design was simplified when the first prototype was made. The simplified design can be seen in the **Figure 2.20** and **Figure 2.21**. The ballast electronics can be seen above the lamps. **Figure 2.22** shows a partially exploded view of the UV system, with one side of the lamp bank removed. The supporting runners for the lamp bank can be seen.

Figure 2.23 shows the assembly drawing for the system and the system's dimensions. End and side panels were mounted on the system to reduce the emission of stray UV light from the system.

2.4.1 UV output irradiance measurements

Before the final subsystem was built (**Figure 2.18** to **Figure 2.20**), a UV germicide lamp system (3 x 40 W at 254 nm) was built above the roller table (which was used to transport the produce). This is shown in **Figure 2.24**. This allowed the effectiveness of the minimal processing system to be evaluated. Experiments were conducted with this set up to deduce the optimal threshold irradiance for minimal processing for the fabrication of the UV system.

A MacCam UV meter was used to monitor the irradiance of the UV lamp assembly (3 x 40W) at a distance of 30 cm from the lamps. The lamps were turned on for varying lengths of time up to 60 min. The detector was perpendicular to the lamps and this was

checked with a spirit level to ensure that the detector was always normal to the incident light.

2.4.2 The temporal and spatial distribution of UV light

The temporal and spatial distribution of the UV light was measured. The lamp system was integrated above the roller table, and mounted on the adjustable platform. The height of the platform could be changed to provide a wide range of intensities on the substrate surface. The platform could be rotated through 90° so the lamps could be positioned either transversely (across the roller table) or longitudinally (parallel to the roller table). Fans were designed and fabricated to provide temperature control of the lamps.

2.5 H_2O_2

The intended chemical (1% H_2O_2 concentration from Fisher Scientific UK Code 41188-1000 Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG UK) delivery system would consist of an atomizer spray jetted across the conveyor system, and also a complete washing, filtration, recycling and mixing system. However, it was not practical and cost effective to build such a system for the small quantity of produce used in the experiments. Instead, experiments were done in smaller tanks to reduce the volume of H_2O_2 used and its wastage. Manual irrigation and agitation methods were used to mimic the chemical decontamination system that would be used within an industrial context.

2.6 Combination decontamination system

After the mechanical assembly was completed for all of the system components' the electronics, power supplies and control were installed and commissioned. The mechanical system comprised a roller table, housed in a polycarbonate safety enclosure. The enclosure was extended so that there was sufficient workspace to complete the experiments. Struts were mounted above the enclosure to support the subsystem components.

The substrate material was placed at the start of the roller table through the safety latch hatch. The order of exposures were designed as follows: chemical, laser, UV lamp and microwave; after which the vegetables were dropped into a sterile container at the end of

the roller table for microbiological analyses and shelf-life studies.

This order of treatment could be changed but due to the size and complexity of the system this process was time consuming. It was relatively easy, however, to use single treatments when the produce was passed through the system. A safety partition was constructed from 3mm thick aluminium sheet and placed across the conveyor belt, perpendicular to the direction of translation of the produce, to contain the laser radiation. An adjustable platform was constructed above the roller table that was suspended from the safety enclosure. In this way, the subsystems could be mounted above the roller table, without compromising the motion of the produce on the table, and allowing easy access and viewing of the produce as they rolled down the table. A CCTV camera was installed to enable remote and safe viewing of the decontamination operation. A schematic of the subsystems and their connectivity is shown in **Figure 2.25**. The scanning mirror is not shown for the sake of clarity.

The UV lamps were secured to a platform that could be raised or lowered via screw rod that attached the platform to the support struts. The height of the UV lamps could be varied in respect to the vegetables being treated. Various exposure heights were tested so that the irradiance could be optimized.

Figure 2.26 shows the electrical connections driving the subsystem components. The laser was fixed as this was the hardest subsystem to move, so to change the order of the combined system treatments the other subsystems were made more mobile.

A computer interface was built and connected to the laser to provide control of the laser operation. The use of the laser was logged automatically, enabling satisfactory operation management. "DELPHI V" was used to design the dialogue windows and the graphical user interface display mimicked the different functional keys on the laser control panel. The source codes were written in C^{++} builder/Borland software. A voltmeter was connected via the external gate of the laser controller to act as a security check during the actual firing of the laser radiation. Additionally the voltage gave a measure of the laser power, with an output of between 0 and 5 V representing a laser power of between 0 and 2 kW.

An oscilloscope was attached to the X40.16 EXT GND of the laser controller to provide a wave-like display to monitor the laser power. The laser and its control system were integrated with the roller table.

The movement of the scanning mirror was controlled by a 4-phase stepper motor controller. The controller had a variable current amplifier and the motor provided a maximum torque of 60 mN metres; the motor was coupled to a 50:1 gearbox to provide greater torque and control over the motion of the mirror. The motor had a moment of inertia of approximately 200 $rads^{-2}$ and accelerated almost instantaneously, it provided 80 scans per min across the 160 mm rail guide; giving an equivalent translational scanning speed of 21.3 cms^{-1}.

Figure 2.27 shows a screen shot of the graphical user interface to control the laser scanning mirror. The scan distance, average speed and number of scans could be controlled from the computer. The mirror velocity was a user selectable input, as was the number of incremental steps for the stepper motor.

The laser was the largest system component and remained relatively fixed. Consequently, the other subsystem components were designed to be installed around the laser system. The beam was moved across the roller table via the scanning mirror. A computer interface was developed to control the laser. The drive supply to the laser was monitored via an oscilloscope. The laser subsystem was assembled and tested. It is seen that the mark to space ratio and burst time can be set from the computer. There is an emergency stop placed on the computer terminal. This of course is an added safety device and does not replace existing emergency stops. Starting instructions are set on the computer for the operator's convenience. Information about the laser settings can be stored to file, so that a log is generated of the computer use. Different gas fills can be automatically selected. Power down instructions are also provided. If this is not followed correctly then on screen instructions appear.

The microwave system was used in series with the other treatment subsystems. The microwave system was kept in the safety enclosure, close to the roller table and powered from a single-phase supply nearby to the roller table. **Figure 2.28** shows the microwave system installed in the safety enclosure, next to the prototype UV system. The produce

that needed microwave treatment was manually placed into the microwave subsystem as appropriate and in the desired sequence. This obviated the need for complex delivery systems of the microwave radiation to allow processing with continuous flow of produce but enabled flexibility to assess the performance of the microwave system, coupled with the other subsystem components.

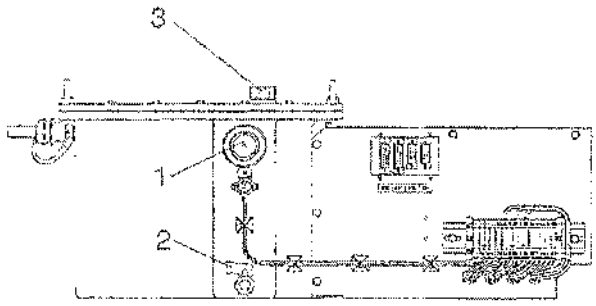
2.7 Commissioning system

Interlocks and micro-switches were installed at appropriate places for control and safety of the system. An interlock switch was installed into the operator box in which the food produce was placed prior to exposure.

A contactor box was installed on the side of the door containing circuit breakers to control the UV lamp (10 amps, single phase), microwave (10 amps, single phase) and the conveyor belt motor (6 amps, single phase). This design enabled close monitoring of the current and was also a safety precaution.

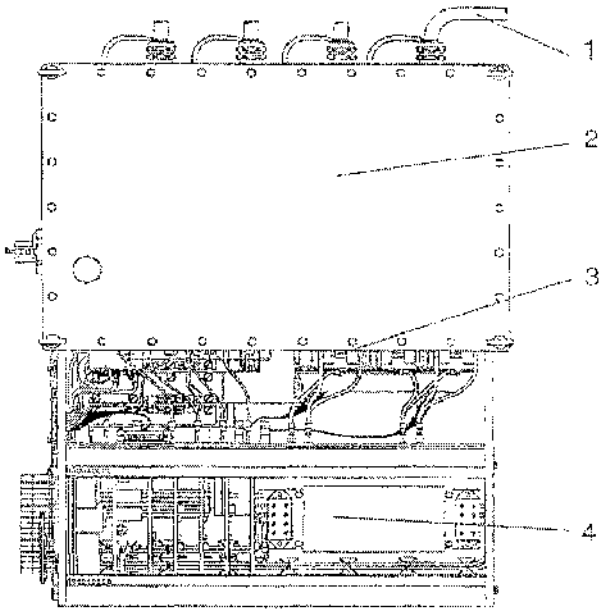
In addition, emergency switches were installed at appropriate places; these were used in to immediately cut off the main power supply to the laser and the integrated subsystems in the event of an emergency. All the sub-systems and the electrical connections were tested and commissioned successfully.

2.8 Figures



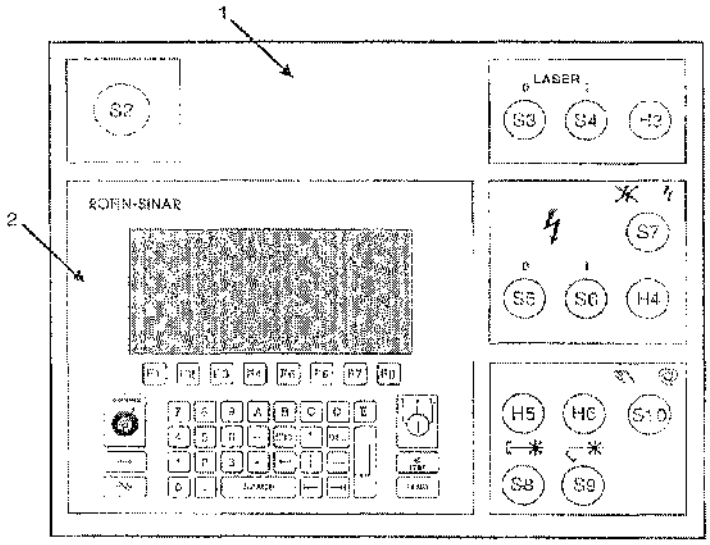
1. Oil inspection glass
2. Oil Drain tap
3. Screw plug for oil filler opening

Figure 2.1 Switched mode power supply (SMPS) [121]



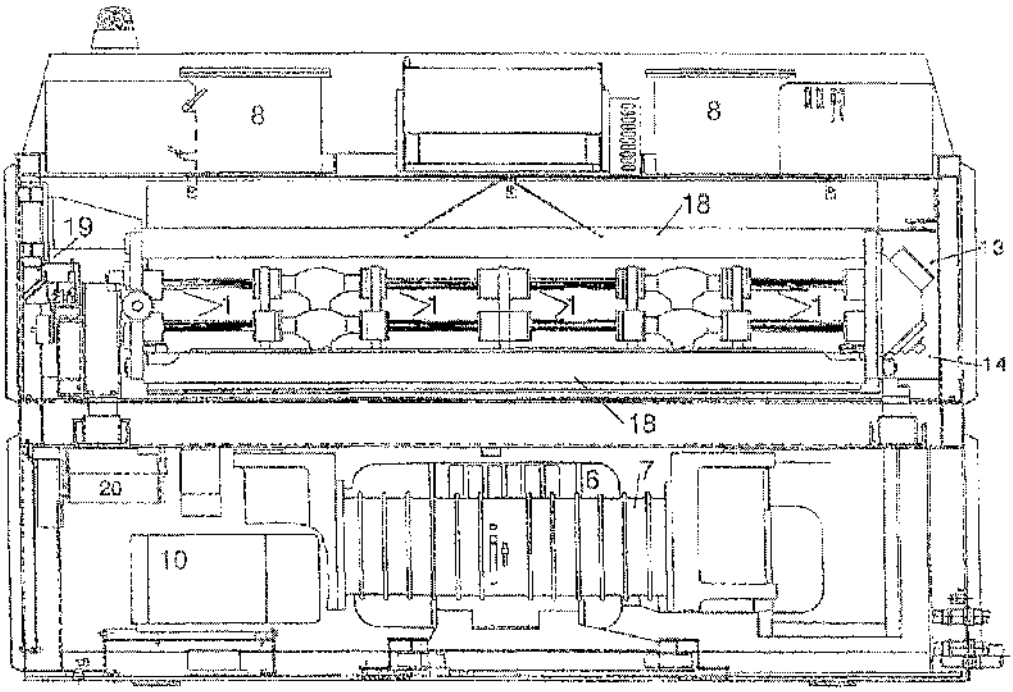
1. Coaxial HV cable
2. Oil box with chokes, HV transformers and rectifiers
3. Power section
4. Control section

Figure 2.2 Switched mode power supply (SMPS) structure [121]



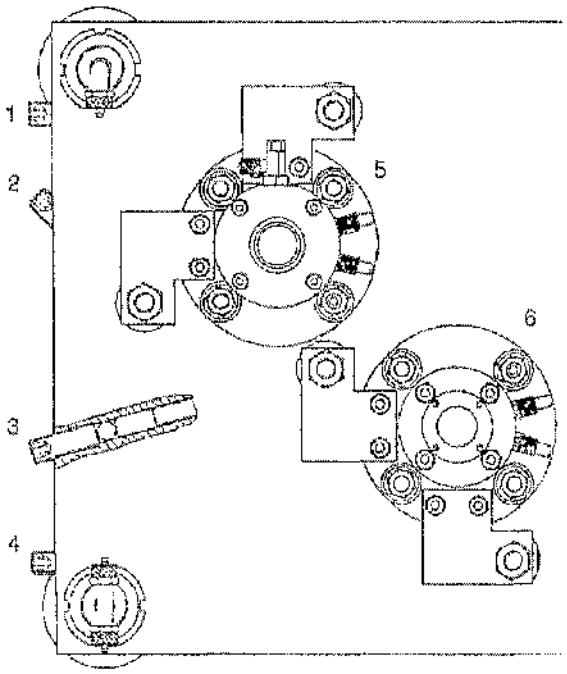
1. Control Panel
2. Display/Keyboard

Figure 2.3 Control panel with display and keyboard [121]



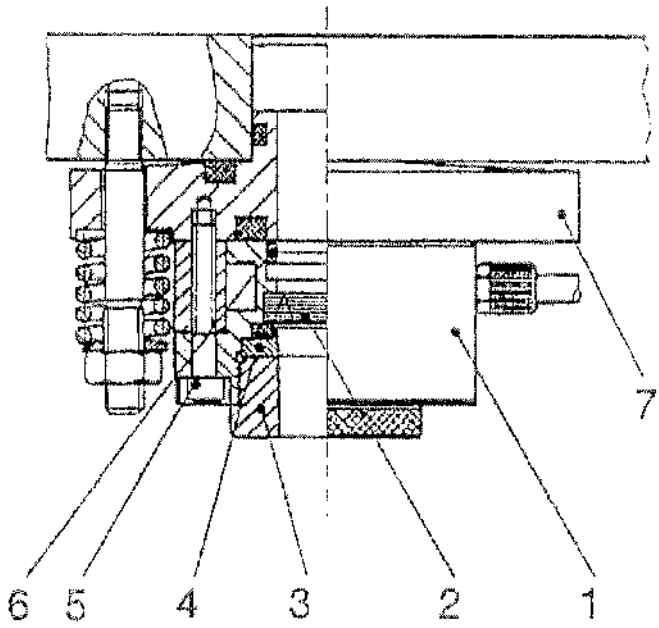
1. Discharge tubes (8sections)
6. Roots blower
7. Heat exchange
8. Power supply
10. Vacuum Pump
13. Adjustable beam-folding mirror
14. Fixed beam-folding mirror
18. Resonator tubes
19. 19 Shutter
20. Gas supply unit

Figure 2.4 Laser structure [121]



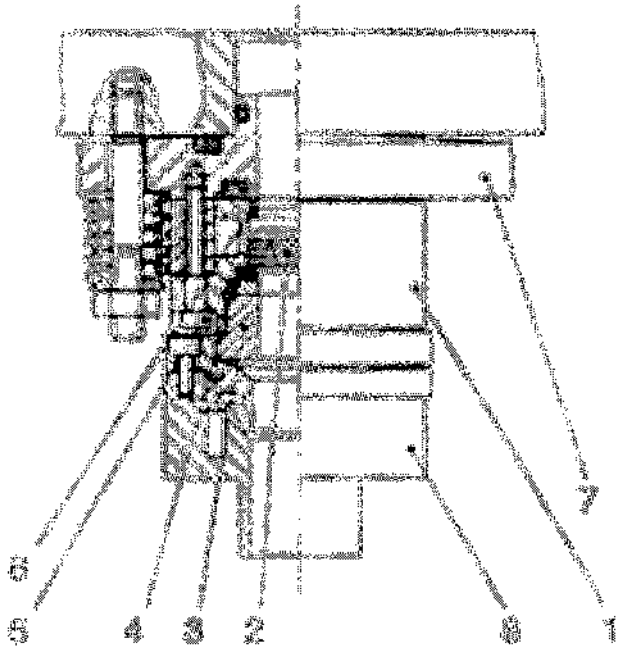
1. Adjustable Screw
2. Adjustable Screw
3. Adjustable Screw
4. Adjustable Screw
5. Output coupler
6. Rear mirror

Figure 2.5 Resonator end plate [121]



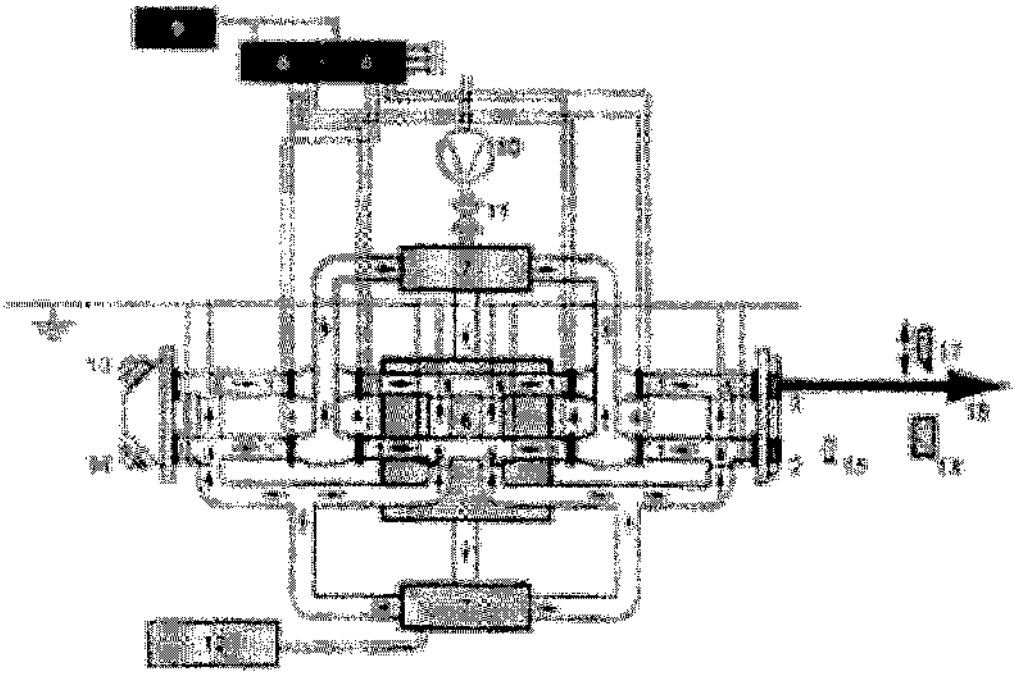
1. Mirror holder
2. Output coupler
3. Crew fitting
4. Supporting ring
5. M5 x 30 pan-head screw
6. Mode aperture
7. Adjusting flange

Figure 2.6 Rear mirror holder [121]



1. Mirror holder
2. Output coupler
3. Crow fitting
4. Supporting ring
5. M5 x 30 pan-head screw
6. Mode aperture
7. Adjusting flange
8. Inert gas shield (optional)

Figure 2.7 Output coupler mirror holder [121]



1. Discharge tubes 8x
2. Rear mirror
3. Output coupler
4. Cathodes
5. Anodes
6. Roots blower
7. Heat exchange
8. Power supply (SMPS)
9. Discharge current control
10. Vacuum pump
11. Evacuation pump
12. Gas supply
13. Adjustable beam-folding mirror
14. Fixed beam-folding mirror
15. Output power sensor
16. Laser beam

Figure 2.8 Schematic diagram of CO_2 laser [121]

Figure 2.9 Assembled laser

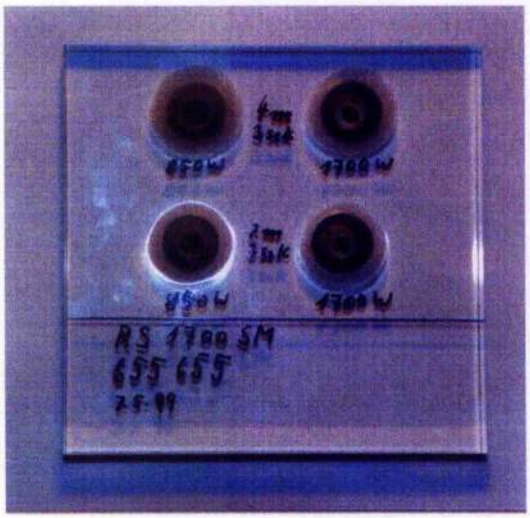


Figure 2.10 Laser beam profiles in perspex sheet

Figure 2.11 Laser burn print of raw beam on thermally sensitive paper

Figure 2.12 Laser chiller

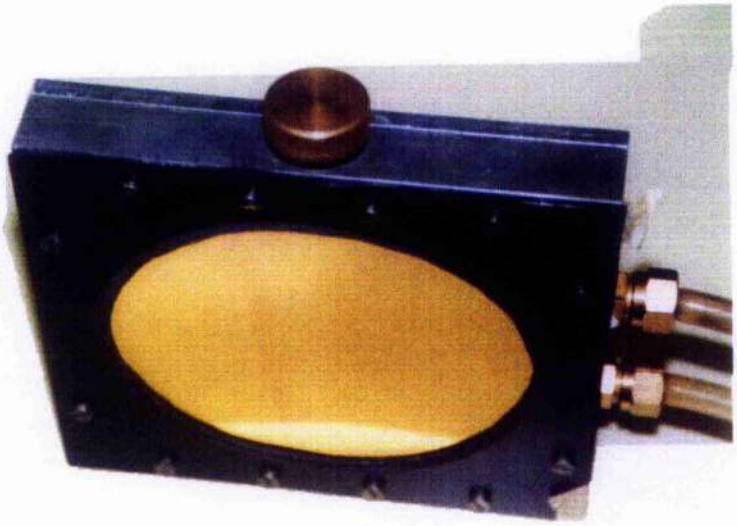


Figure 2.13 Mounted parabolic mirror

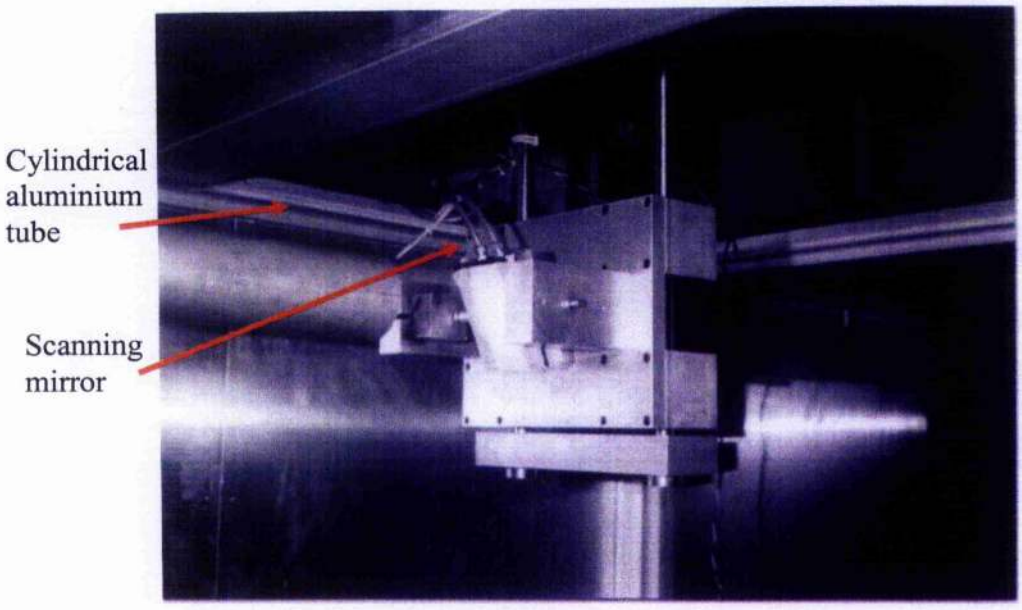


Figure 2.14 Scanning mirror mounting in enclosure

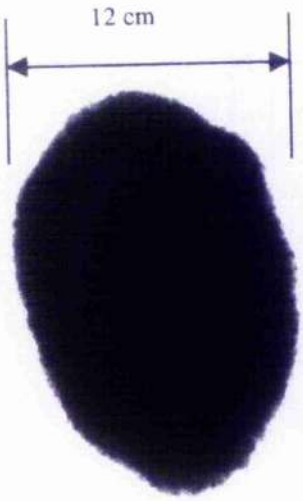


Figure 2.15 Stationary burn print (40% cw laser power with 250 ms exposure time)

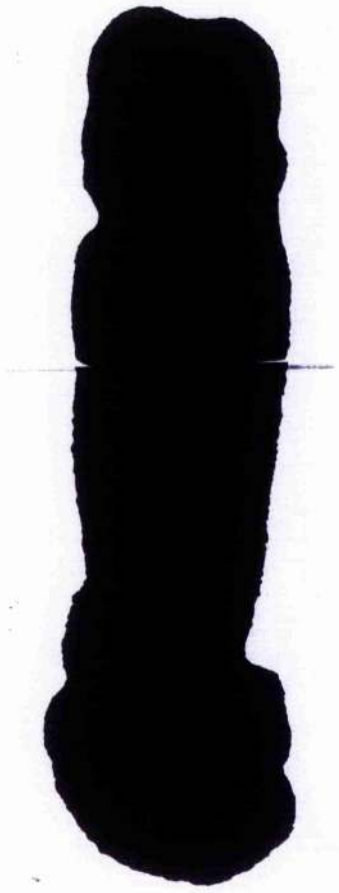


Figure 2.16 Laser burn print with scanning mirror 1.5 cms^{-1}

Figure 2.17 Microwave system

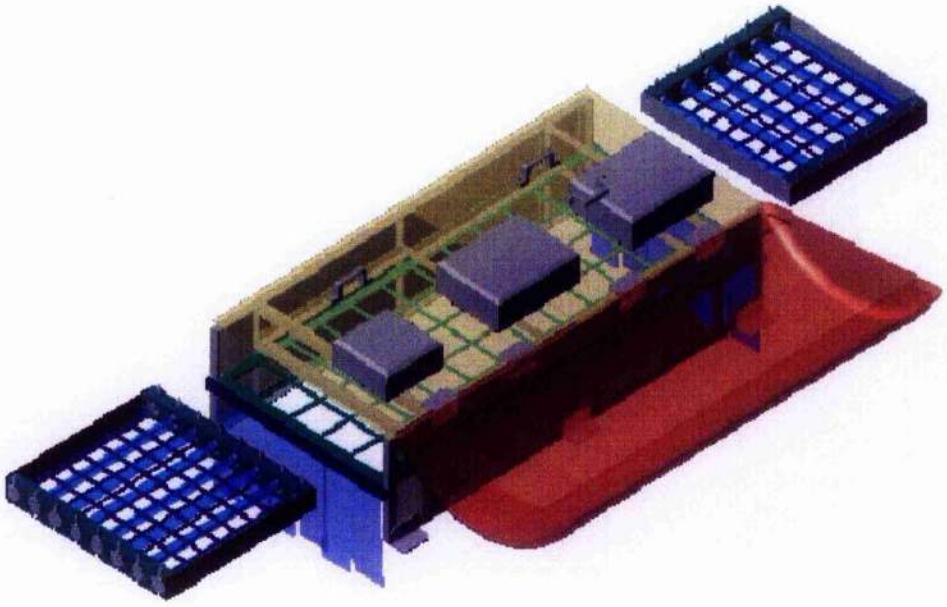


Figure 2.18 Exploded schematic of the original UV system design

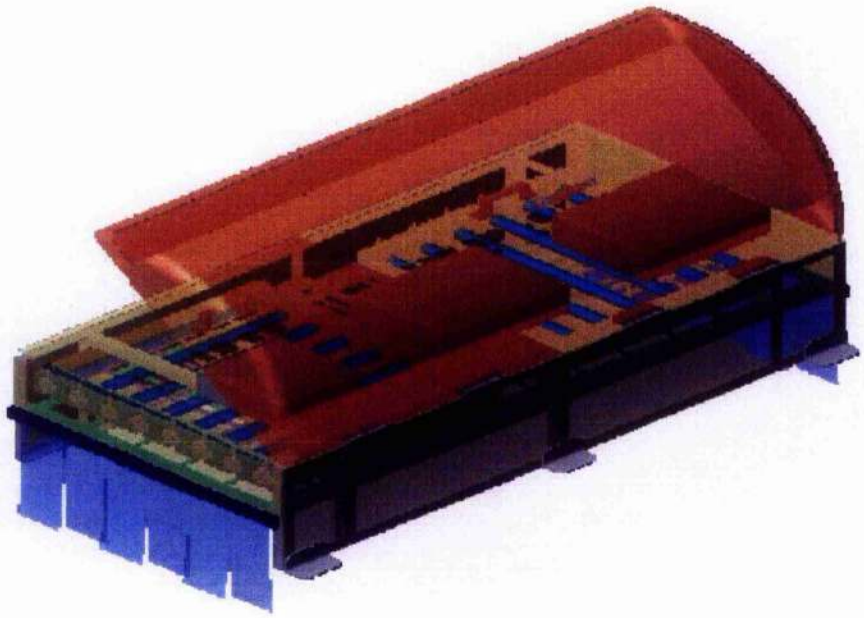


Figure 2.19 Schematic of the original UV system design with dome lid

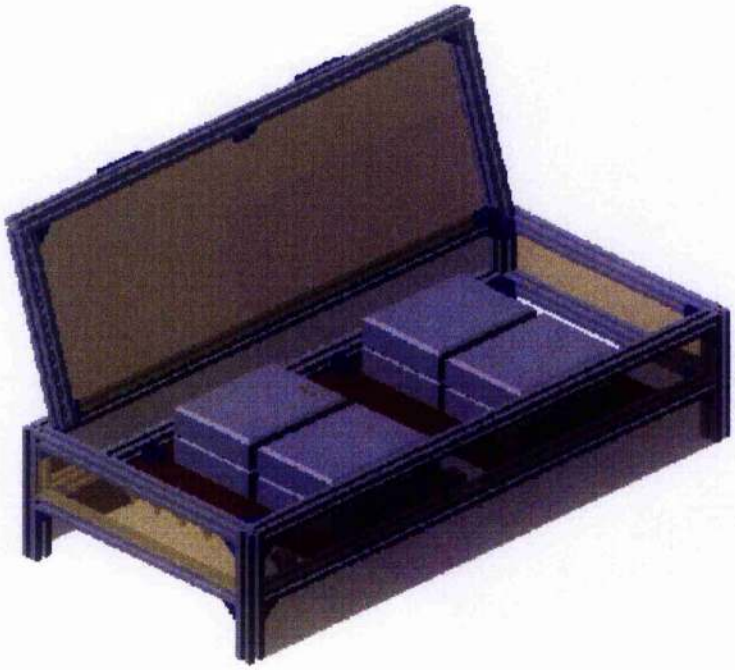


Figure 2.20 Inside view of the first prototype UV system

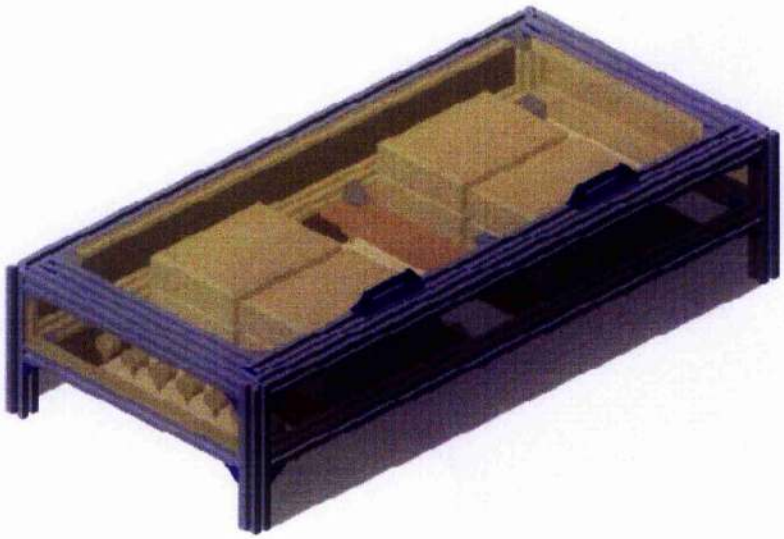


Figure 2.21 Schematic of the first prototype UV system with lid closed

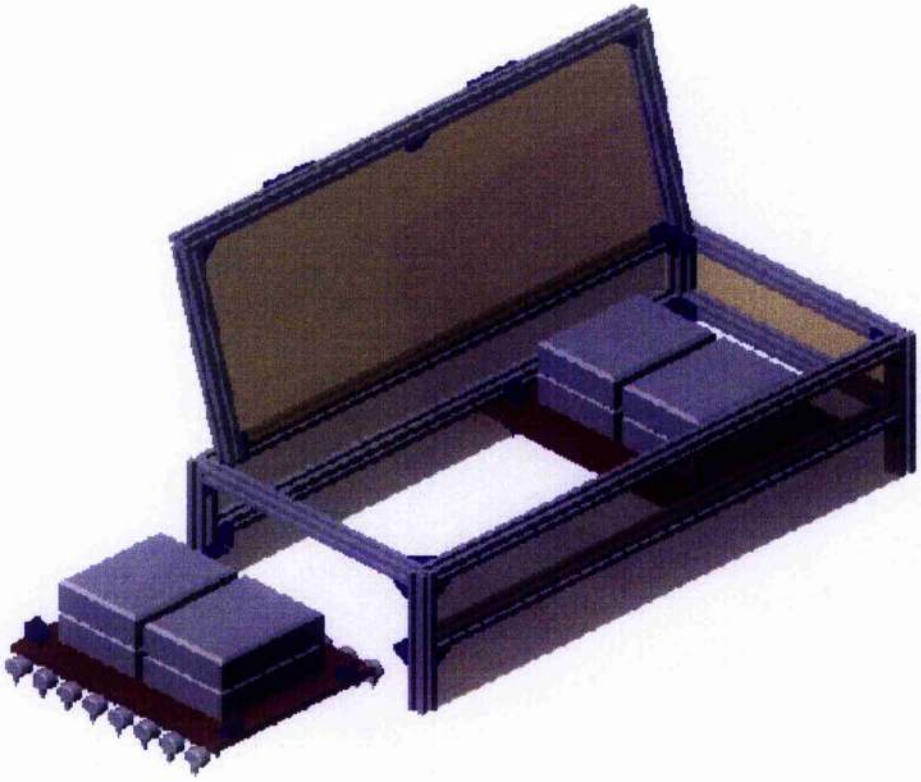


Figure 2.22 Exploded schematic of the first prototype UV system

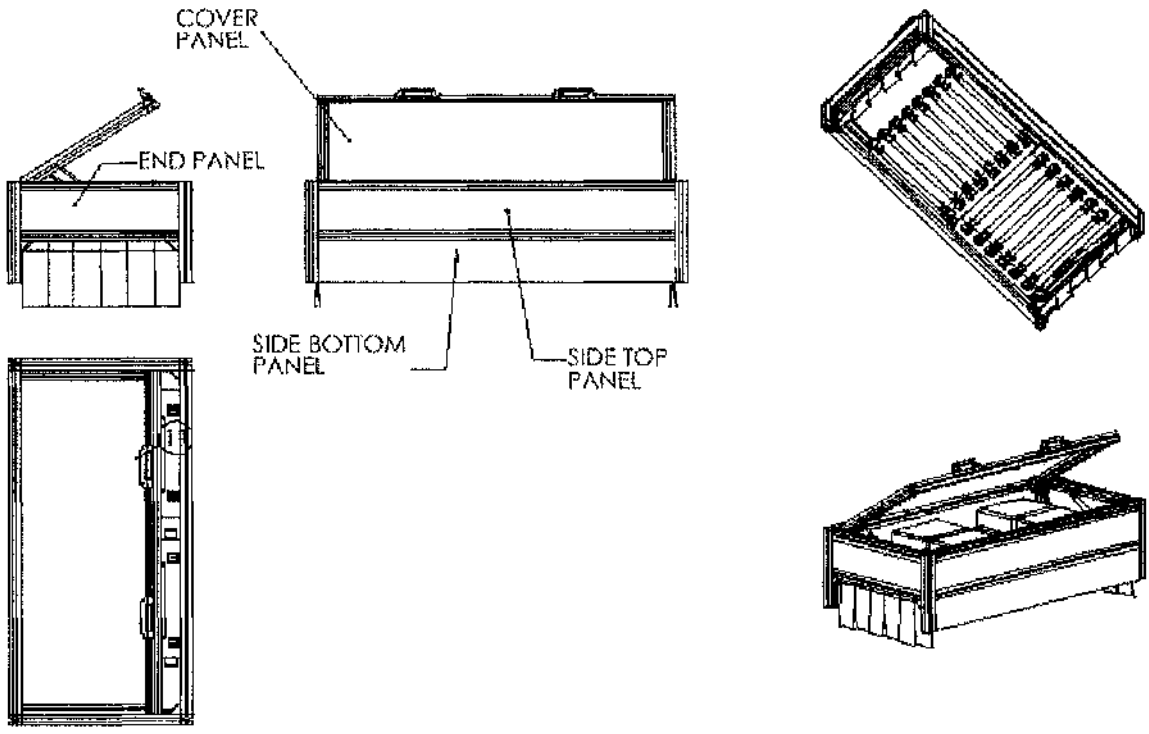


Figure 2.23 Assembly drawing of the new UV system

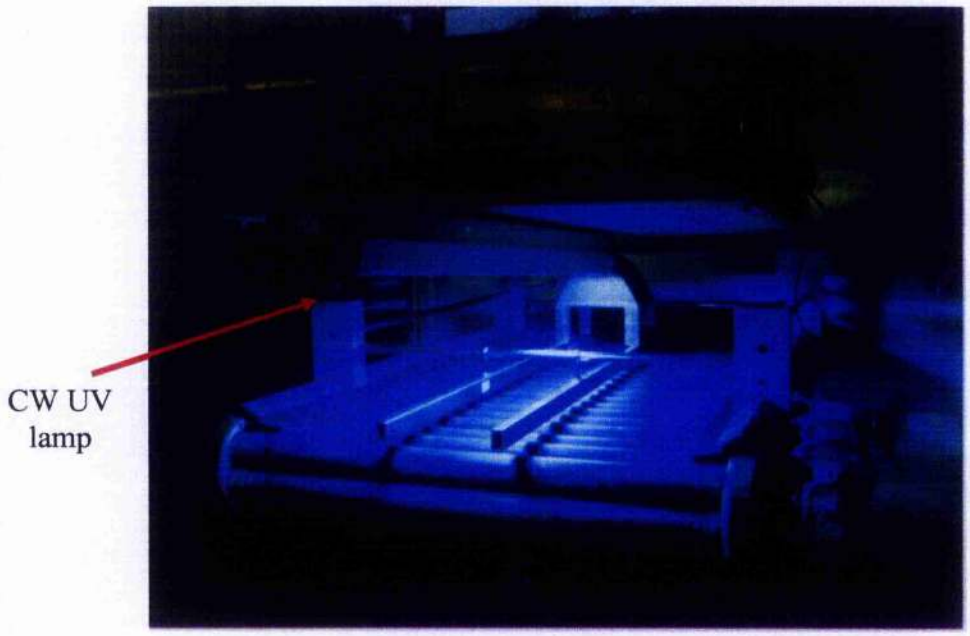


Figure 2.24 UV system, installed above roller table

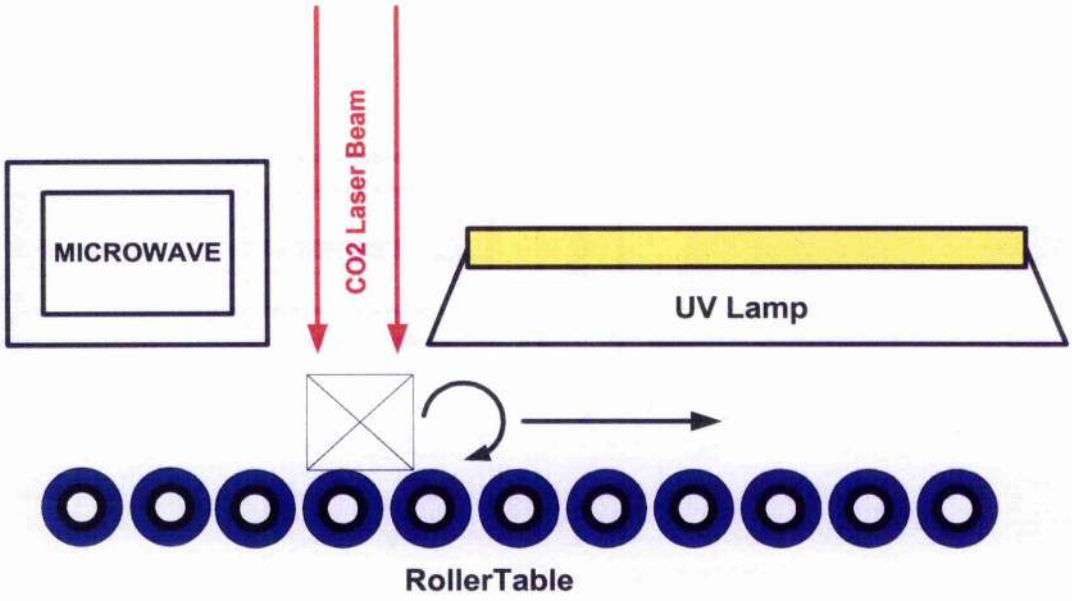


Figure 2.25 Combined treatment system with microwave, laser and UV lamps

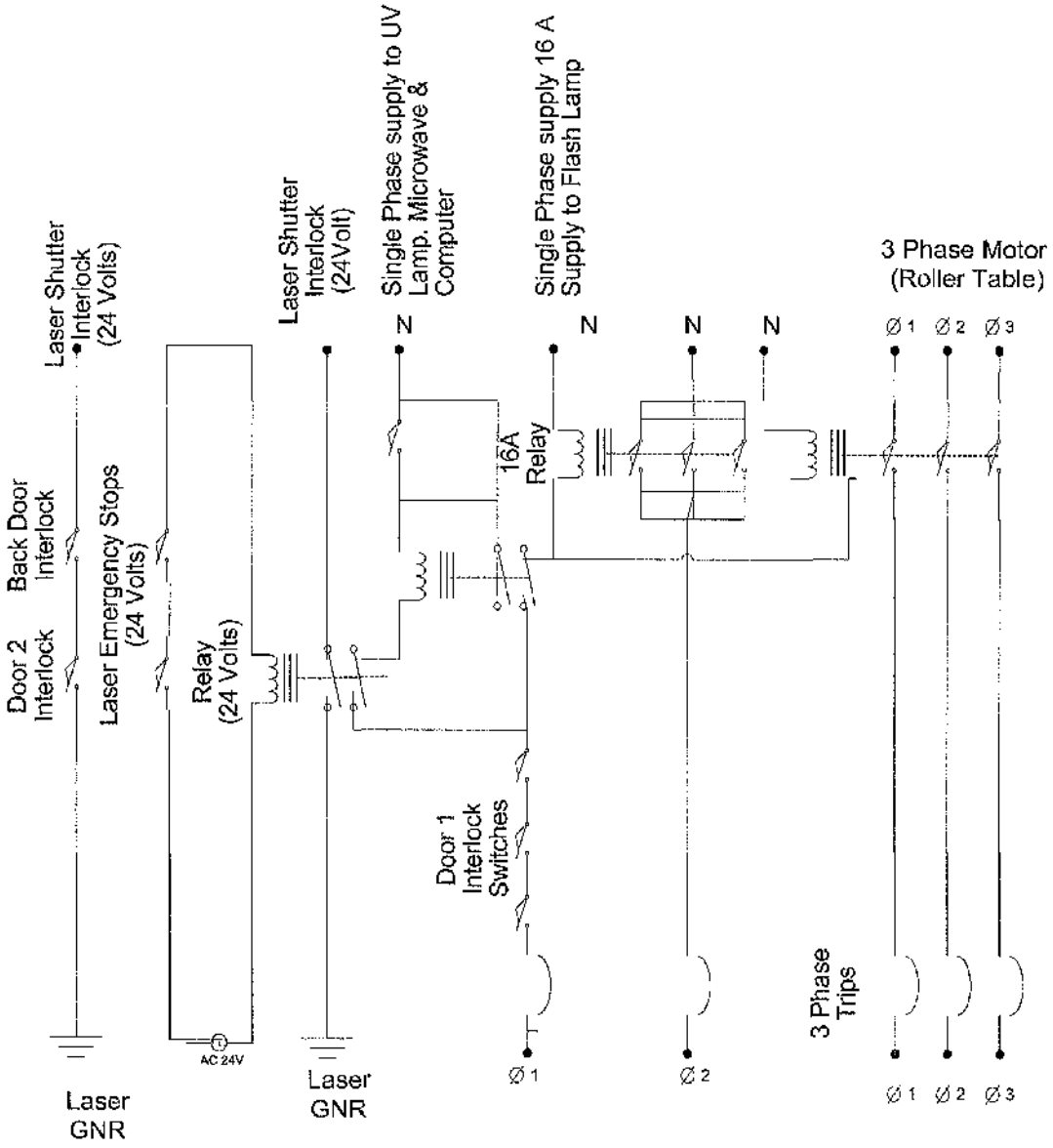


Figure 2.26 Systems integration electrical connections

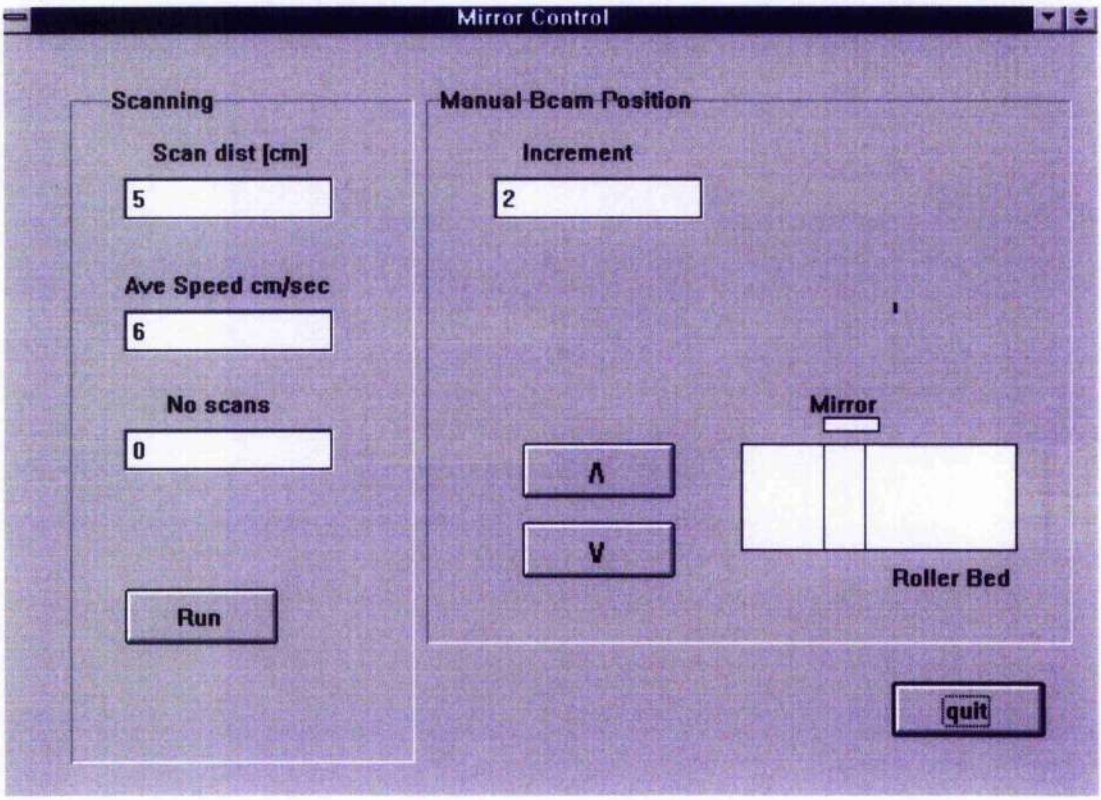


Figure 2.27 Graphical user interface to control the scanning mirror

Figure 2.28 Microwave system installed in safety enclosure next to roller table

CHAPTER 3 Individual and combination treatments

3.1 Introduction

The food industry is in a perpetual race to provide high-quality, "fresh" produce that contains only natural ingredients [122]. This is driven by the consumers' demands for healthy and nutritious produce [123]. Researchers in the food industries have turned to emerging technologies to achieve improved food processing. Emerging technologies such as lasers, UV light, ultrasound, oscillating magnetic fields, pulsed electric field, high pressure processing or ozone have been tried [98;124-126]. There are wide differences between these technologies in terms of their mode of delivery, the source of delivery and their bacterial inactivation mechanism to achieve minimal processing. Unlike conventional heating methods, which can cause protein damage [127], other methods of minimal processing such as UV cause DNA mutations [128]; ultrasound, causes intracellular cavitations and cell rupture [129], and high hydrostatic pressure process denature enzymes. These processes were elaborated in Chapter 1 in more detail. The effectiveness of the treatment can also vary with the type of substrate surface and the bacterial contamination.

A combination minimal processing system that was to be used in the production process of carrots and potatoes was built to investigate the response of *Escherichia coli* on these vegetable surfaces using CO_2 laser, ultraviolet and microwave radiation. Understanding that each technology has its limitation, both individual and combined treatments were also conducted to investigate the possible synergistic effects and improved decontamination when these treatments were applied sequentially. Microscopy analyses were done to investigate the effects of surface topography on the efficiency of these treatment.

3.2 Materials and methodology

3.2.1 Preparation of for *E. coli* suspension

One colony from an overnight culture of *E. coli* (AT1064) on nutrient agar (Oxiod UK CM55) was pipetted into a Universal bottle containing 20 ml of nutrient broth. The bottle was incubated at 37 °C for 18 hr on a shaker rotating at 200 RPM. The bacterial cells

collected by centrifugation were resuspended into sterile distilled water. A colony count was made and the prepared suspension was inoculated onto the food samples just before treatment.

3.2.2 Preparation of carrot and potato samples

For the experimental results to be comparable, the food samples were prepared to a predetermined size of 2.0 x 2.0 x 0.5 cm^3 before the bacterial suspensions were inoculated on them for treatment. Care was also taken during the preparation to prevent cross contamination during handling. The preparation of the potato and carrot samples is described below.

With latex gloves, carrots and potatoes brought from a local supermarket were transferred from the bag onto 90 mm petri-dishes. A thin slice was cut out from the widest surface of the produce. Using a ruler, the slices of potatoes or carrots were cut to the size required for the experiment and placed onto another sterile petri-dish. Before each sample preparation, the rubber gloves and the knife were sterilised with 30% alcohol.

After the food samples were cut to size, they was placed in absolute alcohol for 30 sec, the alcohol was decanted off and replaced with sterile distilled water for 20 min. The samples were washed in fresh distilled water a further four times. The samples were air-dried in the laminar air flow cabinet for 20 min at room temperature.

3.2.3 Decontamination using CO_2 laser

Protocol for the assessment of the laser damage threshold for the roller table

An important aspect of the mechanical system was that the laser did not damage the rollers of the roller table. The rollers were made from hard black rubber. For the laser decontamination process to be usable, it was important that the damage threshold of the rollers on the table was greater than the exposure required to give the desired level of sterility assurance on the carrots and potatoes. The rollers were exposed to a range of energy densities, starting from the lowest value first. Visual inspection for damage of the rollers was made after they were exposed to the laser radiation.

The position of the laser scanning mirror was optimized and the mirror was kept

stationary. The beam size was measured with thermally-sensitive paper, and was approximately 10 x 20 cm^2. The conveyor was set at its slowest speed (0.05 m/s). The laser was then fired at its maximum power settings (2 kW) over the area while the conveyor was turned on. The laser was turned off after the first revolving roller that hit the beam was allowed to travel a complete revolution around the roller table's entire length. Damage assessment was carried out visually after the conveyor was switched off.

CO_2 laser treatment of *E. coli* on carrot and potato segments

Aliquots (20µl) of *E. coli* suspension (5.5 x 10^7 cfu/ml) were placed on the flat surface of sterile skin sections of the carrots and potatoes, and exposed to the laser irradiation. A 2.5 sec exposure and up to 2 kW of laser power was exposed to sections (2.0 x 2.0 x 0.5 cm^3) of carrot and potato; this was done in duplicate, allowing an assessment of the repeatability of the decontamination process. The samples received 0.5, 1.0, 1.5, or 2 kW of irradiation for 2.5 sec. After each exposure, each sample was removed and placed in 10ml of sterile distilled water, a 1 in 10^4 dilution. Three subsequent 10-fold dilutions were made. From these dilutions, 20 µl were drop pipetted onto the surface of nutrient agar plates (in duplicate) that were then incubated at 37 °C for 18 hr. With 20µl of *E. coli* culture placed on the sections, the expected untreated control count should be 5.5 x 10^7 cfu per sample. Colony counts were conducted after treatment by shaking the samples in sterile distilled water for 1 hr before plating them onto agar plates for counting.

The beam area was about 120 cm^2. A larger beam has the added advantage of being able to control the energy density on the produce more accurately, and to reduce the constraints on the scanning system as the optics do not need to scan so accurately or fast, as overlapping of the beam is easier to achieve.

3.2.4 Decontamination using microwave

Microwave killing of *E. coli* on potato segments

The aim was to determine the optimum parameters of microwave treatment for subsequent combination experiments. Sections of potato skin (2.0 x 2.0 x 0.5 cm^3) were prepared as described in section 3.2.2 . Aliquots (25 µl) of *E. coli* suspension at 3 x 10^7 cfu/ml in distilled water were pipetted onto the surface of each section of potato. The contaminated potato segments were exposed to microwave irradiation for 6 - 9 sec. The microwave

power was set at 800W, and the samples were placed at the back of the cavity (centrally aligned). After each exposure, 1 sample was removed and placed in 10ml of sterile distilled water. This was a 1 in 10^4 dilution. Three subsequent 10-fold dilutions were made and, from all of these dilutions, 20 µl volumes were drop pipetted onto the surface of nutrient agar plates (in duplicate) and the plates were incubated at 37 °C for 18 hr.

3.2.5 Decontamination using UV radiation

There were two UV radiation systems used. One system utilised the 120 W (3 x 40 W, 254 nm (Merck) germicide lamps as mention in Chapter 2 section 2.4.1. Developed from the 120 W system, the second UV system was fabricated in-house and optimized to cover the entire treatment area in an attempt to increase productivity and to test the system within a simulated industrial process. Stability measurements were done and the effect of forced cooling of the UV lamps on the output and decontamination efficiency was investigated. For the new optimized UV system (**Figure 2.20**), the spatial decontamination effectiveness was also investigated.

3.2.6 Spatial effectiveness of optimized UV system

The effectiveness of the optimized UV lamp system was investigated by lawning a number of agar plates with *E. coli* and placing them at different positions under the UV system, exposing to the UV light, incubating the plates and subsequently observing them for growth. Particular attention was given to investigating the decontamination performance at the edge of the UV system.

Eight locations for the plates were identified and are shown in **Figure 3.1**. It is seen that the plates were strategically positioned to see how effectively the plates were decontaminated at the sides of the new system, between lamps, and directly underneath a lamp.

At each location, 3 nutrient agar plates were used separately and exposed to 3 different exposures. All of the plates were lawned with 100 µl of bacterial suspension at a concentration of 1.1 x 10^9 cfu/ml. After placing the plates under the UV system, the plates were exposed to UV radiation for 20, 40 and 60 sec. The lamps were located 11 cm from the plates and the roller table was stationary. The lamps were warmed up for 15 min prior

to the experiment and there was a 5 min stabilisation time between each of the different exposure times. After each exposure, the plates were marked according to their location and orientation with respect to the lamps, before incubating them for 18 hr at 37°C. A viable count was carried out to confirm the concentration of the untreated bacterial suspension. A set of agar plates inoculated with 100 µl suspension was not treated and this was the control.

3.2.7 UV system stability measurements

The objective of this experiment was to compare the stability of the existing UV lamp system (**Figure 2.24**) with the new, specifically designed and improved UV system (**Figure 2.20**). The stability and intensity of the UV system was measured and quantified. Also, it was anticipated that these data would prove useful in correlating the decontamination efficiency of the system with the production requirements of a packing house.

Method

The temperature and UV intensity were measured. A 'K' type thermocouple (RS, UK Part no : 219 4309) was used to measure the temperature rise of the surface of the lamp. The thermocouple was securely attached to the lamp with UV-resistant PTFE tape. This ensured positive surface contact and a reliable temperature measurement. Intensity measurements were made using the MaCam UV meter (Glasgow University, Radiation Protection Service). For accurate measurements, the UV detector was mounted on a fixture and located firmly in place with a pair of clamps suspended from a retort stand, shown in **Figure 3.3**. The height was adjusted and optimized as required for the experiments. The new optimized UV system (**Figure 3.2**) is shown mounted above the rollers in the polycarbonate safety enclosure. The thermocouple sensor and MaCam UV meter can be seen. The detector was placed onto the rollers of the roller table.

Before the lamps were switched on, the height between the detector and the lamp was measured and optimized. The angle of the surface of the detector face was checked with a spirit level, to ensure that it was perpendicular to the incident radiation. Temperature and irradiance measurements were taken manually as soon as the lamp was switched on. Both the temperature and UV intensity readings were recorded every 30 sec for the first 5 min, and every 1 min thereafter until the tenth minute. Subsequent readings were taken every

15 min. The final measurement was taken 1 hr after the lamp was first switched on. The irradiance measurements were recorded as done previously with the 3 x 40 W lamp system, at distances of 11 cm and 26 cm. The set-up was identical in each case. The readings from each UV system were compared.

UV output irradiance measurements with and without lamp cooling

The effect of a forced air flow across the lamp on the UV intensity was investigated. The UV lamp assembly was connected to a prototype fan assembly (that was parallel to and just below the lamp). The lamps were switched on and allowed to run for 25 min, with the fan switched off. The intensity of the lamps was measured with the MacCam UV detector at varying distances: 20, 30, 40, 50 or 60 cm. The intensity was allowed to stabilise for 1 min before the readings were taken at each height. This process was repeated but with the fan switched on. A thermocouple attached to the UV lamps was used to measure their temperature which was recorded at the same time as the intensity readings.

3.2.8 UV irradiation treatment of *E. coli* on carrots

A UV lamp (3 x 40 W), situated at a distance of 6.5 cm was used to treat sections (2 x 2 cm) of carrot skin contaminated with *E. coli* suspension. The lamp was in position above the rollers. Aliquots (20 µl) of *E. coli* suspension (6.5 x 10^7 cfu ml^{-1}) were placed on the flat surface of sterile carrot sections and exposed to the UV irradiation for 10, 30, 60, 90, 120, 180 or 240 sec. The experiment was done in duplicate.

3.2.9 UV killing of *E. coli* on potato segments without lamp cooling on 3 x 40W UV lamp system

Once again the optimum parameters of UV treatment for use in subsequent combination experiments were sought. Segments of potato skin (2.0 x 2.0 x 0.5 cm^3) were prepared as described previously.

Aliquots (25µl) of *E. coli* 7.6 x 10^7 cfu/ml were pipetted onto the skin surface of each section. The aliquots were exposed to UV irradiation at the distances of 30, 40, 50 and 60 cm. After each exposure, 1.0 µl was removed and total viable counts were determined by the procedure described above. The UV power was 3 x 40 W and the exposure time was 5 sec.

3.2.10 UV killing of *E. coli* on potato segments with forced cooling on 3 x 40 W lamp system

Segments of potato skin (2.0 x 2.0 x 0.5 cm^3) were prepared as described above. Aliquots (25µl) of *E. coli* were pipetted onto the surface of each segment. The aliquots were exposed to UV irradiation at the distance of 60, 70 and 80 cm. The UV power was 3 x 40 W and the exposure was 5 sec.

3.2.11 Decontamination of whole potatoes and carrots under 3 x 40 W UV lamps

The UV lamps (3 x 40 W), at a distance of 26 cm above the conveyor roller table, were switched on 10 min prior to the experiment. After this period, it was known that the UV output was relatively constant over time. Random groups (n=3) of potatoes or carrots were sent down the roller table at speeds of 0.212 ms^{-1} (fast) or 0.05 ms^{-1} (slow). The UV lamp length was 1.2 m, so the exposures were 6 and 24 sec respectively. The vegetable revolution speeds were 8.5 and 4.5 rpm. The control vegetables were not treated with the UV. The produce was placed on the roller table, passed beneath the treatment and removed from the system while wearing a fresh pair of sterile gloves for each procedure. After placing the produce on the table, the gloves were discarded and a new pair used to remove the produce after treatment.

Duran bottles containing 500 ml of water were weighed and the tare weight recorded. Each vegetable exposed to irradiation was placed in an individual Duran bottle (which was re-weighed to ascertain the weight of the vegetable) and shaken for 1.5 hr (150rpm). A viable count was made from the wash water by filtering the wash water through cellulose nitrate membrane filters (Whatman 0.45µm pore) and placing the membranes on the surface of nutrient agar plates. The plates were incubated at 37°C for 18 hr and the colonies were counted to determine amount of normal flora left behind after treatment.

Presentation of results

A difficulty with whole potatoes or carrots was the variable size of each item. To overcome this problem two values were calculated from the viable bacterial counts:

The results are presented as $cfu g^{-1}$ of carrot or potato.

$$\text{Count per ml} \times 500 \text{ ml} \ / \ \text{wt. of produce (g)} = \text{cfu g}^{-1} \text{ produce.}$$

or as cfu / 500ml to reflect the error introduced by the surface to volume ratio of the vegetable, which does not take into consideration the weight of the produce. The bioburden of the internal layers of the vegetable was considered to be negligible.

$$\text{Count per ml} \times 500 \text{ ml} = \text{cfu in the total 500 ml wash.}$$

3.2.12 Scanning electron microscopy of carrot and potato surfaces

The objective of this analysis was to visually investigate the surface topography of the carrot and potato and to see how this may impact the decontamination process. Carrot and potato samples were sliced into 10 cm square sections with a thickness of about 1 mm, as measured from the top of the skin.

Specimen preparation

The sample preparation included fixation, staining, drying and coating.

Fixation

The specimens were immersed in 2.5% glutaraldehyde in 0.1M phosphate buffer fix for an hour in a bijoux bottle before they were drained and rinsed with 0.1M PO4 buffer fix, thrice.

Staining

The samples were stained to promote a higher level of electron emission in the specimen, and to produce a higher resolution and brighter image. Immediately after the third rinse, the staining agent (1 % osmium tetroxide) was added to each bottle which was set aside for 1 hr. The samples were then rinsed with distilled water 6 times and each time they were suspended in the rinse for 5 min. 0.5 % aqueous liranyl acetate was added next and the samples were suspended for 1 hr in the dark. Aqueous liranyl acetate is sensitive to light, and completing this process in the dark prevented deterioration of the staining agent. Staining was completed after a few quick rinses with distilled water.

Drying the specimen

The carrots and potatoes were dried before SEM viewing to prevent image distortion due to phase changes occurring in the sample under the high vacuum conditions. Drying also

improved the contrast and improved image resolution. Drying was carried out at the critical point where the vapour and liquid phase were indistinguishable. The dehydration medium was acetone. Dehydration was carried out in stages to reduce shock acting on the samples, which would be susceptible to distortion and shrinkage during the transition from their wet to dry state. The concentration of acetone was increased progressively as the specimens became drier (**Table 3.1**).

Table 3.1 Acetone concentration, number of rinses and drying times

Concentration of acetone	No of rinses	Duration of specimen suspended in acetone
30%	2	10 min
50%	2	10 min
70%	1	Overnight
100%	2	10 min
Absolute	2	15 min
Dry absolute	2	15 min

After the last stage of acetone treatment the specimens were transferred into new dry bijoux bottle and left in a desiccator overnight before they were coated and mounted on stubs.

Mounting and coating the specimen

The samples were mounted on the stubs with a pair of tweezers, a paintbrush, stub fixtures and some conductive paint. The samples were carefully handled and located onto the stub, and securely fixed with the conductive paint. The specimens were coated with gold from a gold coating sputtering machine in the usual manner.

Sample viewing in the scanning electron microscope

Finally the samples were mounted on the stubs and loaded into the vacuum chamber of the scanning electron microscope (Leica Electron Optical UK, S360 Stereoscan microscope). After evacuating air from the chamber, the electron beam was switched on and the image formed by the reflection of the electrons was captured on the screen.

3.2.13 Model of the bacterial distribution over a potato

A simple model was developed to investigate the effect of a likely decontamination process on the surface of a potato. The model was used to investigate the effect of the

spatial distribution of bacteria before and after exposure. As input data to the model, samples of eyes and smooth skin were analysed microbiologically.

A number of different assumptions were made for the model. This included for example, that there was no killing on the eyes due to shadowing, complete killing on the skin, equal killing on the skin and eyes, a 1 D value reduction on the skin only, a 1 D value reduction on the skin and eyes. The experimental data were such that the eye material included about 1g of potato flesh. The effect of varying this weight was investigated on the model. The input data included the weight and radius of the potato. It was assumed that the potato was round. Different scenarios were run through the model to investigate the effect of a different number of eyes (and weight) and different size of potatoes.

3.2.14 Combination decontamination systems

Parameter selection for combined experiments

The parameters that were used for the combination experiment were found by analysing all of the available data from previous experiments.

Minimal processing by combinations of laser, UV and microwave irradiation of potato surfaces contaminated with *E. coli*

Segments of potato skin (2.0 x 2.0 x 0.5 cm^3) were prepared as described above. Aliquots (25 µl) of *E. coli* were pipetted onto the surface of each section. The aliquots were exposed to sequential treatments of laser, UV and microwave irradiation. After each exposure, one segment was removed for colony counts.

The experiment was designed so as to incorporate all possible order of combinations of laser, UV and microwave treatment, see **Table 3.2**

Table 3.2 Parameters for combination experiments

Laser	▸ UV	▸ Microwave
Laser	▸ Microwave	▸ UV
UV	▸ Laser	▸ Microwave
UV	▸ Microwave	▸ Laser
Microwave	▸ UV	▸ Laser
Microwave	▸ Laser	▸ UV
No treatment	▸ No treatment	▸ No treatment (control)

Parameters:

Laser	Power 2 kW,	exposure 2 sec, beam area ~120 cm^2
UV	Power 3 x 20 W,	exposure 5 sec, distance 80 cm
Microwave	Power 800 W,	exposure 8 sec

3.3 Results

3.3.1 Decontamination using CO_2 laser

Assessment of the laser damage threshold for the roller table

The susceptibility of roller table to laser induce damage was investigated. No effect was observed when the rollers were moving at the lowest velocity. However, the rollers were not rotating for more than one complete revolution (5 m) so there may be a problem with extended use of the system. Furthermore, there was no produce on the system which would reduce the amount of energy falling onto the table. It may be that when the system was moving there was cooling of the rollers by forced convection. This may have implications for the system's performance when the system is running within a process environment. Assessment was not carried out at faster speeds as the energy density of the beam on any one part of the roller would be lower.

CO_2 laser system killing of *E. coli* on potato segments

The CO_2 laser was used to treat potato segments inoculated with *E. coil* with different exposure times. **Table 3.3** shows the results where the average log kill is shown as a function of the applied energy density, expressed in Jcm^{-2}, for each exposure time.

Table 3.3 Log reductions after laser treatment of *E. coli* on skin of potatoes segments

Exposure time (sec)	Energy density (Jcm^{-2})	Cfu per potatoes segments	Average log_{10} kill
0	0	8.9×10^8	-
0.5	2.2	5.7×10^8	0.2
1.0	4.4	5.6×10^8	0.2
1.5	6.6	2.8×10^8	0.5
2.0	8.8	1.7×10^8	0.7

The results shows that generally, as the laser exposure time increased so did the level of inactivation. With the 2 sec exposure there was a 0.7 D-value decrease. This level of laser irradiation (8.8 Jcm^{-2}) was used for the combination experiments, as it gave the required level of killing for minimal processing and did not damage the potato segments.

CO_2 laser treatment of *E. coli* on carrot and potato segments

The results in the table below show the colony counts recovered from carrot and potato skin after treatment with the CO_2 laser.

Table 3.5 Log reductions after 2.5 sec of CO_2 laser exposure at different powers on carrot and potato segments

Laser Power (kW)	Energy density (Jcm^{-2})	Cfu per carrot section	Average log_{10} kill (n=3)	Cfu per potato section	Average log_{10} kill (n=3)
0	0	2.0×10^7	0	1.8×10^7	0
0.5	4.2	2.1×10^7 1.5×10^7	-0.02 0.12	1.9×10^7 1.4×10^7	-0.02 0.13
1.0	8.3	4.9×10^6 6.5×10^6	0.61 0.49	1.3×10^7 1.6×10^7	0.14 0.05
1.5	12.5	4.3×10^6 4.1×10^6	0.67 0.69	2.3×10^7 2.4×10^6	-0.11 0.88
2.0	16	2.1×10^6 3.6×10^6	0.98 0.74	2.9×10^6 1.4×10^6	0.79 1.11

With the carrot as the test subject, a 90% or 1 log_{10} reduction in viability was observed. Interestingly, the 0.5 and 1.0 kW tests did not produce steam even if the sample remained wet. The 1.5 and 2.0 kW tests, however, produced steam from the carrot surface. The skin was not damaged. When potato sections were used as the test subject, there was

approximately a 90% reduction in the *E. coli* per section. It was noted that a large quantity of steam was produced after exposure to 2kW laser irradiation for 2.5s, but the sample did not dry. There was little difference in the log reductions for the different treatments.

3.3.2 Decontamination using microwave

Microwave killing of *E. coli* on potato segments

The microwave was used to decontaminate potato segments inoculated with 25µl of *E. coli*. The results after microwave treatment for 6, 7 and 8 sec is shown in **Table 3.4**.

Table 3.4 Log reductions after microwave treatment of *E. coli* inoculated on potato segments

Exposure (s)	Cfu per potato segments	Average $\log_{10}$ kill
Control	**4.3×10^8**	-
6	2.1×10^8	0.3
7	2.7×10^8	0.2
8	2.6×10^8	0.2

It was observed that, although there was killing after treatment, the average log reduction did not increase proportionally with respect to the increased microwave exposure.

3.3.3 Decontamination using ultraviolet radiation

Spatial effectiveness of optimized UV system

Agar plates lawned with 100µl *E. coli* at 10^7 concentrations were treated under the optimized UV lamps at different locations and for different treatment times. **Table 3.5** shows a summary of the detected cfu after treatment and incubation as a function of UV exposure and location.

Table 3.5 Summary of the cfu count for different UV exposures and locations

UV Exposure (sec)	Loc 1	Loc 2	Loc 3	Loc 4	Loc 5	Loc 6	Loc 7	Loc 8
	Cfu per plate							
20	4	3	0	4	3	2	4	2
40	1	2	0	1	2	3	1	13
60	0	0	0	0	4	0	0	6

Note : Loc = Locations
See Figure 3.1 for a schematic of the relative positions of the location.

These results are shown schematically in **Figure 3.4** to **Figure 3.7**. **Figure 3.4** shows that a few colonies remained around the periphery of the lamps after UV exposures of 20 and 40 sec. After 60 sec no colonies remained. For the plates placed between the lamps (location 2), only two or three colonies remained after exposures of up to 40 sec. No colonies remained after a 60 sec exposure. For all exposures under the centre of the lamp (**Figure 3.6**) no colonies remained. In locations 5 and 6 a few colonies remained, even after an exposure of 60 sec, **Figure 3.7**.

It should be noted that the only position where colonies remained after exposure of 60 sec was at the very periphery of the lamp bank, where the produce would not pass. Also, in practice, the produce would roll under the lamp system and would not be stagnant under or between one set of lamps, so the produce would receive exposures from different sections of the UV lamp bank.

UV stability measurement results of 3 x 40 W UV lamp system

Figure 3.8 show the temporal evolution of the temperature for the existing and new optimized UV system and their corresponding UV output irradiance profiles, respectively. Interestingly, it is seen that the temperature of the new system rises to a lower value than was measured for the conventional lamp system. This was due to better heat convection from the new system and the improved spacing between the lamps. A further interesting point to note is that the irradiance from the old system characteristically peaked before reducing and stabilising, whereas with the new system, the irradiance increased, over about 10 mins to a stable value. Again, these differences can be attributed towards the differences in the temperature characteristics of the lamp systems.

The irradiance from each system was measured at different heights and compared. The UV irradiance from the 120 W system at 5 cm was approximately 1.6 $mWcm^{-2}$ whereas for the new system it was 1 $mWcm^{-2}$. Importantly, these were at temperatures of about 60 and 23°C respectively. The lower irradiance of the new system was considered to be satisfactory to provide an extension in the shelf life of the produce and the lamp life would be considerably better as it was operating at a lower temperature.

UV output irradiance measurements with and without cooling of 3 x 40 W UV lamps system

Figure 3.9 shows the output intensity of the 120 W UV lamp system as a function of time. The exposure quickly increased to a maximum before reducing and stabilising to a plateau value after about 10 - 20 min. The error bars show the SEM for 5 readings.

Figure 3.10 shows the irradiance as a function of distance from the source with and without the fan operating. There was an increase in intensity with the fan operating, this difference was more pronounced at 20 cm from the source. The corresponding temperatures of the UV lamps, taken at the same time as the intensity readings (**Figure 3.10**) are shown in **Figure 3.11**. The temperatures with and without forced cooling are shown. The temperature of the lamp was lower when the fan was operating, with a difference of about 10 to 20°C. This difference was less when the lamps were higher above the substrate. This was probably due to the increased heat convection for these greater heights and subsequent greater heat losses.

Figure 3.12 and **Figure 3.13** show the 3-D spatial irradiance distribution from the lamp and a side view respectively. It is seen that the energy density profile closely follows the lamp dimensions. This data was acquired by marking a grid on a piece of paper, placing the grid under the lamp, and moving the detector underneath the lamp into each square of the grid, and monitoring the UV irradiance.

UV irradiation treatment of *E. coli* on carrot segments using the 3 x 40 W UV system

As 20µl of *E. coli* culture was placed on the sample, the expected untreated control count should be 1.3 x 10^6 cfu per sample. **Table 3.6** shows the log reduction of *E. coli* after UV treatment for different exposure from the 3 x 40 W UV system. The lamps were placed 6.5 cm away from the treatment surface.

Table 3.6 Log reduction after *E. coli* inoculated on the surface of carrot skins were treated with 3 x 40 W UV lamp system

UV exposure time (sec)	Cfu per carrot section	log_{10} kill
Control	1.6×10^6	-
10	0	Btl
	0	Btl
30	4.0×10^2	3.6
	0	Btl
60	0	Btl
	3.1×10^3	2.7
90	1.0×10^3	3.2
	0	Btl
120	0	Btl
	0	Btl
180	0	Btl
	0	Btl
240	0	Btl
	0	Btl

Note: Btl – Below detection limit 10^2

The UV system provided excellent inactivation of the carrot sections. There was some survival of the *E. coli* for exposures of up to 90 sec, where a 3 log reduction was observed, but for times greater than this no counts were observed above the detection limit. Compared to similar treatments on plates it was only slight less effective. This was probably due to the carrot surface providing some protection from the irradiation due to its topography.

UV killing of *E. coli* on potato segments without lamp cooling

These are the results of 5 sec UV treatment of 25 µl of *E. coli* at 10^8 what $cfuml^{-1}$ concentrations, inoculated on the surface of potato skin, without lamp cooling. **Table 3.7** shows the average log kill as a function of distance from the 3 x 40 W UV lamp system with distances from 30 to 45 cm and **Table 3.8** is from 40 to 60 cm.

Table 3.7 Log reduction of UV treatment of *E. coli* inoculated on surface of potato skin without lamp cooling experiment 1

Distance (cm)	Cfu per sample	Average $\log_{10}$ kill
Control	3.1×10^7	-
30	2.9×10^6	1.1
40	1.1×10^4	3.5
45	1.2×10^5	2.5

Table 3.8 Log reduction of UV treatment of *E. coli* inoculated on surface of potato skin without lamp cooling experiment 2

Distance (cm)	Cfu per sample	Average $\log_{10}$ kill
Control	1.2×10^7	-
40	1.9×10^6	0.8
50	1.5×10^5	1.3
60	2.3×10^6	0.7

The tables above were the results obtained on two different occasions. Both experiments showed that in general, as the UV irradiance decreased with increasing distance, the killing rate reduced. The results in **Table 3.7** (40cm) show a much greater $\log_{10}$ reduction that the corresponding distance in **Table 3.8**. This inconsistency is likely to be due to the natural micro-flora on the potato samples and possibly the uneven distribution of organisms samples.

Table 3.9 shows the log reduction after the *E. coli* on potato skin were treated with UV irradiant at different distant and with the cooling fan turned on.

Table 3.9 Log reduction of UV treatment of *E. coli* inoculated on surface of potato skin with lamp cooling

Distance (cm)	Cfu per sample	Average $\log_{10}$ kill
Control	1.1×10^8	-
60	2.2×10^6	1.7
70	6.9×10^6	1.2
80	1.7×10^7	0.8

It is seen that the log reduction in viability reduced as the distance between the lamp and

the sample was increased. The log reduction at 60 cm (1.7) was about a log greater then when no cooling was used (0.7, in **Table 3.8**)

Detecting ozone

The presence of ozone from the 3 x 40 W UV lamp system was detected with ozone detector cards. A silver protective covering was pulled from the card to reveal the sensor. The sensor changed colour depending on the level of ozone detected, there were four scales. These were between 20 - 90 μgm^{-3}, 90 - 150 μgm^{-3}, 150 - 210 μgm^{-3} or greater than 210 μgm^{-3}. The card was left in the environment of the lamps with the lamps turned on for about 20 min, until a colour changed was observed. After 20 min of exposure, the ozone concentration was between 20-90 μgm^{-3}.

Decontamination of whole potatoes and carrots under 3 x 120 W UV lamps

The potatoes were initially moved along the roller at a distance 26 cm (12.1 μWcm^{-2}) from the lamps. The reason the lamps were at this lower height was to ensure that more than a 1 log reduction in viability was achieved. The results are shown in **Table 3.10.** In this experiment, the total count of the natural microbial bioburden was obtained after UV treatment of whole potatoes at fast and slow speed settings of the roller table.

Table 3.10 Log reduction of bio-burden of potato after UV treatment with the lamp at 26 cm

Treatment	Cfu g^{-1}	Average $cfug^{-1}$ (SEM n=3)	Log reduction
Control 1	9.8×10^5		
" 2	7.3×10^5	9.4×10^5	-
" 3	1.1×10^6		
Fast 1	6.8×10^5		
" 2	1.4×10^6	1.1×10^6	-0.07
" 3	2.7×10^6		
Slow 1	3.4×10^5		
" 2	1.3×10^6	6.3×10^6	-0.83
" 3	5.5×10^5		

There was no log reduction in the intrinsic microbial bioburden on the surface of the

potatoes. This showed that the UV irradiance was not sufficient for treating whole potatoes although it was sufficient to treat *E. coli* on agar plates. As a result, the same experiment was repeated with the UV lamps lowered to a distance of 23 cm (12.8 μWcm^{-2}). These log reduction results are shown in **Table 3.11**.

Table 3.11 Log reduction of bio-burden of potato after UV treatment with the lamp at 23 cm

Treatment	Cfu g^{-1}	Average $cfug^{-1}$ (SEM n=3)	Log reduction
Control 1	2.7×10^6		
" 2	4.2×10^6	3.2×10^6	-
" 3	2.7×10^6		
Fast 1	2.8×10^6		
" 2	2.4×10^6	2.0×10^6	0.2
" 3	1.3×10^6		
Slow 1	3.0×10^6		
" 2	3.0×10^6	2.5×10^6	0.11
" 3	2.3×10^6		

These experiments showed that the UV exposures were still insufficient to reduce the microbial bioburden on the whole produce even with the lamps being moved 3.0 cm nearer to the produce.

Failing to obtain effective inactivation, another experiment was conducted with the UV height fixed at 23 cm (12.3 μWcm^{-1}) but the vegetables were stationary below the lamps for 6 or 10 sec. After treatment, the potatoes were shaken in 1500 ml distilled water and the viable counts were determined. The results are shown in **Table 3.12**.

Table 3.12 Log reduction of bio-burden of a stationary potato after UV treatment with the lamp at 23 cm

Treatment		Cfu g^{-1}
Control trial 1		TMTC
Control trial 2		TMTC
Control trial 3		TMTC
Exposure 6s, trial 1	UV	1.1×10^3
Exposure 6s, trial 2	"	-
Exposure 6s, trial 3	"	2.3×10^3
Exposure 10s, trial 1	"	1.6×10^3
Exposure 10s, trial 2	"	4.2×10^3
Exposure 10s, trial 3	"	1.7×10^3

TMTC: Too many to be counted, the inherent contamination of the potatoes with organisms derived from the soil produced counts in excess of those that could be quantified.

Since the amount of organisms derived from the control sample was too many too be counted (using the same dilution during colony counting), it can be assumed that the organisms per g^{-1} was more then 10^6 compared to that of previous experiment. Therefore, the log reduction in this experiment can be estimated to be between 2 to 3.

3.3.4 Investigation into the bacterial distribution over whole carrots.

The UV distance was kept at 23 cm (12.1 μWcm^{-1}) and the carrots were rolled down the table at the slowest speed. The results are shown in **Table 3.13**. Note that the carrot sections were shaken in 200 ml of water.

Table 3.13 UV treatment of carrots moving along the roller table

Treatment	Cfu /200ml	Cfu g^{-1}	Average $Log_{10}g^{-1}$ (SEM n=3)
Control 1	7.3×10^6	4.3×10^4	
" 2	8.3×10^6	4.6×10^4	4.5 (0.11)
" 3	5.3×10^6	2.1×10^4	
Fast 1	6.5×10^6	2.7×10^4	
" 2	5.5×10^5	3.5×10^3	4.1 (0.27)
" 3	4.0×10^6	1.8×10^4	

Surprisingly, the UV system did not appear to be effective at reducing the bacterial load on

rolling whole carrots. Nevertheless the shelf-life of carrots was substantially increased after UV treatment (See chapter 4).

3.3.5 Investigation into the bacterial distribution over whole potatoes

The spatial distribution of bacteria over the surface of the potato, in the skin and eye areas, was determined. The results are shown in **Table 3.14.**

Table 3.14 The relationship between bacterial load and spatial distribution on whole potato

Area	Cfu/200ml	Cfu g^{-1}
Smooth skin	5.8×10^5	1.4×10^4
Eyes	1.8×10^6	5.8×10^4

It was seen that the eyes had approximately four times the bacterial contamination than the smooth skin. These data were used as input into the model to estimate the effect of different decontaminants levels.

The Model

The calculations were either based on weight or surface area. The calculations based on weight gave a greater reduction than those based on area, but with similar trends in the results for each set of assumptions. Different scenarios were put into the model to investigate the effect of a different number of eyes (and weight) and different size of potatoes. **Figure 3.14** shows the percentage kill as a function of an assumed D-value reduction. Here the potato was 140g, 30 mm radius with 10 eyes and 1 g/eye (experimental determination). It is seen that there are two assumptions. First of all the reduction is assumed to be on the skin only, and secondly, the reduction is assumed to be on the skin and eye region. It is seen that for above a 1D reduction, there is little increase in the percentage killed for the model where killing was assumed to occur only in the eye. Whereas, practically complete killing is observed for the case where there was a reduction on both the eye and skin. **Figure 3.15** shows the same graph as the previous figure but with only 5 eyes and only 0.1g/eye. It is seen that the effect of the eyes contributing towards residual contamination is greatly reduced, and there is near equivalence between the two assumptions.

Figure 3.16 and **Figure 3.17** show the effect of increasing the size of the potato. In each

case the density of the potato was kept the same to within about 0.5%. It is seen that the percentage killed was slightly greater for the larger potato. It is worthy to note that the number of eyes and weight was kept constant for this analysis, and so the fraction of the potato covered with eyes would have reduced, leading to a more effective decontamination for the larger sample.

3.3.6 Scanning electron microscopy of carrot and potato surfaces

Figure 3.18(A-G) show the SEMs of the carrot and potato samples from a top view (from exterior surface). The topography of the potato and carrot are dissimilar, carrots have smaller and denser grain surfaces, while the skin of the potato was rough with large grains and deeper crevices. **Figure 3.19(A-G)** shows the SEMs obtained from the carrot and potato samples from a side view. From the side, the potato skin appeared to be more porous then that of the carrot skin. Generally the carrots under the SEM appeared to be denser than the potatoes, this could have explained why treatment on carrots appeared to be much more effective than on potatoes.

3.3.7 Combination decontamination systems

Parameter selection for combined experiments

For the combination decontamination experiments the value of the parameters of each treatment were chosen based on the damage threshold of carrots and potatoes. These were determined from previous experiments where the treatments were conducted individually. The parameters that were used for the combination experiment are detailed below in **Table 3.15**.

Table 3.15 Parameters for combination experiments

Treatment	Power (W)	Exposure (sec)	
Laser	2000	2	-
UV	3 x 40	5	Distance 80 cm
Microwaves	800	8	-

These parameters were selected as they each gave less then 1 D-value reduction in viability. The combination experiments were designed to combine these sub-optimal levels of killing to minimally process the surface of the produce.

Minimal processing by combination of laser, UV and microwave irradiation on potato surfaces contaminated with *E. coli*

In the combination experiments, the carrots and potatoes segments were treated sequentially in 7 combinations as shown in **Table 3.16**. In the same table, results from the experiments are shown for the first, second and third treatments as the cfu surviving the treatment process in brackets.

Table 3.16 Sequential treatment of samples and number of *E. coli* surviving at each stage

Treatment (cfu surviving per sample)					
First		**Second**		**Third**	
Laser	(1.1×10^{11})	UV	(1.5×10^{10})	Microwave	(1.2×10^{10})
Laser	(1.2×10^{11})	Microwave	(1.0×10^{11})	UV	(9.8×10^{9})
UV	(8.8×10^{10})	Laser	(1.3×10^{10})	Microwave	(4.6×10^{7})
UV	(1.2×10^{11})	Microwave	(3.0×10^{9})	Laser	(1.2×10^{7})
Microwave	(2.1×10^{11})	UV	(4.2×10^{10})	Laser	(1.6×10^{10})
Microwave	(1.7×10^{11})	Laser	(9.0×10^{10})	UV	(5.8×10^{9})
Control	(1.9×10^{11})	Control	(1.8×10^{11})	Control	(1.2×10^{11})

The above experiments were repeated three times to improve the statistical reliability of the experiments. A total of 4 sets of similar experiments were conducted. The above data from the 4 sets of data show the colony counts were included in the analysis of combination experiments. Accumulative and individual log reductions of the combination experiment are tabulated in **Table 3.17.**

Table 3.17 Sequential treatment of samples and number of *E. coli* removed at each stage

Treatment ($\log_{10}$ kill)[SEM n=4]					
First		**Second**		**Third**	
Laser	(0.2) [0.09]	UV	(1.3) [0.23]	Microwave	(1.8) [0.65]
Laser	(0.5) [0.13]	Microwave	(1.5) [0.81]	UV	(2.0) [0.89]
UV	(0.5) [0.21]	Laser	(1.3) [0.28]	Microwave	(2.4) [0.52]
UV	(0.4) [0.25]	Microwave	(0.9) [0.37]	Laser	(3.2) [0.77]
Microwave	(0) [0.05]	UV	(0.6) [0.25]	Laser	(1.1) [0.39]
Microwave	(-0.1) [0.08]	Laser	(1.0) [0.25]	UV	(1.9) [0.59]

The combination treatment showed that the order of treatment had an effect on the final

log. The best synergistic effect was produced by first treating the samples with UV followed by microwave and finally by laser.

3.4 Discussion

3.4.1 Decontamination using CO_2 laser

Assessment of the laser damage threshold for the roller table

Importantly, there were no significant effects of the laser radiation on the roller table. It may be that prolonged use of the table exposed to laser radiation may have resulted in some heating. If this was the case, then measures could be taken to cool the table within an industrial environment. The laser output power could be controlled depending on the produce density on the rollers.

Assessment of the laser damage threshold for carrots and potatoes

It was clear from these experiments that if the exposure was sufficiently high the laser damaged the carrots or potatoes. This is not surprising as laser irradiation can be focused to high values over small areas. The carrots became white which indicated cellular damage and dehydration. The potatoes crackled, indicating vaporisation of water from the potato, eventually the skin charred, indicating burning of the produce.

CO_2 laser treatment of *E. coli* on carrot and potato

It was found that the delivery of laser radiation to potatoes had no significant effect on their appearance. This was important as discolouration of the produce would affect its saleability. The colour of the potato is a quality attribute. For higher energies the potato was charred, which is not suitable for the process. However, these energy densities were higher than those needed to achieve decontamination. It was seen that a 90% reduction was achieved. An important aspect to the laser subsystem is how effectively this could be done on a processing line, justifying its cost. The beam size was 120 cm^2, and this would have to be scanned across the production line. The scanning system again adds to the system cost.

It was clear from the results that the laser offered less effective killing on carrots than on plastic [130]. It was assumed that this was due to the topography of the carrot and the roughness of its surface. Similar results were observed on potatoes. Subsequently SEM analysis of the surfaces of carrot and potatoes was done to attempt to answer these questions. Iwanami found that microorganisms adhere more effectively to damaged sites [131].

The carrot became white after an applied energy density of 60 Jcm^{-2}. This was a large exposure, and lower values were needed to get over a D-value reduction. With the laser system, typically a log reduction was observed for an exposure of about 2.5 sec at 2.5 kW of power. The beam area was about 120 cm^2, yielding an applied energy density of about 50 Jcm^{-2}.

3.4.2 Decontamination using microwave

The damage threshold of the potato governed the selection of the microwave power. An exposure of 9 sec caused a "cooking smell" so this exposure was reduced. For the combination experiments, an exposure of 8 sec was used, as this was the maximum exposure which did not cause damage to the potato. It is seen that with this exposure, less than a D-value reduction in the microbial burden was achieved.

3.4.3 Decontamination using ultraviolet radiation

Spatial effectiveness of optimized UV system

The results indicated the effectiveness of the optimized UV system. The plates positioned directly under the lamps showed no trace of bacterial survival, even at the minimum UV exposure.

Although the length of each lamp was 435 mm, the full UV glow only existed within an effective length of 321 mm. Most of the colonies were found in the regions behind the lamp connectors and the ineffective edge part of the lamp. The number of surviving colonies reduced as the exposure was increased.

The system was designed to suit a roller table of width of 600 mm, and the produce was expected to pass the effective region of the lamps, given the specifically designed lamp

configuration. It is seen from the results at location 3 that with a 20s exposure, 100% killing was achieved, and it was concluded that the UV system was effective.

UV system stability measurements results

The results showed that the intensity radiated from the lamps of both systems varied with respect to temperature and time. A warm up period of about 8 to 10 min was required before the UV output stabilised. The original UV system, which comprised 3 lamps rated at 40 W, stabilised at about 72 °C, with an output irradiance of 1.4 mW/cm^2 at 50 mm. The UV intensity rose to a peak value of 1.6 mW/cm^2 after four min from when the lamp was switched on. After this peak, the irradiance gradually reduced to a stable value.

The improved system comprised 14 lamps each of 15W, mounted separately on 2 insulating boards. These lamps were evenly spaced on the boards over an area of 1.16m^2. Out of the 14 lamps, 8 were mounted on a board measuring 0.6 m in length and 0.47m wide and they were arranged in the longitudinal direction with respect to the flow of produce. The remaining lamps were evenly spaced in the transverse direction (See **Appendix I**). This board was 0.6 m in length and 0.5 m wide. This lamp geometry radiated about 1 mWatt/cm^2 at 5 cm after warming up the lamps for 10 min.

Ozone a possible contributing factor of inactivation during UV treatment

There was significant variation in the results from using or not using forced cooling. It was considered that this may have been due to ozone gas produced by the UV lamps or temporal variation in the lamp output due to temperature variations. If the quantity of ozone produced varied on a daily basis, this would affect the killing efficacy of the lamps. The presence of ozone was detected with ozone detector cards. After 20 min of exposure, the ozone concentration was between 20 - 90 μgm^{-3}. It was decided to quantify the decontamination effect of the lamps and any possible effect of the ozone gas by removing it with a fan.

UV killing of *E. coli* on potato segments with forced cooling

UV treatment at 80 cm tests gave a 0.8 D-value reduction in viability. This distance was used for the combination experiments. The intensity of the UV irradiation increased when the fan was used to blow air across and below the lamp. This might be due to removal of ozone or the optimisation of the lamp's operating temperature. The optimum UV output

varied with temperature, and the temperature was affected by the fan action. The lamps function at their optimum output at ~40°C. Consequently, it was decided that more cooling was needed. A system was designed and fabricated to provide cooling to the prototype system, comprising a bank of UV lamps. The recorded irradiance profile of the lamps provided data to feed into the design of the UV system.

Decontamination of whole potatoes and carrots under UV lamps

The results presented did not correlate with the results found previously, by artificially contaminating the surface of potato skin with *E. coli* where a D-value reduction was recorded after a 5 sec exposure at a distance of 80 cm from the UV lamps.

The apparent reduction in UV killing efficiency could be due to a number of factors. The first of these could merely be that the natural micro flora found on an unsterilised potato were more resistant than the *E. coli* used, for example, there would be resistant bacterial spores present. It could also be due to the shadowing effect that prevent UV irradiance treatment on bacteria resident between crevices and areas around spouting "eyes".

The experiments with segments of potato treated under the UV lamps showed a reduced bio-burden. There was a reduction to countable numbers after 6 and 10 sec of UV exposure, in the observed number of colonies compared with the confluent growth on the untreated controls.

The indications were that the actual UV exposure time, of a given area of potato rolling down the conveyor roller table, was insufficient. This could be overcome by lengthening the housing and number of UV lamps, increasing the power of the lamps, or reducing the height of the lamps above the produce. The height of the lamps above the produce was constrained by possible jumping of the produce from energy gained by rolling down the table and colliding with other produce, which if the height was insufficient could lead to lamp breakage and glass spoilage of the produce, which is unacceptable.

On the other hand, the surface topography of the vegetables, for example 'eyes' in the potatoes or 'crevices' in the carrots prevented adequate exposure for the UV irradiation to be effective in these regions. The possibility of this effect was investigated with carrot and potato samples viewed under the scanning electron microscope. SEMs were taken of the

surface topography of carrots and potatoes. This enabled a detailed investigation into the likely spatial distribution of microorganisms on the surface of potatoes and carrots, this is reported below. The physical surroundings of microorganisms on a whole vegetable may also play a part in their apparent resistance to UV irradiation i.e. the organisms sited in 'crevices, pores or eyes' may also be protected by particles of soil. This would provide them with additional protection from the sterilizing effects of the UV lamps and other forms of electromagnetic treatment.

As additional investigation into the spatial distribution of bacteria on potatoes, different parts of the produce were microbiologically analysed and this data were used as boundary conditions for the model.

3.4.4 Experimental investigation into the bacterial distribution over the potato surface

The mass of material in each case was approximately the same: 42 g of smooth skin and 31 g of eye material, but it was found that the bacterial load of the eyes in the potatoes was about four times that of the smoother skin. Clearly, if the bacteria are protected by surface imperfections, the sterilizing system may require different optimisation procedures.

3.4.5 Scanning electron microscopy of carrot and potato surfaces

Interestingly, early results obtained from minimal processing carrots and potatoes with UV lamps, indicated that there were significant differences in grain structure, roughness, thickness and porosity. The variation in the results between carrots and potatoes was believed to be due to the different topology of the samples, and the depth of the crevices existing on the sample's surface, which would tend to shield some of the microorganisms from UV radiation during treatment.

Surface shadowing was suspected to reduce the effectiveness of the UV treatment. Under the microscope, the carrot's fibres were found arranged in a longitudinal direction along the circumference of the produce. The width of each fibre was between about 10 to 15 μm. Each skin cell on the potato was measured to be about 50 μm.

The images obtained showed that there were less topographical variation in depth in carrots as compared to potatoes. The amount of the electron beam energy reaching deeper regions

is less and these regions appear darker, emitting much lower electron numbers than the top surface of the produce which appears brighter. Large variations in contrast were seen, this showed the differences in the depth were much greater for potatoes than with carrots.

Interestingly, around the eye region of potato skin, semicircular pockets were observed. There was, therefore, a significantly high probability that bacteria underneath this pocket were shielded from the UV radiation during the treatment process. This phenomenon was not observed on carrot surfaces. On potato skins, porosity was visible under a much higher magnification (1000x and above). Triangular shaped holes were seen in between the boundaries of the skin cells. This might be the reason why treatment on carrots was more effective than on potatoes since the larger triangular shaped holes were more likely to trap and shield organisms from treatment.

From the images of the side view of carrot and potato skin, it was observed that the carrot skin layer was much denser than that of potato. The skin cells were made of larger cells with hollow space, which would absorb water. This was observed at magnification higher than about 100 times. The average skin thickness of the carrots was about 250 μm, which was about 70 μm thicker than the potato skin.

The individual systems were used to kill *E. coli* inoculated on the surface of potatoes. These results produced the parameters used for the combination experiments. With the microwave subsystem, approximately 0.3 log kill was observed for an exposure time of about 8 sec. The damage threshold of the potato was a limiting factor in the exposure time. It was important that the potato was not cooked. It should be realised that the initial count for the control on the potato was extremely high, about 10^{11} per sample. For the laser treatment, a similar reduction was observed for a laser operating power of about 2 kW and beam area of 400 cm^2. A 2 sec exposure produced an average log kill of about 0.7. The UV lamp produced about 1 log kill. The effect of distance and exposure was evaluated. After the parameters were selected, the effect of different combinations was found. Interestingly, if the UV treatment was first, then there appeared to be a greater effect. The greatest decontamination was observed with the combination of UV, microwave and laser. Here there was approximately a 3 log reduction. Interestingly, this was above the summation of the individual treatments.

There was some evidence to suggest that the system was reducing bacterial loads of the added organism, but perhaps not the background organisms that naturally exist on the potato due to contact with soil some of these may have been spore-forming organism and therefore likely to be more resistant to the various treatments. These experiments showed that the UV system was capable of killing most of the organisms on the potato. The Gram-negative bacteria were killed within 20 sec.

With positive results from treating organisms on plate, it was important to investigate the treatment effect on the whole produce, this represents the real problem. The majority of the experiments reported in this chapter were done on cut samples while the rest were conducted on whole produce. At 23 cm away form the UV source, there was approximately a 2 log reduction observed after exposure of the potato to the UV irradiance for 10 sec. It was clear that spore formers were producing experimental results that were difficult to quantify as there were too many colonies to count.

3.4.6 Model of the bacterial distribution over a potato

The modelling of the bacterial contamination showed that the contamination found may be entirely due to residual contamination within the eye region. Even if more effective systems can be introduced, it is important that the system is effective at reducing contamination in the eye in order to reduce the overall microbial burden on the potato.

3.4.7 Combination decontamination systems

Parameter selection for combined experiments

It was important to find the exposures that gave minimal killing. In this sense, it was considered that the combined effect of the treatments would be less damaging to the substrate.

Minimal processing by combinations of laser, UV and microwave irradiation of potato surfaces contaminated with *E. coli*

From these results it was apparent that there was a reduction of *E. coli* after each treatment. There was also an indication that if the first treatment was UV, followed by microwave and then laser, the effect was greatest, with a 3.2 log reduction overall. This was compared to a 1.1 log reduction for a treatment order of microwave, UV and laser.

The combination treatment shows that the sequence or order of treatments has an effect of the final log reduction of the combination treatment.

3.5 Figures

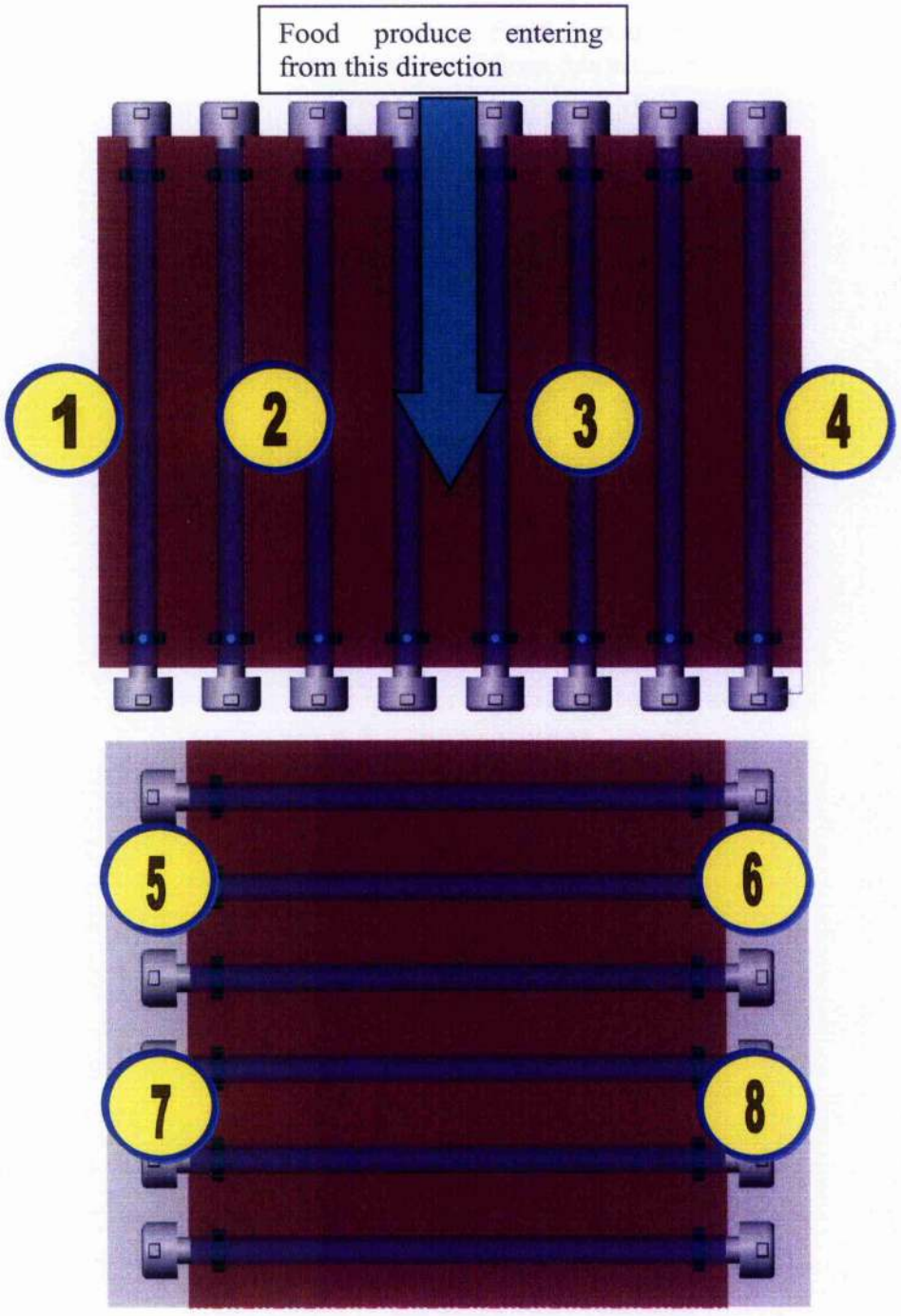


Figure 3.1 Numbered location of agar plates during spatial analysis of the UV system

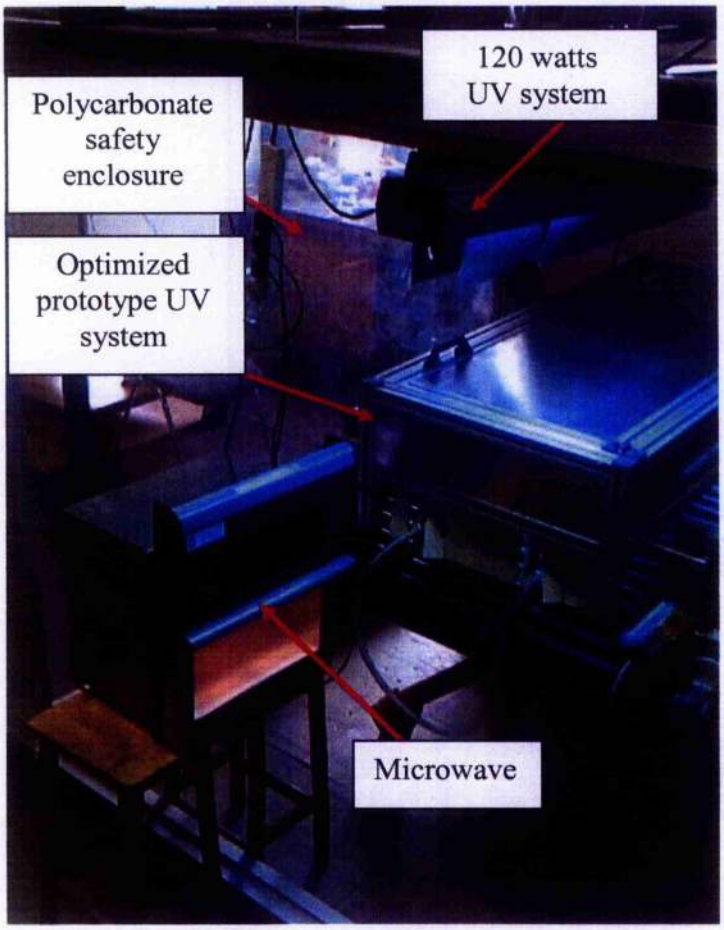


Figure 3.2 The 120 W UV, optimized prototype UV and microwave system

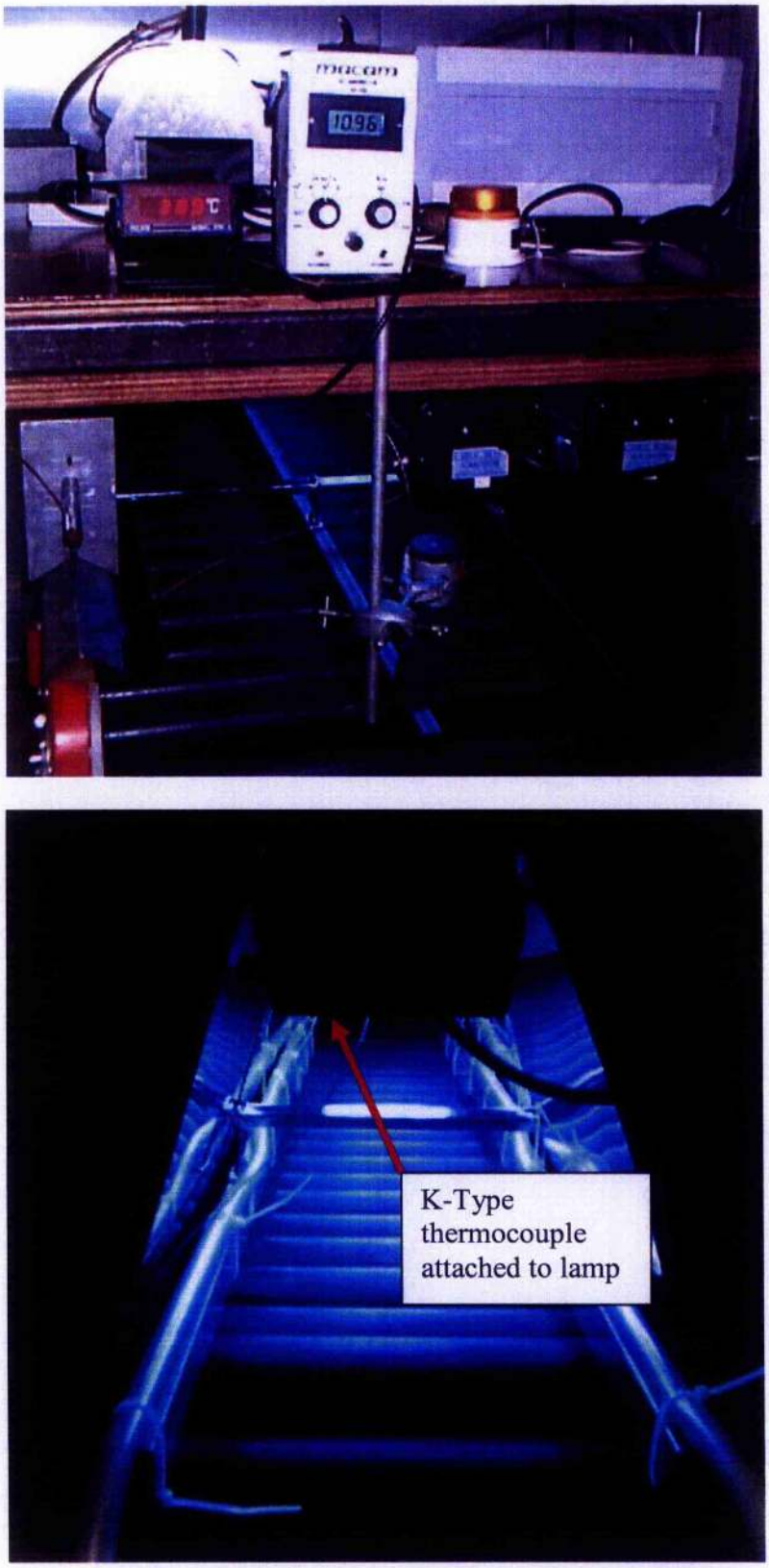


Figure 3.3 Experimental setup for UV stability measurement. (Mounting of thermocouple was repeated on the optimized UV system for stability measurement)

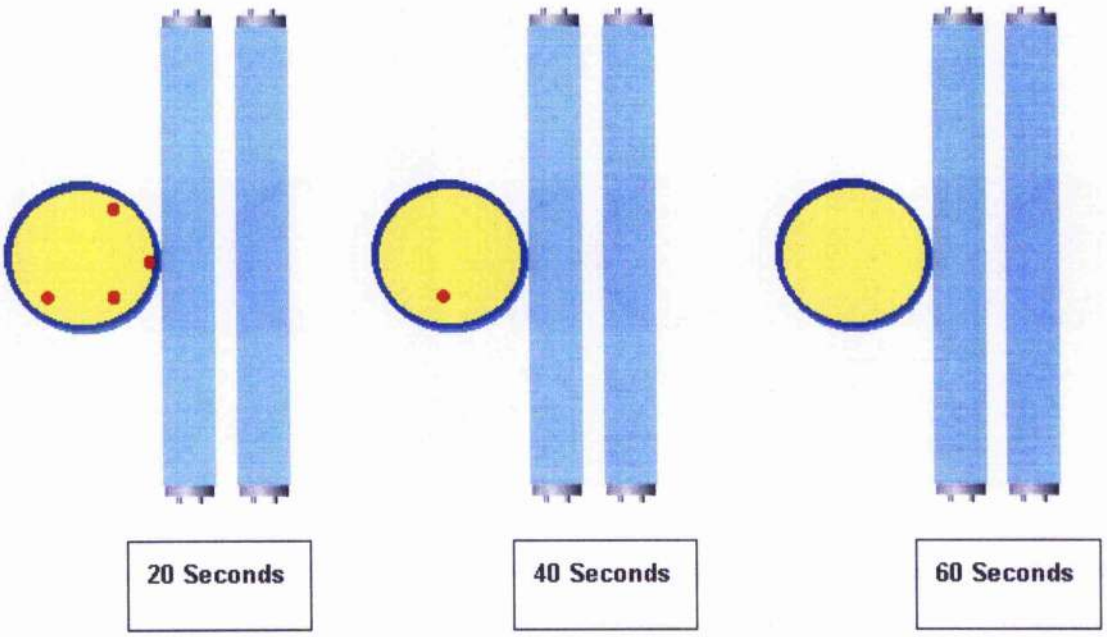


Figure 3.4 Colony counts after treatment at location 1 and 4

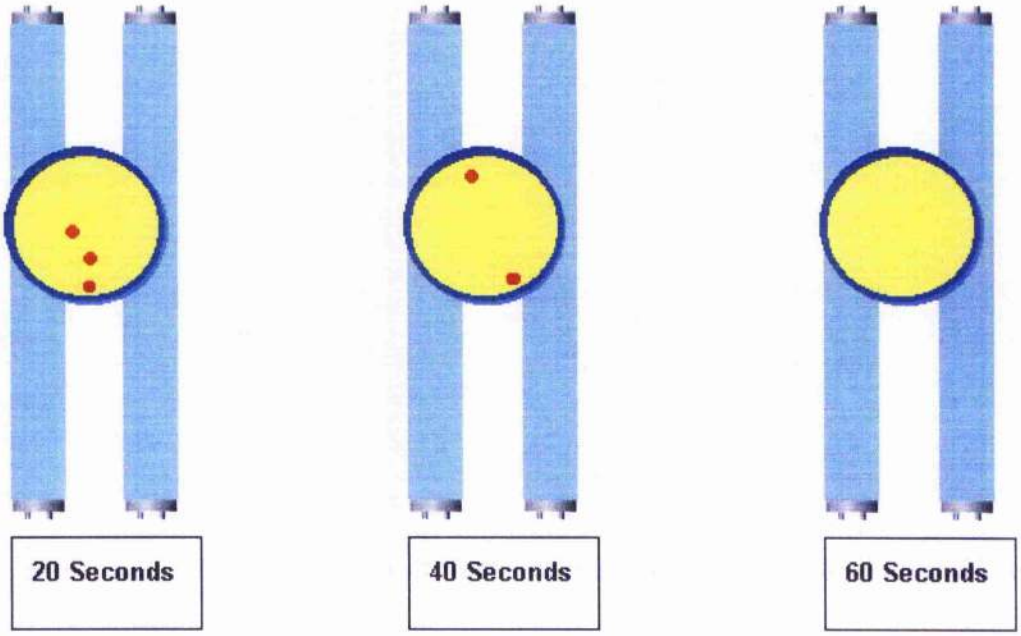


Figure 3.5 Colony counts after treatment at location 2

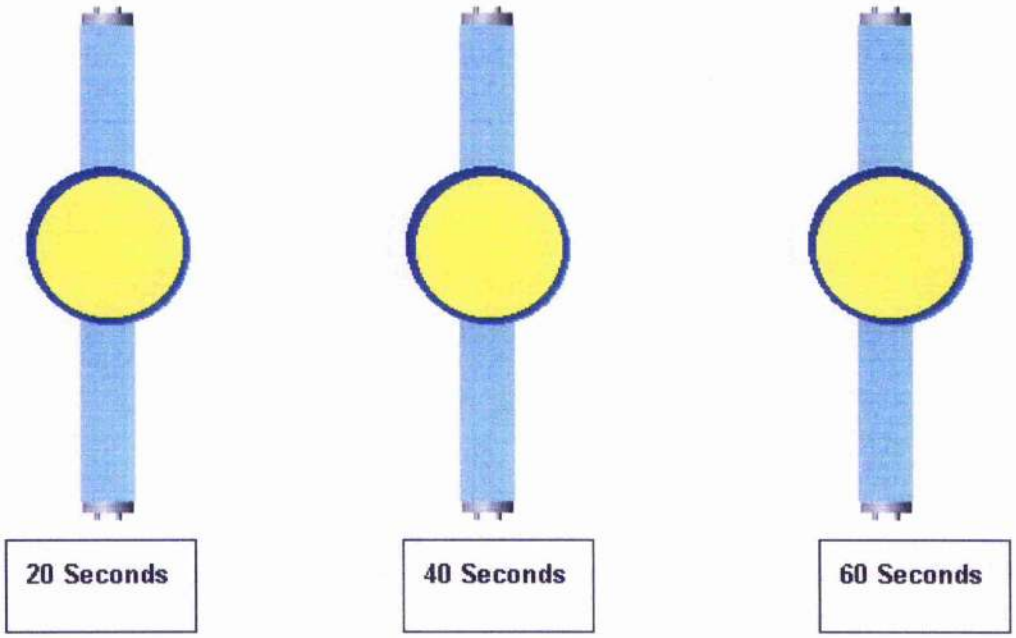


Figure 3.6 Colony counts after treatment at location 3

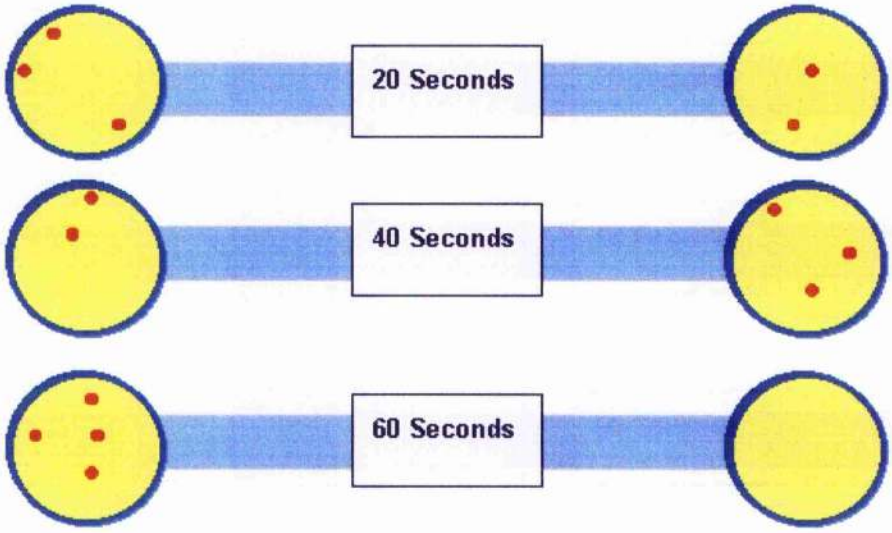


Figure 3.7 Colony counts after treatment at location 5 and 6

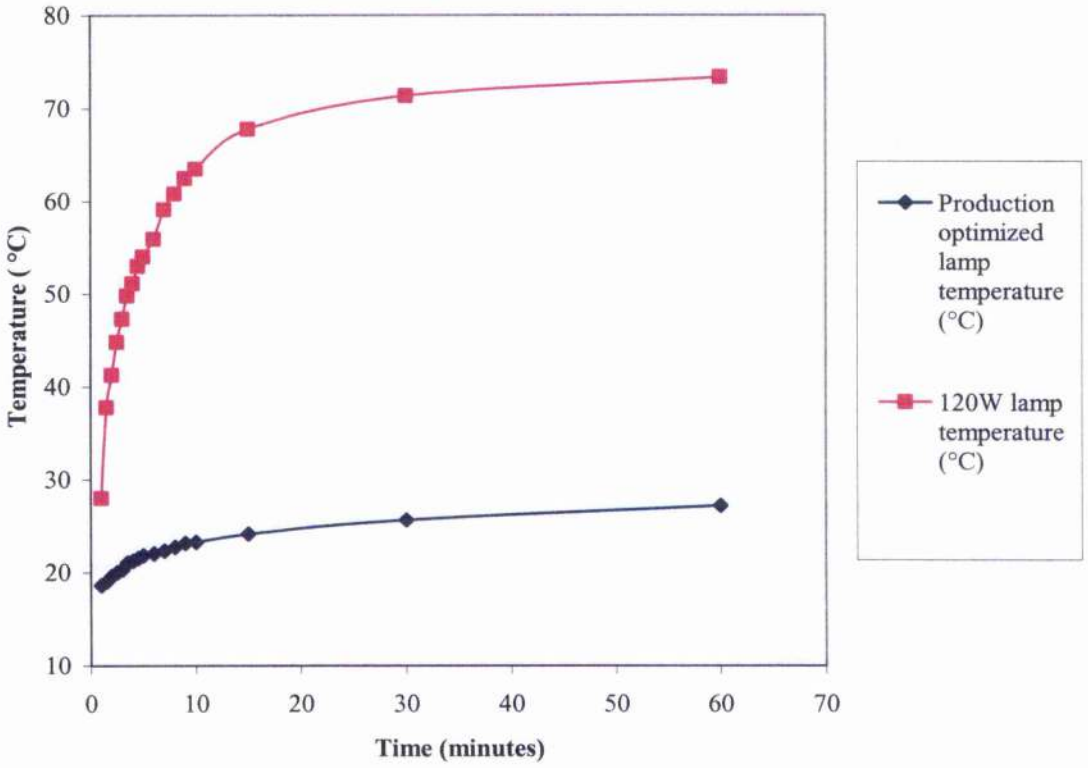


Figure 3.8 Transient temperature of both 120W and production optimized UV lamp system

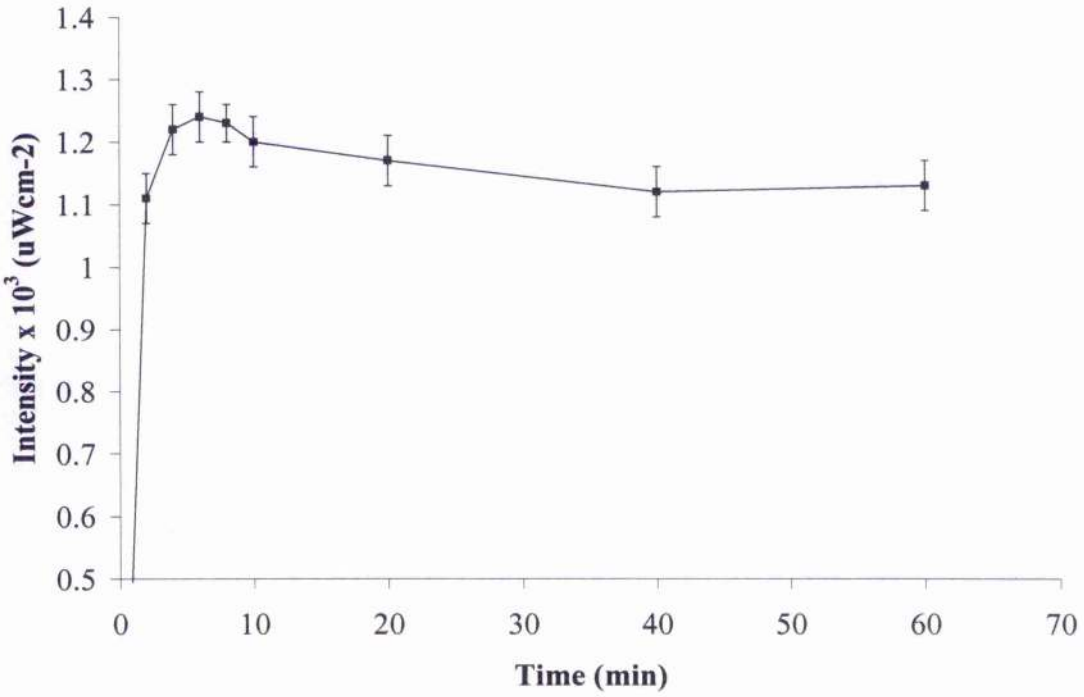


Figure 3.9 Temporal fluctuations in 120 W UV lamp output and the standard error of the mean of 5 measurements

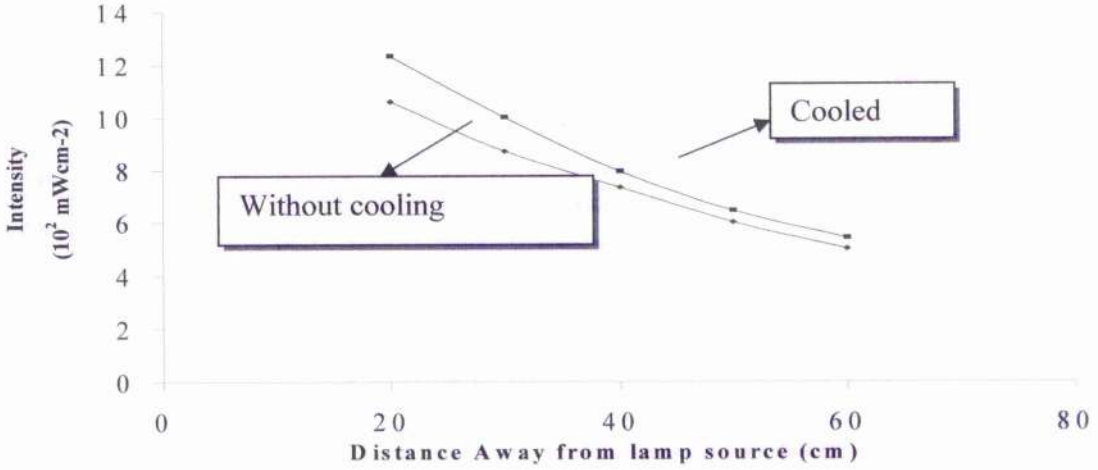


Figure 3.10 The output intensity of 120W UV system with and without cooling

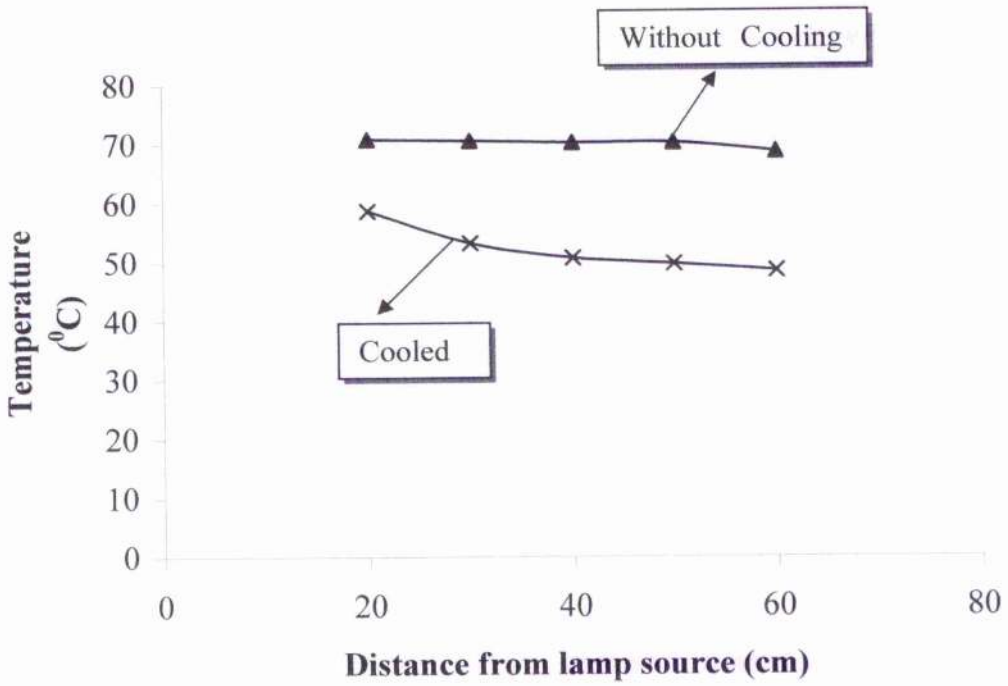


Figure 3.11 Temperature of the UV lamps, taken at the time as the intensity readings in Figure 3.10.

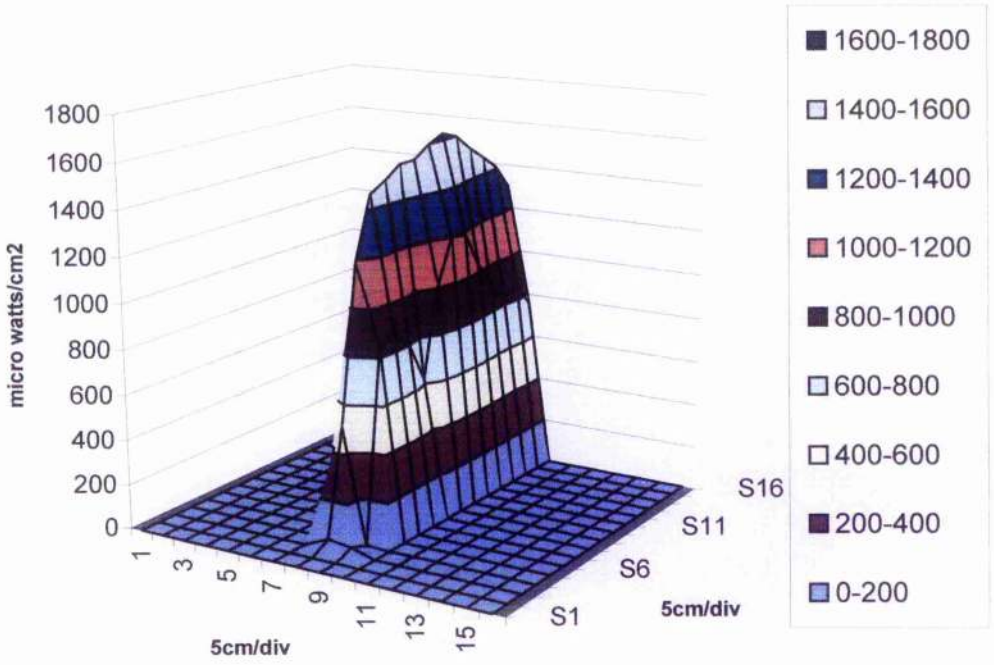


Figure 3.12 Spatial resolution and irradiance profile of 254 nm UV lamps (3 x 40 W)

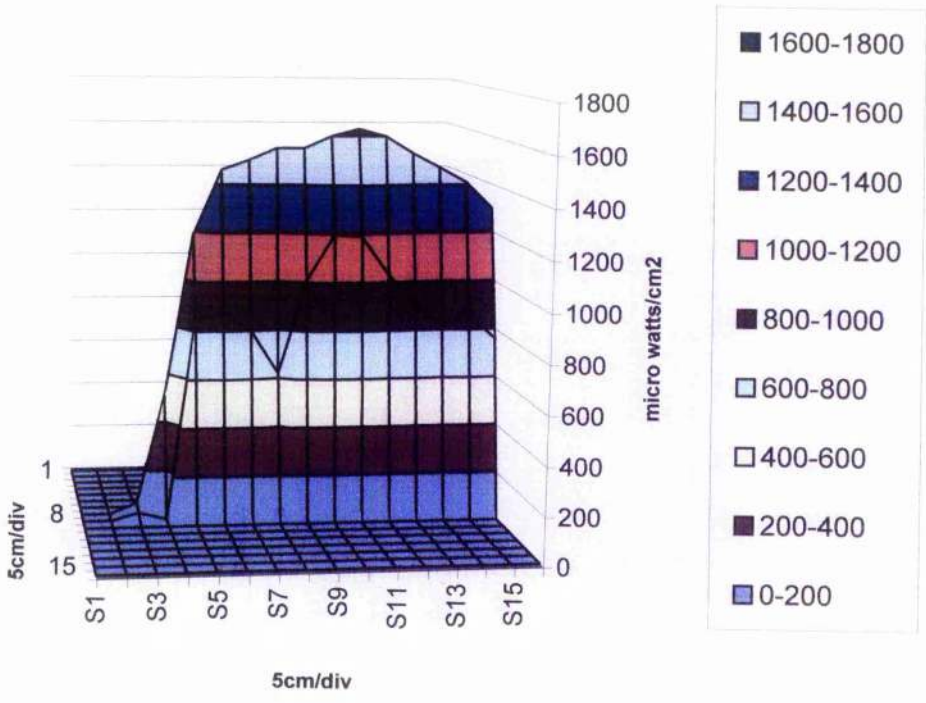


Figure 3.13 Spatial resolution and irradiance profile of 254 nm UV lamps (3 x 40 W side view)

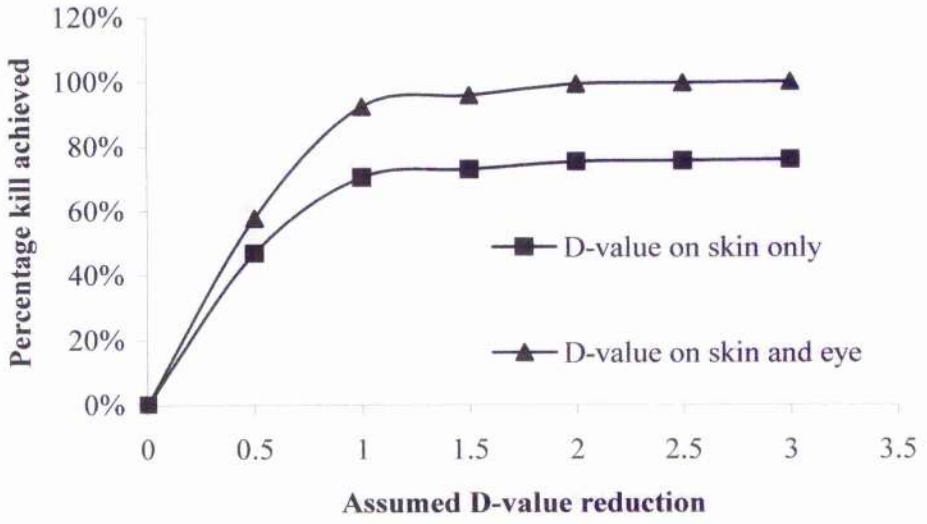


Figure 3.14 Model of potato inactivation showing percentage of organisms killed after different assumed log reductions on skin and eyes (140g, 30 mm radius, 10 eyes, 1 g/eye)

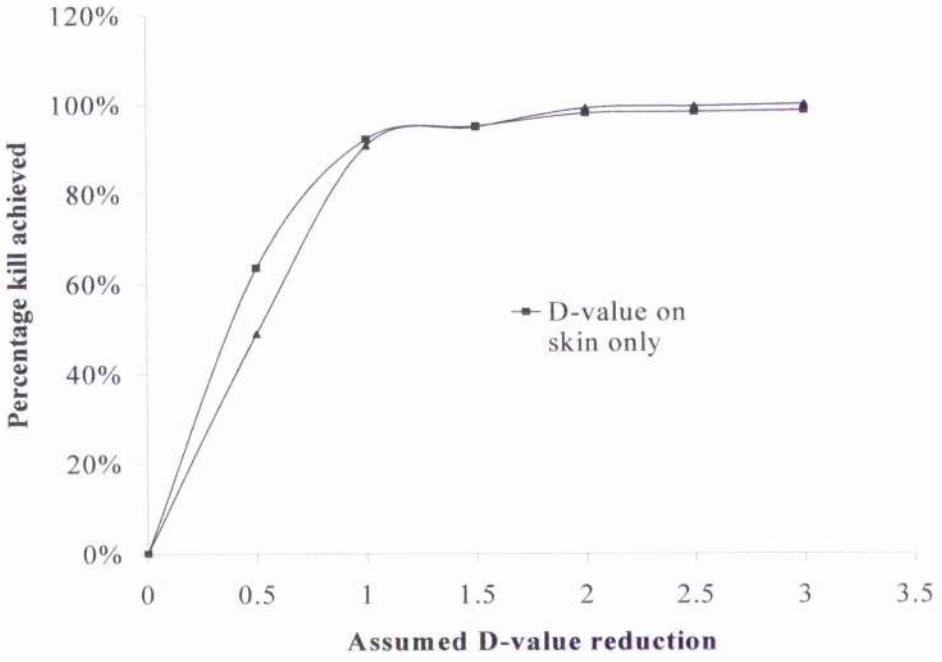


Figure 3.15 Model of potato inactivation showing percentage of organisms killed after different assumed log reductions on skin and eyes(140g, 30 mm radius, 5 eyes, 0.1 g/eye)

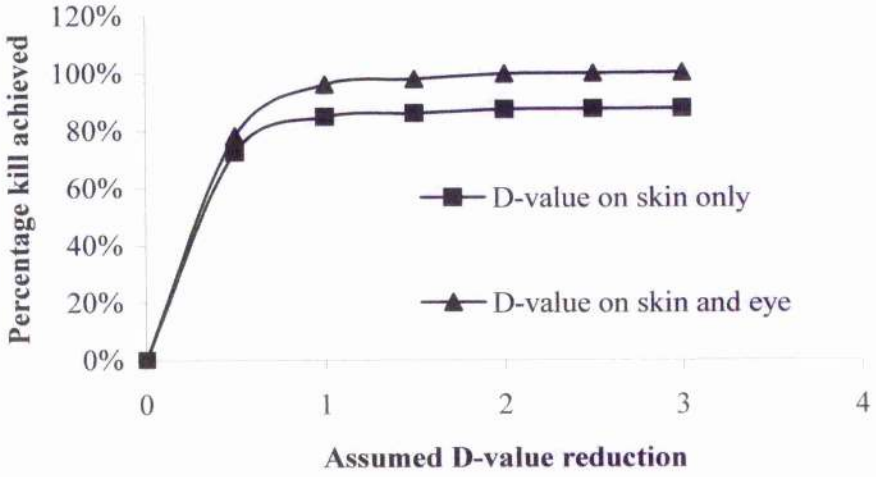


Figure 3.16 Model of potato inactivation showing percentage of organisms killed after different assumed log reductions on skin and eyes (300g, 38.7 mm radius, 10 eyes, 1 g/eye)

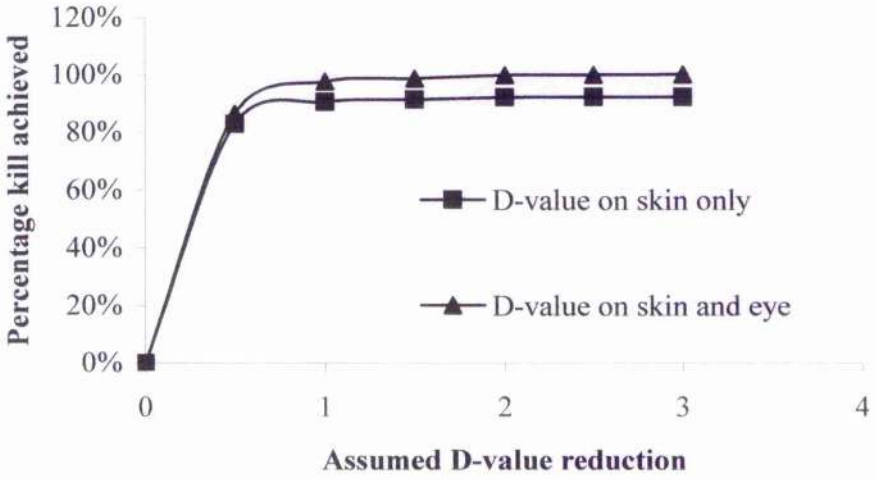


Figure 3.17 Model of potato inactivation showing percentage of organisms killed after different assumed log reductions on skin and eyes (500g, 45.9 mm radius, 10 eyes, 1 g/eye)

Figure 3.18 Top view of carrots and potato skin (10 -10 000 x magnification)

Carrot Skin	Potato Skin

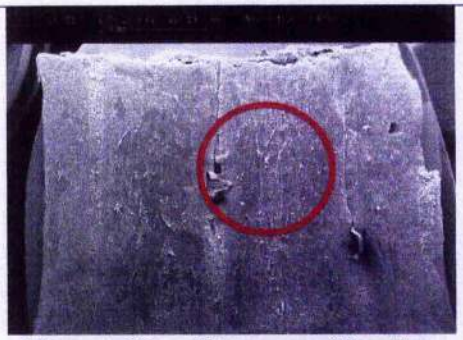
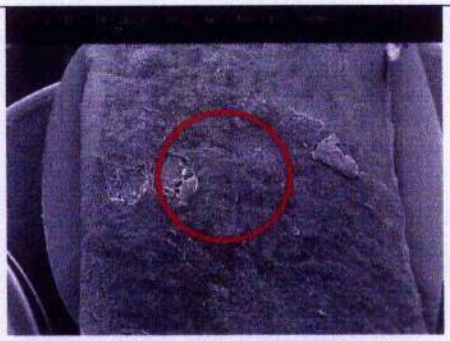

	

Figure C1 – 10 x magnification	Figure P1 - 10x magnification

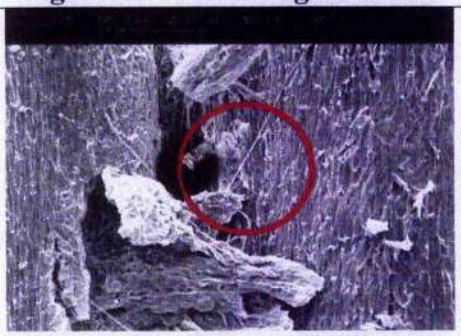
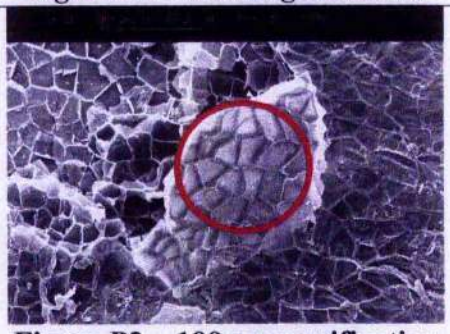
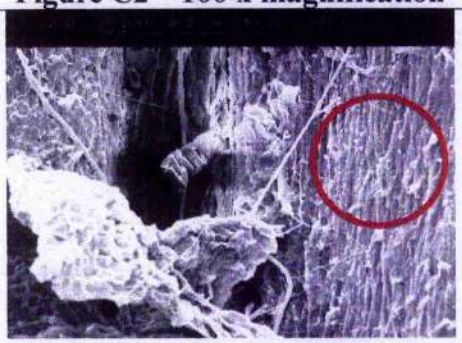
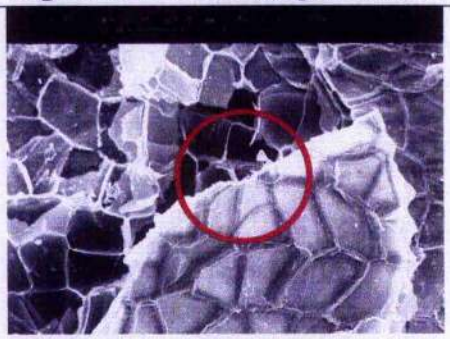

	

Figure C2 – 100 x magnification	Figure P2 – 100 x magnification
	
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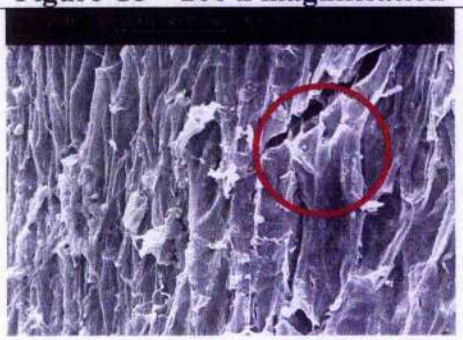
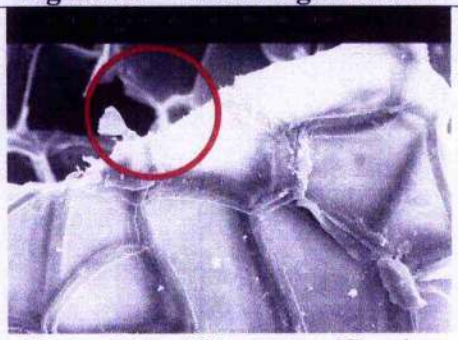
	

Figure C4 – 500 x magnification	Figure P4 – 500 x magnification

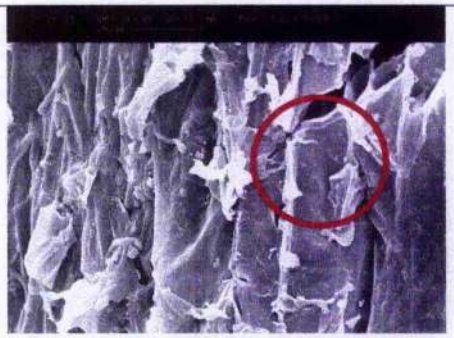
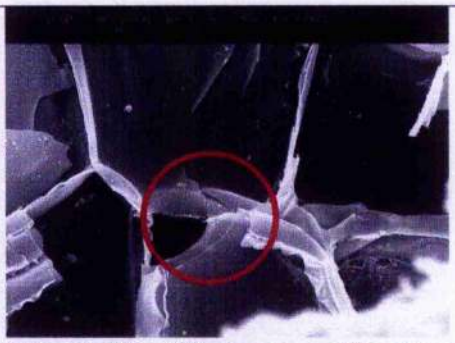


Figure C5 - 1000x magnification



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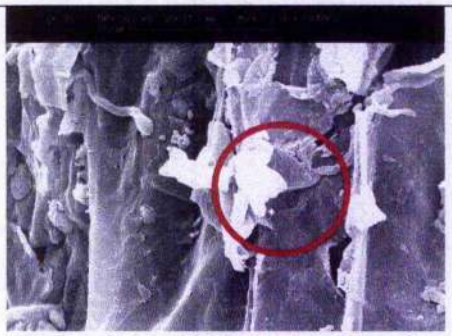
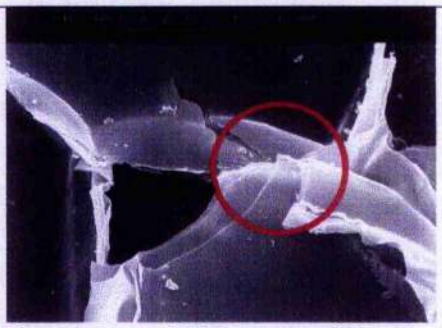


Figure C6 - 2000x magnification



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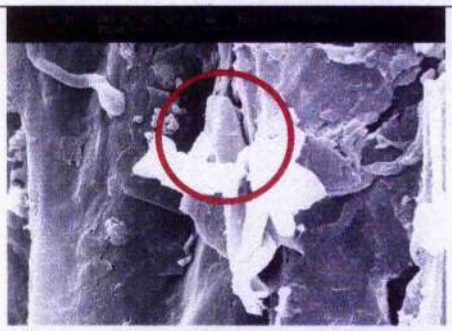
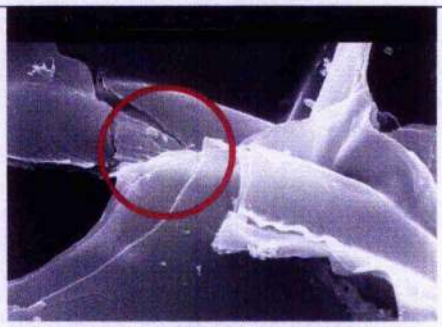


Figure C7 - 3000x magnification



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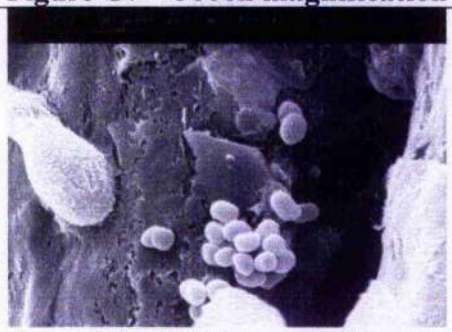


Figure C8 – 10 000x magnification

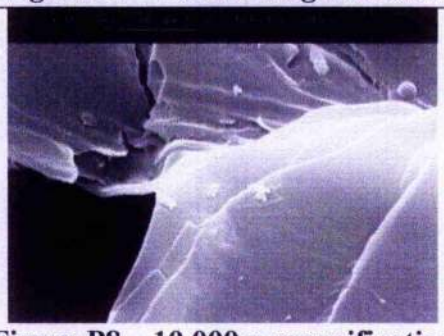


Figure P8 – 10 000 x magnification

Figure 3.19 Side view of carrot and potato skin at the boundary between the skin and flesh (10 – 10 000 x magnifications)

Carrots	Potatoes

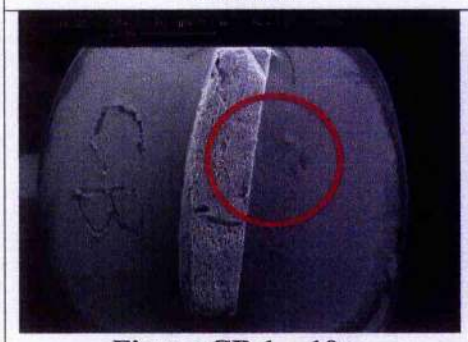
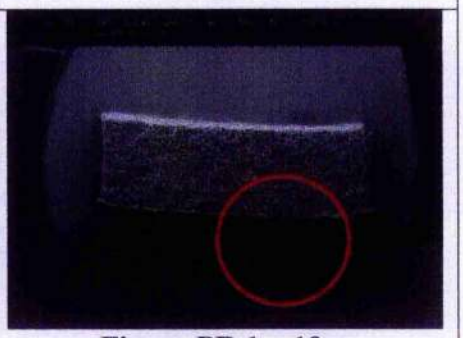

	

Figure CB 1 – 10 x magnification	**Figure PB 1 – 10 x magnification**

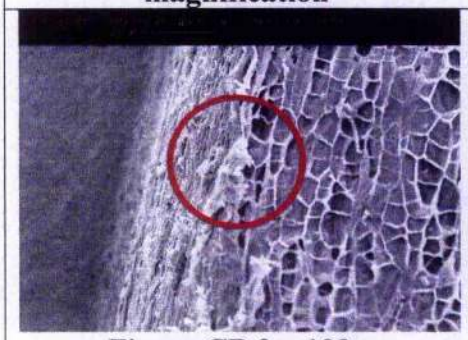
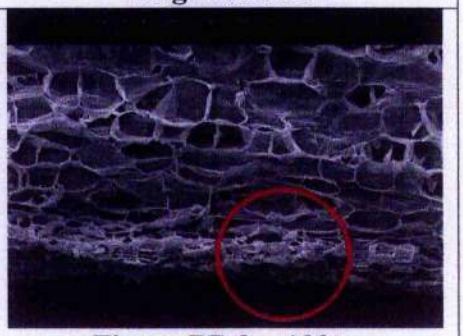

	

Figure CB 2 – 100x magnification	**Figure PB 2 – 100 x magnification**

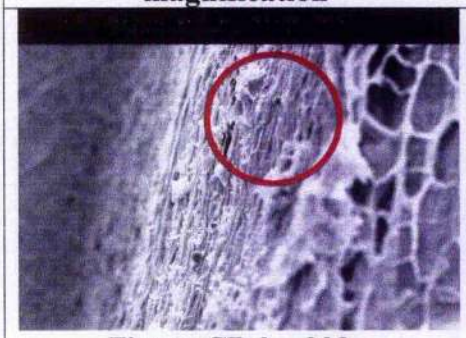
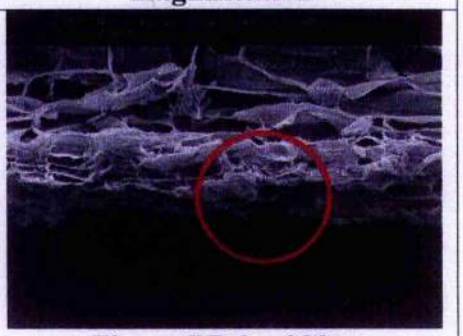

	

Figure CB 3 – 200x magnification	**Figure PB 3 – 200 x magnification**

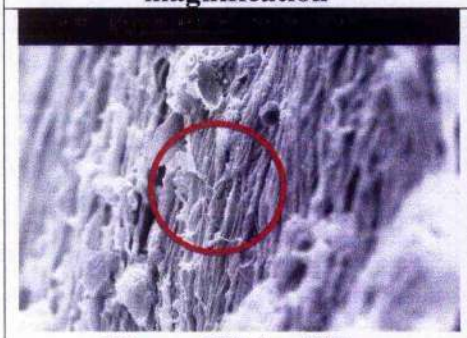
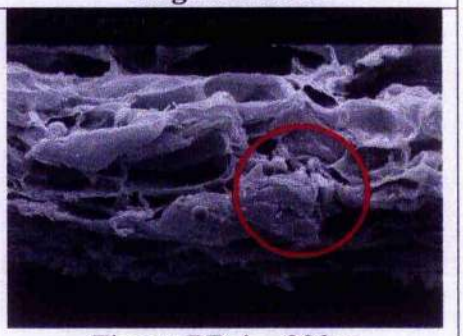

	

Figure CB 4 – 500 x magnification	**Figure PB 4 - 200 x magnification**

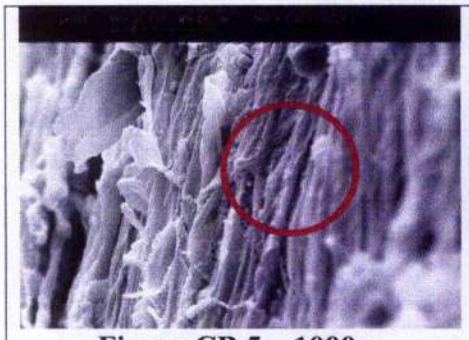


Figure CB 5 – 1000 x magnification

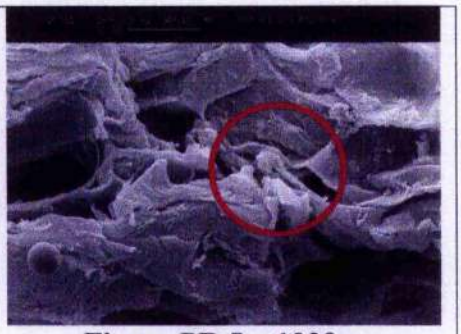


Figure PB 5 – 1000 x magnification

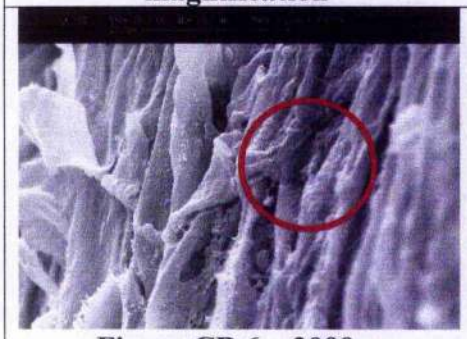


Figure CB 6 – 2000 x magnification

Figure PB 6 – 2000 x magnification

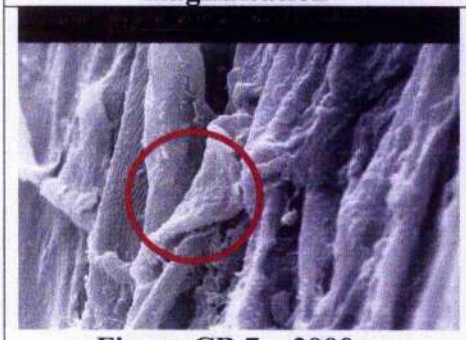


Figure CB 7 – 3000 x magnification

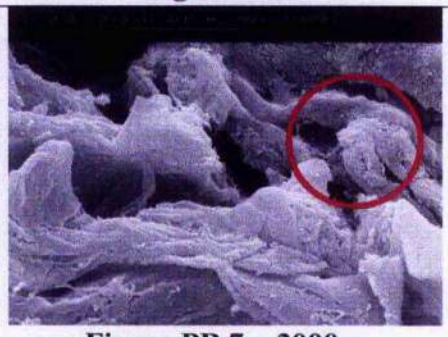


Figure PB 7 – 3000 x magnification

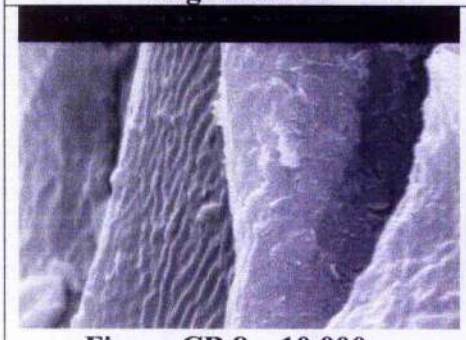


Figure CB 8 – 10 000 x magnification

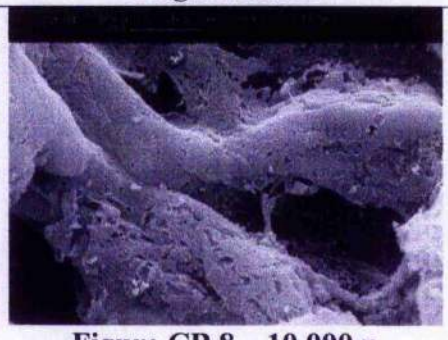


Figure CB 8 – 10 000 x magnification

CHAPTER 4 The impact of combination and individual treatment on shelf life

4.1 Introduction

Many perishable products have a very short shelf life. The shelf life of ready to eat vegetables, processed by manufacturers is usually 7 to 14 days depending on the type of vegetable [78]. In addition, the shelf life does not necessarily reflect the physical condition of produce but often depends on the consumers' perception. This may lead to a reduced marketable life which is much shorter than the produce shelf life determined through biological decay [132]. For these reasons, food retailers and suppliers of today's food industry are caught between meeting the ever increasing demands for ready-to-eat produce [122] and constantly trying to provide them fresher [133] and for longer.

In this emerging market, processes such as modified atmosphere packaging, edible films, coatings and minimal processing are being used to increase shelf life so businesses can stay competitive. Looking into minimal processing in more detail, Ahvenaninen [133] highlighted that minimally processed produce tends to deteriorate because of physiological ageing, biochemical changes and microbial spoilage, which results in degradation of colour, texture and flavour of the produce. This chapter describes the investigation into the physiological changes such as colour and the extension of shelf life of whole carrots, potatoes and tomatoes after treatment with UV, laser, microwave and H_2O_2 treatments, individually and in combination treatments Chapter 3 considered the effect of treating sections of carrots and potatoes. In all cases control sample was not treated but were kept in identical conditions to the treated samples.

Carrots contain the highest amount of β-carotene of common fruits and vegetables and potatoes are a good source of vitamin C. It was reported by Stephane [134] that as much as 25% of β-carotene acting as a pro-vitamin A, or anti-cancer compound, was lost during processing and storage [134]. By measuring β-carotene before and after minimal processing of carrots, the degree of any adverse effects can be determined. The vitamin C content of the potatoes was measured to determine any possible degradation in their nutrient provision.

4.2 Protocols for shelf life experiments

4.2.1 Individual treatment with ultraviolet radiation

There were 2 UV systems developed and used. The single unit UV system (**Figure 2.24**) and the optimized UV treatment system (**Figure 2.20**). The single unit UV system utilised two variations, the 120 W (3 x 40 W) and 90 W (3 x 30 W) systems. These units could be mounted longitudinally or transversely over the conveyor during treatment. In the optimized UV system, a constant UV source at 16 x 15 W irradiated an area of approximately 0.48 m^2. The produce was passed through the system at a speed of approximately 0.05 ms^{-1}. The vegetables to be treated were fed under the lamps via the roller table. At all times, handling of the produce was carried out whilst wearing sterile gloves, and the produce was treated whole.

Effect of irradiation from UV lamps arranged longitudinally on the shelf life of carrots and potatoes and subsequently placed into sealed bags

A number of treatments were used in an attempt to identify the ideal process to extend the shelf life of carrots and potatoes. This included the use of the 120 W (3x40W) UV system, an industrial prototype UV system comprising 3 tubes of UV lamps, the effect of placing the produce into sealed or open bags after treatment, effect of the length of the UV lamp and geometry and exposure on the shelf life were determined. Additionally, the effect of laser, microwave, chemical, and chemical and UV combined treatments was investigated and the effect of these treatments on the vitamin C content in potato and ß-carotene in carrots.

Two variables were considered in this investigation; namely, the rate that the produce was fed passed the treatments (translation velocity) on the roller table and storage condition. The irradiance of the UV was constant and there were eight different treatments given per produce.

All food produce was handled with sterile latex gloves to prevent further contamination. Each treatment was done in triplicate with three samples of vegetable and these were left in the respective storage conditions for 3 weeks or until visible differences or signs of decay were evident. After treatment, the produce was stored in autoclavable plastic bags, sealed and placed immediately in ambient or refrigerated conditions.

Treatments 1 and 2

These treatments acted as control, with no exposure from any treatment being given to the samples. The produce was placed into the autoclavable disposal bags.

Treatments 3 and 4

The roller table was set to rotate at their fast speed at 0.21 ms^{-1}, denoted as UV (fast). The produce was moved along the conveyor belt and exposed to UV irradiation.

Treatments 5 and 6

The roller table was set to rotate at their slowest speed at 0.05ms^{-1}, denoted as UV (slow). The produce was moved along the conveyor belt and exposed to UV irradiation.

Treatments 7 and 8

These were done on the stationary roller table to assess the effects on produce exposured to a prolonged period of UV radiation, i.e. 10 min.

Effect of irradiation from UV lamps arranged transversely on the shelf life of carrots and potatoes and subsequently placed into open bags

The rate of feed of produce along the roller table was varied between fast and slow. The exposure intensity of the continuous ultraviolet irradiation was kept constant. Two UV systems were used: 3 X 40 W ultraviolet germicidal lamp (operating at 254 nm) or 3 X 30 W ultraviolet germicidal lamp (operating at 254 nm). **Table 4.1** shows the treatment matrix.

Table 4.1 Treatment matrix

No.	Treatment	Conveyor speed	Storage condition
1	Nil (Control)	-	Ambient
2	40 + 30 W	Slow	"
3	40 + 30 W	Fast	"
4	40 W	Slow	"
5	40 W	Fast	"
6	30 W	Slow	"
7	30 W	Fast	"

All food produce was handled with sterile latex gloves to prevent post-treatment

contamination. Each set of treatments contained three samples of produce for statistical reliability and each series was left in ambient storage conditions at 18 °C ± 2 °C for 3 weeks or until visible comparison was evident.

Treatment 1

These were controls. The food produce was placed into the autoclavable bags at the start of the experiment.

Treatments 3, 5 and 7

The roller table was set to rotate at 0.21 ms^{-1}, denoted as UV (fast). The produce translated along the roller table and was exposed to UV irradiation before being packed into open bags and stored as stated.

Treatments 2, 4 and 6

The roller table was set to rotate at 0.05ms^{-1}, denoted as UV (slow). The produce translated along the roller table was exposed to UV irradiation before being packed into bags.

Effect of irradiation with UV lamps arranged transversely on the shelf life of carrots and potatoes and subsequently placed into sealed bags

The exact procedure as was described above was used, with the exception that the vegetables were placed into sterile plastic bags that were sealed immediately after the different treatments.

Effect of the length of the UV lamps on the shelf life of carrots placed into sealed bags

In this experiment the potatoes and carrots were exposed to continuous UV radiation with the lamps placed longitudinally with respect to the movement of the carrots. The aim was to test the effect of different energy densities applied on the shelf life of the vegetables. The rate of feed of the produce along the roller table and the storage conditions were fixed at 0.21 cms^{-1} and ambient respectively. The energy density applied to the produce from the CW UV lamps was varied by covering different lengths of the lamp housing; such that full, ¾, ½ and ¼ lengths of the lamp were used. In this experiment, only 3 x 40W CW UV lamps were used. The treatment matrix can be seen in **Table 4.2** below.

Table 4.2 Treatment protocol

No.	Treatment	Roller table speed	Storage condition
1	Nil (Control)	-	Ambient
2	40 W; full length	Fast	"
3	40 W; ¾ length	"	"
4	40 W; ½ length	"	"
5	40 W; ¼ length	"	"

All tests were done in triplicate and the produce was handled as previously described above.

Detailed investigation into the effect of UV treatment on the shelf life of carrots

The experiment investigating the effect of the UV lamps on the shelf life of carrots was repeated to provide greater evidence for the positive effect of the UV. Class I and class II carrots were used. General Class I carrots are larger, more regular in shape and cleaner, where as Class II carrots are usually smaller and not so clean. The UV lamp system comprised 3 x 40 W germicidal lamp, operating at 254 nm. The lamps were positioned above the roller table, as previously described.

The size of each carrot was measured to evaluate any physical differences in their size, which may have contributed to variations in observation after treatment. For example, the larger the diameter of the carrot then the lower the revolutions per minute. Three measurement were taken, these were the overall length, the largest diameter of the carrot recorded at the thickest end of the carrot, and the minor diameter, recorded 1 cm away from its sharpest end.

These carrots were treated with 2 different UV exposures by varying the speed of the roller table from its maximum to minimum speed. In total, 3 sets of parameters were used and this was repeated in triplicate and the produce observed over a period of 28 days. Although this time was longer than required from the experiment it was anticipated that useful information might come from such long observation of the rotting process. Of the three parameters, one set was the control, where no treatment was given. These carrots were allowed to roll along the conveyor at the minimum speed without any UV exposure,

before they were transferred into the autoclave bags.

For the second set, the conveyor was adjusted to its maximum speed 0.21 ms^{-1}. The lamp was turned on and warmed up until its output was stable. The lamp length was 120 cm and consequently the carrots were exposed to approximately 5.7 sec of UV irradiation at 120 W. With the third set of carrots, the table was turned to its lowest velocity of 0.05 ms^{-1} and the UV lamp was turned on. The carrots were exposed to the radiation for approximately 24 sec. The lamps were turned on for at least 15 min prior to the experiment to allow the lamp to warm up and stabilise. A warm-up period was also applied in between experiments to sterilise the roller table and to maintain UV stability.

All carrots were handled with sterile latex gloves to prevent cross contamination. In both experiments involving UV radiation, the vegetables were fed onto the conveyor at a distance shielded away from the hazardous light source and allowed to translate along the roller table into and out of the treatment zone. This simulated a real processing system. They were collected at the other end of the roller table when they dropped from the edge. To reduce the statistical variation, 3 carrots of similar weight and size were randomly picked from the same batch of carrots for all of the experiments.

4.2.2 Individual treatment with microwave

The power level of the microwave system was set to 800 W. Both carrots and potatoes were treated with 5 sec exposures before they were sent to Reading University for ß-carotene and vitamin C quantity for analysis.

4.2.3 Individual treatment with laser

The carrot was attached to a motor via a skewer, placed under the laser beam such that it was evenly treated over its entire surface. The speed of the motor was calibrated to 4 sec per revolution, which was the same as the roller table. This allowed greater control over the position of the carrot with respect to the beam. The laser power was set at 95% with a mark to space ratio of 50 percent and a frequency of 50 Hz (20 ms period), the carrot was irradiated 4 sec while the vegetable was rotating.

After treatment the produce was removed and packed into sterile autoclave bags with a pair of sterile gloves. The carrot was then dispatched to Reading University for ß -

carotene and vitamin C quantity analysis.

4.2.4 Individual treatment with H_2O_2

H_2O_2 was poured into a chemically inert plastic container, sufficiently deep enough to accommodate 3 samples at the same time without having to stack them. The carrots were immersed into the bath; allowing H_2O_2 to act for the required treatment time, while the container swayed from side to side at about 60 cycles per min. With a pair of sterile tongs, the treated carrots were removed and stored at ambient temperature in an open sterile autoclaved bag. Observations were conducted periodically and the results recorded.

4.2.5 Shelf life of carrots and potatoes after being treated with either the optimized UV system, H_2O_2, laser or microwave system in a simulated industrial processing condition

The same protocol was as used for individual treatment with laser and the optimized UV system (**Figure 2.20**). However, instead of using the skewer to hold the vegetable, they were fed through the system by the roller table. The treatments were carried out at 2 different feed-rates of the conveyor. At the fast speed setting, the feed rate was at 0.22 ms^{-1} and at the slow setting the feed-rate was 0.05ms^{-1}. In each set of treatment experiments, 3 samples (i.e. 3 carrots, 3 potatoes and 3 tomatoes) were placed aseptically onto one end of the conveyor and fed into the treatment area. After passing through the treatment zone, the vegetables dropped into an open sterile autoclave bags at the other end of the conveyor and the sample were left at ambient temperature for observation at day 0, 2, 5, 10 and 18. The presence of condensation on the bags was checked, as was the state of decay, change in colour of the produce, number of shoots and shoot growth. The state of decay was determined by taking the ratio of the surface area of the decayed part of the produce to its total surface area. A rule with a millimetre accuracy as used.

Since the integrated H_2O_2 spray system was not completed, the shelf life experiment of all vegetables were treated with the dip protocol described previously (**Section 4.2.4**) and left at ambient temperature for observation. The presence of condensation on the bags was checked, as was the state of decay, change in colour of the produce, number of shoots and shoot growth after 10 and 18 days. For these experiments, Class I and II carrots were used and small organic potatoes and Safeway's family pack of tomatoes.

4.2.6 Shelf life after UV and H_2O_2 combination treatment

The combined treatment of UV and H_2O_2 was done sequentially, first with 20 sec of H_2O_2 followed by UV treatment. The treatment parameters were kept the same as that used previously for the UV and H_2O_2 stand-alone treatments (**Section 4.2.4**). H_2O_2 treatment on the vegetable was carried out by dipping them in the chemical bath until the entire vegetable was submerged. No drying was performed after H_2O_2 treatment or handling; during the transfer from one treatment to another the transfer time was kept to a minimum.

4.2.7 Protocol for determination of β-carotene in carrots

This was done by Reading University for determination of β-carotene in carrots. The carrots were finely grated then approximately 20g saponified with 50% potassium hydroxide. The unsaponifiable material was extracted with a 50/50 mixture of diethyl ether and petroleum ether. The resulting extract was evaporated to dryness on a rotary evaporator and the residue redissolved in 10ml acetone, containing 0.1% butylated hydroxytoluene. Quantification of β-carotene was by RP-HPLC with UV/VIS detection (λ=440nm). The purity of the β-carotene standard was determined spectrophotometrically.

The method is based on UKAS accredited method for the determination of vitamin A as retinol in milk based products, fats and oils (BR13), [135].

4.2.8 Protocol for determination of vitamin C in potatoes

This was done by Reading University for determination of the potato vitamin C content. The potatoes were diced. Vitamin C was extracted by blending approximately 10g of diced potato with 100ml water containing dithiothreitol. The dithiothreitol was added to prevent oxidation of vitamin C during extraction. The extract was centrifuged and the supernatent filtered. Quantification of vitamin C was as ascorbic acid by RP-HPLC with UV detection (λ= 244nm).

This method is based on UKAS accredited method for the determination of vitamin C in beverages (BR9), [136].

4.3 Results

4.3.1 Shelf life after treatment with 3 x 40 W and 3 x 30 W UV system

Effect of UV irradiation with lamp exposure on the shelf life of carrots and potatoes subsequently placed into sealed bags

Carrots

Carrots treated under treatments 1, 3, 5 and 7 (see **section 4.2.1**) when kept in the refrigerator did not show any loss of quality after 10 days, except that the bags contained some condensation.

The effect of ambient storage is shown in **Table 4.3a** and **4.3b**

Table 4.3a The effect of UV treatment on carrots after 5 and 8 days storage

Treatment No.	Treatment	Appearance of carrots after 5 days	Appearance of carrots after 8 days
2	Control	The carrots showed some rot at the tips	Definite evidence of rot
4	UV, fast	The carrots remained firm	The carrots remained wholesome
6	UV, slow	The carrots remained wholesome	The carrots remained wholesome
8	UV, 10 min	No decay	The carrots were wet in the bag but not rotten

Figures 4.1A and **B** show the control and UV slow exposed carrots after 20 days. The decayed state of the control was visible after 5 days, whereas with the treated produce there was no decay up until day 20.

Potatoes

After treatments 1, 3, 5 or 7 (kept at 4°C) the potatoes did not show any degeneration after

day 10 when the monitoring process was stopped. The potatoes stored at room temperature showed no visible changes other than some sprouting, **Figures 4.1C** and **D** show the control and treated potatoes. There was little difference in the samples, but the control did appear to darken on storage.

Table 4.3b The effect of UV treatment on potatoes after 10 days storage

Treatment No.	Treatment	Appearance of potatoes after 10 days (All 3 potato samples)	
2	Control	No visible decay	4 sprouts observed
4	UV, fast	"	4 sprouts observed
6	UV, slow	"	2 sprouts observed
8	UV, 10mins	"	2 sprouts observed

Effect of irradiation with UV lamps arranged transversely on the shelf life of carrots and potatoes subsequently placed into open bags

In this experiment the UV lamps were arranged transversely while, the vegetables to be treated were fed under the lamps via the roller table. For each of the experiments, the intensity of the UV lamps was recorded; this is shown below in the **Table 4.4**. The intensity was recorded during the middle of the experiment, which was approximately 35 min from the start. From the results of the previous work, it was known that the UV output was highest after about 6 min, and then declined to steady plateau after about 10 min.

Table 4.4 UV intensity measurement results

DATE	UV lamps	Irradiance $x10^2$ (μWcm^{-2})
31/7	3 X 30 W	13.02
	3 X 40 W	9.8
9/8	3 X 30 W	15.5
	3 X 40 W	11.5

Carrots

The effect of the UV irradiance with the UV lamps arranged transversely can be seen in **Table 4.5**.

Table 4.5 The effect of UV treatment on carrots after 9 days storage in open bag

No.	Treatment	Observation	
		Decay	Appearance
1	Nil (control)	None	dry
2	40 + 30 W (slow)	Slight	"
3	40 + 30 W (fast)	"	"
4	40 W (slow)	"	"
5	40 W (fast)	"	"
6	30 W (slow)	"	"
7	30 W (fast)	None	"

It can be seen that for both the control and treated samples there was none or little decay after 9 days

Potatoes

Similarly, potatoes were treated and stored for 9 days and the appearance of each was noted; the potatoes showed no decay and were still dry. There was evidence of some sprouts on each of the potatoes but as these were also seen on the controls it was likely they were not the result of the processing, **Table 4.6.**

Table 4.6 Effect of UV irradiation from lamp arranged transversely on the sprouting of potatoes in open bag

No	Treatment	Sprouts observed No on each potato	Total no of sprouts
1	Nil (Control)	3,4,6	13
2	40 + 30 W (slow)	4,6,4	14
3	40 + 30 W (fast)	2,4,4	10
4	40 W (slow)	4,1,3	8
5	40 W (fast)	3,4,4	11
6	30 W (slow)	4,7,6	17
7	30 W (fast)	4,3,2	9

Effect of irradiation with UV lamps arranged transversely on the shelf life of carrots and potatoes, subsequently placed into sealed bags

Similar to previous experiments, the intensity of the UV lamps was recorded in the middle of the experiment, which was approximately 35 min from the start of the experiment. The results are tabulated in **Table 4.6** below.

Table 4.6 UV intensity measurements

Day	UV lamps	Intensity (10^2 μWcm^{-2})
1	3 X 30 W	15.5
	3 X 40 W	11.5
6	3 X 30 W	14.4
	3 X 40 W	12.3
8	3 X 30 W	14.7
	3 X 40 W	11.2

Carrots

The carrots were passed through the irradiation from both the 3 x 40 W and 3 x 30 W lamps arranged transversely, at fast and slow speeds before they were placed into sealed bags for storage and observation. Separate treatments using one set of lamps on carrots were done at both fast and slow roller table speeds with either the 3 x 30 W UV lamps or 3 x 40 W lamp system.

Table 4.7 Effect of UV irradiation from UV lamps arranged transversely on the sprouting of potatoes in sealed bag after 6 days storage

No.	Treatment	Appearance of 3 carrots
1	Nil (Control)	Semi-soggy with 2 out of 3 rotting
2	40 + 30 W (slow)	Dry and fresh
3	40 + 30 W (fast)	Dry and fresh
4	40 W (slow)	Small black dots (fungus) on 1 carrot out of 3
5	40 W (fast)	Dry and fresh
6	30 W (slow)	Dry and fresh
7	30 W (fast)	Semi soggy with 1 out of 3 rotting at tip

Figure 4.2A shows the control after 6 days, and mould is evident which can be seen on the middle carrot. **Figure 4.2B** shows the carrots treated with UV at the slow roller table velocity (**Figure 4.2B**). There was has no decay or significant condensation. The produce was stored in ambient conditions. **Figure 4.2C** shows the control after 8 days, and the first sign of decay on the treated samples was evident, **Figure 4.2D**. Clearly, the controls had decomposed significantly more than the treated samples.

Potatoes

Table 4.8 below, shows the results from observing the appearance of the potatoes after 6 days.

Table 4.8 Effect of UV irradiation from lamp arranged transversely on the sprouting of potatoes in sealed bag after 6 days

No	Treatment	Sprouts observed, No. on each potato	Total no of sprouts
1	Nil (Control)	3,2,4	9
2	40 + 30 W (slow)	5,3,4	12
3	40 + 30 W (fast)	2,3,2	7
4	40 W (slow)	4,4,5	13
5	40 W (fast)	2,2,4	8
6	30 W (slow)	2,3,2	7
7	30 W (fast)	4,4,5	13

Figures 4.2E and F and show the control and UV exposed potatoes after 8 days. There was no visible difference between the samples.

Effect of the length of the UV lamps on the shelf life of carrots placed into sealed bags

The intensity was recorded in the middle of the experiment, which was approximately 35 min from its start, see **Table 4.9**.

Table 4.9 UV irradiance measurements

Number	UV lamps	Irradiance (10^2 μWcm^{-2})
1	3 X 30 W	14.9
	3 X 40 W	10.2
5	3 X 30 W	15.3
	3 X 40 W	11.7
7	3 X 30 W	14.5
	3 X 40 W	12.0

Carrots

The effect of UV irradiation on carrots is shown after 5 days in **Table 4.10**, and after 7 days in **Table 4.11**.

Table 4.10 Effect of UV irradiation from UV lamp arranged transversely on the sprouting of carrots in sealed bags after 5 days storage

No	Treatment	Observations		
		Moisture	State of Decay	Appearance
1	nil (Control)	slight	Slight	black moulds seen
2	40 W; full length	none	None	dry and fresh
3	40 W; ¾ length	none	minimal	fry; only 2 small
4	40 W; ½ length	slight	slight	black moulds on all
5	40 W; ¼ length	slight	slight	black moulds on all

Figures 4.3A to **E** show the control and after treatment effect of the UV exposure on the carrots after 5 days in ambient conditions for the different length of lamp treatment.

Figure 4.3A shows the control carrots, **Figure 4.3B** shows the carrots after ¼ length UV lamp exposure , **Figure 4.3C** shows the carrots after ½ length UV lamp exposure, **Figure 4.3D** shows the carrots after ¾ length UV lamp exposure and **Figure 4.3E** shows carrot after full length exposure. Quite clearly all samples, except the full-length treatment, showed signs of decay. The produce was stored in ambient conditions.

Table 4.11 Effect of UV irradiation from UV lamp arranged transversely on the sprouting of carrots in sealed bags after 7 days storage

No.	Treatment	Observation		
		Moisture	State of Decay	Appearance
1	Nil (Control)	Wet	advanced	all carrots were rotted
2	40 W; full length	None	minimal	dry with only 1 out of 3 carrots slightly mouldy
3	40 W; ¾ length	None	minimal	dry with only 1 out of 3 carrots slightly mouldy
4	40 W; ½ length	Wet	advanced	All carrots rotted
5	40 W; ¼ length	moist	advanced	All carrots rotted

Table 4.12 shows the results of carrots sprouting due to UV irradiation for different exposures by varying the lamp length. Observations on sprouting were after storage for 5 and 7 days.

Table 4.12 Effect of UV irradiation from lamps arranged transversely on the sprouting of carrots stored in sealed bags for 5 and 7 days

No	Treatment	No of sprouts on each of 3 carrots	
		5 days	7 days
1	Nil (Control)	4,4,3	4,4,6
2	40 W; full length	2,3,4	4,4,5
3	40 W; ¾ length	4,3,4	4,4,3
4	40 W; ½ length	2,3,4	3,4,5
5	40 W; ¼ length	3,3,3	3,4,4

Note that the potatoes were still dry and showed no visible signs of decay.

Effect of UV treatment on the shelf life of tomatoes

Table 4.13 shows the effect of the UV treatment for the fast and slow velocity of the roller table, on the shelf life of tomatoes.

Table 4.13 Progressive state of the tomatoes after UV treatment at fast and slow translation speed.

	Control	UV treatment at fast speed	UV treated at slow speed
Day 4 :			
Mould/Rotting Condition (%)	10%	5%	5%
	Hair like mould found growing only on the stem.	Similarly, mould was found but was sparse compared to those found on control. It was also much shorter.	Similarly, mould was found but was sparse compared to those found on control. It was also much shorter.
Changes in skin colour	Nil	Nil	Nil
Texture and rigidity	Shiny and rigid	Shiny and rigid	Shiny and rigid
Other remarks	Unlike carrots there was little or no condensate found in the autoclave bags after 4 days.		
Day 8 :			
Mould/rotting Condition (%)	20%	10%	8%
	Mouldy area grew denser and bigger.	Mould growth found on the fruit.	Growth appeared to reduce.
Changes in skin colour	Nil	Nil	Nil
Texture and rigidity	Lost shininess and rigidity on some areas.	Lost shininess,. slight rigidity was lost compared to control.	Lost shininess and rigidity, slightly similar to those in UV fast treatment.
Other remarks			Only the stem was mouldy the fruit was left untouched
Day 28 :			
Mould/Rotting Condition (%)	80%	70	30%
Changes in skin colour	Turned darker	Turned darker	Not much difference compared to the skin colour on the 8th day
Texture and rigidity	Dull and soggy	Dull and soft	Dull, lost stiffness further but not as bad as control and fast UV treated.
Other Remarks	The fruit had ruptured and totally lost its shape.	Although still intact there was tomato juice in the bag.	Remained intact and dry.

Figure 4.4 shows a plot of the percentage decay of the tomato as a function of the number of days from treatment. It is seen that the UV-slow treated samples had a significantly reduced rate of decay. Further work would need to be done to identify the increase in shelf life of the treated samples. **Figure 4.5** shows the tomatoes after the treatment process.

All the previous results for carrots, potatoes and tomatoes treatments were with 3 x 120 W UV system and the optimized UV lamp systems. Based on these results a optimised UV treatment system was fabricated (**See** Appendix **1**); this was essentially an industrial prototype.

4.3.2 Shelf life of carrots after UV treatment with the optimized UV system in sealed bags

The optimized UV treatment system was designed and built based on industrial specification and requirements. The results obtained using this system were similar to those from the earlier experiments with the simple UV lamp system with the 3 x 40 W, germicidal lamp. Mould and bacterial growth was first sighted on the surface of untreated carrots kept at room temperature. At the minimum UV exposure, with the roller table moving the produce at $0.21 ms^{-1}$, there was retardation of the bacterial growth, see **Figure 4.6.**

Table 4.14 shows the shelf life results after the UV treatment on carrots. It is seen that there was no change in the colour of the produce and the treated samples retained their firmness during the 18 day experiment, even when they were covered in mould after 18 days. Condensate was present on the inside of the bag after all treatments, except on day 0. After 5 days, the control had decayed by about 10%, whereas the UV fast and slow treatment had not decayed at all, **Figure 4.6**. The rate of decay, once decay had started on the UV treated samples, was equivalent to that observed for the control. This is evident from the graph in **Figure 4.6**, where the gradients are about the same after 5 days.

Figure 4.7 shows the carrots for each stage of the experiment. Interestingly, the number of shoots appeared to be less on the UV treated samples.

Table 4.14 Shelf life and sprouting results of carrots after UV treatment

Treatment Parameters	Sample	No of days	Observations				
			Change in colour due to UV radiation	Presence of condensate	State of decay %	Number of shoots	Shoot growth (mm)
Control	Carrots	0	--	No	0%	0	0
	Carrots	2	--	Yes	0%	8	1-2
	Carrots	5	--	Yes	10%	25	1-22
	Carrots	10	--	Yes	45%	30	5-70
	Carrots	18	--	Yes	100%	Sprouting stops	
UV Fast 16 x 15 Watts	Carrots	0	No	No	0%	0	0
	Carrots	2	No	Yes	0%	0	0
	Carrots	5	No	Yes	0%	9	6-26
	Carrots	10	No	Yes	30%	16	3-50
	Carrots	18	No	Yes	80%	Sprouting stops	
UV Slow 16 x 15 Watts	Carrots	0	No	No	0%	0	0
	Carrots	2	No	Yes	0%	0	0
	Carrots	5	No	Yes	0%	8	5-20
	Carrots	10	No	Yes	35%	18	3-40
	Carrots	18	No	Yes	70%	Sprouting stops	

Table 4.15 shows the shelf life and sprouting results for the potatoes after UV treatment. The potatoes can be seen in **Figure 4.8**. There was no significant visual differences in any of the potato samples before or after treatment over a period of 18 days. A few short sprouts were observed on the UV fast treated sample.

Table 4.15 Shelf life and sprouting results of potatoes after UV treatment

Treatment Parameters	Produces	No of Days	Observations				
			Change in colour due to UV radiation	Present of Condensate	State of Decay %	Number of Shoots	Shoot Growth (mm)
Control	Potatoes	0	--	No	0%	0	0
	Potatoes	2	--	No	0%	0	0
	Potatoes	5	--	No	0%	0	0
	Potatoes	10	--	No	0%	0	0
	Potatoes	18	--	No	0%	0	0
UV Fast 16 x 15 Watts	Potatoes	0	No	No	0%	0	0
	Potatoes	2	No	No	0%	0	0
	Potatoes	5	No	No	0%	0	0
	Potatoes	10	No	No	0%	0	0
	Potatoes	18	No	No	0%	4	1-2
UV Slow 16 x 15 Watts	Potatoes	0	No	No	0%	0	0
	Potatoes	2	No	No	0%	0	0
	Potatoes	5	No	No	0%	0	0
	Potatoes	10	No	No	0%	0	0
	Potatoes	18	No	No	0%	0	0

4.3.3 Shelf life after H_2O_2 treatment

Table 4.16 shows the results from treating the carrots with the H_2O_2. The shelf life of the carrots was almost 10 days, after which there was only a 5% covering in mould. There was no change in the colour or appearance of samples after the treatment. **Figure 4.9** shows the percentage decayed plotted as a function of the number of days from treatment. It should be noted that the rate of decay for the control and treated samples was practically the same after 10 days.

Figure 4.10 shows the H_2O_2-treated carrots and **Figure 4.11** shows the H_2O_2-treated potatoes.

Table 4.16 Shelf life and sprouting results for carrots after H_2O_2 treatment

Treatment Parameters	Produces	No of Days	Observations				
			Change in colour due to UV radiation	Presence of Condensate	State of Decay %	Number of Shoots	Shoot Growth (mm)
Control	Carrots	0	–	No	0%	0	0
	Carrots	2	–	Yes	0%	8	1-2
	Carrots	5	–	Yes	10%	25	1-22
	Carrots	10	–	Yes	45%	30	5-70
	Carrots	18	–	Yes	100%	Sprouting Stops	
20 Sec H202 Treatment	Carrots	0	No	No	0%	0	0
	Carrots	2	No	Yes	0%	0	0
	Carrots	5	No	Yes	0%	2	2-4
	Carrots	10	No	Yes	5%	6	2-25
	Carrots	18	No	Yes	50%	Sprouting Stops	

The effect of the chemical treatment on the potato samples can be seen in **Table 4.17**. There was no adverse effect on the colour or appearance of the potato after treatment. After 18 days, 4 shoots were observed on the treated sample but none were seen on the control.

Table 4.17 H_2O_2 treatment of potato

Treatment Parameters	Produces	No of Days	Observations				
			Change in colour due to UV radiation	Present of Condensate	State of Decay %	Number of Shoots	Shoot Growth (mm)
Control	Potatoes	0	--	No	0%	0	0
	Potatoes	2	--	No	0%	0	0
	Potatoes	5	--	No	0%	0	0
	Potatoes	10	--	No	0%	0	0
	Potatoes	18	--	No	0%	0	0
20 Sec H202 Treatment	Potatoes	0	No	No	0%	0	0
	Potatoes	2	No	No	0%	0	0
	Potatoes	5	No	No	0%	0	0
	Potatoes	10	No	No	0%	0	0
	Potatoes	18	No	No	0%	4	1-5

4.3.4 Shelf life after laser or microwave treatments

Table 4.18 shows the shelf life results for carrots after they had been exposed to the laser and microwave treatment. The laser treatment caused a slight darkening of the carrot after storage for 5 days. This colour did not worsen after longer periods. There was no colour change evident from the microwave exposure. The shelf life results were excellent using the laser treatment. The bacterial growth was retarded for about 6 to 8 days without refrigeration. It was about 2 days longer than with the UV treatment alone. After this period, the laser treated sample was only 10% decayed compared to about 30% for the microwave treated and 45% for the control sample, see **Figure 4.12** where the percentage decayed is plotted as a function of the number of days from treatment. There was about 35% to 50% reduction of decay between the 10^{th} and 18^{th} day of the experiment. It is seen that the laser was more effective at preventing rotting than the microwave, but its major disadvantage was the discolouration it produced. **Figure 4.13** shows the samples after laser or microwave treatment.

Table 4.18 Shelf life and sprouting results for carrots after laser and microwave treatment

Treatment Parameters	Produces	No of Days	Observations				
			Change in colour due to UV radiation	Present of Condensate	State of Decay %	Number of Shoots	Shoot Growth (mm)
Control	Carrots	0	--	No	0%	0	0
	Carrots	2	--	Yes	0%	8	1-2
	Carrots	5	--	Yes	10%	25	1-22
	Carrots	10	--	Yes	45%	30	5-70
	Carrots	18		Yes	100%	Sprouting stops	
4 sec laser treatment at 1 KW	Carrots	0	No	No	0%	0	0
	Carrots	2	No	Yes	0%	0	0
	Carrots	5	Turns slightly darker	Yes	0%	4	1-2
	Carrots	10	Remains the same	Yes	10%	11	1-4
	Carrots	18	Remains the same	Yes	50%	Sprouting stops	
5 sec microwave treatment at 800 Watts	Carrots	0	No	No	0%	0	0
	Carrots	2	No	Yes	0%	0	0
	Carrots	5	No	Yes	0%	10	2-5
	Carrots	10	No	Yes	30%	18	4-5
	Carrots	18	No	Yes	80%	Sprouting stops	

Table 4.19 shows the results from treating potatoes with the laser and microwave system. The laser tended to turn the potato a dark brown colour (**Figure 4.14**). This was evident on day 0, but the colour change did not worsen after the 18th day of observation. The potato were stored in ambient condition. Interestingly, the potato appeared a light greenish colour after treatment with the microwave system. No condensate was evident within the bag for either the laser or microwave treated sample. Shoots were observed for the laser treated samples but not for microwave treated samples. No decay was evident for any of the treated samples.

Table 4.19 Shelf life and sprouting results for potatoes after laser or microwave treatment

Treatment Parameters	Produces	No of Days	Observations				
			Change in colour due to UV radiation	Present of Condensate	State of Decay %	Number of Shoots	Shoot Growth (mm)
Control	Potatoes	0	--	No	0%	0	0
	Potatoes	2	--	No	0%	0	0
	Potatoes	5	--	No	0%	0	0
	Potatoes	10	--	No	0%	0	0
	Potatoes	18	--	No	0%	0	0
4 sec laser treatment at 1 Kw	Potatoes	0	No	No	0%	0	0
	Potatoes	2	Turns dark, brownish in colour	No	0%	0	0
	Potatoes	5	Remains the same	No	0%	4	1-2
	Potatoes	10	Remains the same	No	0%	4	1-4
	Potatoes	18	Remains the same	No	0%	8	1-5
5 Sec microwave treatment at 800 Watts	Potatoes	0	No	No	0%	0	0
	Potatoes	2	No	No	0%	0	0
	Potatoes	5	Slightly green	No	0%	0	0
	Potatoes	10	Slightly green	No	0%	0	0
	Potatoes	18	Slightly green	No	0%	0	0

4.3.5 Shelf life after combination of UV and H_2O_2 treatment

The chemical and UV combination treatment produced excellent results. With the chemical and UV slow treatment the decay was only 5% after 10 days and this only doubled to 10% after 18 days. The results are tabulated in **Table 4.20**.

Table 4.20 Shelf life and sprouting results for carrots after H_2O_2 and UV treatment

Treatment Parameters	Produces	No of Days	Observations				
			Change in colour due to UV radiation	Present of Condensate	State of Decay %	Number of Shoots	Shoot Growth (mm)
Control	Carrots	0	--	No	0%	0	0
	Carrots	2	--	Yes	0%	8	1-2
	Carrots	5	--	Yes	10%	25	1-22
	Carrots	10	--	Yes	45%	30	5-70
	Carrots	18	--	Yes	100%	Sprouting stops	
20 sec H202 treatment and UV exposure at Fast conveyor speed	Carrots	0	No	No	0%	0	0
	Carrots	2	No	Yes	0%	0	0
	Carrots	5	No	Yes	0%	22	2-32
	Carrots	10	No	Yes	30%	35	6-50
	Carrots	18	No	Yes	80%	Sprouting stops	
20 sec H202 treatment and UV exposure at slow conveyor speed	Carrots	0	No	No	0%	0	0
	Carrots	2	No	Yes	0%	0	0
	Carrots	5	No	Yes	0%	18	1-16
	Carrots	10	No	Yes	5%	28	15-50
	Carrots	18	No	Yes	10%	Sprouting stops	

The results are plotted in **Figure 4.15** where the percentage decay is shown as a function of the number of days from treatment. It is interesting to observe the different gradients for the 3 different plots. As previously observed, the control had rotted 100% after 18 days. The chemical and UV fast treatment were rotting at the same rate as the control after 5 days, but the chemical and UV slow treatments rotted at a much lower rate. This gradient was the lowest that was observed in all of the treatments and was excellent for non-refrigerated conditions. **Figure 4.16** shows the treated and control carrots after different times of storage.

The effect of the combined treatment with the potatoes was similar to that previously observed, and can be seen in **Table 4.21** and the produce in **Figure 4.15**. No decay was observed.

Table 4.21 Shelf life and sprouting results for potatoes after H_2O_2 and UV treatment

Treatment Parameters	Produces	No of Days	Observations				
			Change in colour due to UV radiation	Present of Condensate	State of Decay %	Number of Shoots	Shoot Growth (mm)
Control	Potatoes	0	--	No	0%	0	0
	Potatoes	2	--	No	0%	0	0
	Potatoes	5	--	No	0%	0	0
	Potatoes	10		No	0%	0	0
	Potatoes	18	--	No	0%	0	0
20 Sec H2O2 Treatment and UV exposure at Fast conveyor speed	Potatoes	0	No	No	0%	0	0
	Potatoes	2	No	No	0%	0	0
	Potatoes	5	No	No	0%	0	0
	Potatoes	10	No	No	0%	6	1-2
	Potatoes	18	No	No	0%	10	1-6
20 Sec H2O2 Treatment and UV exposure at Slow conveyor speed	Potatoes	0	No	No	0%	0	0
	Potatoes	2	No	No	0%	0	0
	Potatoes	5	No	No	0%	0	0
	Potatoes	10	No	No	0%	0	0
	Potatoes	18	No	No	0%	0	0

4.3.6 Comparison of vitamin C in potatoes and *β* carotene in carrots before and after various minimal processing treatments

This investigation was carried out to determine if there was any adverse affect on the β-carotene level of the carrots due to the different treatments. The samples were sent to Reading university for β-carotene level determination. The vegetables were subjected to the same treatment and period of irradiation to that of the shelf life experiments described in **sections 4.2.5** and **4.2.6** . **Table 4.22** shows the β-carotene for the carrots that had undergone the different minimal processing treatments, for laser, UV, H_2O_2,UV and H_2O_2, microwave and the control samples.

Table 4.22 Summary of β-carotene (μg/100g) content for carrots

Treatment	β-carotene	Average
Control	2940	2936.7
Control	2670	
Control	3200	
Laser	4310	3918.3
Laser	3290	
Laser	4155	
UV	2815	3535.0
UV	3915	
UV	3875	
H_2O_2	4025	3435.0
H_2O_2	3300	
$H2O_2$	2980	
UV + H_2O_2	3810	3881.7
UV + H_2O_2	3820	
UV + H_2O_2	4015	
Microwave	4175	3598.3
Microwave	3210	
Microwave	3410	

All ß-carotene concentrations of the treated samples appeared to be slightly higher than the control samples that were not treated. This showed that there was no adverse effect that damaged the β-carotene after any of the treatments. Generally, the results showed higher β-

carotene levels. This does not suggest any of the treatment used were promoting the increase of β-carotene levels, it was more likely due to statistical variation in the β-carotene content in carrots samples.

The investigation of vitamin C content of potatoes was carried out after similar treatments as used for the shelf life experiments described in **section 4.2.5** and **4.2.6** . **Table 4.23** shows the vitamin C of the potatoes that were treated with the different minimal processing treatments and the controls.

Table 4.23 Summary of vitamin C (mg/100g) content of potatoes

	Potatoes	
Treatment	**Vitamin C**	**Average**
Control	14	14.3
Control	13	
Control	16	
Laser	11	15.0
Laser	15	
Laser	19	
UV	12	13.0
UV	14	
UV	13	
H_2O_2	14	16.0
H_2O_2	14	
$H2O_2$	20	
UV + H_2O_2	15	15.3
UV + H_2O_2	16	
UV + H_2O_2	15	
Microwave	15	12.7
Microwave	11	
Microwave	12	

The results showed that neither laser, UV, H_2O_2 or microwave treatment caused degradation of the vitamin C. On average, the measurements were very similar to the control sample which was not subjected to any form of treatment.

4.4 Discussion

4.4.1 UV treatments

Effect of UV arranged longitudinally exposure on the shelf life of carrots and potatoes placed into sealed bags

Through the various combinations of speed and UV exposure, it was seen that the treatment had a significant effect on the shelf life of the carrots. In general, the results were favourable and encouraged further investigations into this avenue of research. There was some indication that the rate of sprouting increased slightly after exposure to the UV light, but this was not investigated further.

Effect of UV lamps arranged transversely on the shelf life of carrots and potatoes placed into open bags

This experiment showed that the shelf life could be extended with all the treated vegetables. The producers participating in the FAIR CT 98-9522 [119] minimal processing research project considered that an extension of 1-2 days would be an advantage and in this experiment the shelf life was extended for much longer periods. Similar experiments were conducted with the carrots placed into sealed bags.

Effect of UV lamps arranged transversely on the shelf life of carrots and potatoes placed into sealed bags

Carrots

UV had an enhanced effect on extending the carrots's shelf life and after treatment it was beneficial to seal the bags to prevent aerial re-contamination. The rate at which the carrots showed deterioration after 8 days depended on the age of the carrots bought at the supermarket. Nevertheless, the controls acted as a very good comparison to justify the effects of UV treatment on the food produce.

In this experiment, the transverse position was not as effective as placing the CW UV lamps parallel to the conveyor since the energy density was much less (the ratio of the UV tubes dimension (width/length) was 90/1200; i.e. 7.5 % in this case).

Potatoes

The control potatoes did not rot as distinctively as the carrots. However, the experiment showed no visible adverse effects after exposure of the potatoes to UV. Justification of this promising inactivation process with UV was checked by microbiological tests that proved to be satisfactory in the removal of much of the Gram-negative population. This was reflected in the First year report FAIR CT98-9522 on Minimal processing with combined emerging unit operations – laser, UV, microwave and chemical for microbial killing and improved food quality [137].

Effect of the length of the UV lamps on the shelf life of carrots placed into sealed bags

From the previous experiments, it was observed that with the conveyor roller table speed at $0.21cm^{-1}$, the carrots still showed an enhanced shelf life effect after UV treatment. Therefore in this experiment, the length of the lamps exposed was varied; namely at ¾, ½ and ¼ length to reduce the energy density and thus the amount of UV applied on the food produce. This was an important experiment since it was designed to determine the minimum energy density required for enhancement of the shelf life of the treated produce. An enhanced effect was achieved when the length of the lamp was set to no less than ¾ length, the shelf life was then increased by 2 days. This will be important in the future design of a suitable machine.

4.4.2 Shelf life experiments

Shelf life after UV treatments

Carrots

The experimental results showed an improvement in the shelf life of the treated carrots over the control. The rate of decay and shelf life was between 4 to 8 days without refrigeration, where the vegetables showed no traces of mould or baterical formation.

Class I carrots had a longer shelf life compared to class II carrots, even without treatment. UV treatment was equally effective on both classes of carrots, with an observed 30 to 40% reduction of decay.

Although information was recorded on the effect of UV on sprouting of carrots there was little useful information gained from this work. On class I carrots, sprouting was less rapid after treatment whereas on class II carrots sprouting was a little faster than the contol

samples. It was apparent that UV exposure reduced the microbial content on the carrots. There was no colour change after treatment or any other significant adverse effects of the exposure. Random sprouting showed that the UV exposure did not change or alter any growth behaviour of the carrot. The treatment, however, was effective at improving the shelf life of the produce.

Shelf life after H_2O_2 treatment

Carrots

The experimental results showed that treating carrots with H_2O_2 was slightly more effective then using the UV lamps. H_2O_2 treatment extended the shelf life to about 10 days compared to treating the carrots with UV, which only produced 6 days of shelf life extension, see **Figure 4.9** and **Figure 4.10**. The sprouting rate was moderately slower than carrots treated with UV.

Potatoes

Similar to that of the UV the treatment on potatoes, there were no visible adverse effects after H_2O_2 treatment. No significant difference in the shelf life extension was observed between the treated and control samples.

Shelf life after laser or microwave treatment

In both laser and microwave experiments, despite achieving a shelf life extension, undesirable discolouring affected the potato surfaces. Although no visible damage was observed immediately after treatment, potatoes irradiated by laser turned a dark brown colour after 2 days.

When potatoes were treated with 5 sec of microwave at 800W, a different discolouration was observed. The vegetables turned light green when compared to the untreated ones.

Shelf life of carrots after UV and H_2O_2 combination treatment

The UV and chemical treatment produced an extension in shelf life of longer than 3 days. Interestingly, the rate of decay for periods longer than 10 days was reduced as compared to the control. This was with the UV slow treatment. Carrots exposed to UV treatment at the fast roller table speed seemed to decay at about the same rate as the control after 10 days.

In **Figure 4.15**, the gradient is significant in that it can be used to indicate what treatment processes could be used to extend the shelf life even further. In the present industrial context this was not considered relevant as an extension of even a day or two was considered a success. It is clear that the treatments can offer this capability.

Shelf life findings

In general, the treatment processes for the carrots produced an extension in the shelf life of about 3 days. Clearly, although the laser treatment prevented rotting of the produce by bacterial or mould contamination, there was the disadvantage that it produced a browning of the carrot. This would, of course, be unacceptable to the consumer. Additionally, the laser part of the system was considered to be too expensive for this application. The microwave results indicated that it was capable of extending the shelf life but there were technical difficulties in producing a reliable system that could run safely within a production line environment. The chemical or UV treatment alone was effective, but their combination was most effective.

For the potatoes, comparison between the shelf life treatments was not possible as there was no moulding, signs of decay, change in texture or reduction of rigidity on either the treated or untreated samples. Further investigation may be necessary with a longer storage time.

Experimental results showed significant improvement in the shelf life of tomatoes over the untreated samples. There was no change in colour or appearance after prolonged UV exposure. Mould grew on the treated and control samples around the stalk end of the tomato but was retarded on the treated samples. However, this made it difficult to clearly state the exact increase in shelf life, but it was likely to be more than a day.

4.4.3 Effects on vitamins after treatments

In all treatments, the β-carotene (vitamin A) level of the treated carrots were similar, or greater than the untreated ones. This indicated that the treatments were an effective minimal process and did not degrade the carrot's nutritious value. On potatoes, the investigation of vitamin C level also indicated that its nutritious quality had not been degraded with any of the treatments.

4.5 Figures

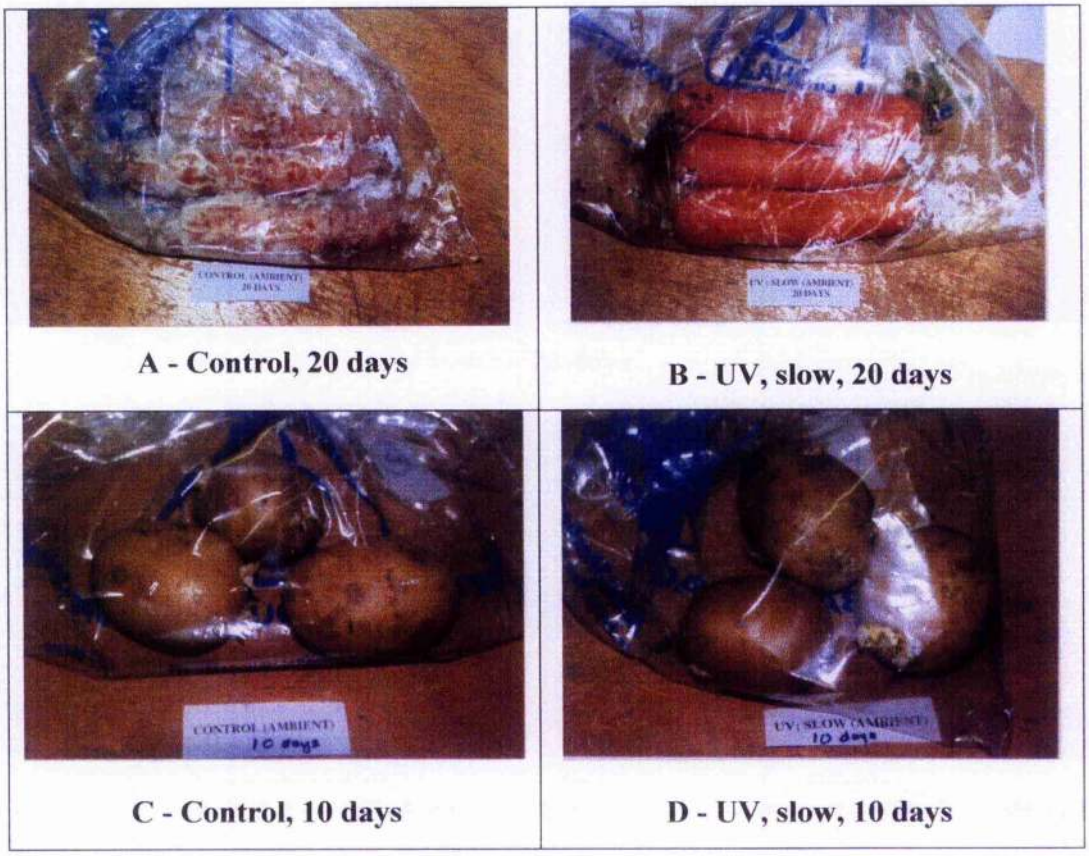


Figure 4.1 Effect of UV exposure on the shelf life of carrots and potatoes placed into sealed bags. 3 x 40 W UV system. Slow translation velocity 0.05 cms^{-1}

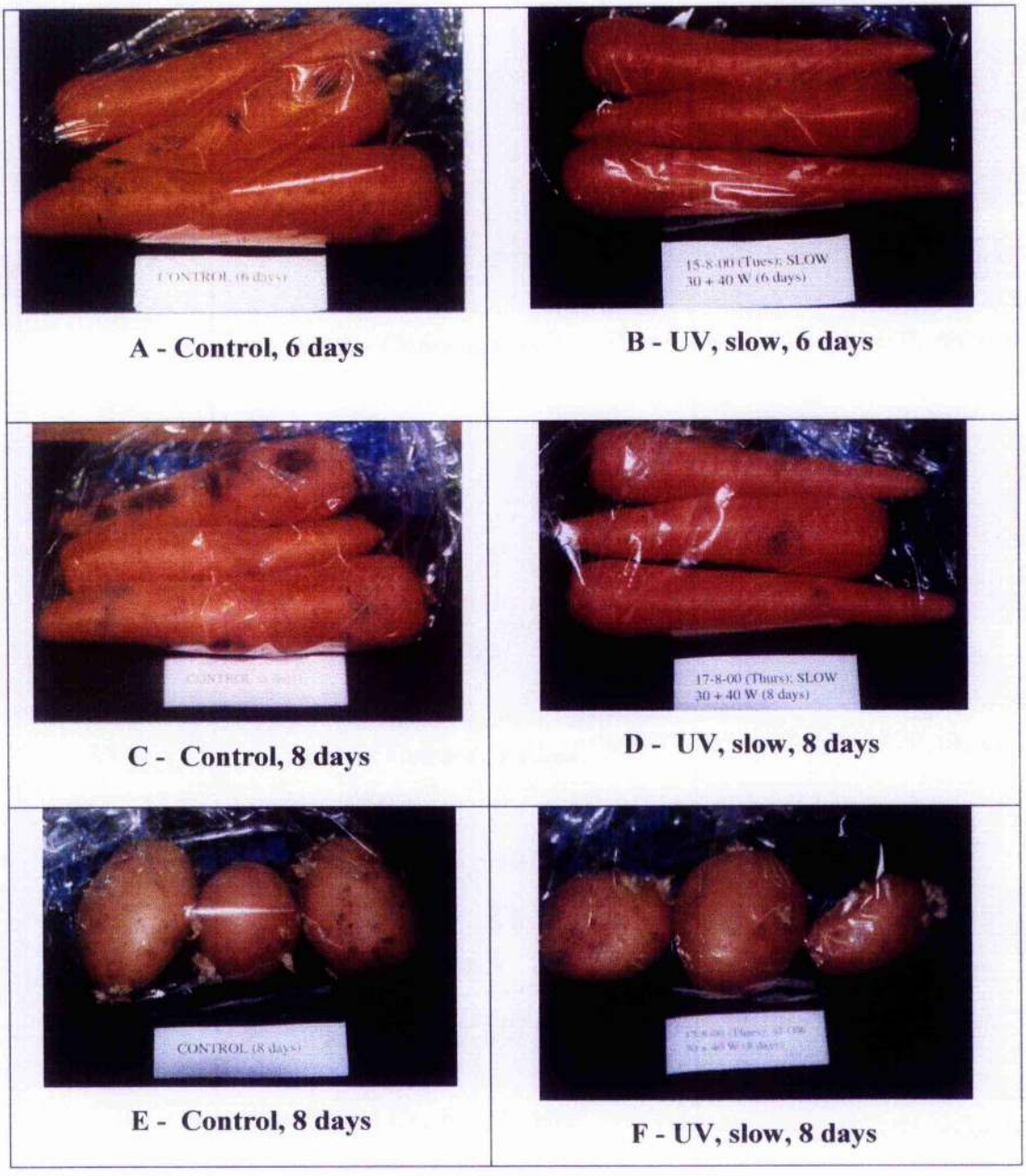
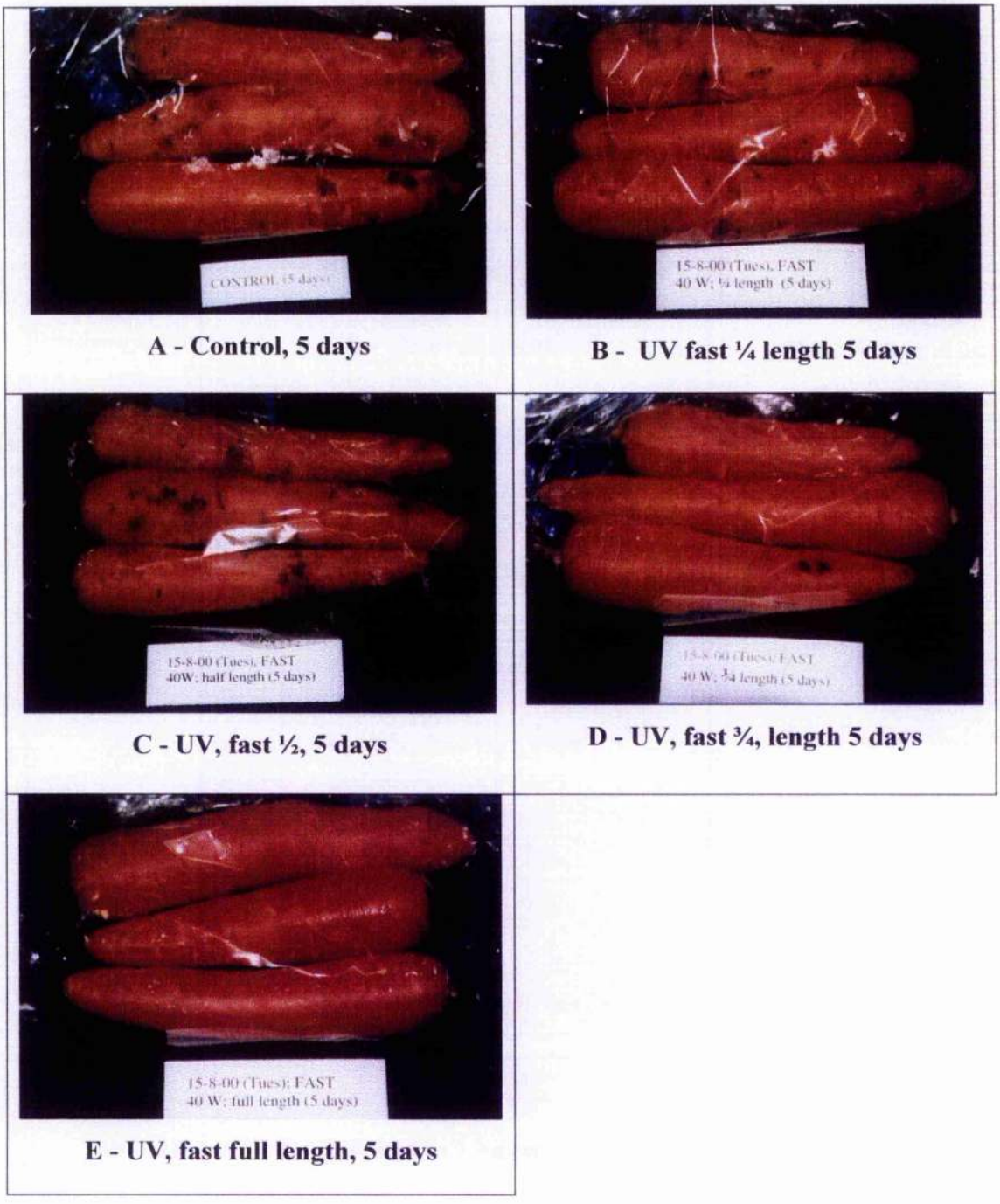


Figure 4.2 Effect of UV lamps arranged transversely on the shelf life of carrots and potatoes placed into sealed bags. 3 x 40 and 3 x 30 W UV system, arranged transversely. Slow translation velocity 0.05 cms^{-1}



A - Control, 5 days

B - UV fast ¼ length 5 days

C - UV, fast ½, 5 days

D - UV, fast ¾, length 5 days

E - UV, fast full length, 5 days

Figure 4.3 Effect of the length of UV lamps on the shelf life of carrots placed into sealed bags. 3 x 40 W UV system, arranged longitudinally. Fast translation velocity 0.21 cms^{-1}

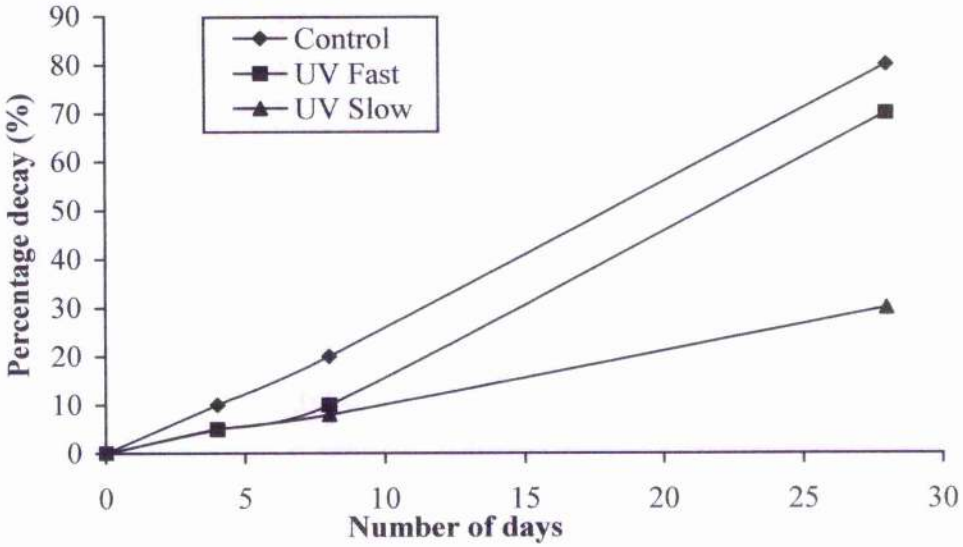


Figure 4.4 Percentage of the tomato decayed as a function of the number of days from treatment for the control, fast and slow translation velocities

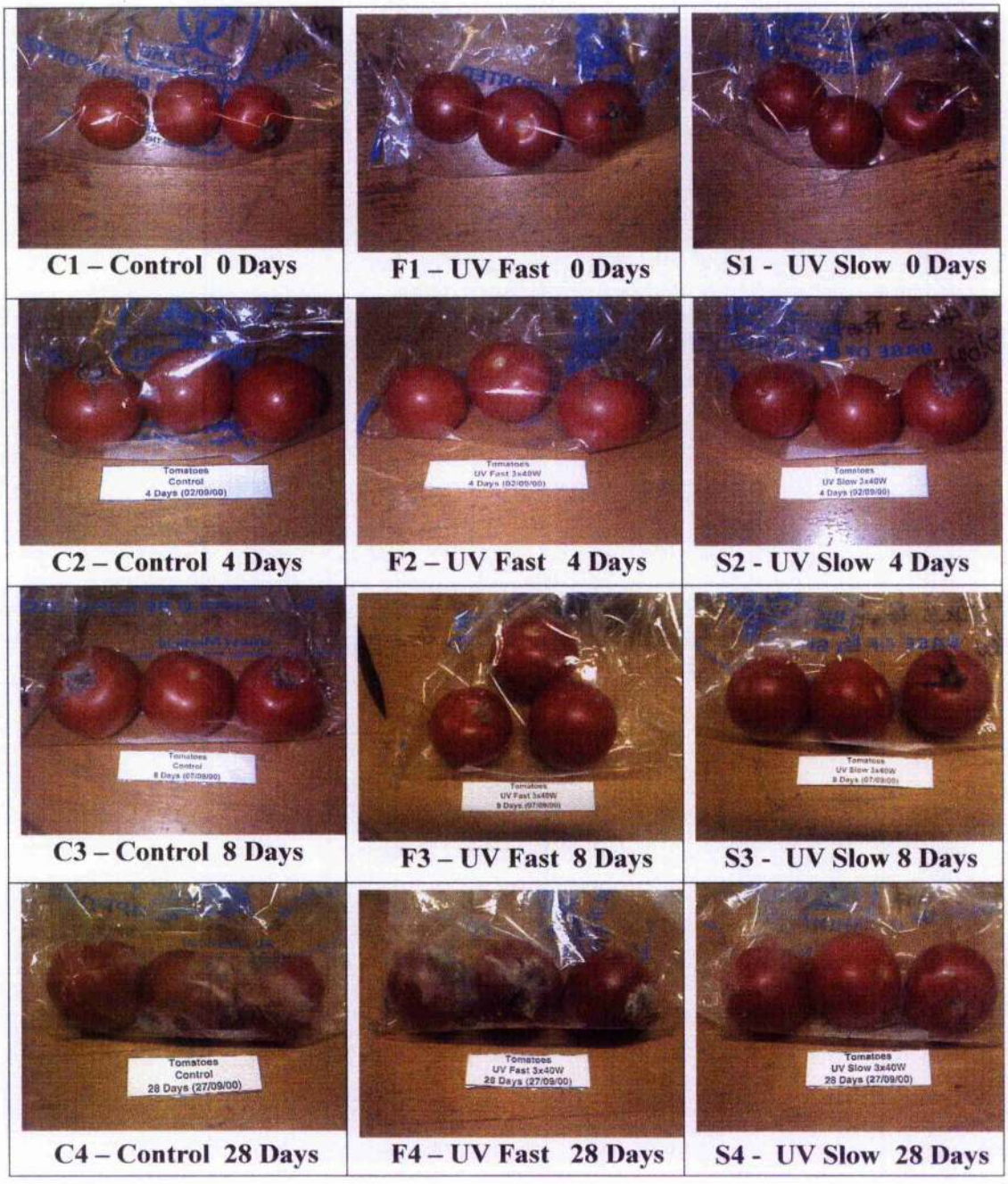


Figure 4.5 UV treated tomatoes at slow and fast translation velocities

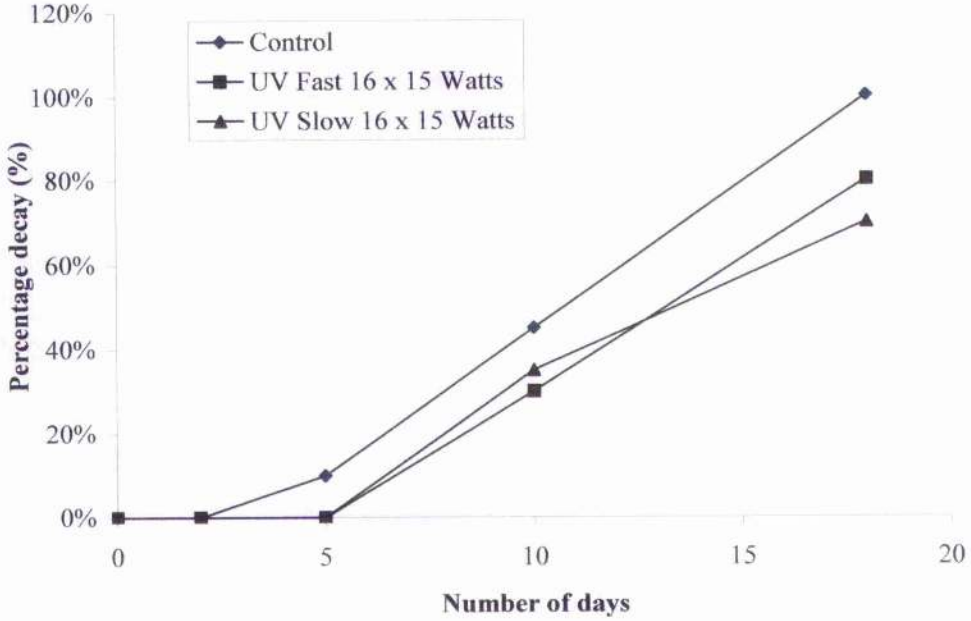


Figure 4.6 State of carrots after UV treatment

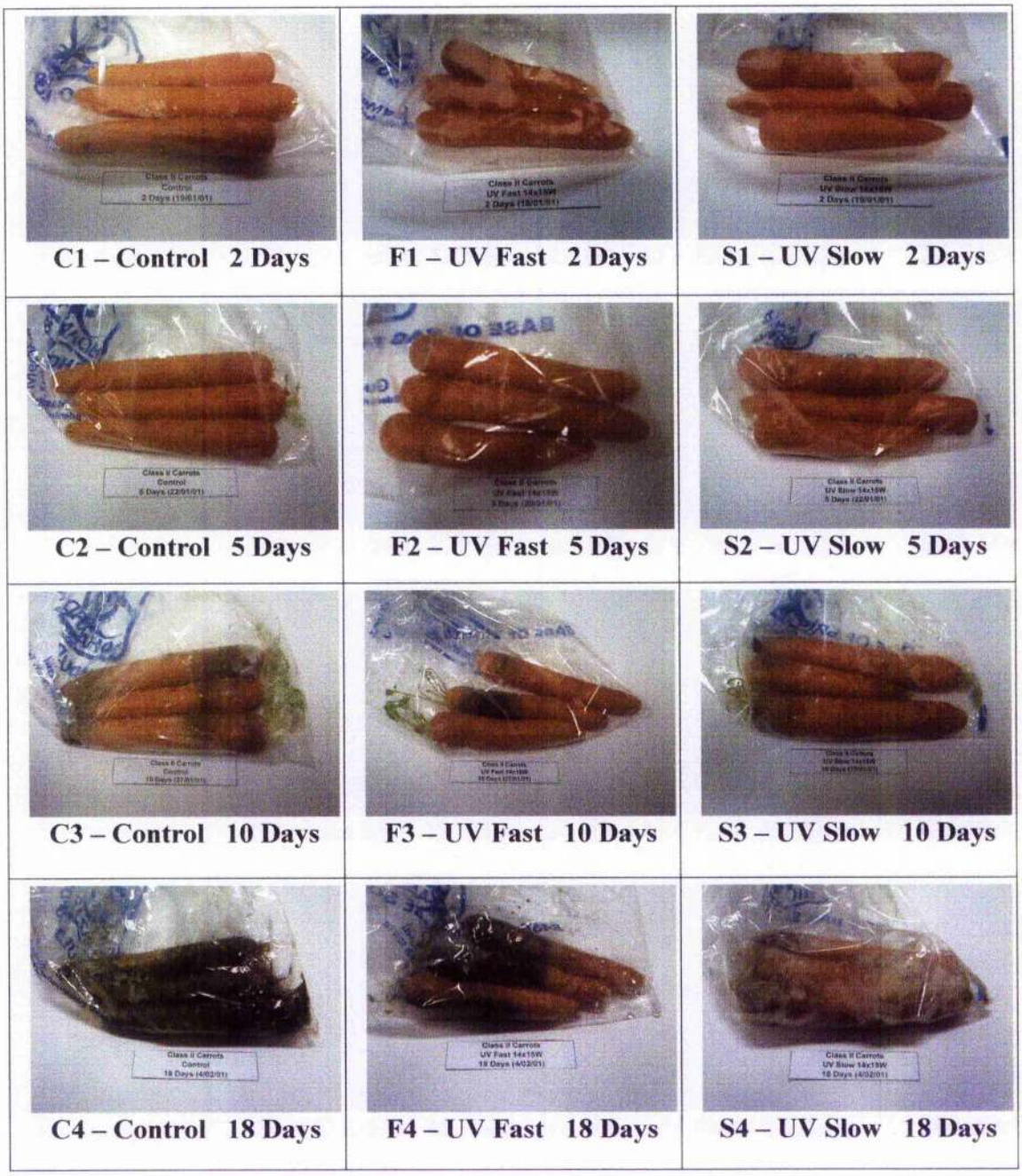


Figure 4.7 Carrots treated with optimized UV system

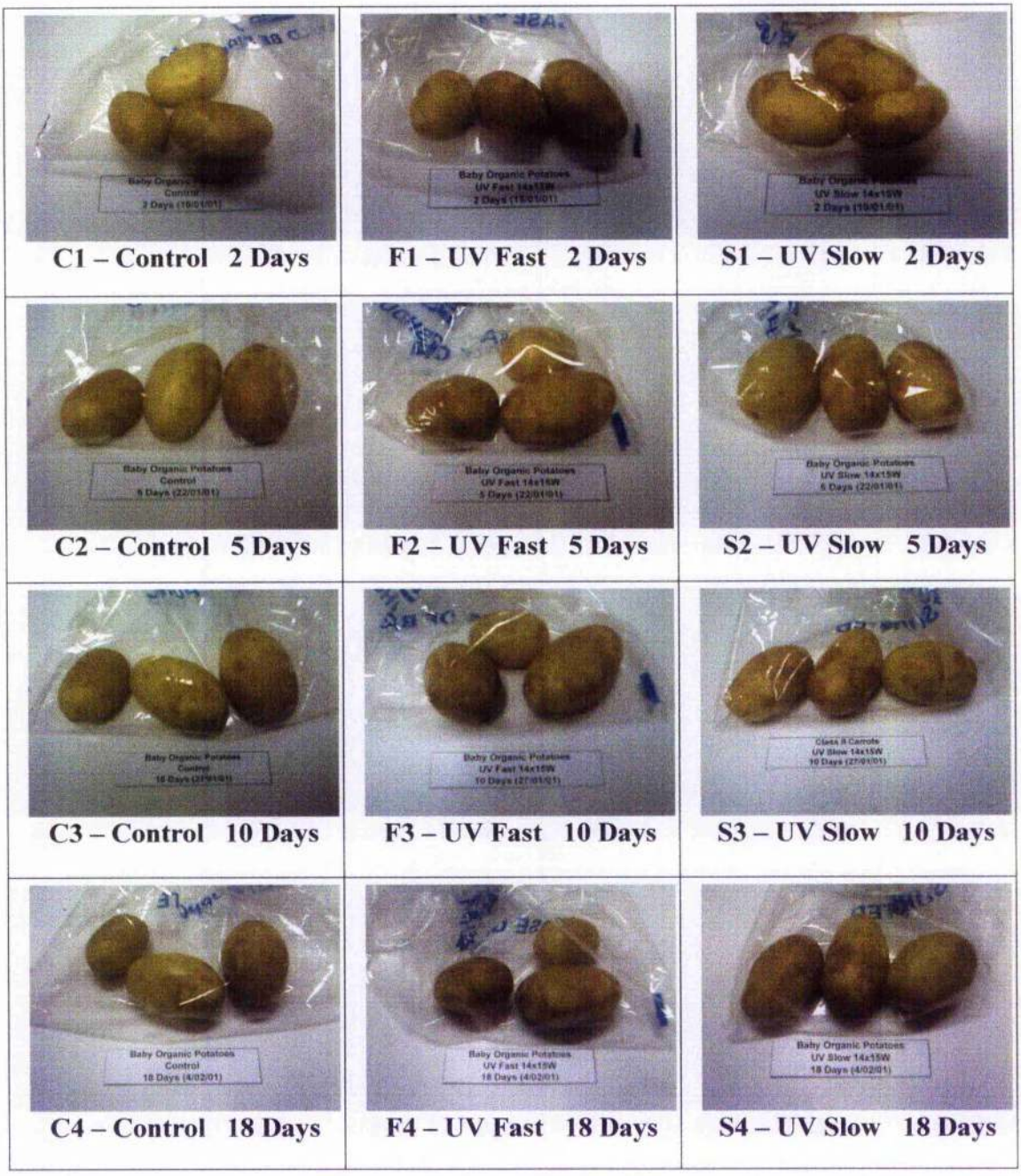


Figure 4.8 Potatoes treated with optimized UV system

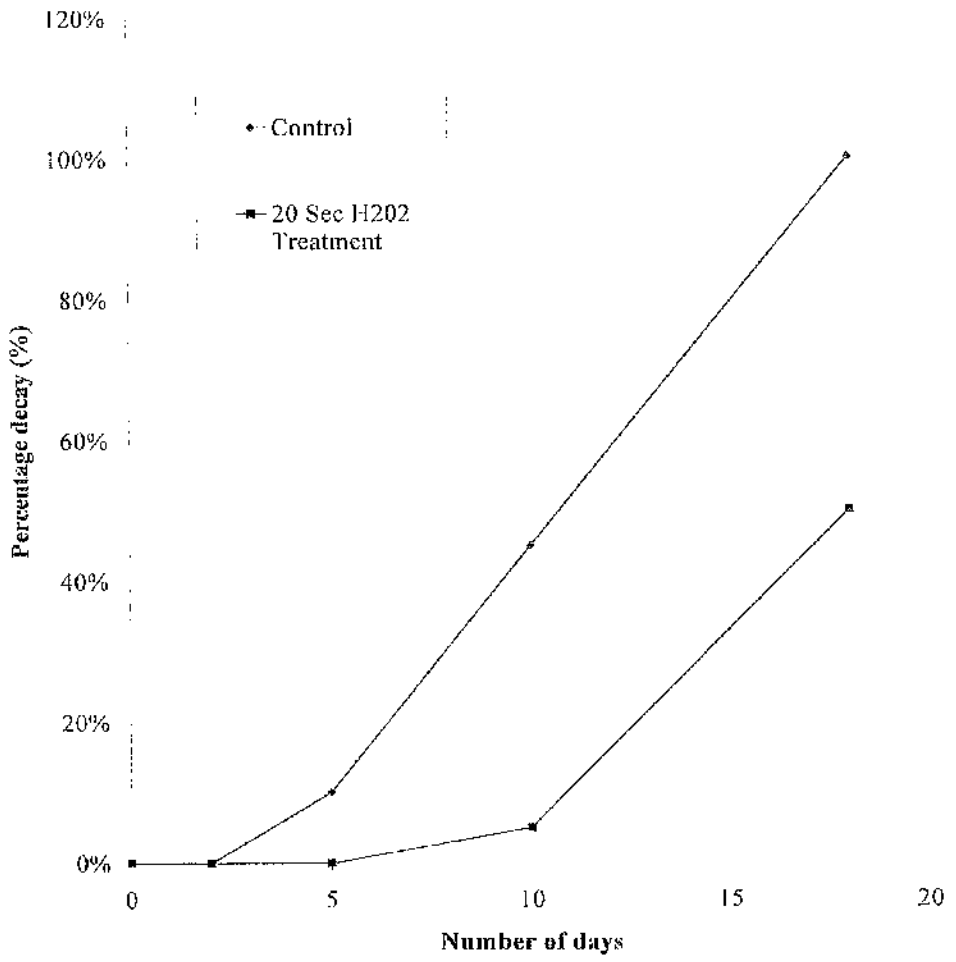


Figure 4.9 Percentage decay of carrots as a function of days after H_2O_2 treatment

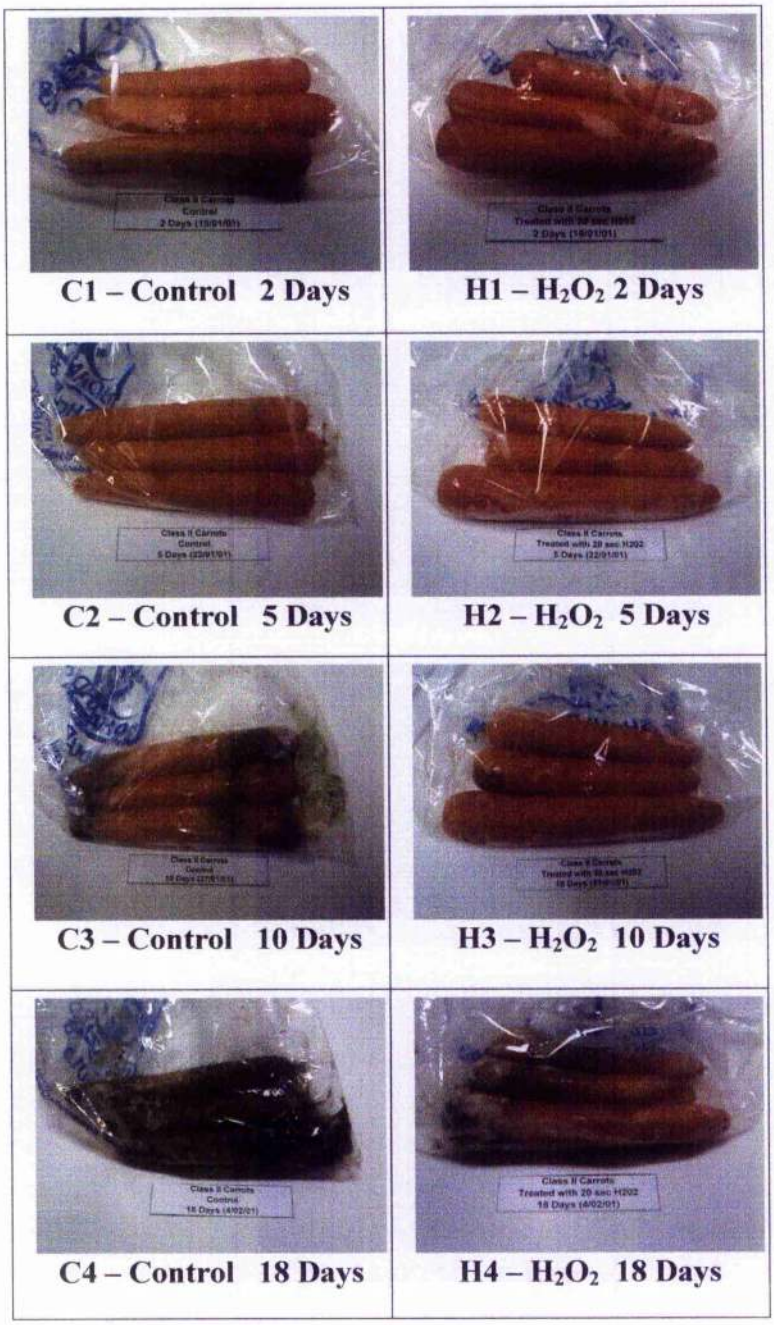


Figure 4.10 H_2O_2 treated carrots

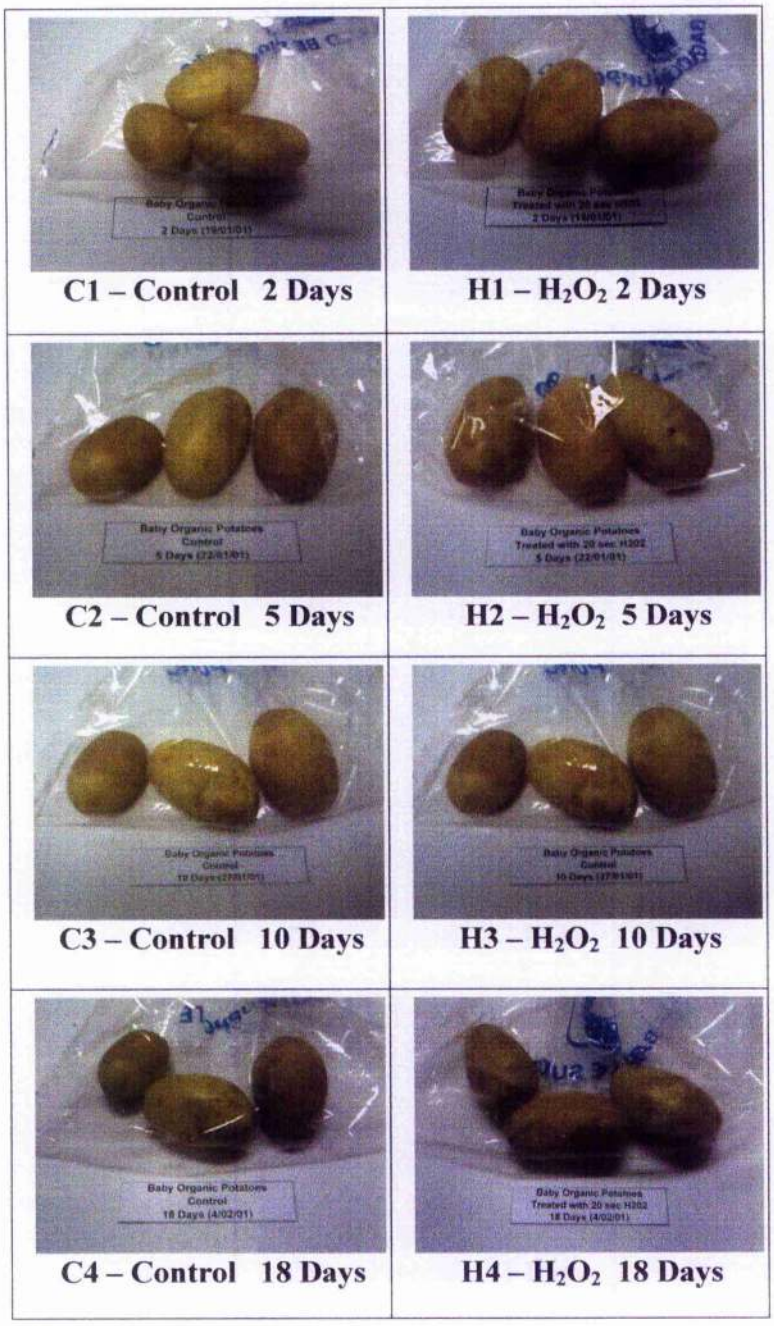


Figure 4.11 H_2O_2 treated potatoes

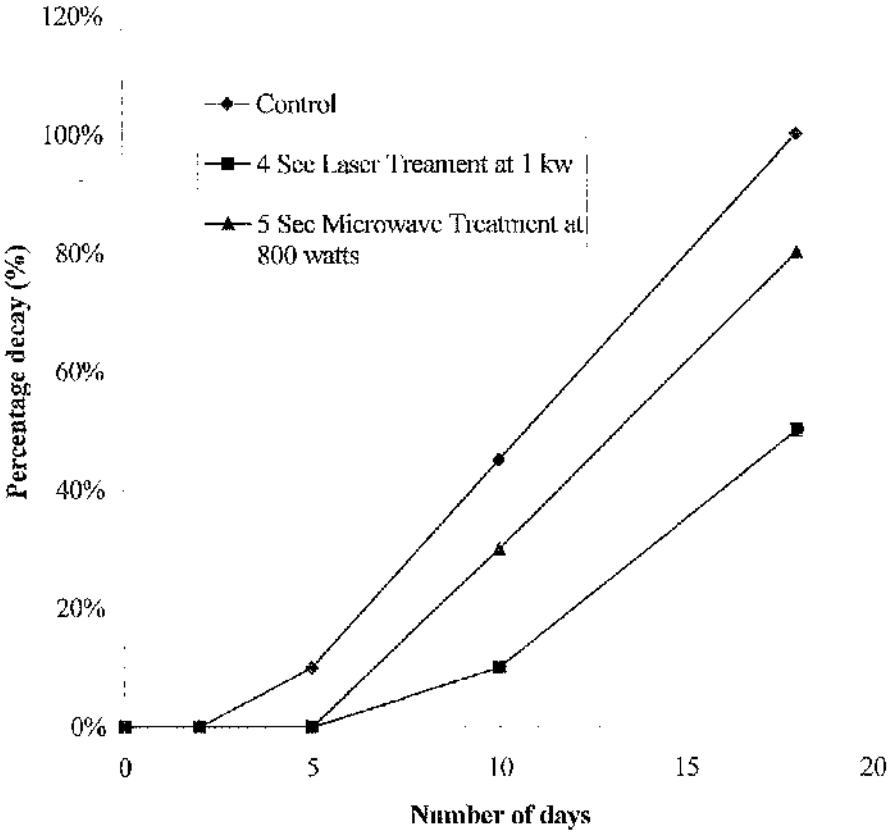


Figure 4.12 Shelf life of carrots after laser or microwave treatment

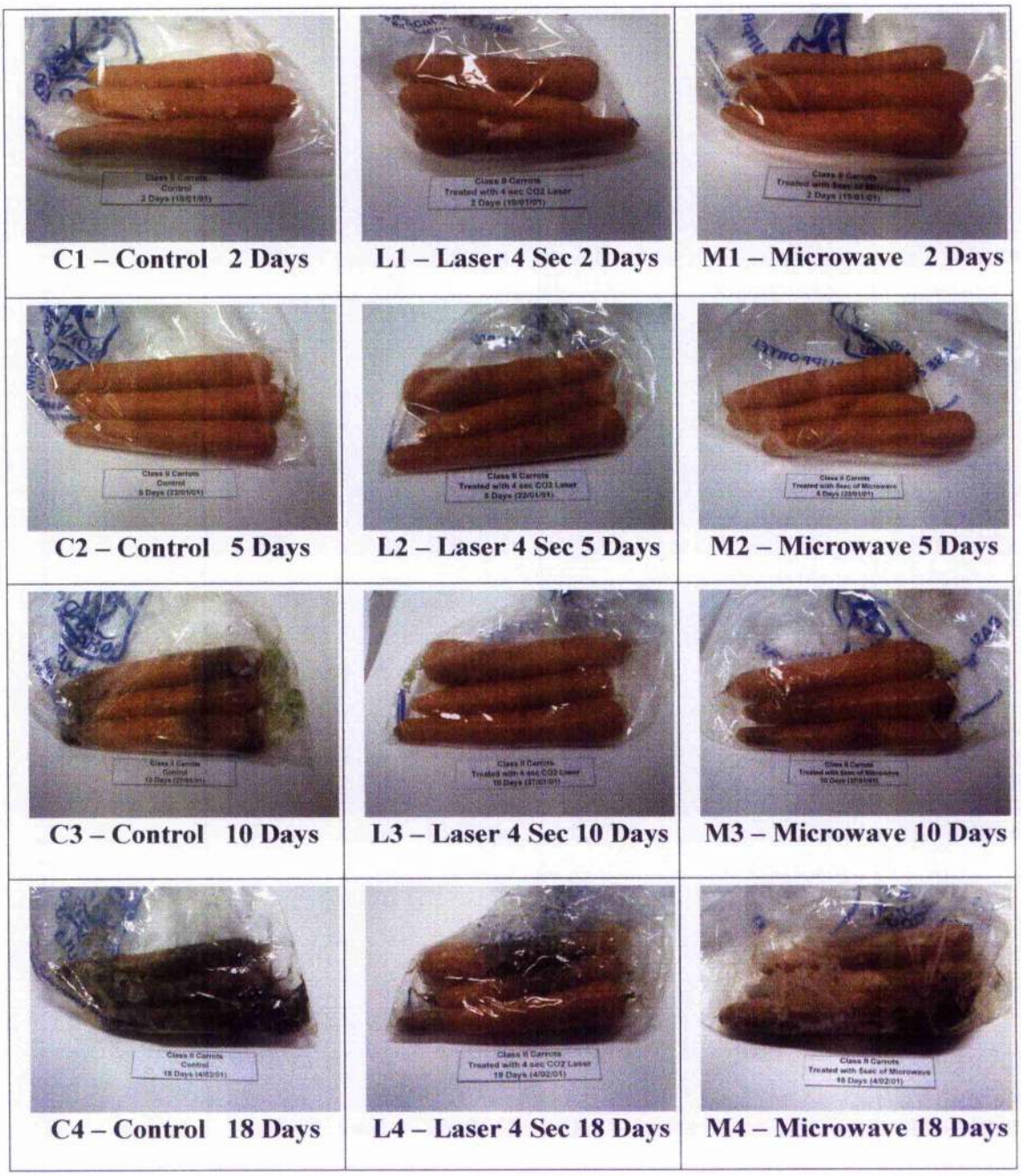


Figure 4.13 Laser or microwave treated carrots

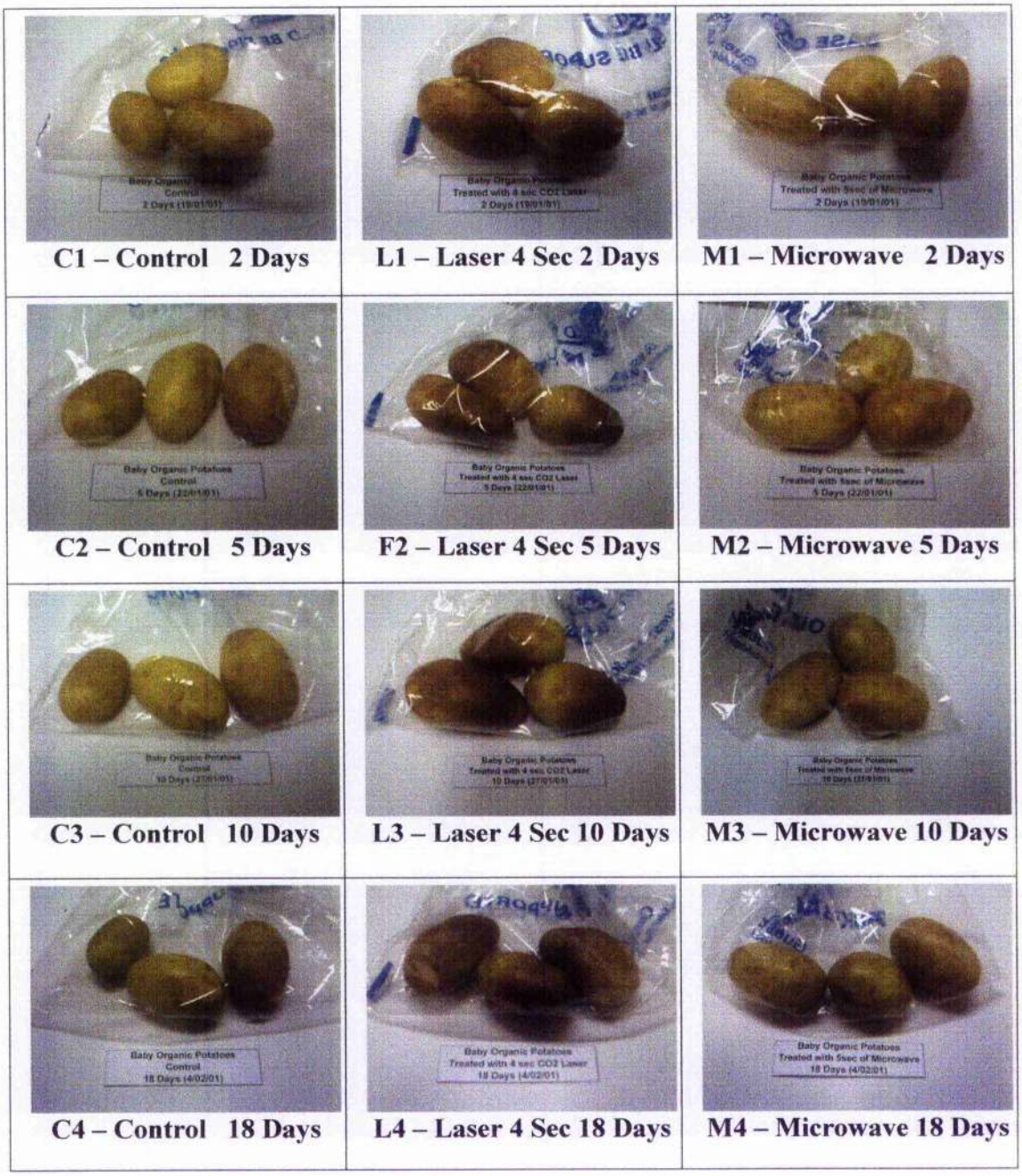


Figure 4.14 Laser and microwave treated potatoes

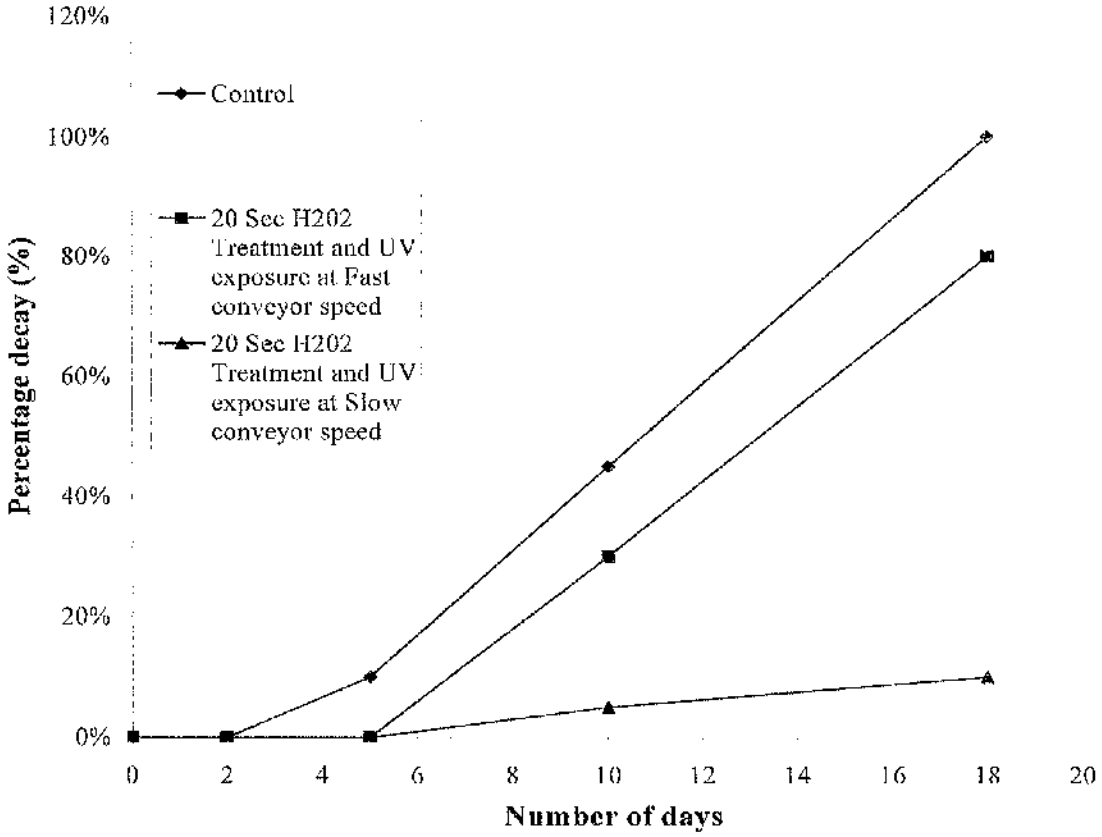


Figure 4.15 Shelf life of carrots after combined H_2O_2 and UV treatment

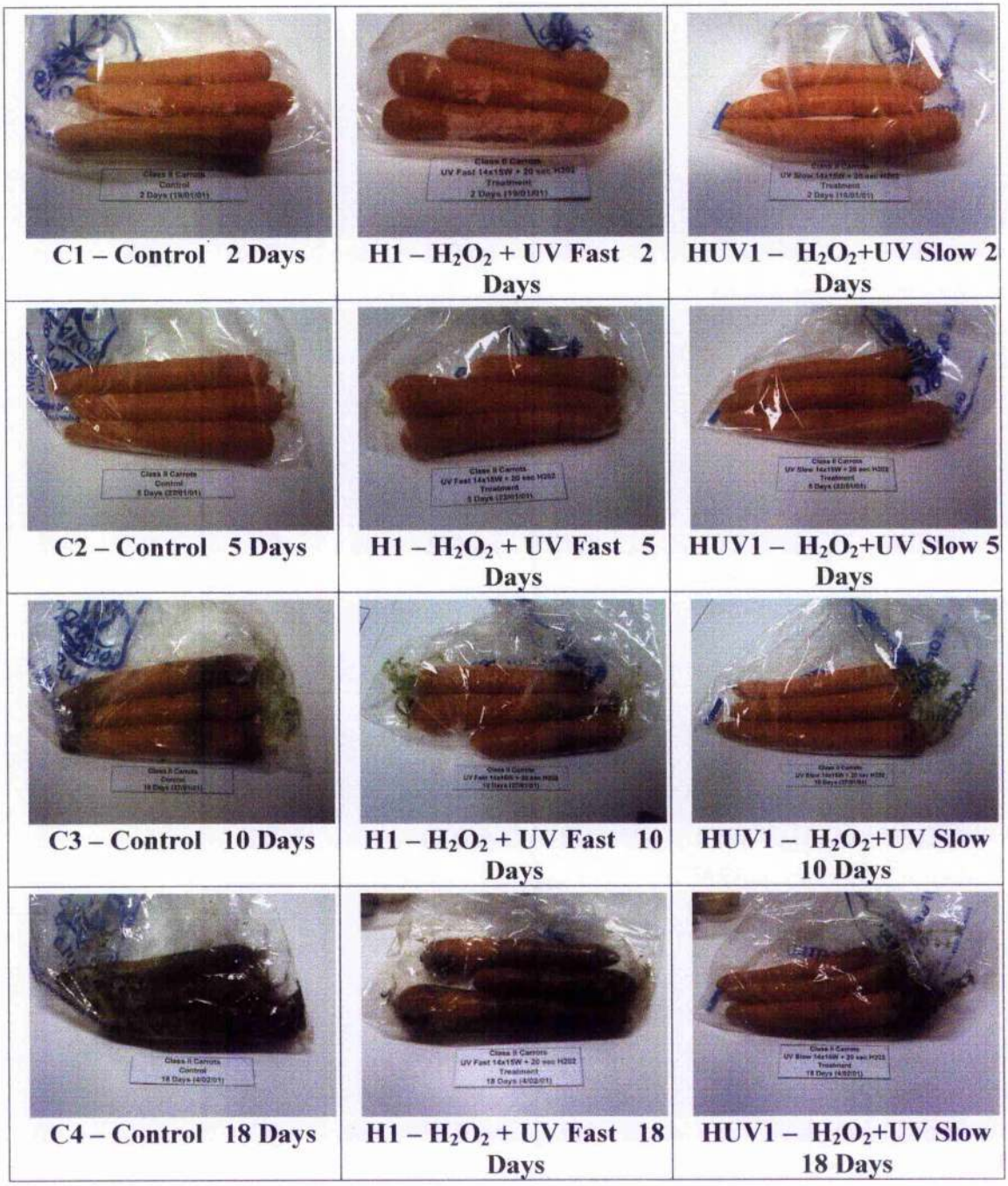


Figure 4.16 H_2O_2 and UV combined treated carrots

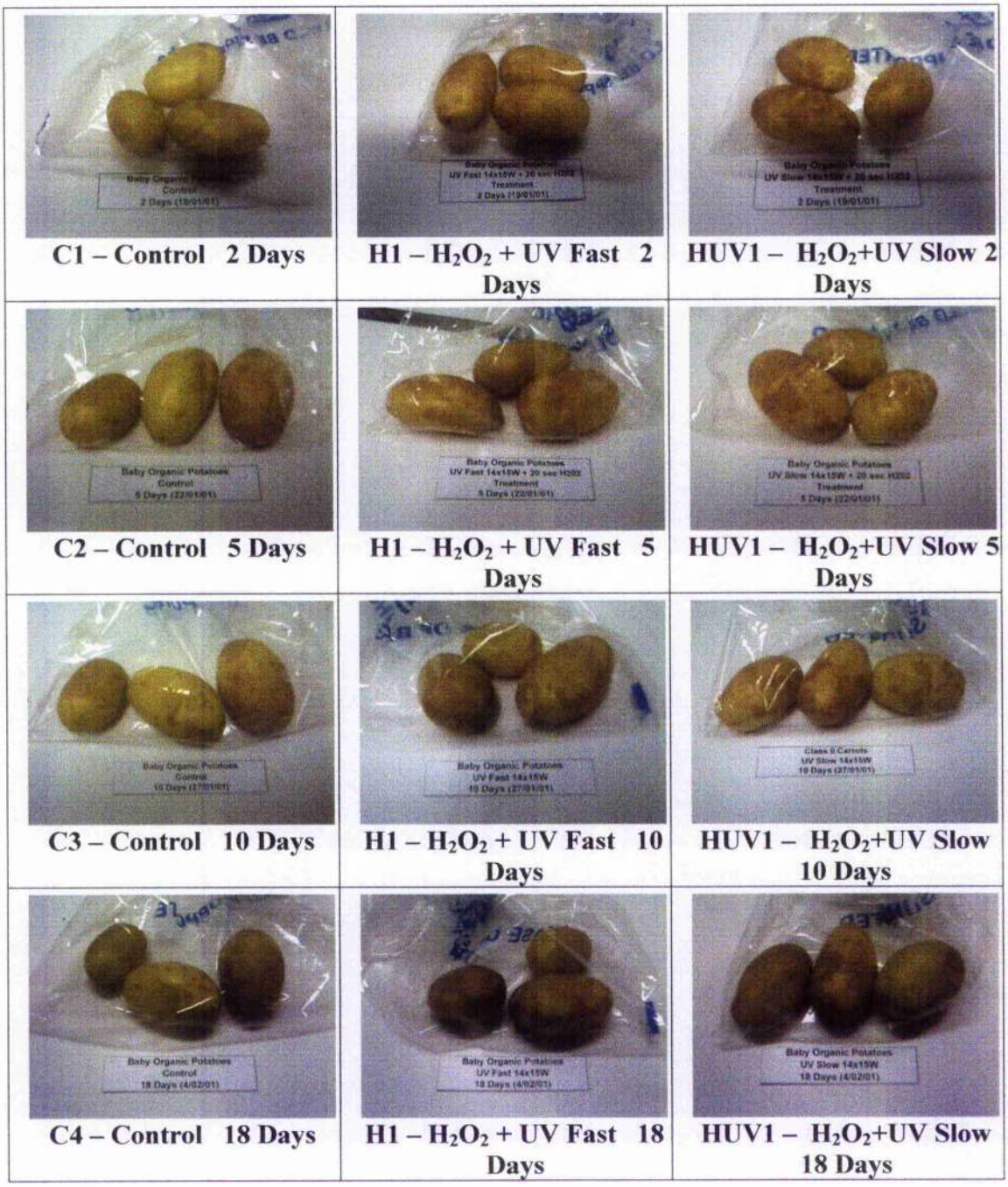


Figure 4.17 H_2O_2 and UV combined treated potatoes

CHAPTER 5 Bioluminescence detection and measurement

5.1 Introduction

There is continued interest in developing systems to reduce microbial bioburdens on a range of surfaces, which may include living cellular surfaces or inert substrates. Whatever the surface or substrate medium, there is a need to quantify the effect of the process efficiently and rapidly. Any microbiological experiment investigating bacterial growth or death kinetics generally relies on total viable counts (TVC) as a measure of the microbial contamination. This is the method that was used in Chapter 3. TVC methodologies are expensive and time consuming, often requiring 24 hr of incubation before any results are known. Furthermore, uncertainties may be introduced into the counts with regard to the actual contamination levels through assumptions about bacterial adherence recovery and errors at each stage of the dilution and counting process.

With some decontamination systems, there is a wide range of physical variables that need optimisation. It is desirable therefore, to complete a detailed set of experiments to yield the optimized settings. However, environmental factors may influence these results significantly. A system capable of real time monitoring of viability would be useful in the design and optimisation of decontamination systems. In the present case, an instrument was fabricated to investigate the effect of ozone on killing of a bioluminescent construct of *Escherichia coli* (*E. coli* lux) lawned onto agar plates or inoculated into a liquid suspension.

Ozone is often used in disinfection systems for waste water [138], because of its high decontamination efficiency. If TVC are made, then this involves a significant effort to map the effect of the system parameters, environmental conditions and even bacterial type on the system's decontamination efficiency. One method to reduce the data set is to do multifactorial experiments; however, assumptions are then made regarding the interpolation between points and there is still a lag for viable counting between the experiments and results. In some circumstances such as food poisoning cases, such delays may be unacceptable, and faster, more efficient processes are needed.

Some efforts in the field of light imaging measurement have been made to provide rapid data. For example, a low level imaging system was fabricated to investigate photo-oxidative stress response in leaves [139]. Site specific responses to reactive oxygen species was assessed by analysing the chlorophyll fluorescence. Images were recorded over 60 min and a peltier cooled camera was used. Single photon imaging was used for metabolic mapping of biological tissue with a spatial resolution at the cellular and subcellular level, with applications to clinical oncology; yielding metabolic results "within a few min after removal of the biopsy" [140].

Ozone has an absorption band around 4.5 eV and its presence in the atmosphere is vital for life on earth via absorption of harmful UV light [141]. Further work has investigated absorption properties in the vacuum ultraviolet (VUV) [142] and at higher frequencies (12-21eV) [143]. There has been considerable interest in the literature modelling effects of atmospheric ozone because of the concerns over global warming. Ozone, however, is also a pollutant, and high concentrations at terrestrial levels is degrading agricultural crops [144;145]. Because of its reactive nature, ozone is an extremely effective biocide and has been used to sterilize a range of substrates including air and water [138;146]. Ozone can be generated in number of ways including DC coronas, dielectric barrier discharges, or silent electric discharges [147-149].

The effectiveness of ozone treatment of bacteria is strongly dependent on parameters such as ozone concentration and ozone treatment period. The treatment results can be quantified either against an absolute or relative measured value of the ozone concentration. Ozone causes damage to the DNA and cell lysis which kills the organism. These mechanisms were discussed in Chapter 1.

Detection and measurement of ozone is a fundamental challenge. Ozone (O_3) is a highly unstable, colourless gas. Formation of O_3 is a result of photochemical dissociation of the normal oxygen molecule. Alternatively, the formation of O_3 can occur when oxygen molecules detach in the presence of the corona effect [150], where the oxygen molecule is excited by a non-uniform electric field generated from a high voltage, source and the dissociation occurs when the excitation energy exceeds the molecular bond strength of the oxygen (4.5eV). These free O atoms can then combine with an oxygen molecule to form

O_3.

The measurement and detection of ozone rests upon the importance of ozone's ability to absorb ultraviolet (UV) radiation. The amount of radiation absorbed by O_3 at a specific wavelength depends on two factors: the number of molecules in the path of the light, and the absorption cross-section of the ozone molecule.

Atmospheric O_3 concentration is commonly determined using UV spectroscopy due to ozones' photoabsorption properties between 200-310 nm, which is called the Hartley band region. [142;151]. Wavelengths at the much weaker band system, called the Huggins band, in the range of 310-360nm [152] are also used. Calculations using the absorption spectra of ozone in the Hartley band were investigated by Bruce *et al.* [153]. With these parameters, the ozone concentration can be determined using the photodissociation methodology reported by Baljit and Gejo [154;155]. In the present case, the ozone concentration was calculated from the absorption of UV radiation by ozone. The attenuation of UV light at 253.7 nm is solely due to ozone rather then the presence of oxygen [156].

Bioluminescence is the production of light by living organisms through an internal chemical reaction. Bioluminescence is found among some insects, fish, ctenophores comb jellies and annelid worms. Some of the most dramatic light-producing creatures are found in marine environments [157]. It is well known that bioluminescence can be utilised to rapidly and specifically detect bacteria [117]. In an ideal world, a real time detection process would enable the effect of all the system and physical variables to be assessed through instantaneous measurement of the changes in the contamination level. Such a process creates possibilities to introduce control and optimisation of the decontamination system in real time, ensuring that the level of contamination is below the level of sterility assurance that is required. Furthermore it is likely that such systems will provide greater scientific understanding of bacterial death kinetics.

5.2 Materials and methods

5.2.1 Bacteriological protocols

Preparation of the bioluminescent strain

The bioluminescent (*E. coil lux*) was strain DH5α containing plasmid PT7-3. This plasmid encoded ampicillin resistance and contained the lux ABCDE genes from Xenorhabdus luminescent and was kindly provided by Professor E. Meighen (From the Department of Biochemistry, McGill University, Montreal, Quebec II3G 1Y6, Canada). For use they were grown at 37 °C for 18 – 24 hr on nutrient agar (Oxoid CM55) or in nutrient broth (Oxiod CM3). Agar plates were prepared using Oxiod CM55 nutrient agar powder; 28g of the powder was added to distilled water, mixed and boiled, before placing in an autoclave for 15 min at a temperature range between 121 to 124 °C. The sterilised nutrient agar was allowed to cool for 2 hrs in a 36°C water bath. At pouring temperature, the agar was transferred into a fume cupboard where 2.5 ml of ampicillin stock at 10mg/ml concentration was added into 500 ml of liquid agar and poured into petri dishes. A burner was used to remove any air bubbles on the surface after the agar was poured. After the agar was set and cooled, the defrosted culture was spread over the nutrient agar, containing ampicillin, using the 16 streaks technique. The plate was incubated for 18 hrs at 37 °C in a hot room to obtain single colonies. A single colony was re-suspend into nutrient broth with ampicillin added and the culture was incubated for 24 hr at 37 °C.

Preparation of *E. coli* lawned plates

A single colony obtained by the above procedure was inoculated into 100 ml of nutrient broth, which was incubated for 24 hrs at 37 °C. 100 µl of the resulting *E. coli* culture was pipetted onto a nutrient agar plate and spread evenly with a sterile L-shaped glass rod. The lawned plates were incubated at 37 °C for 10 hrs to maximise the light output from the bacteria.

Preparation of *E. coli* suspensions

The nutrient powder was suspended in distilled water, to a ratio of 13 g of powder to 1 litre of distilled water. The mixture was boiled to dissolve the powder and autoclaved for 15 min at a temperature between 121 and 124 °C. The prepared nutrient broth was stored at room temperature. 2.5 ml of ampicillin at 10 mg/ml was added to 500 ml of the broth

just before use. For preparation of the bacteria suspension, a single colony of *Escherichia coli*, obtained from the prepared culture, was inoculated into 1 litre of nutrient broth. The suspension was incubated for 10 hrs at 37°C on a shaker at 200 rpm. It was then distributed into 10 duran bottles, each containing 100 ml of suspension.

5.2.2 Determining ozone concentration and ozone absorption at 254 nm.

The transmission of UV light from the source to the detector was measured. The light passed through a known length of ozone gas occupying the volume between the source and detector. The signal from the UV detector was passed out of the chamber, amplified, captured on an oscilloscope and downloaded to a computer, where the ozone concentrations were subsequently calculated using Beer-Bouguer-Lambert's law [158].

The irradiance detected as a function of time $I(t)$ is given by

$$I(t) = I_o \exp\left(-\sigma[N(t)]x\right) \tag{5.1}$$

Where I_o is the irradiance with no ozone present, σ is the absorption cross-section for ozone at 254.3 nm, [N(t)] is the total number of molecules per cm^3 as a function of time and x is the path length. The temporal distribution of ozone may be simply found from the temporal distribution of the UV light. The number of ozone molecules was, therefore, determined by rearranging (5.1) giving,

$$-[N(t)] = \frac{1}{\sigma x}\ln\left(\frac{I(t)}{I_o}\right) \tag{5.2}$$

However, to get meaningful data on the decontamination efficiency of the ozone the temporal profile and evolution of the ozone concentration is important, as this affects the ozone efficacy. It is useful, therefore to integrate the temporal ozone concentration function from (5.2) over time T to give the integrated ozone concentration and a measure of the dose-time treatment of the ozone.

$$[N(T)] = \int_0^T |N(t)|dt = -\frac{1}{\alpha x}\int_0^T \ln\left(\frac{I(t)}{I_0}\right)dt \qquad (5.3)$$

The cross-section was taken over the Hartley band as 11.5 x 10^{-18} cm^2, see **Figure 5.1** [155;156;159;160]

The concentration of ozone (gm/cm^{-3}) can be determined by equation (5.4) below. Where the concentration of ozone is denoted by C_O, N is the number of molecules, A_v is Avagodro's number, M_m is molar mass and V is the volume over which the measurement is taken.

$$C_O(t) = \left(\frac{N(t)}{A_v}\right)\cdot\left(\frac{M_m}{V}\right) \qquad (5.4)$$

5.2.3 The ozone concentration measurement system and the fabrication of the UV meter and UV detection system

The O_3 detection system comprised a stainless-steel chamber, 15 Watt UV source, a hermetically sealed ultraviolet sensitive silicon photodiode (RS, UK, Type OSD5.8-7Q) with enhanced responsivity in the 190 - 400nm spectral range, and a signal conditioning and amplifying circuit. The UV lamp and the detector were aligned in the chamber where the gas analysis was done. A band pass filter was placed in front of the UV lamp to limit the spectral width to 255.3 ± 5.1nm (Melles Griot UK, Part No: 3 FIM 016). The experimental setup for the ozone concentration is illustrated in **Figure 5.2.**

The UV meter used for the ozone detection system was designed and manufactured in-house. The UV meter was powered by 230V at 50Hz. In an attempt to reduce the noise and impove stability, the source was rectified to a DC voltage using a two stage bridge and two sets of capacitors. The detector converted the UV irradiance into current, which was converted into a voltage signal with the AD711 operational amplifier as shown in **Figure 5.3**. The converted voltages were amplified with another AD711 in an inverter amplifier with x1, x10, x100 and x1000 selectable gain. These gain factors were achieved by varing the resistance of resistor R3, located across the amplifier, in relation to the resistance of

R1, in the inverting amplifier configuration. Immediately after amplification, the voltage signal was impedance matched to 50Ω, using the MAX405CP amplifier, for the oscilloscope connection.

A GPIB card (National Instrument Model: NI TNT GPIB) was used to interface the oscilloscope to the computer and the graphical user interface software was written in CVI (Graphical programming software from National Instruments). An average of the RMS (root mean square) value over 100 readings, at 1 minute intervals was taken. The relative values obtained from this setup were calibrated against the MaCam UV meter.

5.2.4 Calibration of the UV Meter

After the equipment was setup up as shown in **Figure 5.2**, the UV light source was turned on to warm up for about 5 min, before the recording was commenced. For calibration purposes both the in-house detector and MaCam UV meter were aligned in parallel, facing the UV lamp located 12 cm from the other end of the chamber. Both outputs were wired to the input channel of the Tektronic oscilloscope. The gain of the in-house UV meter was adjusted so the output mapped the output signal from the MaCam meter. The resistor values of the in-house detector were trimmed so the outputs were identical. Ensuring that the calibration was consistent over a variation of UV irradiance, both measurements were compared during the attenuation of UV when ozone was present. The following experimental procedure was adopted for this comparison. The oxygen flow to the ozone generator was set to 100 l/hr, the gain of both meters was set at 100x and the computer logged the data captured from the oscilloscope. The ozone generator was switched on, suppling the O_3 flow into both chambers via a T-joint and the drop of irradiance detected by both sensors was recorded. At the highest ozone concentration, where the UV was recorded at its lowest value, the ozone generator was switched off while measurements were still taken until the UV irradiance returned to its initial value and there was no ozone in the chamber.

5.2.5 Measuring the absorption of UV irradiance through O_3

The protocol for measuring the absorption of the UV irradiance through O_3 at different concentrations was identical to the calibration process described in the section above. However, instead of using both meters only the calibrated UV meter was used.

5.2.6 Dissociation of ozone in the measuring chamber

The measurement of ozone dissociation was required to determine how long ozone remained in the chamber after the ozone supply from the ozone generator was switched off. The values were used to determine treatment duration for the ozone. Using the setup as shown in **Figure 5.4** O_3 was first allowed to flood both chambers at 100 l/hr. After 5 sec, both the ozone generator and the O_2 supply were cut off. With the chamber still sealed, data were collected, recording the increase of UV irradiance. When the signal reached the same level as the starting UV irradiance the measurement was stopped. Ozone in both chambers was thoroughly vented to ensure no residual O_3 remained before the next set of experiments were conducted. This experiment was repeated to measure the ozone dissociation rate with the chamber charged with 5, 10 and 15 sec of ozone treatment.

5.2.7 Real time bioluminescence measurement

Figure 5.4 shows a schematic of the real time monitoring and detection system. The O_3 delivery and detection system comprised an ozone generator (Fisher 53 Bonn-Bad Godesber 1), two ozone chambers, 15 Watt UV source, an ultraviolet sensitive silicon photodiode with enhanced responsivity in the 190-400nm spectral range and a signal conditioning circuit. The UV lamp and the detector were aligned in the chamber where the gas analysis was done.

Instead of a single chamber, two chambers were used to optically isolate ozone treatment and ozone measurement. This prevented accidental exposure of the bacterial sample to biocidal UV. The ozone gas was generated via a controlled flow of oxygen through a corona discharge in the ozone generator. The ozone was fed into both chambers where the ozone measurement, ozone treatment and bioluminescence detection was done. Each chamber was light proof and the experiments were done at an ambient room temperature of 20 ± 2° C.

The agar plates were mounted in an identical stainless steel chamber to the one that was used for the ozone detection system. The agar plates were placed in-line and perpendicular with the photomultiplier tube PMT (Hamamatsu, PMT R928) that converted the faint light output from the *E. coli* lux growing on the surface of the agar into an

amplified electrical signal. A constant voltage of 400 V was applied to the PMT which had a gain of 10^4. A mechanical chopper was placed between the agar and the PMT. A lock-in amplifier (Scitec Instruments, Model 410, UK) was used at a chopping frequency of 37 Hz, a time constant of 10 s and an input voltage of 10 mV. This reduced errors in the detected signal due to temperature fluctuations and drift, which may be severe with high gain amplifiers.

The data were measured in decibels to eliminate possible errors due to temperature drift. The maximum current measured from a fresh, untreated lawned plate was used as the reference current (I_r) and measured after the system was allowed to warm up for half an hour. This represented the maximum bioluminescent output. The bioluminescent output, B, was therefore given by

$$B = 10 \log \frac{I_m}{I_r} \text{ dB} \qquad (4)$$

Where I_m is the measured current at a later time.

The UV absorption data was collected as a function of time as the ozone filled the chamber; the corresponding bioluminescent output from the *E. coli* bacteria was collected simultaneously. The transmission of UV light from the source to the detector was measured. The light passed through a 12 cm length of ozone gas occupying the volume between the source and detector. The signal from the UV detector was amplified, captured on an oscilloscope and downloaded to a computer. After 15 min, the ozone generator was turned off and the plates were left in the chamber for a total of 200 min until the ozone had decayed. The plate was removed and incubated at 37°C for 18 hr and assessed for growth. The ozone concentration was calculated using Beer-Bouguer-Lambert's Law and from knowledge of the absorption cross-section of ozone in the Hartley band about 254 nm.

Controlled experiments were done where the bacteria did not receive any ozone treatment but were placed in the chamber and treated in an identical fashion. These measurements were done in duplicate to evaluate the validity of the monitoring and detection process.

5.2.8 Effect of ozone on bacteria in suspension

Using a sterile stainless steel tube with an internal diameter of 6mm, ozone was bubbled through 100ml of *E. coli* lux suspension in nutrient broth for 2, 5, 10 and 15 min. The ozone generator was set to the same parameters as for the other experiments. At each time interval, 2 ml of the suspension was removed for viable counting and bioluminescence measurement (conventional luminometer model 1251, running on multi-use software Bioorbit Oy, Turku, Finland). The bioluminescence readings from these measurement were not only used to test the efficacy of the ozone generator, but also to provide data for comparison with respect to the ozone treatment of the agar plates and to compare the different methods of measurement. These experiments were done in duplicate only because of the limit of 25 samples on loading the carousel of the luminometer. The relationship between the relative light output from the bacteria as a function of colony count was determined.

5.2.9 Imaging system analysis

As well as using the bioluminescence chamber and the luminometer, a set of plates was analysed directly after treatment using a conventional high sensitivity CCD imaging system (Digital Pixel Advance Imaging System 3000 - CCD video camera with KAF 1600 sensor of 1500 x 1024 with 9 micro pixels) to allow comparison of the analysis methods. The experiment was repeated 5 times. The plates were treated as described in the exposure of plates to ozone treatment for 15 min (**section 5.2.7**) and then placed into the imaging system, contained within a light proof box. The gain of the system was adjusted to try and optimise the resolution and imaging of the plates.

5.2.10 Dark current measurement

As the light levels were extremely low, it was prudent to assess the optical noise or dark current associated with the measurements in the bioluminescence chamber. Additionally, because of the low light output from the bacteria, possible fluorescence from the agar or petri dishes may have contributed to a significant error in the bioluminescence measurement. Using the same apparatus as was used for the real-time monitoring system, two experiments were carried out to determine the possibility of stray fluorescence signals

from the agar and/or the petri dish, after they were exposed to daylight for 1 hr. The plate containing the agar was placed in the bioluminescence chamber first and the chamber was sealed light tight. The light was detected and recorded over 1 hr. The plate was removed from the chamber and replaced with an empty sterile petri dish and again the dark current was monitored over 1 hr and converted into decibels. The first 100 readings over 1 minute were taken and averaged to give the zero reference reading for the dark current measurements.

5.3 Results

5.3.1 UV calibration result with MaCam UV meter

Figure 5.5 shows the measurement from both the manufactured and the MaCam UV meter over the maximum and minimum ozone concentration over a period of 2500 min. The signals were almost identical after calibration. However, there was a difference of about 5 $\mu W/cm^2$ (1 %) for the lowest UV irradiance, where the ozone concentration was at its highest. This was most probably due to the different sensitivity response between the two photodetectors.

5.3.2 Determining UV absorption due to ozone at 253.7 nm

During photodissociation, UV energy is absorped by ozone molecules causing a reduction of the detected signal. **Figure 5.6** shows a typical graph of the UV light that was detected by the photodiode as a function of time as the chambers were flooded with the ozone for 15 min, where the ozone generator was then switched off allowing the ozone in the chamber to decay over 180 min. **Figure 5.7** and **Figure 5.8** show the corresponding ozone concentration over this period, calculated from the temporal variation of the UV irradiance and the integral of the ozone concentration. It is seen that as the ozone entered the chamber, the UV irradiance reduced. This continued reduction in the UV light was indicative of the corresponding increase in absorption of the UV light by the increasing ozone concentration. The UV irradiance had dropped from 549 $\mu W/cm^2$ to 20 $\mu W/cm^2$. When the ozone generator was turned off after 15 min, the ozone concentration was at its peak. The UV irradiance gradually increased up to near its original level after about 180 min from when the ozone first flooded the chamber. This indicated the decay of ozone

from the chambers. Using the UV irradiance data obtained at the maximum and minimum, the O_3 concentration was determined. After 15 min, the O_3 concentration was found to be 3.986×10^{-6} g/cm^3. Detailed calculations are given in Appendix I

5.3.3 Ozone decay rate of system

The ozone decay rate in the chamber was investigated to give an indication of how long the ozone would remain effective in providing treatment. Three fill times were used of 5, 10 and 15 sec. For each of these times, the final ozone concentration was calculated to be 0.04468, 0.086338 and 0.1199 μg/cm^3 respectively.

The total decay time was taken as being the time from when the ozone generator was turned off to when no ozone remained in the chamber. It was found that the ozone remained for 21, 39 and 50 min for the fill times of 5, 10 and 15 sec respectively. The experiments showed that the ozone concentration decayed exponentially. Taking the appropriate ozone concentration and dividing by the decay time, it was found that the ozone decay rate reduced with increasing fill time. For the 3 experiments, the ozone decay rate was calculated to be 470 (5 sec), 451.7 (10 sec) and 417.0 mins/μg/cm^3 (15 sec). The results are shown in **Figure 5.8**.

5.3.4 Response of the *E. coli* in suspension to ozone treatment

Figure 5.9 shows the Relative Light Output (RLO) for the different exposure times for the ozone treatment of the *E. coli* in suspension. For clarity, the percentage reduction in the RLO is shown in **Figure 5.9** above the exposed time. It is seen that as the contact time increased, the RLO from the bacteria reduced, indicating the increased biocidal nature of the ozone with time. However, after exposure of 5 min and above, the RLO were below the detectable threshold of the luminometer. **Figure 5.10** shows the RLO from the bacteria plotted as a function of their concentration (cfu/ml). It is seen that as the bacteria concentration is reduced the RLO reduces; this is true down to levels of about 1×10^4 cfu/ml, below which there was no change in the detected light as the signal was into the noise of the luminometer system. This graph also demonstrates the linearity of the relationship between the light detected and the bacterial concentration for concentrations

above $3x10^4$ cfu/ml.

5.3.5 Response of the *E. coli* lawned on agar plates to the ozone treatment

The lawned plates were placed into the chamber which was then sealed and flooded with ozone through an inlet port. After treatment and incubation the plates were observed for growth. **Figure 5.11** shows the RLO from a typical control sample that did not receive any ozone treatment, the RLO is plotted over 3000 min to investigate the bacterial growth and death characteristics. This graph demonstrates the viability and enzyme activity of the bacteria through 60 hr. It is seen that there is initially an increase in the light output, after peaking at about 350 min. The light output then declines steadily as the bacteria become less metabolically active or loses viability. This graph gives the standard response of the control bacteria.

Figure 5.12 shows the temporal UV light detected and the corresponding light output from the control sample and those samples treated with ozone. It is seen that the sample treated with ozone showed a rapid reduction in light output, simultaneously with the reduction in the UV irradiance and increase in ozone concentration.

The light output was monitored for a further three hrs in the chamber before the plates were removed and incubated and assessed for growth. There was no growth found after incubation. This indicated that total inactivation was achieved as predicted from the very low bioluminescence readings after the ozone treatment (see **Figure 5.11**).

Figure 5.13 shows the first 10 mins of the evolutionary development of the bioluminescent output from the bacteria (B), the corresponding normalised ozone concentration $[O_3]$, the integral of the ozone concentration ($\int[O_3]dt$) and the ratio of the RLO from the *E. coli lux* over the integral of the ozone concentration $B/\int[O_3]dt$. It is seen that the integral of the ozone concentration increased with the corresponding reduction in the UV light. The bioluminescence output, B, was relatively constant during the first three min, but began to reduce as the ozone concentration increased.

5.3.6 Imaging system results

Figure 5.14 shows the results of using the high sensitivity CCD imaging system on the treated and control plates; the ozone exposure was 5 min. Two sets of images are shown for the same plates. The top image is for a low gain and the bottom image is for high gain. For each image, the control is on the right and the treated plate is on the left. It is seen that in the low gain picture, the culture was emitting little light, whereas in the high gain picture, the control plate is saturated, but with slightly greater contrast on the treated plate.

5.3.7 Dark current measurements

The results of the light output from the agar and plate and the plate only are shown in **Figure 5.15** and **Figure 5.16** respectively. It is seen that the peak-peak light output for the agar and plate varied by only about 0.10 dB. Interestingly, there appeared to be an exponential decay for the RLO from the fluorescent plates with an estimated half-life of about 27 min. Because of the magnitude of the signals, this decay was not evident on the agar and plate measurements, where the much higher signal was attributed to the agar.

5.4 Discussion

5.4.1 Ozone measurement and detection

After calibration, the signal response from the in-house detector and amplifier system were comparable to the reference design of the MaCam UV meter. Strong UV absorption was observed during the introduction of O_3 into the path between the UV source and the detector. A steady decline of the UV irradiance from 549 $\mu W/cm^2$ to 20 $\mu W/cm^2$ was seen when the O_3 was at its highest to lowest concentration, respectively.

The ozone concentration was determined from the amount of UV irradiance absorped using Beer-Bouguer-Lambert's law. The O_3 concentration was found to be 3.986×10^{-6} g/cm^3. This value appeared to be comparable to other ozone generator specifications that were available. Unfortunately, a calibrated O_3 reference source was not available through the period of research.

Factors that governed the rate of O_3 dissociation were temperature, pressure [151;161] and other external factors such as ventilation and leakage. The amount of time O_3 will be effective in a chamber effects the efficacy of the ozone treatment. Results shown **Figure 5.8** indicated that the decay of O_3 was exponential. At lower concentrations ($0.04468\mu g/cm^3$) the decay rate was about 470 min/μg and the decay rate gradually increased. At 15 min, the O_3 level reached 3.986×10^{-6} g /cm^3 and it took about 224.5 min for the silicon detector to measure the same UV irradiance as when there was no O_3. The decay rate was calculated to be 56.32 min/μg.

5.4.2 Evaluation of bioluminescence detection using conventional bioluminometer and CCD camera

A conventional bioluminometer and a high resolution CCD camera were used to compare their performance to the real time monitoring system.

Ozone was also treated in liquid suspension by bubbling the ozone through the liquid and using the bioluminometer to assess the samples for growth. It was found that the system could only indicate killing down to just less than 1% or nearly three logs. It was shown that the light output was linear over this range by taking Total Viable Counts. From **Figure 5.9** the bioluminescence measurements indicated that the detection limit for the bioluminometer was between 10^4 and 10^5 cfu/ml. It was seen that the ozone effectively killed more than 99% of the bacteria in liquid solution after a 5, 10 and 15 min treatment. It was not possible to be more precise due to the limitations of the detecting system.

The high resolution CCD camera was used with the agar plates lawned with *E. coli* lux. The system, however, was not able to resolve the relatively large dynamic output between the treated and the control plates, where there was about a 5-6 log kill. It was clear that there were significant differences in the images obtained from the high sensitivity CCD camera for the treated and untreated samples. It was difficult, however, to optimise these images on the treated and untreated plates by varying the gain on the CCD camera without losing resolution. This was due to the large differences in the number of bacteria, and hence the light output, for the control and treated plates. At a gain level set for a good resolution for the untreated plates, the image for the treated plate was almost lost due to

the low bacterial numbers. On the other hand when the gain of the CCDs was increased to achieve a clear image of the treated plate, the image on the untreated plate was totally saturated. Because of the problem of image saturation and reduced resolution, numerical quantification on even digitised images was difficult. Hence, this assessment method only gave a quick indication of the viability, but it was not good at providing a large dynamic range for the amount of contamination used. It was not possible to view one plate at a time as an accurate comparison between the control and the treated plate could then not be made. The bioluminometer and the high resolution CCD camera are off line systems and are not able to be used within a real time situation. The dynamic response of both systems is low.

5.4.3 The real time bioluminescence detection system

A real time detection system has been fabricated that allowed the effect of ozone gas on *E. coli* lux to be monitored. The system comprised two chambers, an ozone monitoring system was housed in one chamber and the bioluminescent measurements were done in the other. This allowed the effect of the ozone on *E. coli* lux to be ascertained without the UV light adversely affecting the results. The ozone concentration was measured via the absorption about 255.3 nm (Band pass filter 255.3 ± 5.1nm), and from knowledge of the path length and the ozone absorption cross-section. The ozone concentration and its integral with respect to time were calculated and normalised. The light output from the bioluminescent *E. coli* lux was measured as a function of time both with and without ozone present. The light output was then compared for the untreated and treated samples to ascertain the difference in the samples. The untreated sample was assessed over 3000 min where the characteristic curves of the growth and death phase could be seen. This was contrasted with the treated samples where cell death or changes in metabolism were noted by the reduction in light output from the bacteria over a 200 min period. Data from the bioluminescent output and the ozone concentration were plotted to highlight the cell death processes.

The dark current was measured with either just a petri dish in the system or a petri dish and agar. It was found that there was an exponential decay of the light output from the plastic and agar combination with a half life of about 27 mins. The light output from the petri dish was only 0.15 dB compared to the bioluminescence signal which was typically 5

dB. The noise levels, however, were insignificant with a peak-peak variation of only 0.1 dB.

Figure 5.13 demonstrated the system performance with a temporal plot of the ratio of $B/\int[O_3]dt$. This ratio gives a measure of the phase lag between the light output from the bacteria and the ozone concentration. Initally, the value of B is close to 0, representing the maximum light output from the bacteria, as the bacteria become inactivated with the increasing ozone concentration, the value of B becomes more negative and the light output reduces. Additionally, in the early stage of the experiment, the fractional increase in the integral of the ozone concentration is significant which also contributes to the first minimum. B gradually decreases and $\int[O_3]dt$ increases, leading to the positive gradient.

It is seen that after about 3.5 min the UV irradiation had decreased appreciably and B is just beginning to reduce; which is indicative of the ozone concentration required to achieve an effect on the bacteria. Also $\int[O_3]dt$ is now beginning to increase appreciably. From this point the reduction in B is almost linear with the increase in the ozone concentration. After about 5 mins the gradient of the ratio becomes positive as $\int[O_3]dt$ has now increased considerably. It is interesting to note the significant phase difference between the UV spectrum and B.

From these graphs, it is apparent that the ozone produces a cumulative effect, which is particularly marked after a treatment time of about 4 min. There is a delay of about 30 sec between the reduction of B and UV then the gradient of the ratio $B/\int[O_3]dt$ becomes negative, indicating a relatively high increase in the reduction of B for a given increase in $\int[O_3]dt$. This trend continues until $\int[O_3]dt$ dominates for times greater than about 5 min.

The new instrument has proven to offer real time capability and enabled a comparison to be made between the light output from the bacteria as the ozone concentration is increased. Such a system will be a useful tool to gain further insight into the death kinetics of bacteria under different conditions. It is possible to vary the temperature and pressure of the chamber, and other physical variables to assess the mechanisms involved. The *E. coli* lux is a useful bacterial source against which the effect of different treatments can be rapidly evaluated. Knowing the relative response of these bacteria compared to other

bacterium will allow rapid development of decontamination systems, processes and methodologies.

5.5 Figures

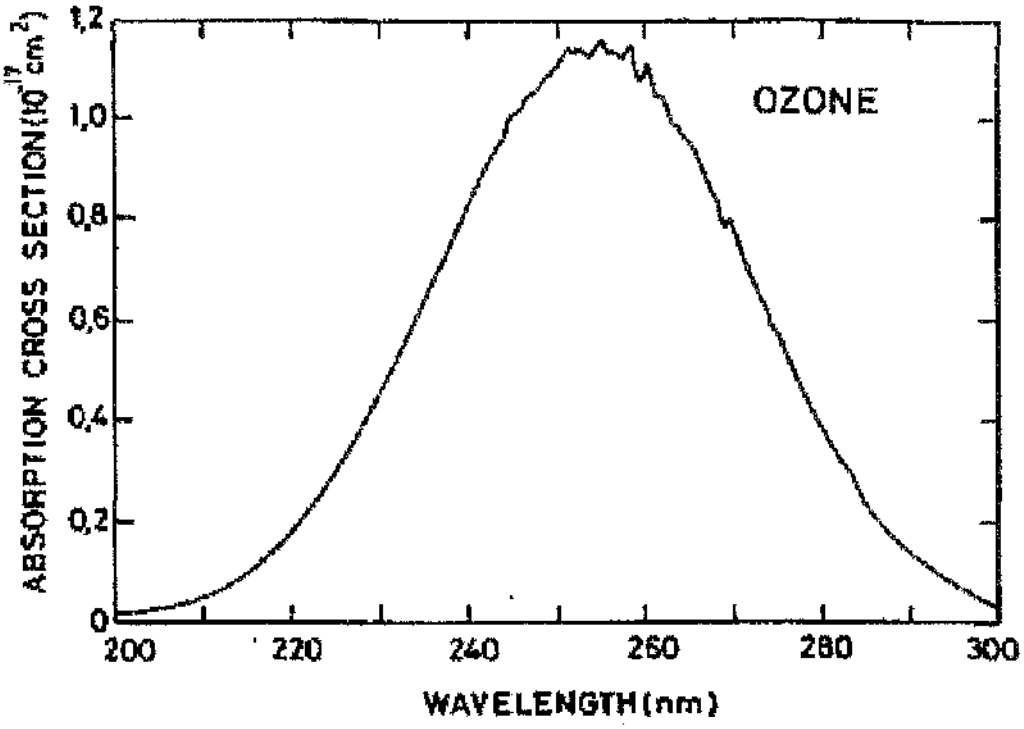


Figure 5.1 Absorption cross-section of ozone across the Hartley band [156]

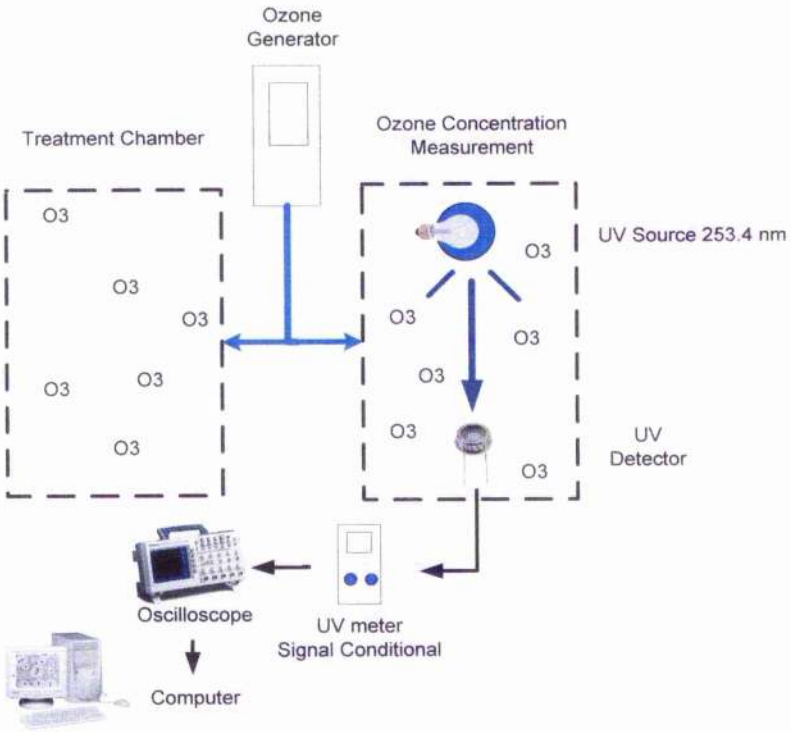


Figure 5.2 Experimental setup for ozone concentration, ozone decay rate and bioluminescence measurement

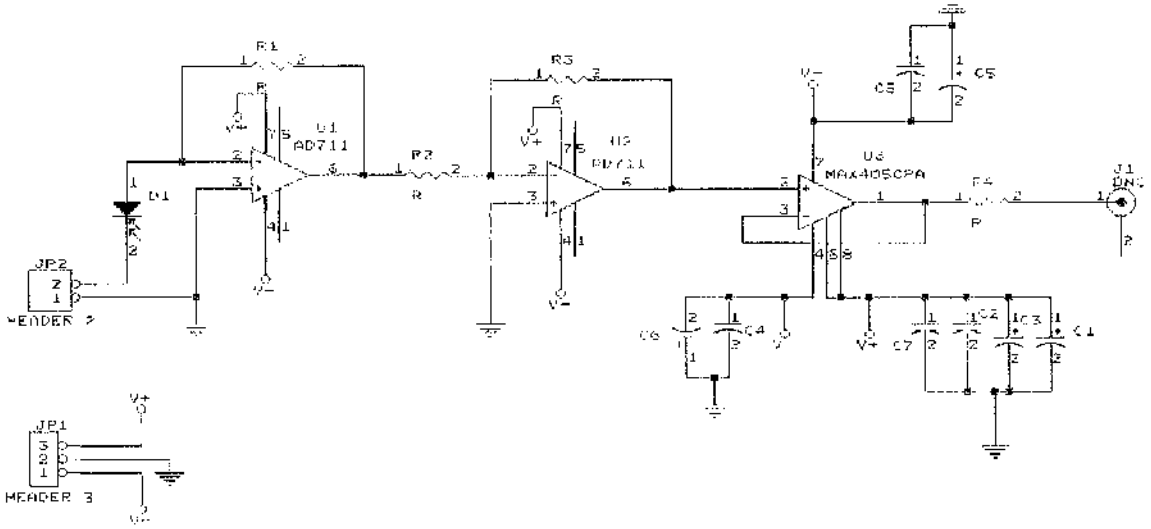


Figure 5.3 Circuit diagram of the UV meter

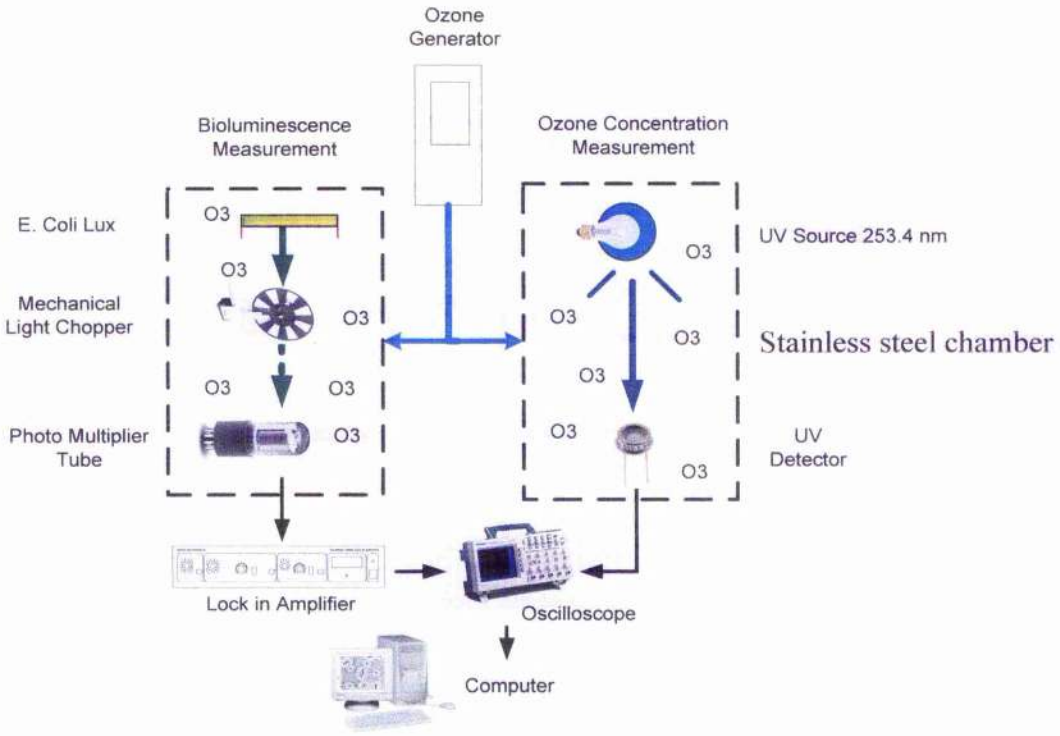


Figure 5.4 Real-time bioluminescent monitoring system for assessment of the effect of ozone on *Escherichia coli* lux.

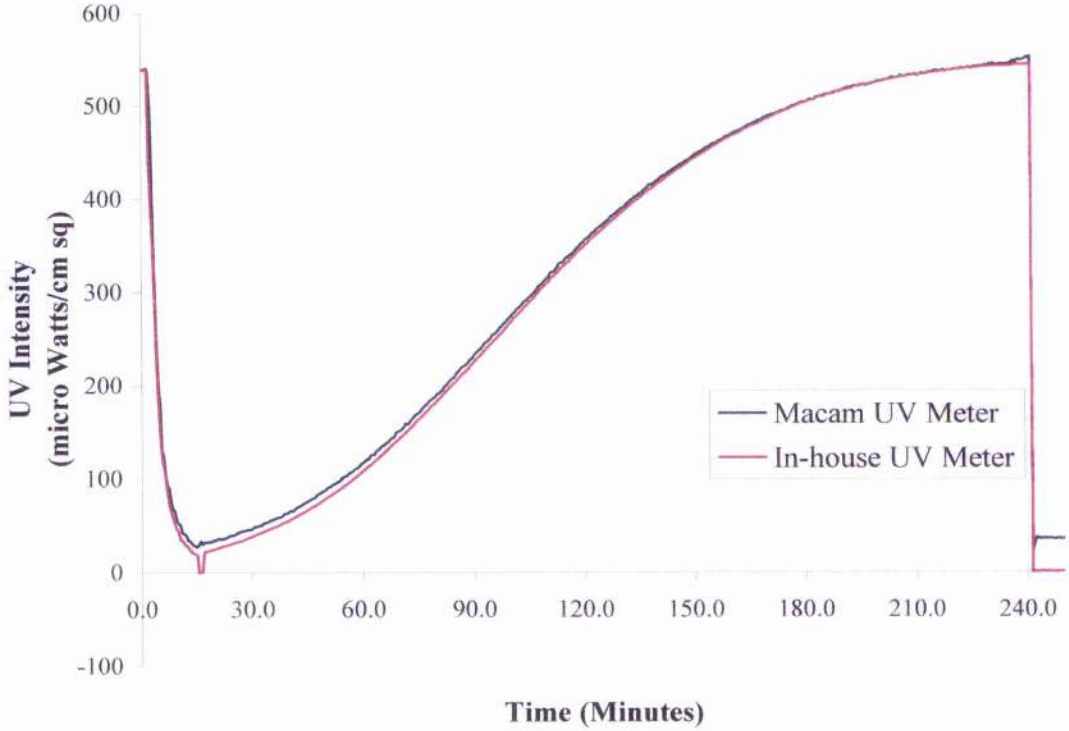


Figure 5.5 Measurement of manufactured UV meter reading compared to the reference reading from the MaCam meter.

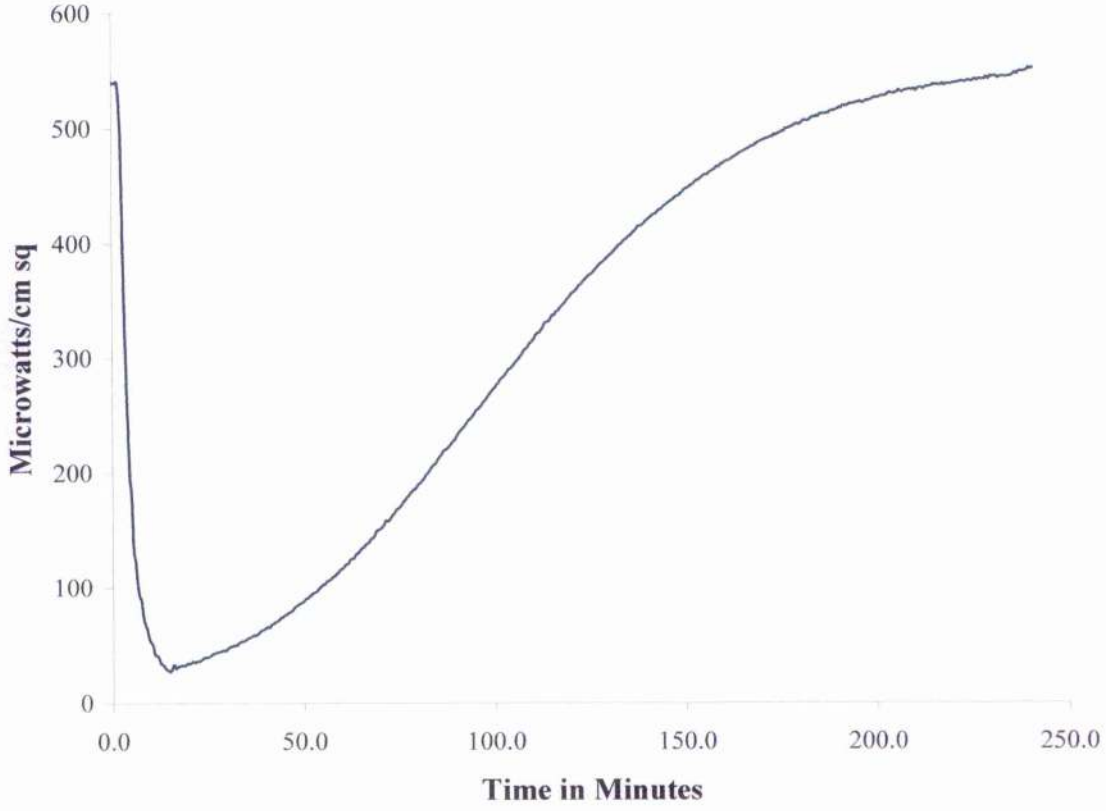


Figure 5.6 Ultraviolet light (253.7nm) absorption through ozone gas in the measuring chamber during ozone filling up to 15 min and subsequent decay of the ozone and increase in UV light detected after the ozone generator was turned off

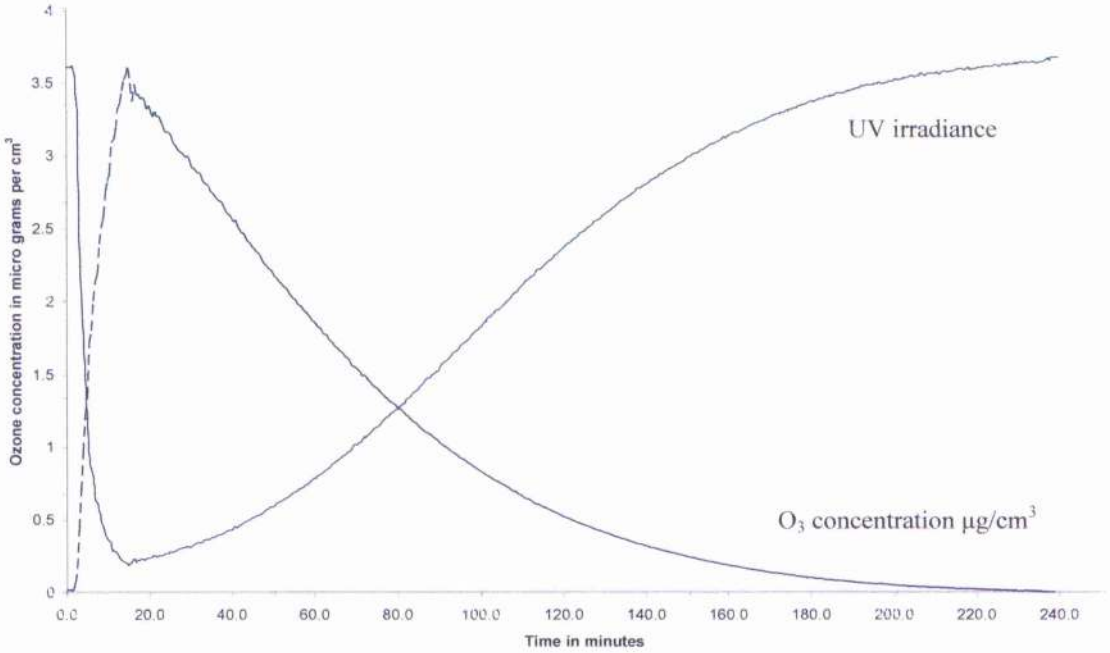


Figure 5.7 Calculated ozone concentration plotted against time

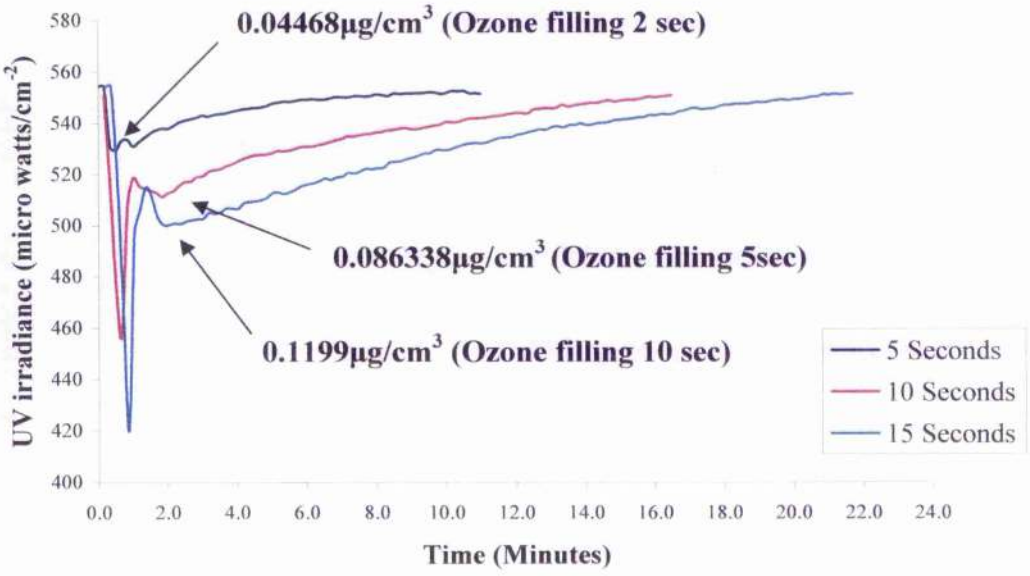


Figure 5.8 Ozone decay rate at different ozone concentrations

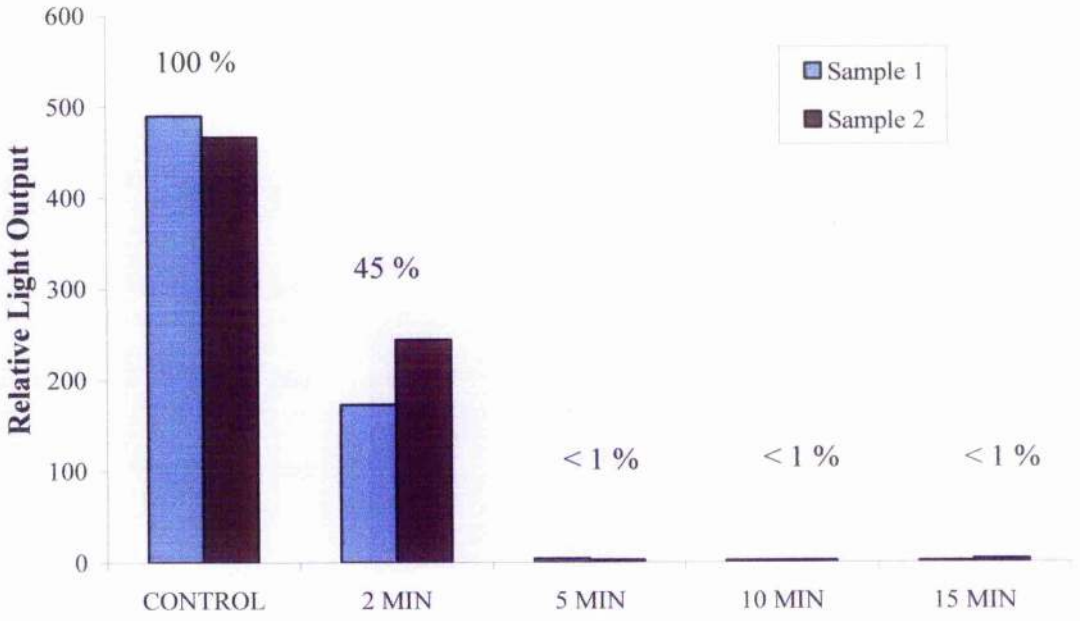


Figure 5.9 Viability results after applying ozone treatment to *E. coli* suspension (percentage reduction is shown above the exposure time)

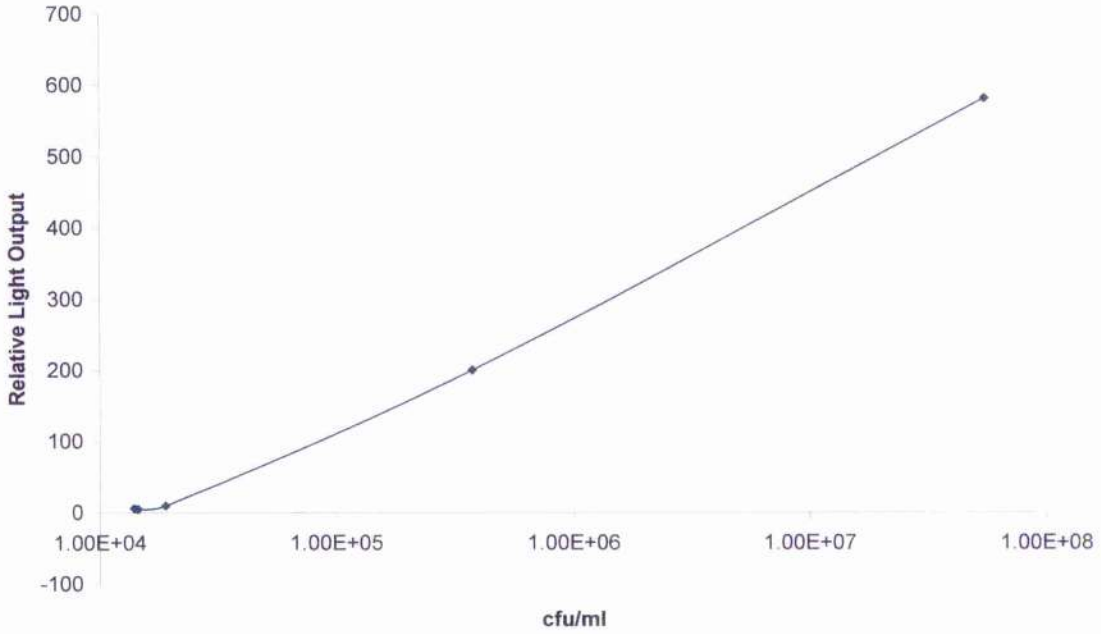


Figure 5.10 Relative light output as a function of the cfu/ml of *E. coli* suspension

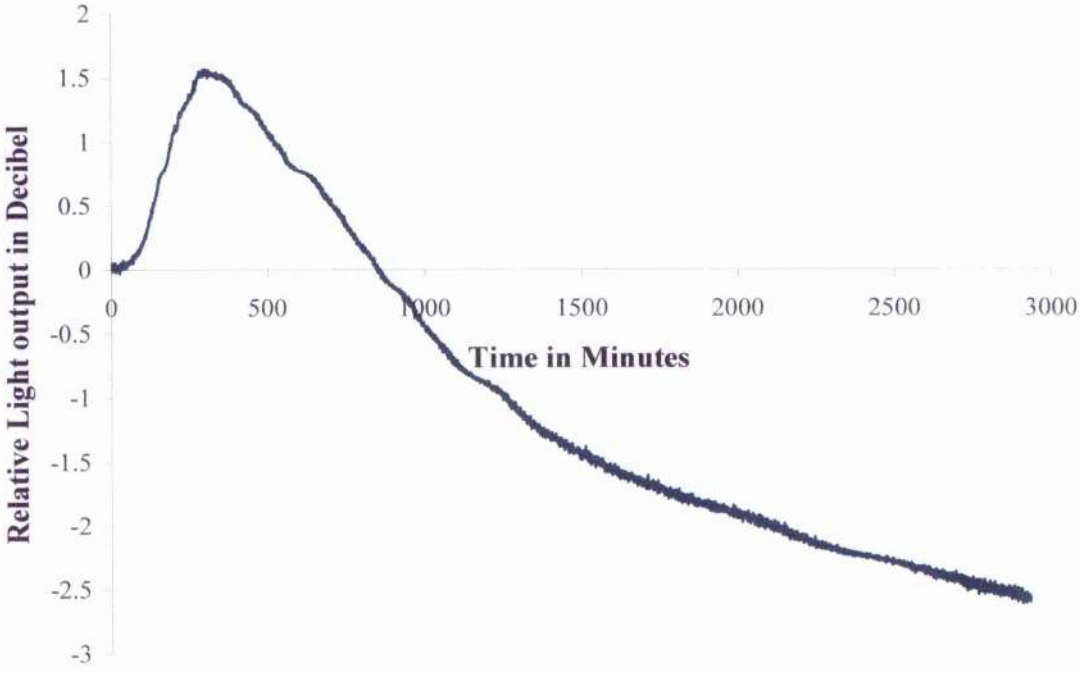


Figure 5.11 Real time viability results of untreated culture on agar plates.

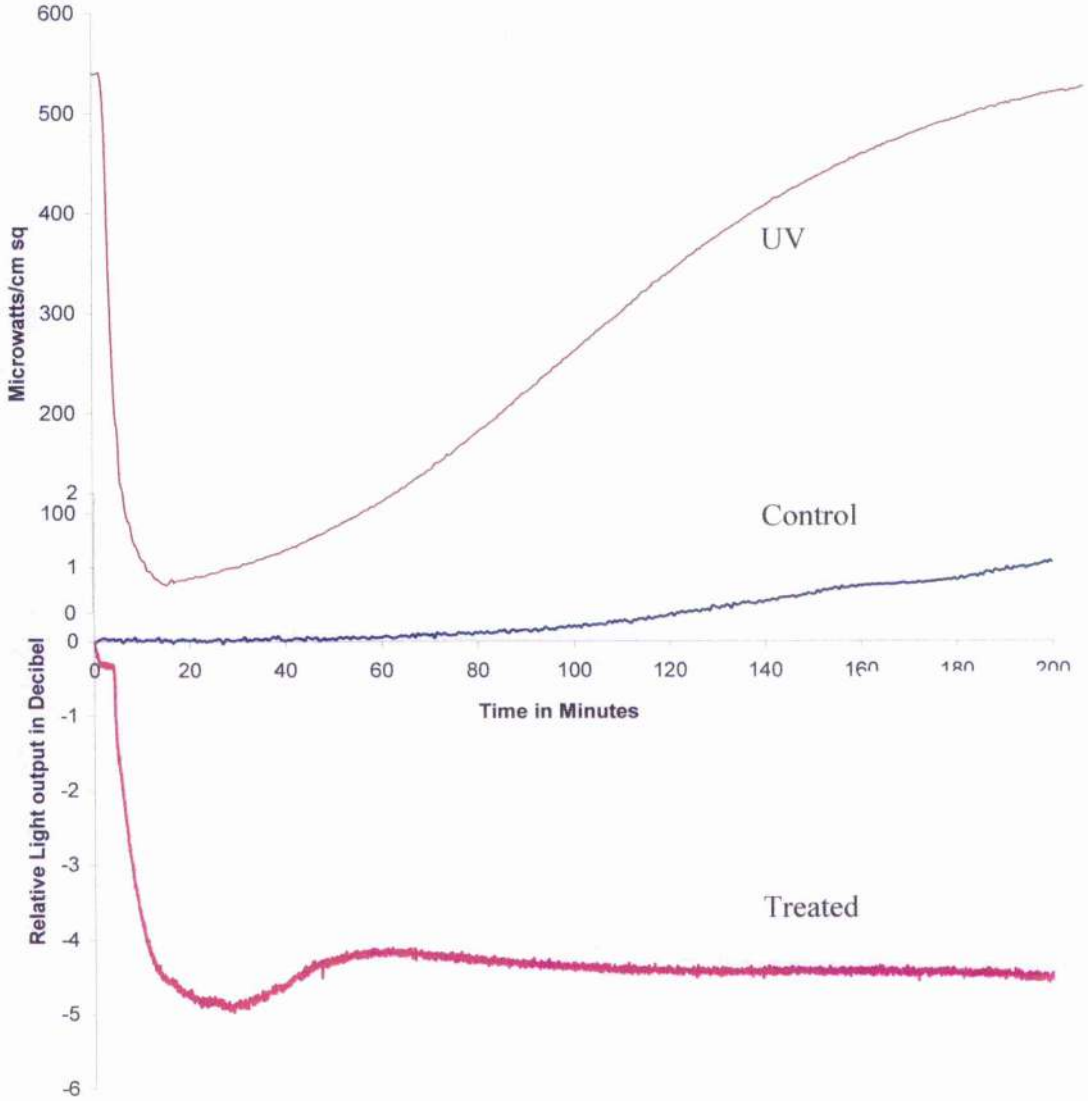


Figure 5.12 Relative light output, as a function of time, from lawned agar plates either treated or untreated with ozone and the corresponding UV measurements (μWcm^{-2}).

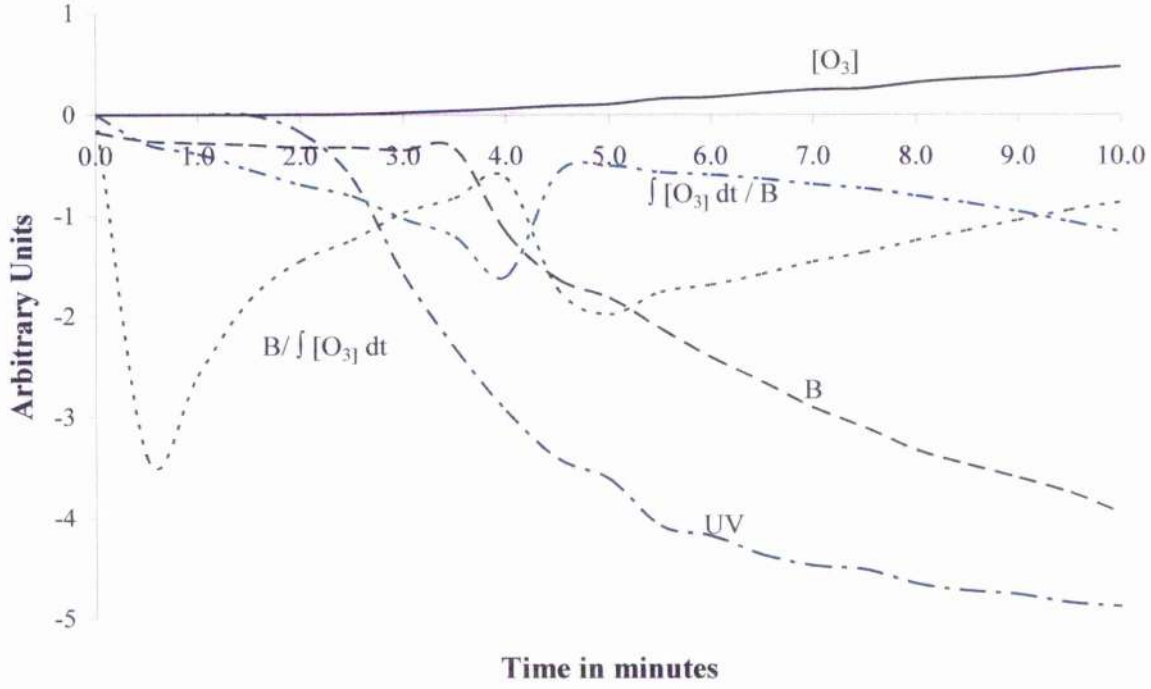


Figure 5.13 Evolutionary development of the bioluminescent output from the bacteria (B) corresponding normalised ozone concentration ($[O_3]$), the integral of the ozone concentration ($\int[O_3]dt$) and the ratio of the bioluminescent output over the integral of the ozone concentration ($B/\int[O_3]dt$)

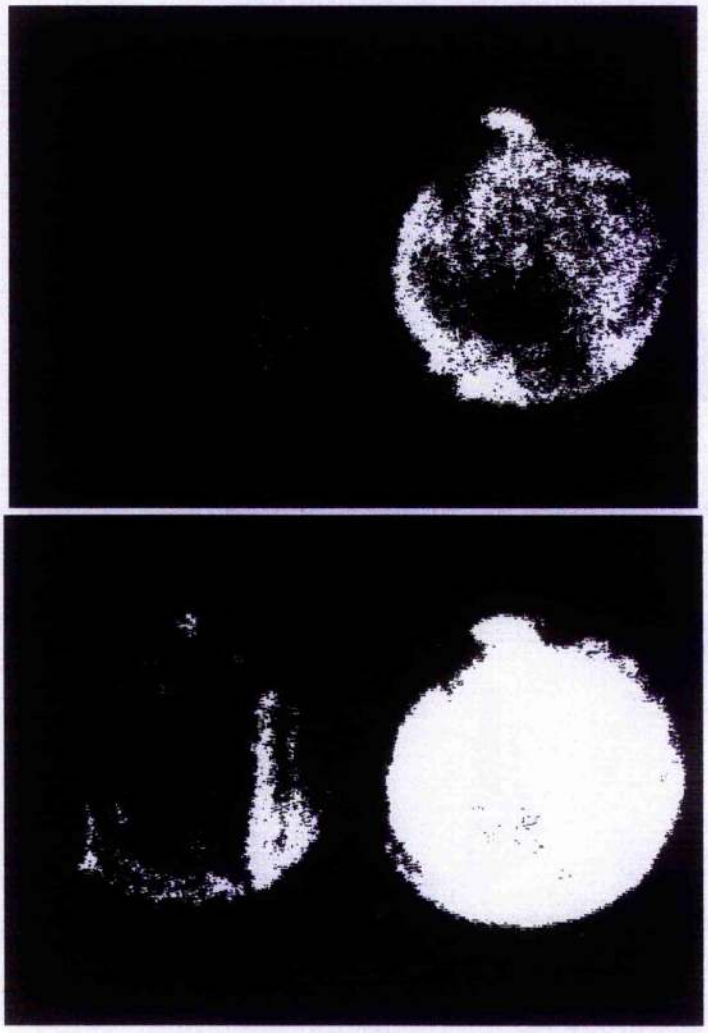


Figure 5.14 Shows the same set of luminous *Escherichia coli* under the high sensitive CCD camera at different gains. The top image was captured at low gain while the bottom image was taken at high gain. On both images, the left hand plate was treated with ozone for a duration of 5 min and the right hand agar plate was the control.

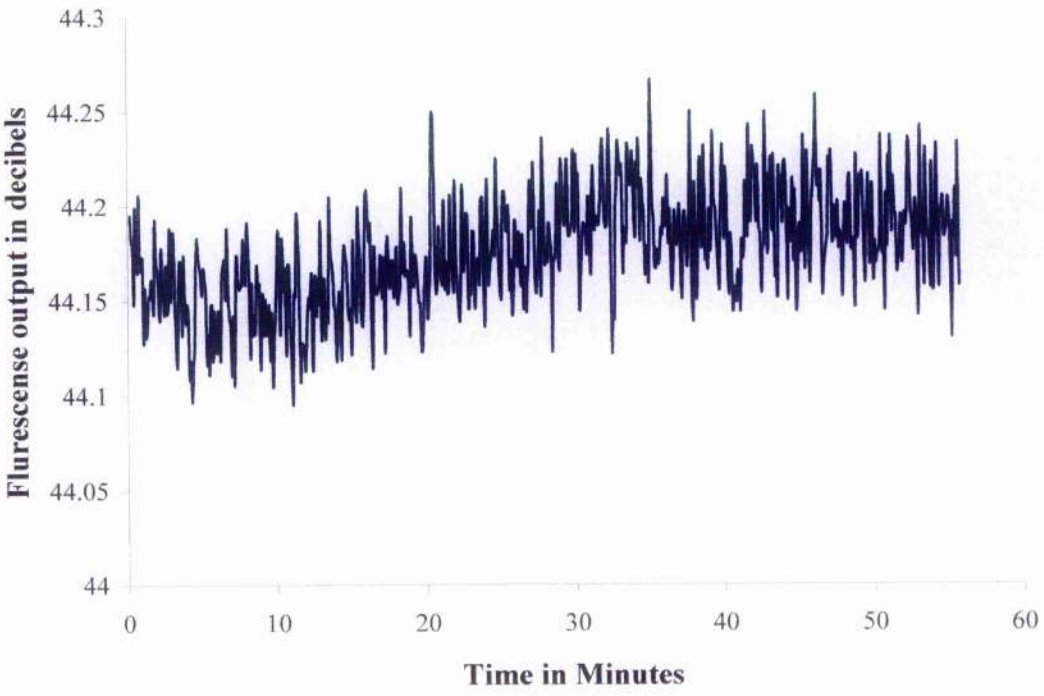


Figure 5.15 Result of agar fluorescence measurement

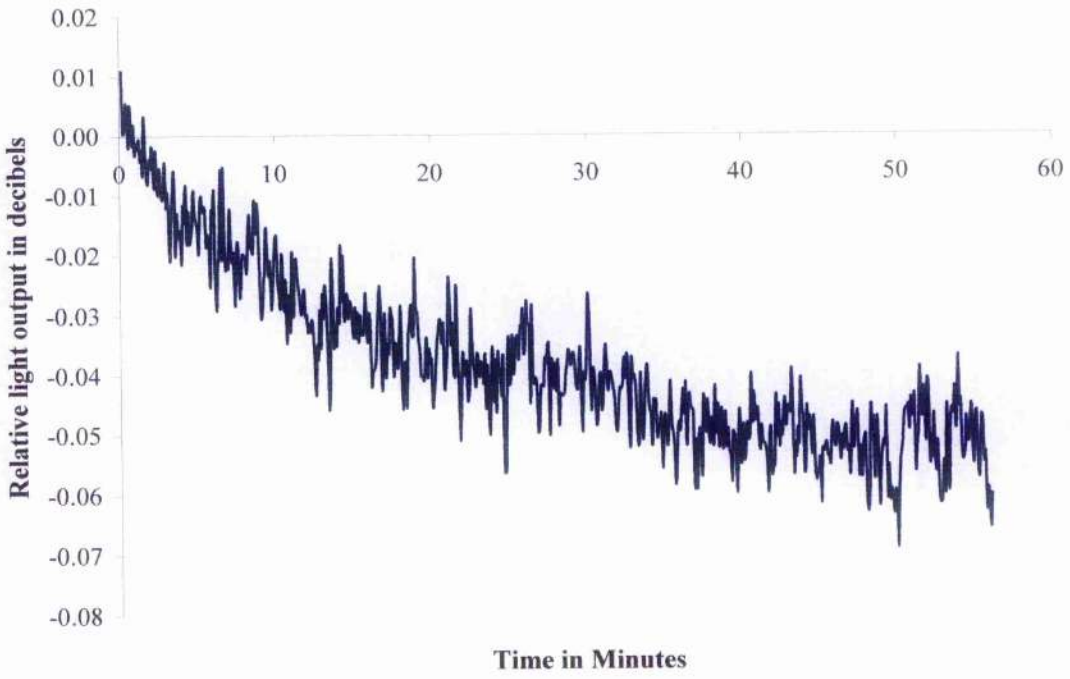


Figure 5.16 Plastic plate and agar temporal fluorescence measurements.

6.1 Introduction

According to the World Health Organisation, of a total of 52.2 million deaths in 1997, 17.3 million were attributed to microbial infections and parasitic diseases, of which diarrhoea was responsible for 2.5 million deaths [162]. Food-borne pathogens such as *Campylobacter, Salmonella, Escherichia coli* and *Staphylococcus aureus* can be disseminated from infected food such as fresh chicken or preparation surfaces. In the Republic of Ireland in 1999, *Campylobacter* was identified as the main cause of food poisoning [163]. Dufrenne *et al* [164] reported *Salmonella* and *Campylobacter* were the most likely mode of infection in raw poultry. Food poisoning can also result from food intoxication by enterotoxin producing organisms such as *Staphylococcus* [165]. With fish, one of the most common pathogenic bacteria is *Listeria monocytogenes*. In a 9 year study conducted in Switzerland between 1992 to 2000, 2053 samples were examined and of these 282 were found to be contaminated with *Listeria* and marinated fish had the highest prevalence [36].

In an attempt to guard against human exposure to contaminated food and reduce the number of food poisoning cases, a number of minimal processing techniques have been developed herein. This has included the use of laser, UV and microwave. Chemical processes such as ozone gas have also been reported to be used in the food processing industry [166]. The current work was conducted using ozonation to inactivate *Listeria monocytogenes, Salmonella typhimurium, Campylobacter jejuni, Staphylococcus aureus, Escherichia coli* and *Bacillus cereus* on agar plates, chicken and salmon. Bacteria were either plated or inoculated onto chicken or salmon, allowing a comparison between the viability of the different organisms on different substrates after exposure to ozone. The benchmark results were obtained from ozone treated plates. In order to evaluate the bacterial response, both Gram negative and Gram positive bacteria were chosen. Among the organisms used, 3 strains were Gram positive including a spore forming type and 3 were Gram negative. They were chosen because they contribute to cases of food contamination on salmon and chicken produce. *Escherichia coli* was used as a reference bacterium and is commonly used as an indicator of faecal contamination in the food and

water industries.

Although sporing organisms like *Bacillus cereus* and *Bacillus anthracis* are not usually found on chicken and smoked salmon, it was useful to determine the effect of ozonation on such organisms that have a robust cell structure and can retain their viability in harsh conditions. *Bacillus anthracis* is known as a potential biological weapon and its use was prohibited in current experiments because of associated dangers. However, *Bacillus cereus* has similar characteristics to *Bacillus anthracis*. *Bacillus cereus* produces endospores, which are much tougher than their vegetative cells. These spores have a very resistant structure that is able to withstand exposures to temperature as high as 80°C for long periods. Also, the spores are more resistant to various other forms of treatments; for example radiation, heat and chemicals. They are difficult to kill even in harsh conditions. The effectiveness of the ozone treatment was evaluated by comparing the viability before and after ozone treatment of *Bacillus cereus* and the other, non-sporing bacteria. The results obtained from the ozone treatment of *Bacillus cereus* may be useful for the evaluation of ozone as a bactericidal agent for *Bacillus anthracis*.

In the concluding part of the ozone treatment experiments, the effect of ozone treatment on the quality attributes of chicken and salmon were considered. The effect of ozone on the sample's colour and aesthetic qualities were determined.

6.2 Medium and culture preparation

6.2.1 Nutrient agar preparation

28g of nutrient agar powder medium (CM55) from Oxiod was added to 1 litre of distilled water and boiled until dissolve, before placing in an autoclave for 15 min at a temperature range between 121 to 124 °C. The sterile molten agar was allowed to cool in a 50°C water bath, and transferred into a sterile cabinet cupboard then aseptically into petri dishes. A bunsen burner was used to remove any air bubbles on the surface after the agar was poured.

6.2.2 Nutrient broth preparation

The nutrient broth was prepared in a similar manner to the agar. The broth powder was suspended in distilled water, 13g of broth powder to 1 litre. The suspension was boiled

until the powder dissolved and the suspension was autoclaved for 15 min at a temperature between 121 and 124°C. The nutrient broth was stored at room temperature.

To support the growth of the desired bacteria and inhibit the growth of others, a number of different selective media were prepared. The protocols for the preparation of these media are described below.

6.2.3 Selective medium for *Campylobacter jejuni*

Based on the manufacturer's directions, 500 ml of blood agar base No: 2 (Oxoid) was made and sterilized. 2 ml of sterile distilled water was added aseptically to a vial of *Campylobacter* growth supplement (SR 084E, Oxoid) and the contents were added to the medium at 45°C. The contents of a vial of *Campylobacter* selective media (SR098E, Oxoid) and defibrinated sheep's blood 5% v/v final concentration were also added to the medium. The medium was mixed well and 25 ml was pipetted into each petri dish and allowed to dry.

6.2.4 Selective medium for *Listeria monocytogenes*

18 g of powder of *Listeria* enrichment broth base (CM862, Oxoid) and 27.75g of powder *Listeria* selective agar base (CM856, Oxoid) was suspended in 500 ml of distilled water and boiled until completely dissolved. The medium was then sterilised at 121°C for 15 min. The contents of a vial of *Listeria* selective enrichment supplement (SR 141E) and a vial of Oxford *Listeria* selective supplement (Merck) were added aseptically to the medium at 45°C. They were mixed well and 25 ml of the medium was pipetted into each petri dish and allowed to dry.

6.2.5 Selective medium for *Salmonella typhimurium*

Mannitol selenite broth base powder (CM 3998, Oxoid, 19g) was mixed in 1 litre of distilled water and boiled until completely dissolve. After sterilization and cooling down, Bismuth sulphate agar powder (CM 201, Oxoid, 20g) was dissolved in the broth and heated gently with frequent agitation until the medium just began to boil and was simmered for 30 sec to dissolve the agar. 25 ml of the medium was pipetted into each and allowed to dry.

6.2.6 Selective medium for *Staphylococcus aureus*

Based on the manufacturer's directions, 1 litre of the Baird-Parker agar (CM275, Oxoid) was made. 58 g of Baird-Parker agar power were suspended in 1 litre of distilled water. The mixture was autoclaved for 15 min at 121 °C and then cooled to 45-50 °C. 50 ml of Egg-yolk Tellurite Emulsion and 50 mg sulfamethazine were added before being transfer into the petri dished. .25 ml of the medium was pipetted aseptically into each plate and allowed to dry.

6.2.7 Preparation of culture media and culture for ozone treatment on plates

Salmonella typhimurium 509*, *Listeria monocytogenes R479a*, *Staphylococcus aureus*, and *Escherichia coli

One colony was pipetted into a Universal bottle containing 20 ml TSB (BO0124M, Oxoid, Tryptone soy broth). The bottle was incubated at 37 °C for 18 hr on a shaker at 200 RPM. The suspension was then centrifuged at 3000g for 5 min in Heraeus Sepatech Megafuge 1.0. The supernate was removed and the cells were resuspended in normal saline. The OD was measured at 600 nm against saline and a colony count was made. The concentration of the culture and the measured optical density before plating are tabulated in **Table 6.1**. For the ozone treatment of inoculated plates, the plate cultures were prepared by pipetting 100 µl of the suspension onto the agar surface. The suspension was spread evenly over the agar and left to dry for 15 min on the bench before treatment. In each ozone treatment experiment, 7 plates were required, 3 plates were used as control, and the rest of the plates were placed in the treatment chamber as shown in **Figure 6.1**.

Campylobacter jejuni

14 g of powder of Brucella broth (Difco) was suspended in 500 ml of distilled water and boiled until it was completely dissolved. The broth was then sterilised at 121°C for 15 min. 2 ml of distilled water was added aseptically to a vial of *Campylobacter* growth supplement (SR 084E, Oxoid) and the contents were added to the broth at 45°C. The broth was cooled and stored at 4 °C and used for making the bacterial suspension. 1 to 2 colonies of the bacterium were pipetted into a 100 ml sterile bottle containing 50 ml of the broth. The suspension was then placed into an anaerobic jar under microaerophilic conditions (80% nitrogen, 15% co_2 and 5% oxygen) for 36 h. The culture was then centrifuged at 4000 RPM for 7 min. The supernate was removed and the cells were

resuspended into normal saline. Colony counts were taken and are tabulated in **Table 6.1**. 100 µl of the suspension was pipetted onto the *Campylobacter* selective agar surface and spread. The plates were left to dry for 15 min on the bench before treatment. Again, for each set of treatments, 7 plates were prepared, 3 for control and 4 for treatment.

Bacillus cereus

From stock cultures or nutrient agar slopes, *Bacillus cereus* was inoculated into a 100 ml Duran bottle filled with sterile nutrient broth. The culture was incubated for 24 hrs at 37 °C to give a good yield of spores. 25 ml of culture was then transferred into a 30 ml Universal bottle and centrifuged at 3000g in a Heraeus Sepatech Megafuge 1.0, for 5 min. The pellet was washed 3 times in sterile distilled water then resuspended with 25 ml sterile distilled water. Finally, the cell suspension was then placed in an 80°C water bath for 10 min to inactivate any vegetative cells that may have been present, to leave a viable spore suspension. The presence of spores was confirmed by Gram-staining. During Gram-staining the chemical composition of the cell wall and the colour combine, leaving a purple colour stain behind the Gram-positive vegetative cells while the spores remain unstained. Under a microscope at about 100x magnification, unstained spores can be differentiated from the purple vegetative cells.

Table 6.1 Colony counts from bacterial culture before ozonation

Bacterium	Gram	Colony Counts cfu/ml
Salmonella typhimurium 509	-ve	8×10^7
Listeria monocytogenes R479a	+ve	6×10^7
Staphylococcus aureus	+ve	4×10^7
Escherichia coli	-ve	4×10^7
Campylobacter jejuni	-ve	2.4×10^7
Bacillus cereus	+ve	0.5×10^7

6.3 Effects of ozone treatment on *Bacillus cereus*

6.3.1 Experimental protocol

A series of 10-fold dilutions were made to determine the viable count of the *Bacillus cereus* spore suspension. For each dilution, 2 x 20 µl spots were plated onto a nutrient agar plate. By averaging the cfu counts per level of dilution, the dilution was calculated to be about 0.5 x 10^7cfu/ml.

8 x 20 µl spots of *Bacillus cereus* spore suspension were lawned onto each of 4 agar plates. Another 4 plates were inoculated with 40 µl of *Bacillus cereus* and spread evenly over the surface of the agar with a sterile glass spreader. One dish from each group of the 4 plates was used as the control, and the remaining 6 were treated with ozone.

After connecting the ozone generator to a hose system as shown in Chapter 5 **Figure 5.4**, all 6 petri dishes were placed carefully in the chamber and the lid securely replaced. The plates were laid out in such a way that 4 plates were arranged in a square and the remaining 2 plates were placed on top, with each plate of the second layer resting on 2 plates on the bottom layer. The integrity of the vacuum seal of the chamber was checked. The oxygen flow into the ozone generator was set to 100% and the oxygen inlet pressure was regulated to 5 Bar. The ozone generator was switched on for 15 min to fill the hoses and chamber with ozone. Following treatment, the plates were incubated for 18 hr at 37°C before growth of surviving bacteria was accessed.

6.4 Ozone treatment of *Salmonella typhimurium 509, Listerial monocytogenes R479a, Staphylococcus aureus, and Escherichia coli* on agar plates

6.4.1 Ozonation experiment protocol

The experimental ozonation setup was identical to that shown in Chapter 5, section 5.2.7 . **Figure 5.4** shows a schematic of the bioluminescence monitoring system. In this experiment bioluminescence measurements were not done. The experiment was carried out in two chambers, namely the measurement and the treatment chamber. Prior to

treatment, the UV lamp used for the ozone measurement was warmed up for 30 min to minimise variations of the UV irradiance. Both chambers were vented with air before the samples were carefully placed into the treatment chamber. With the samples in position, (see **Figure 6.1**), the lids were immediately sealed. The ozone generator oxygen inlet flow-rate was regulated to 100 litres/hour, just before the main switch was turned on. Oxygen passed through the corona in the generator, rapidly forming ozone gas. The ozone was fed into a common pipe connecting both chambers. A computer recorded the temperature and the drop in UV irradiance as the ozone concentration increased during the entire ozonation process. After the required treatment time, the ozone generator, was switched off. Samples were immediately removed from the chamber and both chambers were vented with a suction fan. Subsequent experiments were conducted identically, varying only the treatment duration. This ensured that the same ozone concentration was used for each experiment.

6.5 Ozonation of food samples (smoked salmon, chicken skin and chicken breast)

To evaluate the effectiveness of ozonation of food samples, a controlled number of bacteria were inoculated onto the samples and they were then subjected to the same protocol as used for the ozonation experiments conducted on agar plates. However, working with actual food had the added complication of other bacteria being already present on the food, and representing the background count. Selective media were required to isolate the bacterium used for treatment in order to determine the colony counts. In this experiment, two bacteria were used. *Salmonella typhimurium* was inoculated onto smoked salmon and *Campylobacter jejuni* was inoculated onto chicken skin and breast.

6.5.1 Experimental protocol

Both *Salmonella typhimurium* and *Campylobacter jejuni* were prepared using the same protocol to that of the ozonation experiments on agar plates. However, selective media were used instead to isolate and allow growth of specific bacteria from the chicken and salmon samples.

Ozonation of chicken breast

Packets of fresh chicken breasts were purchased from a local supermarket and cut with a sterile knife into 4 portions of approximately 50 x 60 mm^2 on a sterile petri dish. Each portion was transferred onto another sterile petri dish. 2 of these portions were used as controls and the other 2 were treated with ozone. Before treatment, all portions were weighed and labelled. The chicken portions from the first breast were labelled C1 and T1 and the other labelled C2 and T2 respectively, breasts with the prefix C denote control and T denotes the treated specimen.

The apparatus and setup used to generate the ozone was identical to that previously described (See **Figure 5.4**). The specimens to be treated were enclosed in a stainless steel chamber, with a perspex cover bolted onto its top with a rubber gasket seal to ensure the vacuum integrity.

With the chicken specimen inside the chamber, the ozone generator was left on for 15 min. Immediately after treatment, the vacuum chamber was vented through a heater to destroy the ozone and the specimens were removed. Together with the controls, all 4 specimens were refrigerated for 8 hrs at 4°C. Observations of the colour of the chicken were recorded after 8 hrs before viable counting was done.

All 4 specimens were transferred into a sterile 100 ml duran bottle with 40 ml of nutrient broth and placed into a mechanical shaker for 1 hour at 200 rpm, the room temperature ranged between 15 to 20°C. A series of 10-fold dilutions in distilled water was made to determine the viable counts from all 4 specimens. The first dilution was carried out by pipetting 2 ml from 40ml of nutrient broth into a bijoue with 18 ml of sterile distilled water. Subsequently, 8 dilutions were carried out by pipetting 2 ml from the previous dilution into bijou bottles. On nutrient agar plates, 2 x 20 µl spots were plated out from each dilution and incubated for 18 hrs at 37°C before total viable counts were taken.

Ozonation of smoked salmon

A total of 11 smoked salmon samples were prepared; 3 were used as control, 4 samples were treated with ozone for 10 min and the rest of the samples were treated for 15 min. The smoked salmon was purchased from "Safeway" supermarket and cut to size with a size 7 cork-borer. The samples were handled with sterile tweezers at all times; the sterile

tweezers and the cork-borer were flamed to maintain sterility between each sample preparation sequence. Each slice of salmon measured about 1 cm in diameter and was transfered onto sterile petri-dishes and weighed. These samples were inoculated with 10 µl of *Listeria monocytogenes* at the concentration of 6 x 10^7 cfu/ml. Ozonation was carried out in the same way as described for earlier experiments. After treatment, the samples were transferred into plastic Universal bottles, and filled with 9 ml of *Listeria* enrichment broth. Using a blender, the samples were homogenised before plating 100 µl onto *Salmonella typhimurium* selective media plates. Colony counts were obtained after incubation for 48 hr.

Ozonation of chicken skin

Similarly as with the smoked salmon experiments, 3 x 12 samples were prepared using a size 8 cork-borer. The samples were about 1 cm in diameter, were weighed and transferred onto sterile petri dishes for treatment. In total, 3 sets of experiments were carried out with chicken skin samples, each with different organisms. The organisms used were *Salmonella typhimurium, Staphylococcus aureus and Campylobacter jejuni*. The cultures were prepared using the protocols described in **Section 6.2.7** . The concentration of the suspensions prepared were 8 x 10^7 cfu/ml for *Salmonella typhimurium*, 4 x 10^7 cfu/ml for *Staphylococcus aureus* and 2.4 x 10^7 cfu/ml for *Campylobacter jejuni*. 10 µl of inoculum was used on each sample. For each set of experiments, 12 samples were inoculated with 10 µl of the same organism. 4 samples were used as controls and the rest of the samples were equally divided for ozone treatments at 3 different durations: 5, 10 and 15 min, with the same fill time.

After ozonation, all the samples were transferred into a plastic universal bottle containing 9 ml brucella enrichment broth and shaken at 200 rpm for 15 min, before plating 100 µl suspension onto *Campylobacter jejuni* selective media. These plates were incubated at 41°C for 36 hrs in an anerobic jar, under microaerophilic conditions with carbon dioxide before the colony counts were taken.

6.6 Results

6.6.1 Ozone treatment of *Bacillus cereus* on plates

By comparing the treated plates with that of the control, it was seen that after 18 hrs of incubation the treated plates showed no sign of growth, 100% killing was evident. The control plate indicated growth on the spots. Total inactivation was achieved after 15 min of ozone treatment. The plates were incubated at 37°C for a further 24 hrs. Again, no colonies were found on the treated plates.

Figure 6.2 shows examples of the control and treated plates after ozone treatment for 15 min and incubation for 24 hrs. Here, 8 spots of 20 µl of inoculum were placed onto each plate. **Figure 6.3** shows the treatment effect after lawning the plate with 40 µl of inoculum. After treatment and incubation for 24 hr, no growth was evident on the treated plate, whilst the control indicated growth.

6.6.2 Gram-staining

Gram-staining was done on the spore suspension for the ozonation experiments to confirm that there were spores present. The results obtained from Gram-staining showed that there were spores present after 24 hrs. This also showed that spores were present at the time of ozone treatment and the treatment was effective on spores as well since no colonies were found after ozone treatment. As the population of cells was not synchronous, it was expected to find spores developed to different stages. After Gram-staining, visual examination under the microscope at about 1000x (100x objective, 10x eyepiece), both spores and vegetative cells were found.

6.6.3 Ozone treatment of bacteria on plates

Results for ozone treatment of *Salmonella* typhimurium 509, *Listeria monocytogenes* R479a, *Staphylococcus aureus* and *Escherichia coli* on agar plates.

Figure 6.5 shows the viability of the treated organisms (cfu/100µl) after different periods of ozone treatment. There is a general trend showing viability reduces as the ozone treatment increases; this was true for both Gram-negative and Gram-positive organisms. It

is evident that increasing the ozone treatment reduced the cell viability. Additionally, there was a considerable difference in the effect of the ozone depending on where the plate was placed relative to the ozone inlet into the chamber. The plates were placed into the chamber in accordance with **Figure 6.1**, for assessment of the effect of the ozone spatial distribution. It was seen that the greatest effect was on plate 4 (closest to the ozone inlet) and the least effect was on plate 1 (furthest from the ozone inlet). **Figure 6.6** shows that in the case of treating the plate with ozone for short durations, the in-flow of gas was unevenly distributed, which resulted in the uneven growth on the treated plates for shorter treatment times. As the treatment time was increased, however, the effect of the uneven distribution was significantly reduced.

6.6.4 Ozonation results on food samples

Ozonation of chicken breast

From the suspension obtained by washing the chicken sample in sterile distilled water, colony counts were carried out and the bacterial concentration in 1 gm of sample was determined. **Figure 6.8** shows the effect of ozonation on chicken breast. Both (C1) and (C2) were minimally processed by ozone showing up to 0.48 log reduction. Other important attributes were also investigated. **Figure 6.9** compares the colour of the chicken breasts before and after ozone treatment. In both samples, no colour changes were observed after treatment and refrigeration for 8 hrs.

Ozonation of smoked salmon

Figure 6.10, shows the viability of *Listeria* before and after ozonation on smoked salmon, recovered, and incubated. After 15 min of ozone treatment half a log reduction was achieved.

Ozonation of chicken skin

Figure 6.11 shows the viability results for *Salmonella, Staphylococcus aureus, Campylobacter jejuni, and Listeria* after inoculation onto chicken and before and after the ozone treatment. Generally for all the bacteria, the increase in ozone exposure reduces their viability.

6.7 Discussion

6.7.1 Ozone treatment on *Bacillus cereus*

No colonies of *Bacillus cereus* were found after 15 min of ozonation and up to 24 hrs of incubation. To prove that the treatment was effective on the endospore, Gram-staining was done on the original culture and it was confirmed that the treated culture was sporing.

The image quality could be improved by using endospore staining methods. One method of staining uses malachite green which stains the spores a different colour from the vegetative cells. Thus, improving the image quality and aiding spore identification and enumeration.

6.7.2 Ozone treatment of *Salmonella typhimurium 509, Listeria monocytogenes R479a, Staphylococcus aureus*, and *Escherichia coli* on agar plates

All the results consistently showed ozone was effective against all strains of the bacteria tested during the experiment. For all the bacteria, viability reduced with increased treatment time. The best results indicated that after 15 min of treatment, the log reduction of the 3 Gram-negative organisms were 3.7 for *Salmonella typhimurium*, 3.6 for *Escherichia coli* and, 3.8 for *Campylobacter jejuni*. The Gram positive organisms showed a 2.9 log reduction for *Listeria monocytogenes* and 3.3 log for *Staphylococcus aureus* after ozonation.

The Gram positive organisms were only slightly more resistant to ozonation than the Gram negative. Looking at the spatial distribution of the colonies growing on the plates for all bacteria treated at 2 and 15 min, it was observed that the ozone distribution was not homogenous at lower gas concentrations and short treatment times. In all experiments carried out at 2 min, plates Q2 and Q4, which were positioned closer to the inlet of ozone gas, appeared to have a larger area of clearing than plates Q1 and Q3, which were located at the far end of the chamber. This effect of the non-homogenous gas concentration appeared to be less significant at higher concentrations and for longer treatment periods; where the gas concentration was believed to be more uniform throughout the chamber.

6.7.3 Ozone treatment of chicken breast

No colour or textural changes were observed on the chicken breast after treatment. The samples were shaken for 30 min in a conical flask, in sterile distilled water before the suspensions were diluted and plated onto nutrient agar plates. **Figure 6.8** showed that the

treatment induced a **0.43 - 0.48** log reduction on the overall counts of non-isolated bacteria present on the chicken breast.

Two different media were used for the cultures, it was discovered that the bacteria that survived the ozone treatment grew much better in the blood culture medium than on nutrient agar. The treatment was effective as it achieved almost 0.5 log reduction, consistently on both specimens. However, comparing the results obtained to that of *Bacillus cereus* cultured on plates that were treated under the same condition where total inactivation was achieved, the treatment on chicken was comparatively less efficient. One possible factor was that the *Bacillus cereus* experiments were on nutrient agar plates, with the culture evenly spread on a flat surface. In the chicken experiment, however, only one surface of the chicken specimen was exposed to the treatment, while the other surface was in contact with the petri dish. The reduction in viability may have been greater if the breasts were suspended or rotated and not placed in contact with the surface, where that part of the breast was not exposed to the ozone. Additionally, the surface of chicken is rough and bacteria may remain within crevices on the chicken.

6.7.4 Ozone treatment of chicken and salmon

In general, the reduction in viability obtained on the food samples were less significant than those on agar plates. For the salmon samples inoculated with *Listeria*, there was 0.9 D-value reduction, see **Figure 6.10**. This was by far less effective then ozone treatment of *Listeria* on plates (see **Figure 6.5**) where the ozone treatment induce 2 – 2.5 D-value.

Figure 6.11 shows a summary of the ozonation on chicken samples for all 4 organisms. With *Salmonella* inoculated on chicken skin, the best result produced only 0.37 D-value reduction after treatment. The most effective results for the food samples were from ozone treatment of chicken skin, inoculated with *Campylobacter jejuni,* where a log reduction of more then 1.5 was achieved. Although all ozone treatment has an effect on both Gram positive and negative organisms, the log reduction found on treating chicken samples was much lower than that obtained from treating the same organism on plates.

6.8 Figures

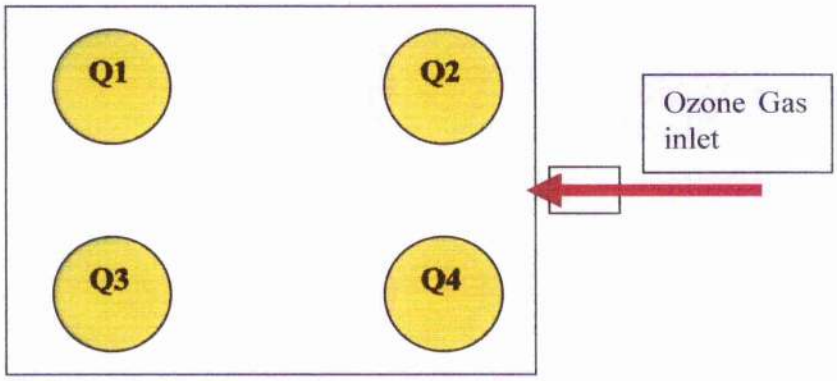


Figure 6.1 Location of lawned agar plates in the treatment chamber during ozonation

Figure 6.2 Control (left) with 8 x 20 µl spots of *Bacillus cereus* and similarly inoculated agar plate (right) after ozone treatment for 15 min.

Figure 6.3 Control (left) with 40 µl *Bacillus cereus* lawned on plate and similarly inoculated agar plate (right) after ozone treatment for 15 min.

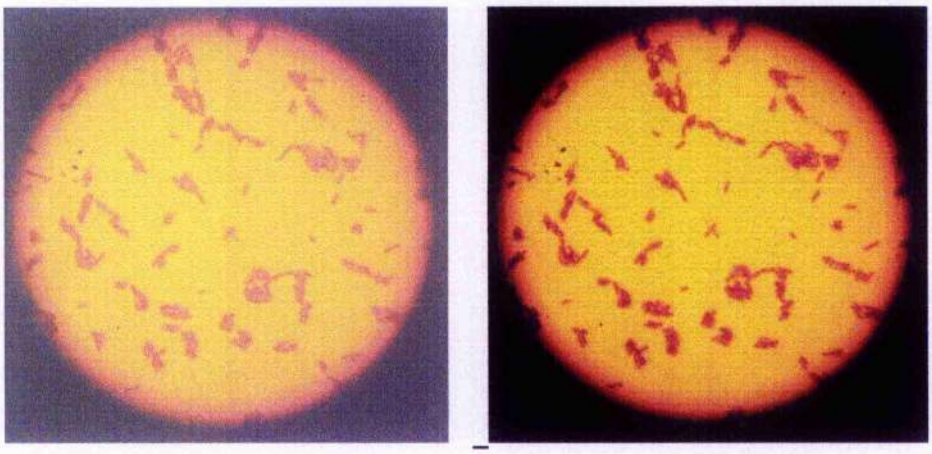


Figure 6.4 The left image shows that there was a mix of spores and vegetative cells at a magnification of 1000 x. On the right, the image was digitally enhanced with lighter purple tones to highlight the spores.

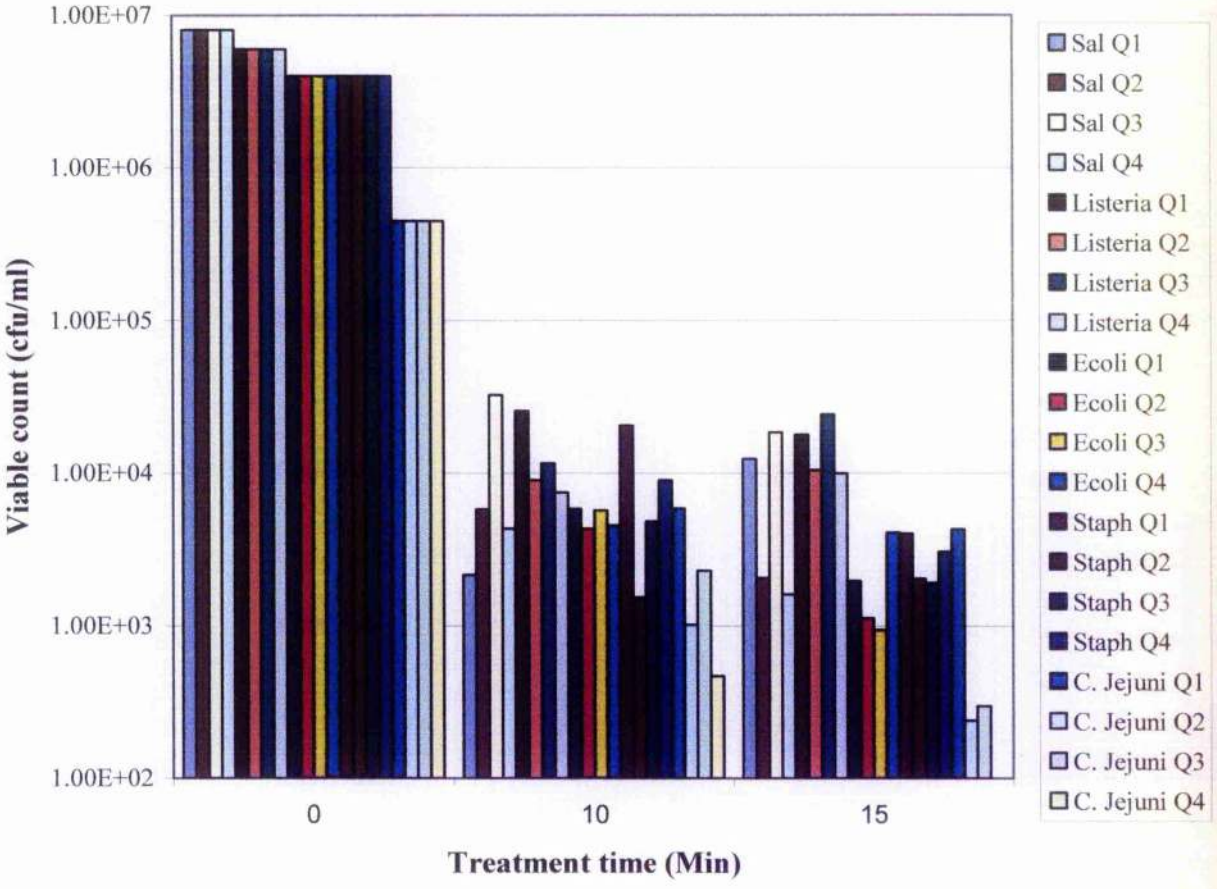


Figure 6.5 Bar graph showing cfu counts of *Salmonella typhimurium 509, Listeria monocytogenes R479a, Staphylococcus aureus, Escherichia coli* inoculated on agar plates after different periods of ozone treatments

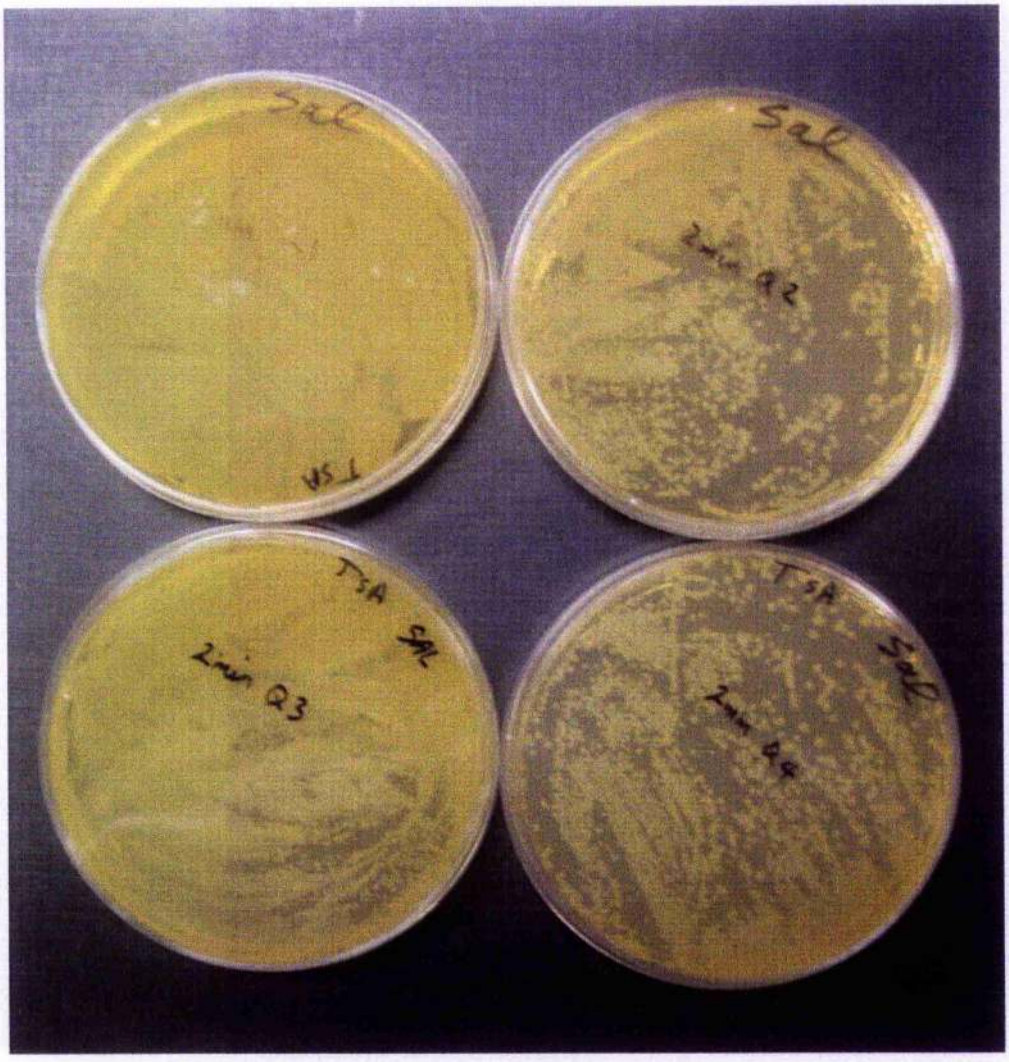


Figure 6.6 Plates after treatment at different positions in chamber. In the case of treating the plate with ozone for short durations, the distribution of gas resulted in an uneven ozone concentration, which resulted in uneven treated plates and uneven killing.

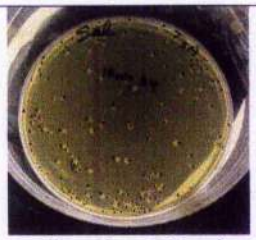
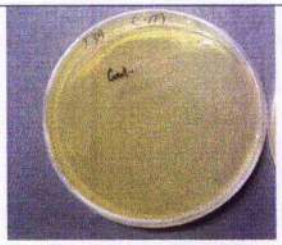
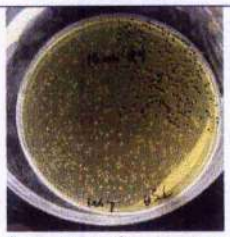
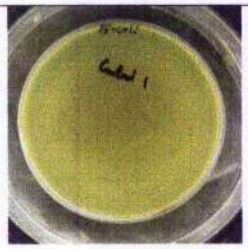
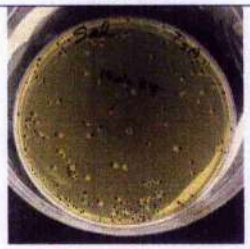
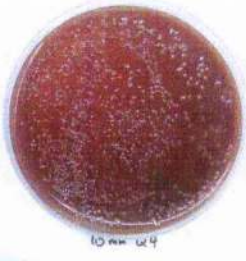
 *Salmonella* control	 *Salmonella* after 15 min ozone treatment
 *Listeria* control	 *Listeria* after 15 min ozone treatment
 *Staph aureus* control	 *Staph aureus* after 15 min ozone treatment
 *E. coli* ontrol	 *E. coli* after 15 min ozone treatment
 *Campylobacter jejuni* control	 *Campylobacter jejuni* after 10 min ozone treatment

Figure 6.7 Innoculated plates of different organisms before and after ozone treatment

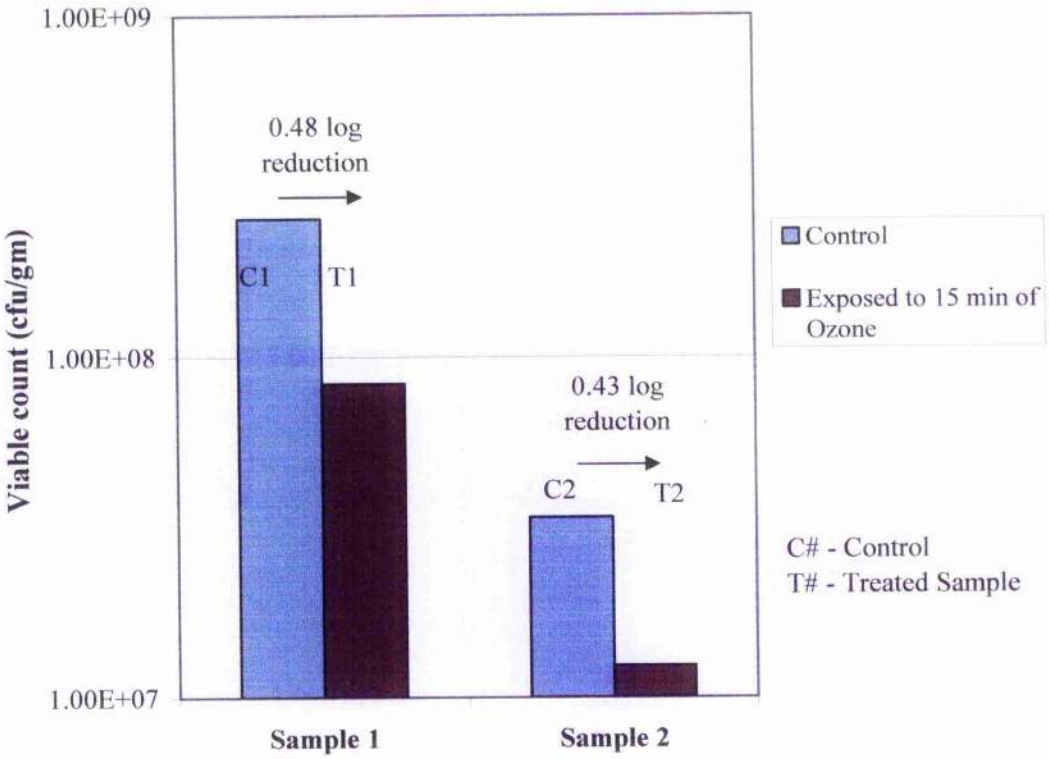


Figure 6.8 Effect of ozone treatment on normal microbial flora of chicken breast samples

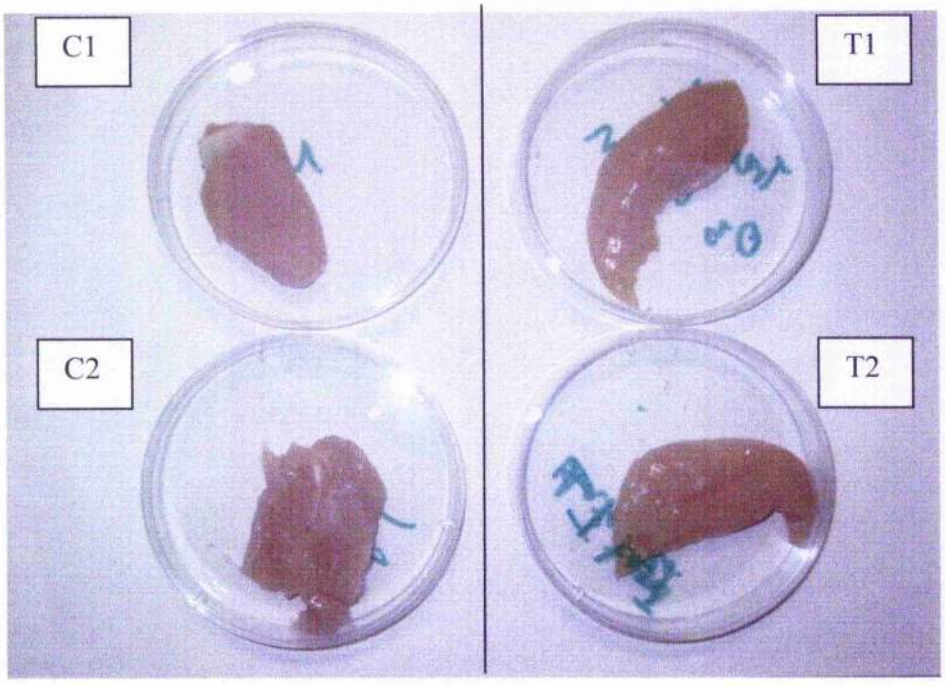


Figure 6.9 The left hand plates show the control (C) chicken samples and the right shows the treated (T) samples after ozonation and for 8 hrs refrigeration.

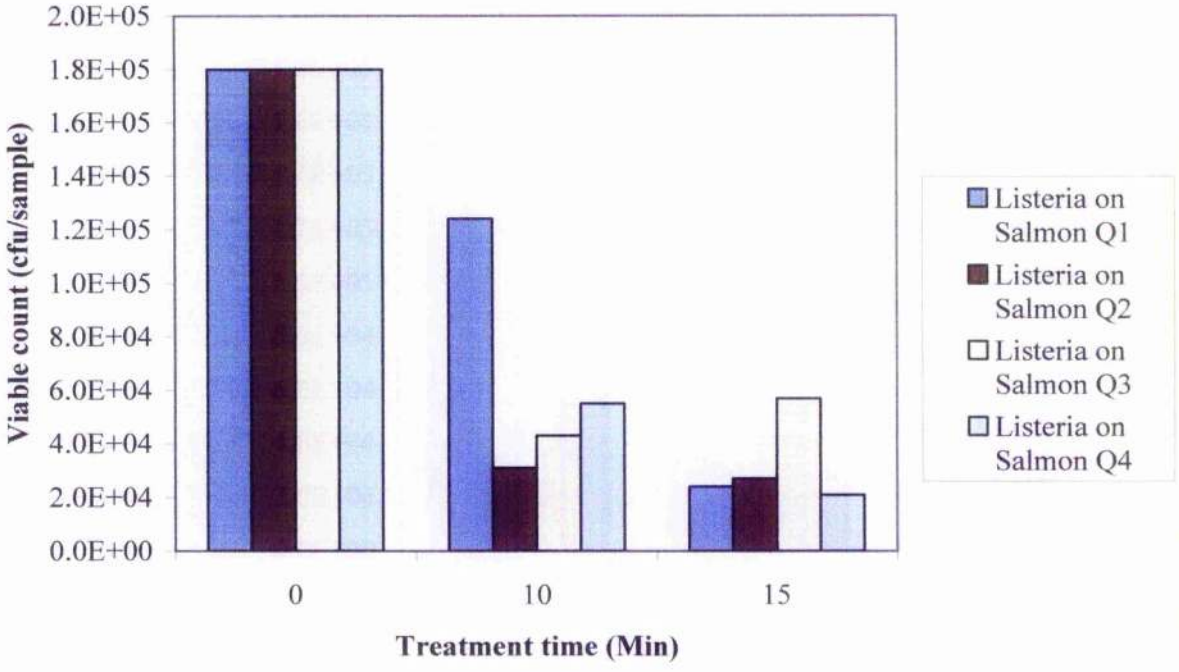


Figure 6.10 Ozone treatment of salmon samples inoculated with *Listeria*

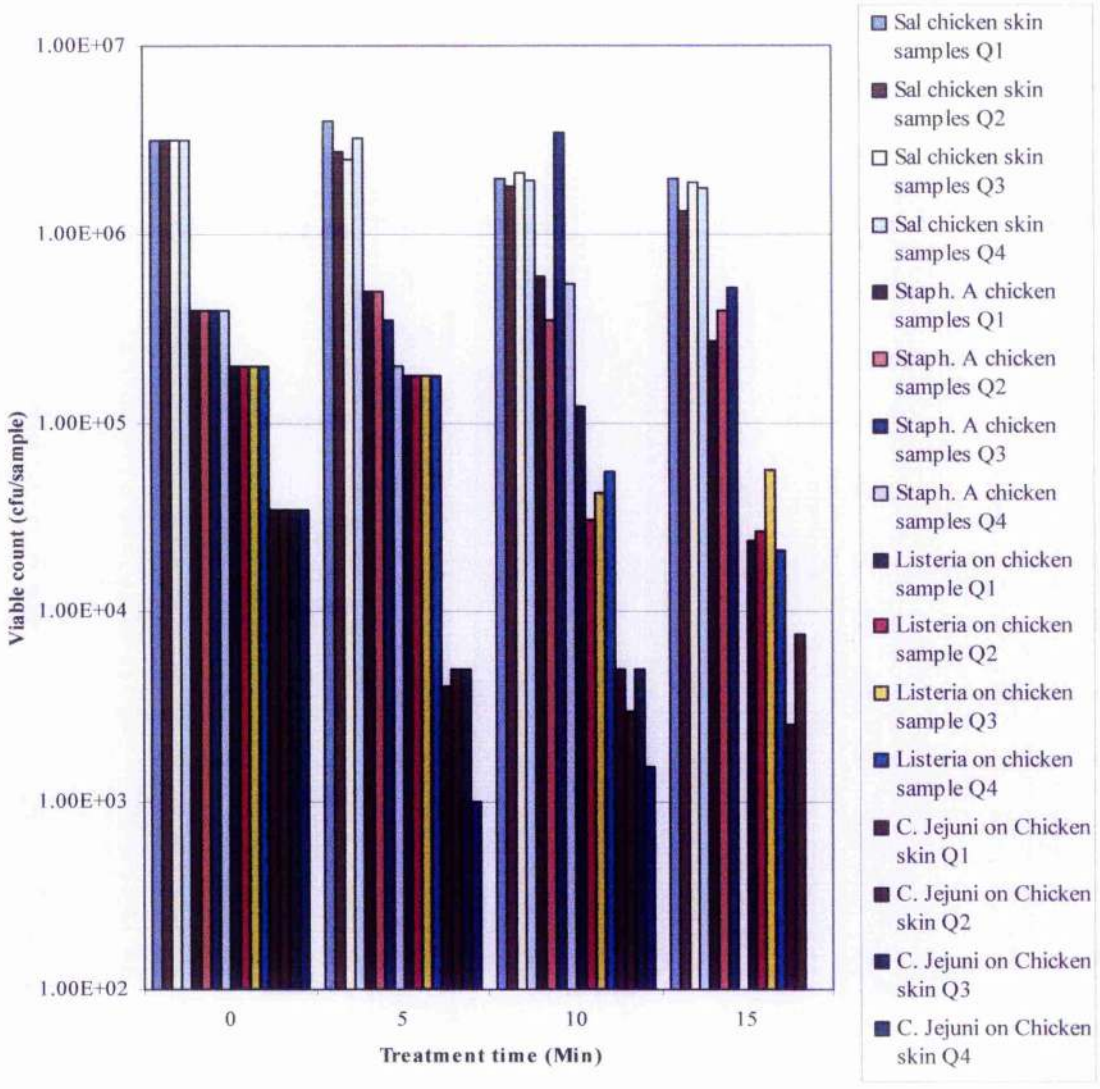


Figure 6.11 Ozone treatment of chicken skin samples inoculated with *Salmonella* (Gram-negative), *Campylobacter jejuni* (Gram-negative), *Staphylococcus aureus* (Gram-positive) and *Listeria monocytogenes* (Gram-positive)

CHAPTER 7 Conclusions

A number of different decontamination systems were fabricated and tested, both individually and in combination. This included laser, UV microwave and H_2O_2. The systems were tested microbiologically and against standard shelf life experiments in a simulated industrial environment. Additional systems were built to determine the real-time effect of ozone on a bioluminescent reporter strain *E. coli* lux, which served as an indicator organism and the effect of ozone on chicken and salmon samples. An ozone detector system was also developed.

7.1 Decontamination systems

For the investigation to determine the impact on shelf life after minimally processing carrots, potatoes and tomatoes, the system comprised a CO_2 laser, UV lamp arrangement, microwave system and H_2O_2 wash process. Additionally, a roller table and transport system were used to simulate an industrial process.

After the system was commissioned, damage threshold experiments for the laser and microwave were done to determine the maximum treatment duration allowed before damage was done to the equipment and produce. At the maximum laser peak power of 2.5 kW and with the roller table operating at the lowest speed (0.05 m/s) no damage was observed. With potatoes, a laser energy density of 8.8 Jcm^{-2} was used for the combination experiments; this was the maximum energy allowed before damage was observed on the produce. With an applied energy density of 60 Jcm^{-2} the carrots became dry and white and applying higher energy densities was not possible without damaging the substrate. The microwave damage threshold of the potato was an 8 sec exposure at 800 W.

This performance of a simple UV system design was initially tested. It comprised 3 lamps rated at 40 W operated at 253.7 nm and providing an irradiance of 1.4 mW/cm^2 at 50 cm. The UV irradiance stability was assessed and it was found that the irradiance output changed as a function of time. The UV irradiance increased to a peak value of 1.6 mW/cm^2 after four min from when the lamp was switched on. After this peak, the irradiance gradually reduced to a stable value.

Based on these experimental results an optimised industrial prototype system was fabricated. This was installed onto the simulated production line and tested for its effectiveness at decontaminating carrots and potatoes and extending their shelf life. The system was designed to suit the industrial roller table of 600 mm width. The produce was made to pass the effective region of the lamps, given the specifically-designed lamp configuration. This lamp geometry produced about 1 mW/cm^2 at 5 cm after warming up the lamps for 10 min. This was slightly less than for the first design but it was an optimized value. Using plates lawned with *E. coli* (100 µl of 1.1 x 10^9 cfu/ml) positioned directly under the lamps, no trace of bacterial survival was evident. At the minimum UV exposure of 20 sec (maximum velocity 0.212 ms^{-1}).100 % killing was achieved within the active area, and it was concluded that the UV system was effective.

7.2 Individual treatments

During laser treatment there were no significant effects on the appearance or the colour of the potato just below the damage threshold at 8.8 Jcm^{-2}. It was seen that a 1 log reduction was achieved in the intrinsic microbial burden. The laser offered less efficient decontamination on whole carrots than the carrot segments artificially inoculated with *E. coli*. It was assumed that this was due to the topography of the produce and the roughness of its surface.

Similarly, the microwave treatment could only be applied just below the damage threshold of the potatoes which was 8 sec at 800 W. For this exposure, less than 1 log reduction in the microbial burden was achieved.

It was found that there was significant variation in the irradiance from the lamp when using forced cooling. It was considered that this may have been due to ozone gas produced by the UV lamps or temporal variation in the lamp output due to temperature variations and the production of ozone. Initial confirmation of the presence of ozone was detected with ozone detector cards. The detector card showed that there was between 20 - 90 μgm^{-3} after the lamps had been switched on for 20 min of exposure with the 120 W UV lamp system.

The presence of ozone prompted further investigations to quantify the decontamination

effect of the lamps and any possible effect that ozone gas may have had by removing the ozone with a fan. The irradiance of the UV output increased when the fan was used to blow air across and below the lamps. The optimum UV output varied with temperature, and the temperature was affected by the fan action.

While decontaminating whole carrots and potatoes, the results presented did not correlate with the results found by artificially contaminating the surface of carrot and potato segments with *E. coli*, where 1 D-value reduction was recorded after a 5 sec exposure at a distance of 80 cm from the UV lamps. The apparent reduction in the UV killing efficiency could be due to a number of factors. The first of these could merely be that some of the many different soil organisms found on unsterilized carrots and potatoes were more resistant than the *E. coli* used as the inoculum. For example, it is likely that there would be resistant bacterial spores present as the intrinsic microbial. The surface topography of the vegetables, for example 'eyes' in the potatoes or 'crevices' in the carrots, would prevent complete exposure to the source. Experiments conducted showed that the bacterial load of the eyes in the potatoes was about four times that of the smoother skin.

A simple model was developed to estimate the effect of non-uniform decontamination over the surface of the potato. This was due to the likely variation in the intrinsic microbial over the potato's eyes and skin. Different assumptions were made about the levels of decontamination. It was determined that even if the bacterial levels dropped significantly on the potato they were sufficiently large to produce an overall high level of contamination.

Scanning electron microscopy was done on carrot and potato surfaces to determine whether there was less topographical variation with carrots as compared to potatoes. The amount of the electron beam energy reaching deeper regions was less and these regions appeared darker, emitting much lower electron numbers than the top surface of the produce which, therefore, appeared brighter. Large variations in contrast were seen on the SEMs of the potato. This showed that the differences in the depth of the crevices were much greater for potatoes than with carrots. Around the regions of the eyes, semicircular pockets were observed. These images showed a significantly higher probability that bacteria underneath the pockets were shielded from the UV irradiation during the treatment process and the

significant differences in the results were believed to be due to the different topology and the depth of the crevices existing on the sample's surface.

7.3 Combination decontamination systems

Combination decontamination experiments were conducted to evaluate possible synergistic effects of laser, UV and microwave treatment. During the combination experiments, minimal processing by sequential treatment with laser, UV and microwave irradiation of potato surfaces contaminated with *E. coli* was done. The results showed that there was a sequential increase in the reduction of *E. coli* after each individual treatment. There was also an indication that if the first treatment was UV, followed by microwave and then laser, the effect was greatest with a 3.2 log reduction. If the treatment order was microwave, UV and laser only 1.1 log reduction was achieved. This result confirms that the possible synergistic effect could be optimised by different treatments orders.

7.4 Shelf life

An improvement in the shelf life of the treated carrots was observed after UV treament, the shelf life was between 4 to 8 days without refrigeration, where the vegetables showed no traces of mould or baterical formation. The shelf life of the control was 2 to 5 days. Class I carrots had a longer shelf life compared to class II carrots, even without any treatment. Random sprouting and no colour changes after treatment showed that the UV exposure did not change or alter any growth behaviour of the carrot or quality attributes.

H_2O_2 treatment was found to be slightly more effective than using the UV lamps. The treatment extended the shelf life from 5 to about 10 days compared to treating the carrots with UV which only produced about 4 to 8 days shelf life. However with both UV and H_2O_2, a significant difference in the shelf life extension was observed between the treated and control samples, with an overall reduced rate of decay.

In the shelf-life experiment with both laser and microwave treatments an undesirable discolouring affected the potato's surface. Although no visible damage was observed immediately after treatment, potatoes irradiated by laser turned a dark brown colour after 2 days. As for potatoes treated with 5 sec of microwave at 800 W, different discoloration

was observed. The vegetables turned light green when compared to the untreated ones, the controls did not turn green.

Although the laser treatment prevented rotting of the produce by bacterial or mould contamination on carrots there was the disadvantage that it produced a browning of the potatoes. Microwave results indicated that it was capable of extending the shelf life but the produce became green.

A comparison between the shelf life of potatoes after treatments was not possible as there was no moulding, signs of decay, change in texture or reduction of rigidity on either the treated or untreated samples.

Investigation of the β-carotene (vitamin A) level of the treated carrots and vitamin C level of the potatoes indicated that the various treatments did not degrade the carrot's or potatoes' nutritional value.

On tomatoes, the shelf life results showed improvement over the untreated samples. Unfortunately, observations could not clearly determine the exact increase in shelf life after treatment, however, an increase of one day in the shelf life was estimated.

7.5 Ozone measurement and bioluminescence detection

Using a measurement system built in-house, the ozone concentration was determined from the amount of UV irradiance absorbed between a UV source and a detector. Applying these experimental data to Beer-Bouguer-Lambert's law, the O_3 concentration was found to be typically 3.9×10^{-6} g/cm^3. The ozone was generated from a commercial generator. The amount of time O_3 was active in the chamber affected the efficacy of the ozone treatment. The system was used to determine the decay rate of ozone in the chamber. At lower concentrations (0.04468 μg/cm^3) the decay rate was found to be about 470 min/μg/cm^3 and the decay rate gradually increased with ozone concentration. Since the ozonation treatment operated for a duration of 15 min, the O_3 concentration reached was 3.9×10^{-6} g /cm^3.

Prior to building a real-time monitoring system, bioluminescence measurements were done using a bacterial suspension on the Bioorbit luminometer and agar plates with a CCD camera. They were evaluated and the results were compared with the real time bioluminescence detection system (RBDS). A series of 10-fold dilutions of the bacterial suspension was made and placed into the Bioorbit luminometer for measurement. The result showed that the relative light output was significant at more than 10^4 cfu/ml. At lower *E. coli* concentrations, it was not possible to be more precise due to the limitations of the detecting system.

A high resolution CCD camera was used with the agar plates lawned with *E. coli* to determine the performance of the CCD camera before and after ozone treatment. However, due to the large dynamic range between the treated and the control plates, where there was about a 5 - 6 log kill, it was not possible to obtain satisfactory images. At the same time it was not possible to obtain a viewable image of the treated plate without losing resolution of the untreated plate. In addition, the luminometer and the high resolution CCD camera are off-line systems and are not able to be used within a real time environment and, furthermore, the dynamic response of both systems is low.

The real time detection system (RBDS) was fabricated to determine the effect of ozone gas on *E. coli*. However, the system can be used with practically any form of biocidal treatment. The ozone concentration was measured via the absorption about 255 nm (Band pass filter 255 nm), and the light output from the bioluminescent *E. coli* lux was measured as a function of time both with and without ozone present. The light output was then compared for the untreated and treated samples to ascertain the difference in the samples.

The RBDS has proven to offer real time capability and enabled a comparison to be made between the light output from the bacteria as the ozone concentration was increased, allowing some possible elucidation of the killing mechanism of ozone.

7.6 Ozone treatment

Bacillus cereus spores were prepared and treated by an exposure to ozone for 15 min. After 24 hrs of incubation no colonies were found. This indicated that the ozone treatment was effective on endospores. Gram-staining was done on the original culture to determine

whether the treated culture was sporing. Images taken at 500 x showed that the culture used during treatment was sporing and confirmed that ozone was effective against *Bacillus cereus* spores.

To show that ozone was effective against other organisms, *Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus,* and *Escherichia coli* these organisms were treated with ozone for 5, 10 and 15 min. For all the bacteria treated, it was found that the viability reduced with increased treatment time. After 15 min of treatment, the log reduction of the three Gram-negative organisms were 3.7 for *Salmonella typhimurium*, 3.6 for *Escherichia coli* and, 3.8 for *Campylobacter jejuni.* The Gram positive organisms showed a 2.9 log reduction for *Listeria monocytogenes* and 3.3 log for *Staphylococcus aureus*. Gram-positive organisms were observed to be slightly more resistant to ozonation than the Gram negative type.

When ozone was used on chicken breast, no colour or textural changes were observed after 15 min of ozone treatment. The treatment produced a reduction of 0.43 - 0.48 log on the overall counts of the intrinsic microbial on the breast. The treatment achieved 0.43 - 0.48 log reduction, consistently on both specimens. This was similar to those results from minimal processing of carrots and potatoes with the laser, microwave UV and H_2O_2. The results obtained on the actual samples were less effective than compared to those of *Bacillus cereus* cultured on plates. Although SEM imaging was not performed on the surface of chicken, the reduced efficiency was thought to be due to bacteria remaining within the crevices on the rough chicken surface. To improve the reduction in viability, it may be beneficial if the breasts were suspended or rotated and not placed in constant contact with the surface, where one part of the breast would not be possibly exposed to the ozone at all. From a geometric point of view a reduction of at most 50% would be expected if the half of the chicken was left unexposed to the ozone.

In general, the reduction in viability obtained on the food samples was less significant than those on agar plates. With salmon samples inoculated with *Listeria* there was a log reduction of 0.9. This was by far less effective than ozone treatment of *Listeria* on plates, where the treatment induced 2 – 2.5 log reductions. With *Salmonella* inoculated on chicken skin, the best result produced only 0.37 log reductions after treatment. The most effective result for the food samples were from ozone treatment of chicken skin,

inoculated with *Campylobacter jejuni,* where a log reduction of more then 1.5 was achieved. Although all ozone treatment had an effect on both Gram positive and negative organisms, the log reduction found on treating chicken samples was much lower than that obtained from treating the same organism on plates.

7.7 Future Work

This work has produced a combination minimal processing system. The development to date has been close to implementation in a real production line and processing plant. In the near future, the construction of decontamination system should be installed in a food processing environment and its performance evaluated. Large scale investigation of shelf life and batch processing can be determined against the treatment parameters investigated in detail within this thesis. It is seen that the UV treated samples had a significantly reduced rate of decay. However more work would be required to identify the increase in shelf life of the treated samples at a higher temporal and spatial resolution. This could be achieved by using photo-digitization to quantify the rate of decay and introduce video monitoring to record the entire decay process. A longer observation time of the potatoes after treatment would be useful as there was no significant visual difference in any of the potatoes samples over an 18 days period. Extension of shelf life in lighted and dark condition may also be another area that could be investigated.

The light output of luminous *E. coli* depends on the oxygen concentration and temperature as well as their viability. Optimization of the RTDS (Real time detection system) for bioluminescence to improve sensitivity, consistency and reduce interference from external factors could be carried out by incorporating temperature and atmospheric control for a more accurate measurement. The detection treatment chamber can be improved with feedback to keep the temperature and humidity controlled during measurement. The RTDS has only been tested on agar plates, monitoring on actual food product has yet to be investigated.

In the ozone experiments, the efficiency of reducing microbial loads by minimally processing fish and chicken produce was much lower than that of processing bacteria cultured on plates. The delivery of ozone, therefore, could be investigated and improved.

It may be possible to suspend the samples in the treatment chamber to improve the treatment efficiency.

CHAPTER 8 References

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CHAPTER 9 Appendices

Appendix I Design report of optimized UV system

I.i Introduction

The continuous UV source at 254 nm has proven to be an effective method for use of minimal processing of produce in the system that was developed during this period of research. Positive results obtain in the UV test rig were optimized and minor improvements were incorporated to this combination processing test rig. Optimization work included the characterisation of the stability of the UV source as well as identification of the optimized parameters and operating conditions. However due to the ease of experiment the UV set-up was deliberately made with great flexibility, so that it could be adapted to various experimental usage to simulate different test conditions. Hence, this test UV system was not suitable for industrial installation in a production line. There is a need to develop a modular UV system that is suitable for industrial applications.

I.ii Problem analysis

Technically there are a number of aspects to consider when trying to install the system into an existing production line of a carrot or a potato factory. First the delivery system used to transport the produce. The bed width of the roller and processing the flat bed conveyor come in various sizes and the speed can be altered on demand. The UV system will have to be effective along its entire width. Space constraints are also an issue as the most liking area to locate the system is currently occupied by operators. As such, the system should be reasonably compact and safe to suit the requirements. Provision for the integration of a chemical cleaning system.

The product design criteria was based on industrial requirements during implementation as well as the research optimisation data to cope with the demands of the product design specification. The needs were judged against the complexity, practicality, manufacturing practice, functionally, operator safety, food handling safety, reliability and the aesthetic characteristics.

I.iii Statement of need

The basic industrial requirement of the system is utilise hardware that is corrosion free using non-corrosive material to avoid contamination of food produce.

The UV lamp system should be able to accommodate a bed width 1.6 m for the conveyor used in Country Crest and 0.77 m for that used in Dew Fresh.

UV irradiance should be sufficient for effective sterilization and shelf extension based on experimental data.

Operation should be simple to keep incurring cost of training to a minimum.

Maintainability is one of the priorities in the design criteria. Most production losses were incurred during unexpected down time in the production line. Improving the maintainability significantly reduces the maintenance overhead as well as the downtime.

To prevent the requirement to do corrective maintenance during production, the system has to be slightly over designed to allow continuous operation in the event of single lamp failure that would result in down time.

The system will have to operate for a duration of at least 8 hrs a day and 12 hrs during peak season, for 6 days per week, and continuously during peak production season.

The mechanical components are required have a long service life.

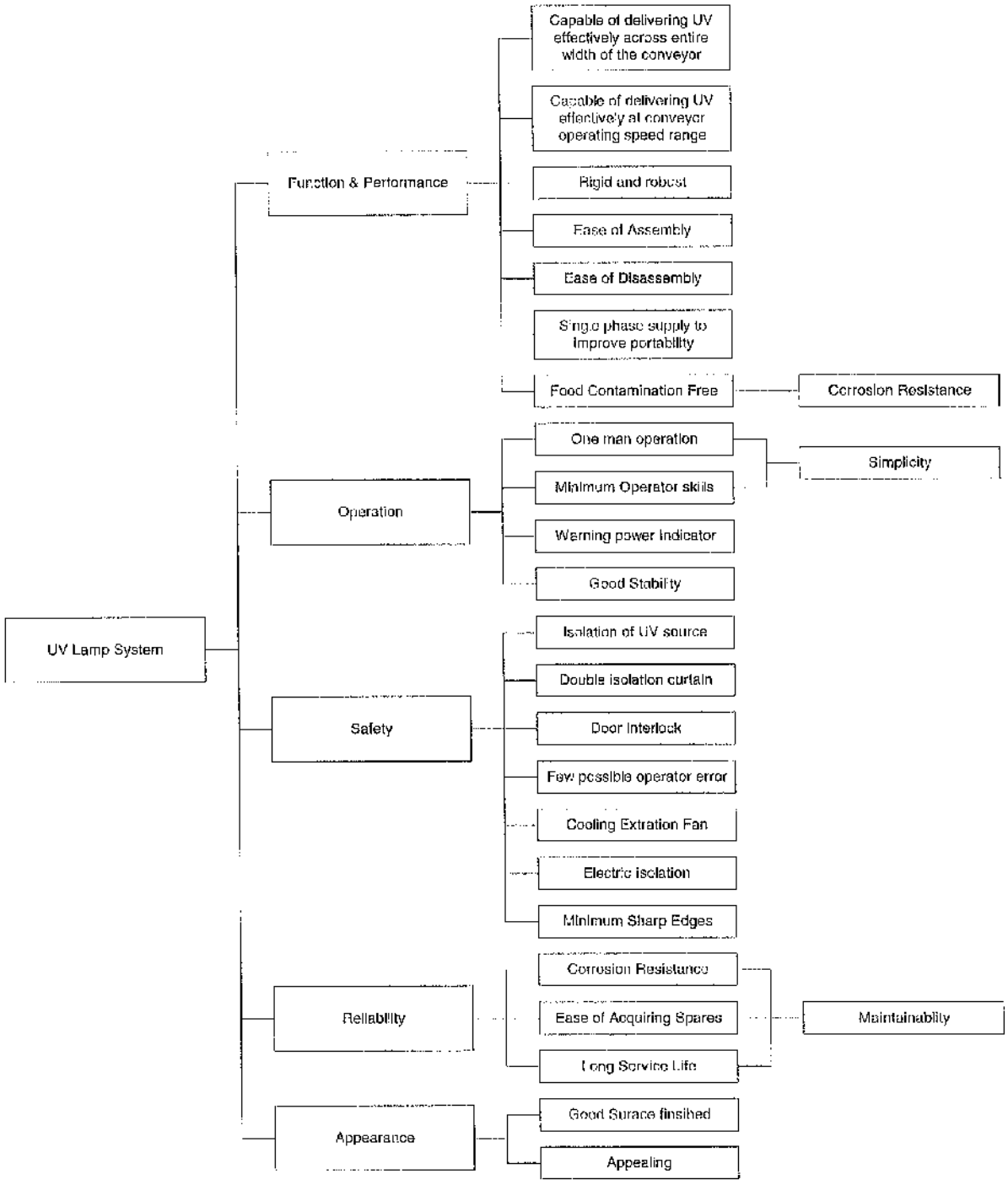
Light proving seal and curtains should be available to isolate hazardous UV light from radiating onto the operator working near the system.

Safety interlocks are required as part of the safety.

A modular design permits integration of other systems such as a chemical cleaning spray, laser and other processes.

I.iv The objective tree

The objective tree was developed to take into consideration all of the requirements stated in the previous section. The objective tree shows the hierarchy and interconnection between the objectives. Although manufacturing limitations may not permit every single objective to be fulfilled, it eases the selection of adequate effective techniques in the process of developing a system that must meet those requirement laid down in the industry.


UV Lamp System
Function & Performance
Capable of delivering UV effectively across entire width of the conveyor
Capable of delivering UV effectively at conveyor operating speed range
Rigid and robust
Ease of Assembly
Ease of Disassembly
Single phase supply to improve portability
Food Contamination Free
Corrosion Resistance
Operation
One man operation
Simplicity
Minimum Operator skills
Warning power indicator
Good Stability
Safety
Isolation of UV source
Double isolation curtain
Door Interlock
Few possible operator error
Cooling Extration Fan
Electric isolation
Minimum Sharp Edges
Reliability
Corrosion Resistance
Ease of Acquiring Spares
Long Service Life
Maintainability
Appearance
Good Surface finished
Appealing

I.v Design specification

I.v.i Function and performance

The main and most important function for this system is the delivery of a continuous source of UV radiation, sufficient to minimally process the food to improve its shelf life, hence reduce the cost of storage and maximise returns. The power required for the use of such a system will be supplied by a single-phase source. A set of ballast then converts and rectifies the required electric supply to light up the UV lamp and supply the power after lighting up.

It is a standard requirement in the industry that the hardware must be robust under stringent environments. Fixed and semi-permanent mounting will be used to achieve both rigidity of the system as well as the ease of Assembly and disassembly during maintenance and relocation. On the production conveyor, produce are spread across the bed width, therefore the effective working area enveloped by the UV light should be large enough and intense enough. In addition the corrosive free and not toxic material is compulsory to ensure the safety of consumers.

I.v.ii Operation

Only a single operator is required to operate the system. Operating guidelines will be made available, enabling the user to operate the device in an easy and simple manner, taking up the minimum amount of time and effort for training. Stability control will be automatically done by conventional temperature control feed-back systems, adjusting the cooling rate to ensure lamp output stability and unnecessary formation of ozone during operation. Laminated warning lights and 7 segment displays will be available within the system control system.

The corrosion resistant structure will have a service life of 10 years while UV seals, UV food curtains and other rubber material will serve up to about 5 years. In which, it will be capable of operating for 8 hrs daily, 12 hrs daily during peak season and 6 working days a week.

I.v.iii Reliability

Practically, the moving parts of the system were kept to the minimum to reduce the chances of mechanical breakdown. Electrically approved power supplies were used to ensure that no overloading occurred. Electric consumption rating of the germicidal lamps were double check not to exceed the overall supplied rated.

I.v.iv Maintainability

Have few mechanical moving parts so the system can be maintained free during operation. Self-lubricated high strength hinges were used for the maintenance door. The lamps used were mounted on quick release clips. Replacing faulty lamps should not take more than 5 min. The period between subsequent maintenances varies from 5.2 months to 6 months before the first lamp fails base on manufacturers' specification who ensure a lamp life of at least 1000 hrs, mean time between failure.

All spare were available locally in Britain. These spares will be delivered within 2 to 30 days upon the order confirmation depending on the availability of stock.

I.v.v Aesthetics

Although lavish aesthetics requirements were not required for this application, basic aesthetic appearance should still be kept in mind. Reasonably appealing equipment appears cleaner and will be better maintained in the long run.

I.vi Design approached

The evolution of the conceptual designs very much depends on the research results, the complexity of manufacturing and the cost of manufacturing. The design factors were compared with the product design specification to meet the final objectives required.

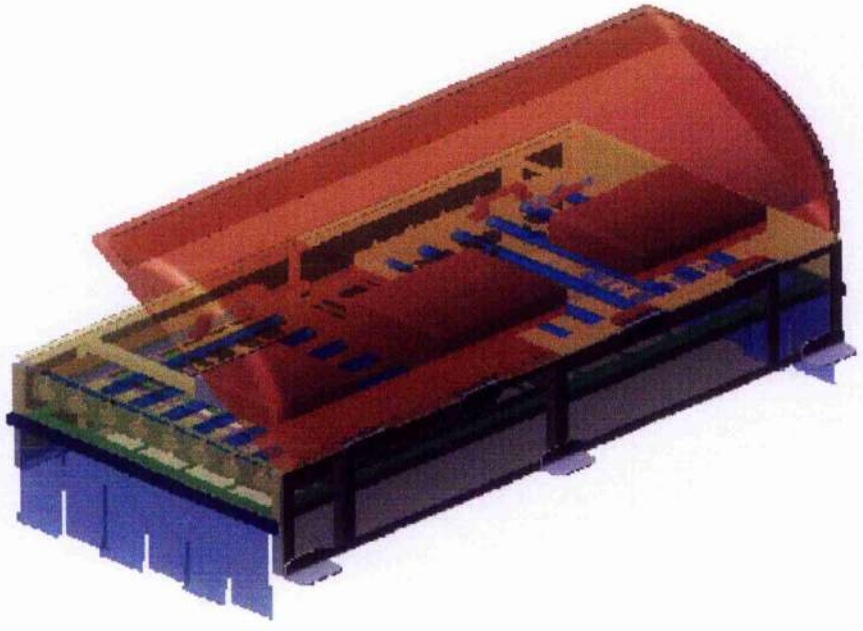
Customised ballast and standard 15 watts germicidal UV lamp supplied by "Inter Marine" were used for both design the lamps. Feasible combination of holding, mounting methods, and frame structure were evaluated and put together into 2 conceptual designs below.

I.vii Conceptual designs

I.vii.i Conceptual design 1

This design uses stainless steel (316 Grade) right angle bar as the main frame structure of the system. The lamps were held together with 2 sets of customised lamp holders that rested on top of 2 fixed tracks, spread across the entire width of the conveyor bed. Sliding movement along the rack made space to facilitate maintenance, such as lamp changing and cleaning. 2 mm think stainless steel sheet was used for the side panels that were fitted onto the frame by capscrews.

The structure was held together mostly by welding, whereas the protective panel and covers were put in place using bolts, nuts and hinges. So spot welding was used in locations were maximum stress such as the handles were fitted onto the panels. For aesthetics and safety reasons, the top cover was included in the system to isolate the control box and the ballast mounted on the top surface of 2 separated pieces of stainless steel panel. Only a single source from a single phase power supply is required to power up the system.



1) Operating Procedure

Operating procedures were kept as simple as possible. There were 2 modes of

operation. Starting the system can be manual or automatic. In a manual mode the operator will only have to switch on the power supply to the system, just like any applicances. Where as in an automatic mode the supply is directly coupled to the conveyor. As soon as the conveyor is switched on the system powers up itself. Having the UV light contained with no motorised mechanical parts safety is not compromised in this automatic starting mode.

2) Advantages

- Simple operation, minimum training required.
- Material Cost is relatively cheap.
- Effective across entire conveyor bed.
- Single phase supply (reduce factory rewiring cost).
- Lamp operating enveloped shield from dirt and grimed from environment.
- Simple maintenance.
- Few mechanical parts, contributing to long service life.

3) Disadvantages

- More machining work will be required to fabricated the lamp mounting.
- High manufacturing cost involving skilled technicians and machinists.
- Extensive welding involved
- No flexibility of adjustment of height.
- No flexibility for the integration of others systems.

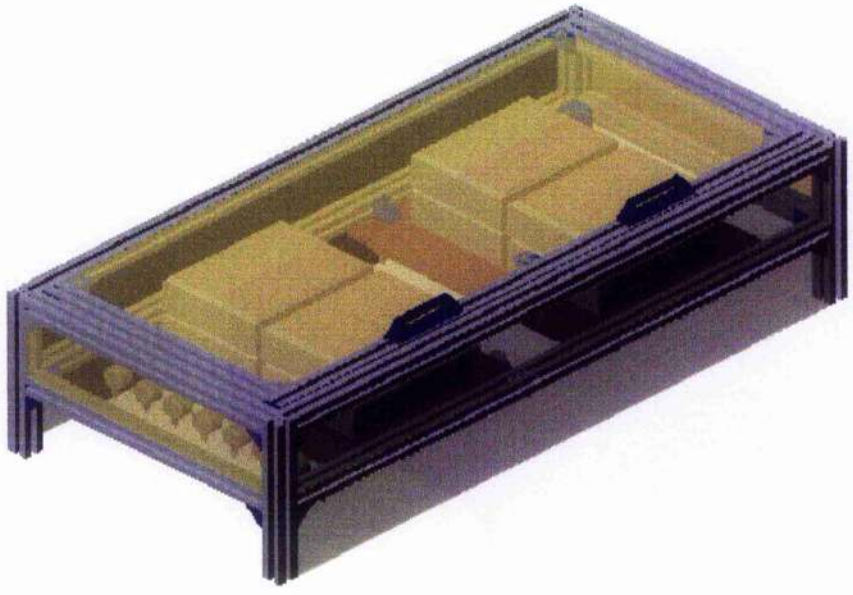
- Fixed structure, modification will be costly.
- Parts are customly made which could be costly for future maintenance.
- On site assembly slightly more complicated as the frame has to be welded and its not dismantable.

I.vii.ii Conceptual design 2

Conceptual design 2 uses conventional aluminium structural profile as its main structure. Unlike design 1, the lamps were held together under a sheet of electrical insulated tufnol board, with quick release springs-clips and the boards were then mounted onto the frame itself with standard angle brackets. No welding was required to build the frame. Structurally the frame is assembled together using standard bracket, T-slot nut and bolts. Minimum machining is required during fabrication making this design highly flexible and versatile. This modular design permits future modification and integration of other systems such as the chemical system, easily using standard beam and bracket extensions.

Corrosion resistant stainless steel plates were used for all side panels. Electrically the control boxes and ballast were integrated on the reverse side of the lamp mounting board, hence not only reducing the complexity of creating an extra platform for the mounting of these components, but it effectively utilised the space which permitted better convection control necessary for the lamps stability.

The lamp arrangement was kept the same. It used the same power supply and was powered from a single phase supply.



1) Operating Procedure

The operating procedure was very much the same as design 1. It consisted of 2 operating modes both manual or automatic with conveyor delivery system integration.

2) Advantages

- Simple operation, minimum training required.
- Minimum fabrication required hence lower manufacturing cost and time.
- Effective across entire conveyor bed.
- Single phase supply (reduce factory rewiring cost).
- Lamp operating envelope shielded from dirt and grime from the environment.
- Simple maintenance.
- Little mechanical parts contributing to long service life.
- Flexible design enables integration, future upgrade and customisation.
- Standard components which significantly cheaper to replace during maintenance.
- Aesthetical appealing to the eye.
- On site assembly possible.

3) Disadvantage

- Higher material cost.

I.viii Evaluation grid

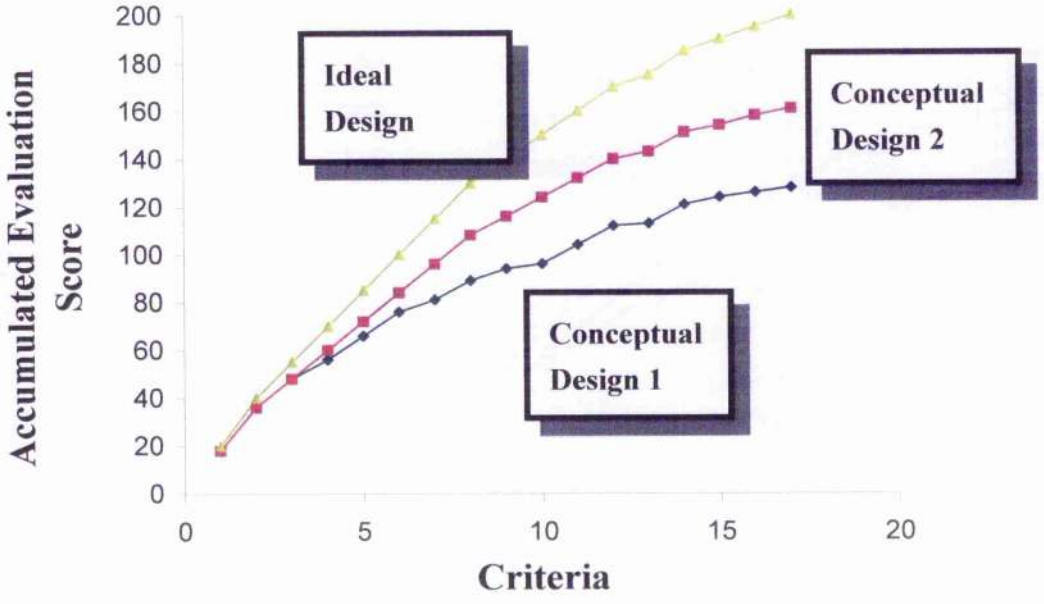
Besides giving an overall summary of the primary system design specification used during the development of all conceptual designs, the evaluation grid enable the numerical evaluation of the advantages, disadvantages and the specification performance numerically. The evaluation grid give a better picture on have each design performance for the selection of the design.

S/No	Concept / Criteria	Design 1	Design 2	Max Score 200
	Overriding Criteria			
1	Delivery UV Across the entire conveyor width	18	18	20
2	Generate enough UV density from minimum	18	18	20
	Primary Criteria			
3	Ease of Operation	12	12	15
4	Ease of Installation	8	12	15
5	Ease of Maintanance	10	12	15
6	Cost of Installation	10	12	15
7	Cost of Fabrication	5	12	15
8	Cost of Maintanance	8	12	15
9	Integration flexibility (module design)	5	8	10
10	Cost of Integration and Upgrading	2	8	10
11	Safety Interlock Mechanism	10	10	10
12	(MTBF) Mean time between failure	8	8	10
13	Portability	1	3	5
14	Corrosion Resistance	8	8	10
15	Long service life	3	3	5
	Secondary Criteria			
16	Aesthetics	5	4	5
17	Equipment Standardisation	2	3	5
	Total Score	133	163	200
	Percentage Score	**66.50%**	**81.50%**	**100.00%**

4)

I.ix Accumulative evaluation chart

From the accumulative evaluation chart it is possible to compare the ideal design to what is the strength and weakness of each criteria accumulatively.



I.x Discussion of final design selection

After some detail of the evaluation of both conceptual designs, comparing the advantages, disadvantages and performance with respect ideal design the conceptual design 2 was chosen as the final design.

Both designs do meet the primary specification and are both equally capable of performing the necessary task of delivering enough UV irradiation for the minimal processing application. However, the complexity of conceptual design 1 was the main deterrence from this being the adopted design.

Complexity during fabrication not only increases cost, it commonly introduce mismatch problems with joints, bad fitting which are inevitable even with skilled machinist and technicians. Welding itself introduce tremendous amount of heat that leads to distortion during expansion and contraction. Additional costs for fixture would be required to minimise distortion. Also welding of stainless steel causes weld decay, a phenomenon whereby carbon combines with chromium to form chromium carbide. This intercrystalline corrosion occurs between 600°C and 900°C at the heat affected zone. To solve this problem post-heat treatment is required. It is usually done by reheating it to well above the austenitic temperature to dissolve all chromium carbide and quenching it at 1050°C. Unfortunate, quenching also contributes to undesirable structural distortion. Eventually it will affect the fit up of the system that reduces the aesthetics appeal and increases unforeseen teething problem, during installation due to misalignment and mismatch.

On the other hand fabrication of conceptual design 2 is much simpler compared to that of conceptual design 1. The only fabrication work necessary to build the frame is to cut the aluminium extrusion profiles to size. Assembly is easily carried out with standard brackets, screws and an allen key. Comparatively the lamp assembly is much more easily made as well as the dimensional accuracy of becoming less important as the mounting clips for the lamps are more accommodative.

Therefore conceptual design 2 was selected as the final design to be used.

Appendix II Calculations for ozone concentrations

II.i Result for ozone concentration using Beer-Bouguer-Lambert's law

The attenuation of UV light in the experiments at the highest ozone concentration was found from the experimental setup.

Area of the detector	:	$0.5cm^2$
Distance between UV source and detector	:	12 cm
Avogadro's Number (1 mole)	:	6.02×10^{23} Particles/molecule
Molecular Wt of O	:	15.99994 g/mole
Volume of Absorption	:	$6\ cm^3$
UV irradiance at max without O_3		
Taken at 12cm from source	:	549 $\mu Watts/cm^2$
After absorption at max O_3 concentration	:	20 $\mu Watts/cm^2$
Plank's Constant h	:	6.626×10^{-34} Joule s
Speed of light c	:	3×10^8 m/s
Wavelength of light source	:	253.7×10^{-9} m
Dimension of chamber	:	36 x 27.5 x 32 cm
Volume of second chamber	:	$63360\ cm^3$

From experiments $I_0 = 549\ \mu Watts/cm^2$ and $I = 20\ \mu Watts/cm^2$ at the highest concentration of the system using (equation 5.4 and 5.5)

$$I(t) = I_o \exp\left(-\sigma[N(t)]x\right) \qquad (5.1)$$

$$C_O(t) = \left(\frac{N(t)}{A_v}\right)\cdot\left(\frac{M_m}{V}\right)$$

$$-[N(t)] = \frac{1}{\sigma x}\ln\left(\frac{I(t)}{I_o}\right) \qquad \text{Take In of 5.1 (5.2)}$$

Therefore N $= \ln (20/549) / (-1.15 \times 10^{-17})$

$= 2.88 \times 10^{17}$ Molecules

Concentration of Ozone C_O = (No Molecules / Avagodro No) x (Molar Mass / Volume)

$= (2.88 \times 10^{17}$ Molecules) / $(6.02 \times 10^{23}$ molecules /mole)

x (49.998 mole / 6 cm^3)

$= 3.986 \times 10^{-6}$ g /cm^3

Ozone production rate at 100 L/hr (O_2 supplied) $= 3.986 \times 10^{-6}$ g/cm^3 x 100000

$= 0.3986$ g/hr

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