

CRANFIELD UNIVERSITY

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Temporal Changes in Vase Water

Applied Microbiology Group,
Cranfield Health

MSc by Research
Academic Year: 2011 - 2012

Supervisor: Dr R. LAMBERT
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ABSTRACT

This study investigated the influence of flower food on vase water quality with the attempt to correlate this with the flowers' appearance and microbial growth occurring in the vase water. A mixed bouquet of different cut flowers was used in this study for the first time instead of the common practice in the literature of using a single cut flower or a single cultivar.

Different combinations of vase solutions; standard water and reverse osmosis water with or without added flower food were used as initial vase solutions and also as the topping up water. The effect of vase solution's pH on microbial growth and therefore flowers vase quality was also examined. Moreover the analysis of sugar content of vase water was conducted using HPLC and LC/MS.

The analysis of vase water in the Cranfield Health laboratory has shown that: Sugar presumably plays a central role in energy for both microbes and plants but the concentration levels present in flower food seems to have no subsequent effect on the growth or otherwise of the microbes even when diluted with top up water. Water uptake by the flowers is little influenced by the presence of flower food or the microbial population. Flower food reduces the pH of Standard water, but not sufficiently enough to inhibit the growth of common pathogens or spoilage organisms. If microbial growth begins, addition of further flower food in the top-up does not inhibit further growth. If reverse osmosis water (ROW) is used with flower food the initial pH is lower than the pH minimum for all common pathogens and the majority of common spoilage organisms. Topping up with ROW with flower food maintains the low pH environment. If growth is initiated due to the presence of microbes capable of growth in the low pH environment, then growth will continue regardless of topping up solution. Microbial growth in ROW with flower food is confined to acidophilic organisms. Addition of weak acid preservatives such as benzoic acid or sorbic acid could control or prevent the growth of such acidophiles, whilst allowing a pH compatible with the flowers to be maintained.

Keywords:

Cut flowers, flower food, microbial growth, pH, reverse osmosis water.

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1 INTRODUCTION AND LITERATURE REVIEW

1.1 General introduction

Vase water provides an environment for cut flowers to die. Lengthening the time of senescence gives a reason for the existence of the emergence and longevity of the cut-flower food industry. Cut flowers require not only water but the energy to appear healthy and, for example, to allow buds to open. A shelf-life of approximately 14 days for cut-flowers is a general standard for consumers. Guaranteeing this is difficult as the variation of the product is large; the supply chain can occur over large distances and the requirements of the flowers to maintain a fresh look are complex. A particular problem which has vexed the industry is the role of microorganisms – bacteria, yeasts and moulds – in the senescence of cut flowers. There are a variety of hypotheses as to the possible role that bacteria have, with experimental evidence to back them, but there are also contrary observations which makes the field difficult to understand.

There is, however, no disagreement on the fact that the vase water provides a good environment for the growth of microorganisms. Prolific microbial growth is seen as an increased cloudiness (turbidity) of the vase water, often accompanied by off-odours depending on the type of microbes growing. The growth of mould on the vase sides or on the cut stems is a very common observation and often produces repugnance by the consumer. The consumer's handling of the flowers may, in fact, dictate the likelihood of such occurrences. Vases are more often placed at room temperature – brightening a room is a principal reason for buying such products - and the vase water is open to the atmosphere, but can also provide anaerobic conditions in the depths of vase water. If flower food is used this provides sugars for extensive bacterial growth, and the plants themselves can also provide nutrients to the bacteria.

Microorganisms have the ability to grow in many diverse environments, but do have limits on their abilities to grow in any particular environment. This knowledge is used, for example, in the food industry to inhibit pathogenic

bacterial growth by placing an upper limit on the pH of certain foods, below this threshold pathogens will not grow. Refrigeration is a very common way of preventing extensive microbial growth, but certain spoilage (e.g. *Pseudomonads*) as well as pathogenic bacteria (e.g. *Listeria*) can grow albeit slowly in such conditions. The food industry cannot simply reduce pH to protect the foodstuff – the low pH may render the food unpalatable. Often, a reduced pH is achieved by the combination of an acidulant, such as citric acid, with a weak acid preservative such as acetic, sorbic or benzoic acids. These combined systems have the effect of increasing the potency against microbial growth for a given pH.

Commercial flower food not only contains sugars, essential for cut-flower maintenance, but also provides a low pH, generally achieved using citric acid. The flower food may also contain antimicrobials to further inhibit growth of the microorganisms.

At an initial meeting between Cranfield and Chrysal International BV it was stated that under the conditions of pH in the vase water achieved by the addition of flower food, the very organisms which were isolated (e.g. *Pseudomonads*) could not grow, i.e. the pH was too low to achieve substantive growth. It was concluded that the vase water environment must change during the period that the consumer displays the flowers, to allow such microbes to grow.

Cranfield University (Dr R Lambert) has an extensive knowledge and database of microbial growth in a variety of environments with particular relevance to the food industry. It was suggested that this knowledge may allow new insights into the microbial ecology of vase water.

1.2 Cut flowers

The term cut flower is often used to describe decorative indoor flowers with stem and/or leaves that have been cut from a flowering plant. Plants producing cut flowers can be grown in the field, climate control glass houses or in the wild.

Fresh cut flowers have a limited vase life, therefore the work on various methods to extend or maintain the freshness and quality of such a product is a continuing process. Scientific and consumer research has improved rapidly in this field in the last few years; the aim behind all research efforts is to reduce waste, maximise profit and satisfy consumers so they can still enjoy fresh cut flowers for longer.

The term “vase life” of cut flowers should refer to the longevity of cut flowers at the final consumer’s home but not including the time of transportation or pre-treatment (Halevy and Mayak, 1981). The interpretation of the results of any vase life experiment may vary depending on pre-harvest and surrounding environment conditions of the cut flowers themselves. Therefore the lack of standard methods or protocols to evaluate vase life hampers efforts to establish a defined cause of shortening that vase life despite the increase of papers published in this field over the last decade (Fanourakis *et al.* 2013).

The cut flower market is driven by a mix of powers; human emotions in any society, rules and regulations, economic situation and of course demand and availability of the product itself. The quality of cut flowers is in continuous demand for improvement to meet consumer expectations and to adapt to the ever changing regulations which governing the market. Parameters to evaluate quality of cut flowers (size, colour and fragrance) are quite different and vary between European and American markets and therefore a great deal of efforts is done by the industry to keep updating and improving these evaluations according to ever changing regulations (da Silva, 2003).

Higher quality product can be obtained by cultivating plants under optimal conditions; hence the frequency and date of harvesting, the physiological or developmental stage of the flower and distance to the target market are all important factors controlling the global trading of cut flowers (Monteiro *et al.*, 2001). For example inadequate lignification in vascular tissue of peduncles resulting in conditions like ‘scape bending’ in gerbera or ‘bent neck’ in roses can be a common sign of flowers being cut too early in the field – the plants simply did not have enough time to form and deposit lignin in cell walls. Cold storage

prior to marketing is also an inevitable practice if flowers have to be transported or shipped for long distance to reach the target market; cold storage is usually used to extend shelf life by slowing down metabolism and also reducing the heat produced by respiring tissues (da Silva, 2003).

There is some evidence from the literature reported by van Doorn and Perik (1990) showing that the blockage of basal parts of cut rose flowers' stem reduces the water uptake by the flowers. However the nature of the occlusion in the stems is not fully understood. Different factors were suggested to explain the cause of the blockage such as the presence of microorganisms, air embolism or the physiological changes in the stem itself triggered by the cutting process, although there is not enough evidence in the literature supporting the latter factor (the physiological changes in the stem).

There is current debate in the literature regarding whether to consider the amorphous plugs found in the xylem of cut rose flowers as a serious problem in the vascular blockage or not, some argued that the plugs were found in only few xylems which does not support the idea of this as a common problem (van Doorn and Perik, 1990).

1.3 Physiology of plant and cut flower

The physiological structure of cut flowers cannot be separated from the physiological structure of the plant from which they have been cut, though the nutrient needs and care are different. Plants get their nutrients from the soil through roots, but cut flowers lose that source as soon as they are cut from the mother plant, therefore the addition of nutrients (such as sugars) to the vase solution could be considered a vital intervention to keep the physiological activities running and to prolong the vase life of cut flowers.

The basic needs for any plant to grow are carbon dioxide, light, water and minerals (including nitrogen). There are some other internal and external factors which affect and regulate the growth of the plant; hormones are the most

important internal factor, while temperature, light direction and gravity are some of the external factors (Raven *et al.*, 2003).

Plants capture the sun light and use its energy to produce organic materials and energy from inorganic materials by photosynthesis. In this process green plants use water and carbon dioxide with the help of energy comes from light and chlorophyll in plant cells to produce oxygen and glucose which is essential for the respiration process. Respiration is the chemical process that uses glucose with the presence of oxygen to release energy needed for the plant to live and grow. The need of carbon dioxide in the photosynthesis process goes together with a high demand of water; plants need to replace the loss of water vapour resulting from stomata opening to uptake carbon dioxide into the leaf surface, therefore the steady flow of water (also called transpiration stream) between plant's roots and leaves through the xylem should not be interrupted or blocked to ensure effective plant growth (Heldt, 2005).

1.3.1 Physiology of cut flowers

Good understanding of the post-harvest physiological requirements of any plant can greatly assist and help the grower to take suitable actions which lead to satisfactory results when the product reaches the hands of the final customer, cut flowers are no exception to this rule (da Silva, 2003).

Mature leaves in plants work as a very important source of sugars through the photosynthesis process. These sugars normally translocate in the plant tissue through the phloem to where they are needed (such as developing flowers). When stems are cut from the mother plant this source is dramatically reduced (Arrom and Munné-Bosch, 2012), however during cut flower senescence part of the flower (in particular petals) may act as a sugar source (Bielecki, 2000).

The senescence physiology of cut flowers is a rather complex one as one inflorescence can consist of different units e.g. androecium, gynoecium, petals, sepals, stem and most of the time leaves. Each unit has its own morphological features. When designing postharvest techniques to handle cut flowers or when

developing products to prolong the vase life of cut flowers consideration of two different and rather conflicting stages of the cut flower development must be examined; the first one is the growth processes such as bud growth and flower opening which need to be stimulated, and the second one is the metabolic processes leading to senescence which need to be delayed (Halevy and Mayak, 1981).

Senescence is a very important physiological process that determines the quality of cut flowers. Understanding this programmed process is vital when it comes to exploring ways to preserve the freshness of cut flower for longer after opening. The process of senescence does not take place in all floral organs at once, the first tissues to show signs of senescence are normally petals whereas the gynoecium (mainly the ovary) remains active and functional all the way through ensuring seed development (Arrom and Munné-Bosch, 2012).

Sucrose in vase solutions is widely known for its effects in increasing energy availability for respiration by floral tissues, improving the water usage and extending the vase life of cut flowers. A recent study has evaluated the role of sucrose in making changes to the hormonal balance of floral tissues and its effect in accelerating flower opening and delaying senescence in lily cut flowers (Arrom and Munné-Bosch, 2012). Results from this study showed the increase of endogenous glucose levels during the flower opening stage and the decrease of the same levels during the senescence stage in all floral organs, despite the fact that sucrose levels increased only during senescence in the androecium and in outer and inner tepals of lily cut flowers.

Although adding sugar to the vase solutions is important as an energy source it can have negative effects on leaf quality in cut lily flowers; van Doorn and Han (2011) showed that the effect can be mitigated by the addition of hormones such as benzyladenine and gibberellins (GA_{4+7}). The role of sugars in delaying ethylene biosynthesis in cut flowers has also been reviewed (Pun and Ichimura, 2003). The authors of this review concluded that there was a possible role of sugars in the delay in ethylene biosynthesis or the drop in sensitivity to ethylene

in cut flowers but there was not enough information about the mechanism of that action.

1.3.2 Water movement

Water naturally moves from a higher potential energy (also called higher water potential) to a lower potential energy (also called lower water potential), the energy controlling the movement of water could be gravity, pressure or the concentration of soluble particles in that water. The latter is an important factor affecting the movement of water in living systems, in this case the molecules of water move from lower solute concentration to higher solute concentration (Raven *et al.*, 2003). Bulk flow and diffusion are the two mechanisms that water and solutes use to move in living systems, bulk flow is mainly used when water, including solutes (such as sugars) moves from one part to another in a multicellular organism, while diffusion is largely used when moving ions and many other molecules from, into or through cells (Raven *et al.*, 2003).

In cut flowers water movement can be hindered by many factors such as the blockage of the xylem caused by high numbers of bacteria (Put, 1990; van Doorn and de Witte, 1995; Loubaud and van Doorn, 2004), by wound reaction by the cut flower stem (van Doorn and Cruz, 2000; Loubaud and van Doorn, 2004; van Meeteren *et al.*, 2006), or by aspired air in the case of dry storage (van Meeteren *et al.*, 2006). The ultimate result of all kinds of blockage is a decrease in water intake by the cut flower and therefore loss of quality, senescence and reduction of vase life.

The quality and pH of water are essential for smooth and balanced water uptake by cut flowers; acidic solutions are more suitable than alkaline and neutral solutions due to their easy and free movement up the stem, also clean pure water is very important in reducing the risk of contamination and stem blockage (da Silva, 2003).

1.4 History of cut flowers

The use of cut flowers has a long history going back more than 5000 years. They were used by ancient Egyptians, Chinese and ancient Greeks and Romans for symbolic or religious purposes. Iris, lotus and rose flowers were widely used by ancient Egyptians, where tiger lily, peony and orchid were the favourite flowers used by the Chinese, the Greeks and Romans used herbs and flowers such as laurel, parsley, rosemary, anemones and roses to celebrate victory in events such as athletic competitions and military battles ([eHow.com, 2012](#)).

The production of cut flowers started in the United States of America with the arrival of the European immigrants and has continuously improved with the improvement of transportation and refrigeration systems ([North Carolina State University, Department of Horticultural Science, 2012](#)).

Nowadays cut flowers are still used for various occasions including religious festivals, new born celebration, to patients, in funerals and to celebrate love all over the globe.

1.5 Cut flowers industry

The annual global trade in cut flowers is estimated to be around € 30 billion. Europe and North America are the leading markets, but with the increasing production cost in these continents a new market to produce cut flowers has started to establish since the 1990s in some developing countries like Ecuador and Colombia in South America, and Ethiopia and Kenya in Africa. The climate conditions and labour costs are more amenable than those same in the Northern hemisphere ([Rikken, 2010](#)).

The UK flowers and houseplant industry is currently worth about £1.7 billion compared to its peak (£2.4 billion) in 2007 according to the Flowers and Plants Association. Figures for 2008 showed that in the UK £121.5 million were spent on wedding flowers, £103.8 million on funeral flowers and £59.55 million on

New Baby flowers. The peak seasonal sales of cut flowers is Valentine’s Day, on which 2.3 million bunches of flowers were bought in 2010 compared to 1.9 million in 2009. Despite the big size of the industry only 5% of UK cut flowers are grown and sourced in the UK, 95% are imported from overseas countries including the Netherlands (77%), Kenya (9%), Colombia (6%), South Africa (1%), Spain (1%) and 5% from other various countries ([Flowers & Plants Association, 2012](#)).

The customer’s preference and favourite cut flower changes with the time, the ten top best-selling flowers in UK in 2009 are different from those ten years earlier as shown in Table 1.

Table 1 Comparison between the ten best-selling cut flowers in the UK in ten years period (source: *Flowers and Plants Association, UK, 2012.*)

Preference	1999	2009
1	Carnation	Roses
2	Chrysanthemum	Mixed Bunch
3	Mixed Bunch	Carnation
4	Daffodil/Narcissus	Daffodil/Narcissus
5	Other single variety	Chrysanthemum
6	Tulip	Lily
7	Rose	Tulip
8	Mixed arrangement	Sunflower
9	Freesia	Alstroemeria
10	Lily	Amaryllis

It was suggested that the European Union (EU) is consuming half of the world’s flowers. On the other hand the Netherlands is the major producer of the cut flowers in the EU with an estimated share of half of the EU total production value of € 4 billion ([CBI Market Information Database, 2009](#)). The total spending by individuals in each European nation buying cut flowers is varied; the Netherlands as a population has the highest consumption of cut flowers per

head followed by Belgium and Austria, but it comes sixth when looking at the total consumption as a country, the biggest consumer of flowers in the EU is Germany followed by the UK and France ([CBI Market Information Database, 2009](#)).

According to the recent US Consumer Floral Tracking Survey (2012), the floral market in the USA is bouncing back despite the decline in sales during the last decade (2000 to 2010). The consumer profile from the survey revealed that the consumers buying fresh cut flowers for home decoration during the current economic climate were from young households (under 35 years old); it is considered that this age group could help the floral market regain its position prior to the current recession, as global economies improve ([P & P Market Report, Floral Market Research.com, 2012](#)).

Cut flowers care products such as flower food is an important sector of the industry. Fresh cut flowers are delicate products, needing careful handling and consideration at the farm and postharvest stage. Choosing the right care product at each stage of production is considered vital to ensure a healthier, cost effective and value for money product for customers.

Chrysal[®] International B. V. (Naarden, The Netherlands) is one of the leading and largest companies in the flowers care industry, the company was founded in 1929 by H. P. Bendien. In 1949, a novel formula that helped to double the vase life of cut flowers was introduced by Ir Camille Buys. Since then the company has strengthened its place in the global market through continual innovation in order to meet the increasing customer demand for the high quality of fresh cut flower. The products of the company are available in over 60 countries worldwide with offices in Europe, Asia, the United States and Colombia ([Chrysal International web site, 2012](#)). Chrysal products are designed to care for flowers from the moment they have been harvested throughout the chain until they reach the final customer's home, these products include flower and plant care products, control and conditioning products, and post-harvest treatments.

Research & Development Division, Floral Solutions, in Chrysal is constantly working to develop new products and improve their existing products for cut flowers care. The division is also responsible for quality monitoring and innovation (Chrysal.co.uk, 2013).

1.6 Growth of microorganisms in vase water

Microorganisms have a substantial presence in the vase water of cut flowers. As time proceeds the vase water, initially clear, becomes turbid or cloudy. As time further proceeds, the turbidity increases and often the vase water, perceived as the whole by the consumer, becomes foul smelling. The growth and metabolism of microorganisms are at the centre of these phenomena.

Microorganisms can be found in almost every place on Earth – from the scalding acid geysers that surround volcanoes to the ice of Antarctica. Many microbes can synthesise their own food, e.g. some carry out photosynthesis, and hence can be found in nutrient limited environments, they can also synthesise compounds to allow them to survive in extreme environments. The vase water environment is not an extreme environment, indeed quite the opposite. The vase water, and often the plant food given is an ideal environment for many types of microorganisms, including yeasts, moulds, spoilage and pathogenic bacteria and viruses too. Indeed the growth of some pathogenic microorganisms in vases might be the reason why flowers were banned from almost all NHS hospitals.

The ban on flowers in the NHS hospitals had started only in few wards (high dependency units), but press reports on total ban in general wards started appearing in 1996 ([Day and Carte, 2009](#)). Since then the ban is growing; the reasons believed to be behind the ban are varied and greatly depend on the individual NHS trusts; this could be anything from:

- 1- Health and safety issues, e.g. preventing staff from the risk of injuries from broken glass vases and avoiding incidents of water spillage over expensive electronic equipment.

- 2- Hygiene and clinical issues, e.g. belief that flowers and/or their vase water may pose a risk of infection to patients or even that they might contribute to the reduction of oxygen in the air from their decomposing material.
- 3- Some hospital staff think that changing vase water and disposing of old flowers is adding more burdens on their already overloaded duties, so they are fully supporting the ban for the sake of some extra duty and pressure relief.

Although all the above are not fully supported with solid evidence from the published literature according to the British Medical Journal article published in December 2009, but the ban on cut flowers in the NHS hospitals is wide spread (Cohn, 2009).

1.6.1 Ecology of vase water

The vase life of fresh cut flowers is normally determined by their physical ability to use xylem to uptake water and nutrients; this ability is often hindered by the amount of bacteria and fungi in the vase water. The vascular occlusion of the xylem can be caused by live or dead bacteria (van Doorn *et al.*, 1991).

Some studies showed that some bacterial strains with counts as low as 10^5 colony forming unit (CFU) /ml have the ability to reduce the vase life of carnation cut flowers, but later experiments showed that although as a result of the increase of bacteria population in vase water leading to vascular blockage, this had relatively slight effect on the time flowers took to wilt. In these experiments when flowers were kept in vase water with, primarily, a low bacterial count (below detection on day 0) for 7 days the number of bacteria in the water increased to 10^7 CFU/ml, then when freshly cut stems were placed in this vase water, the senescence of flowers was not affected. This showed that despite the rather high numbers of exogenous bacteria the vase life of carnation cut flowers was not reduced as previously hypothesised (van Doorn, 1995).

Microbes present or growing on cut flower (stems and/or leaves), on flowers containers and in the water used to sustain the cut flowers are the main sources of the microbial contamination of vase solution (Florack *et al.*, 1996). Different species of bacteria, some yeast and some fungi were isolated from the vase water of cut roses, species such as *Bacillus*, *Enterobacter* and *Pseudomonas* were found to be predominant in such vase water, although other species were also detected but in less frequency such as *Citrobacter*, *Alcaligenes* and *Acinetobacter* (Florack *et al.*, 1996).

It has also been found that the dominant microbial species were different in each stage of vase life. The results of one study showed that the initial dominant bacteria in the vase water of freshly cut flower stems of chrysanthemum, gerbera and rose cultivars were *Enterobacter* and *Bacillus* species alongside fungi, then after 3 days of vase life *Pseudomonas spp.* dominated, in less than 10 days *Enterobacter* dominated again with *Bacillus* and at 10 days or more fungal growth increased greatly (Put, 1990)

In another study the flora of vase water was tested from three different environments (hospitals, restaurants and private gardens), 41 different species of bacteria were identified some of which were multi-resistant to antibiotics. Among the 41 different species identified there were 32 Gram negative; 12 different species of the genus *Pseudomonas*, 4 *Klebsiella* species, and 4 *Enterobacter* species. Results showed that the antibiotic multi-resistant bacteria were indigenous to flowers and did not originate from the environment where the flowers were kept, (Figure 1-1) (Kates *et al.*, 1991).

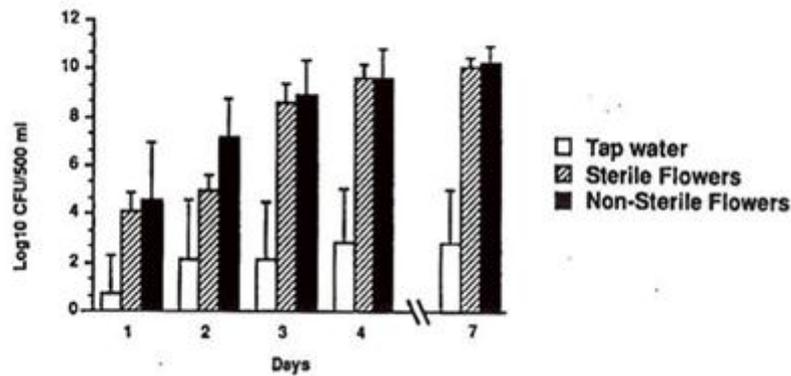


Figure 1-1 *Log₁₀ colony-forming unit (CFU)/ 500 ml of water from tap water, sterile flowers and non-sterile flowers cultured daily for 7 days (from Kate et al., 1991).*

The stem xylem can also be blocked when cutting flowers at harvest time, the blockage is thought to be caused by air embolism trapped into the xylem conduits. Placing cut flowers in vase water containing high numbers of bacteria with the existing air in the xylem conduit could lead to immediate cavitation. This hypothesis has been tested in two cultivars of rose cut flowers (Madelon and Cara Mia) by using ultrasonic acoustic emissions (Bleeksma and van Doorn, 2003). The results showed that although the cavitation was caused mainly by bacterial occlusion, the presence of air bubbles in xylem conduits had contributed to a degree as well. It was also noticed that the air embolism can be eliminated by cutting flowers under water.

In addition to the effect of bacteria some cut flowers have a negative effect on other cut flowers when placed together in the same vase, for example the effect of daffodil flowers on the vase life of cut roses and tulips. In the case of daffodils placed with cut roses the effect was mainly due to its mucilage and the increase of bacteria in the vase water, but when it was placed with tulips the effect was mainly due to mucilage toxicity to tulips flowers (van Doorn, 1998).

Inhibition of water uptake and therefore reduction of vase life has also been observed in cut carnation flowers as a direct effect of high numbers of exogenous bacteria (10^8 CFU ml⁻¹ or more). It was found that a mix of bacteria species (20% each from *Acinetobacter calcoaceticus* and *Alcaligenes spp.* and

50% from *Pseudomonas* spp.) isolated from carnation flower stems kept in water for 10 days or pure culture isolates from *Acinetobacter calcoaceticus* and *Pseudomonas fluorescens* had no effect on permanency of vase life but they had the same effect of inhibiting the water uptake (van Doorn *et al.*, 1995).

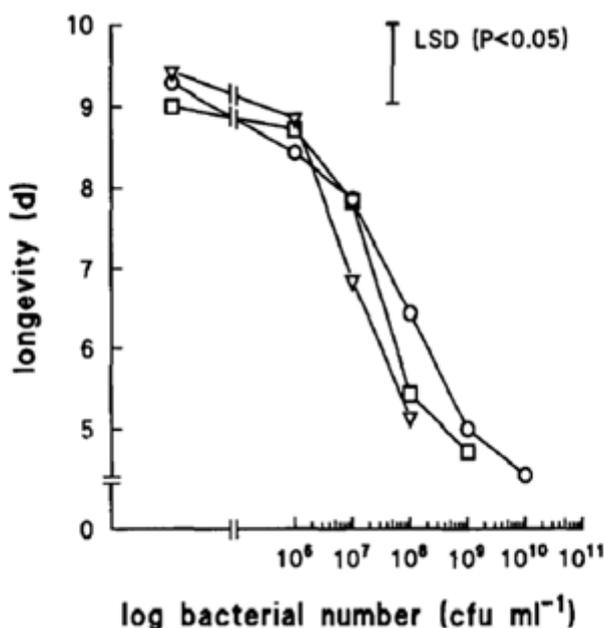


Figure 1-2 The longevity (in days) of carnation cut flowers cv. White Sim after insertion of a number of concentrations of bacteria in the vase solution on day 0. Bacteria were: *Acinetobacter calcoaceticus* (Δ), *Pseudomonas fluorescens* (\square), or a mixed flora from carnation stem held in water for 10 days (\circ) (from van Doorn *et al.*, 1995).

1.6.2 Inhibition of microorganisms

Minimising bacterial growth appears to be essential to extend the shelf life of cut flowers. Certainly reducing the appearance of turbidity and the foul odours would benefit from such a strategy. Many compounds, individually and also in combination, have been used to control the growth of microorganisms in vase water of different cut flowers. These have ranged from simple metal salts (e.g. silver) to weak acids (e.g. acetic), hydroxyquinoline and similar compounds, essential oils and their components, metal ion chelators and antimicrobial peptides to name only a few examples;

Antimicrobial Peptides:

For cut flower roses the toxicity of cecropin B, hordothionin and tachypleisin I peptides were tested and evaluated and found to be effective in controlling the growth of bacteria in vase water (Table 2), in particular tachypleisin I was the most effective of the three to control a population of *Bacillus*, *Enterobacter* and *Pseudomonas Spp.* (Florack *et al.*, 1996).

Table 2 Toxicity of peptides (in µg/ml) in vase water (7 day old) using pour plating, where MIC is the concentration at which the growth is inhibited when using CFU counting, and MBC is the concentration at which 99.9% of the inoculum is no longer able to form colonies after plating (Florack *et al.*, 1996).

Peptide	Tap water		Sterilized tap water	
	Minimal Inhibitory Concentration (MIC)	Minimal Bactericidal Concentration (MBC)	Minimal Inhibitory Concentration (MIC)	Minimal Bactericidal Concentration (MBC)
Cecropin B	12.8-25.6	>25.6	6.4	25.6
Hordothionin	12.8	>25.6	3.2-6.4	25.6
Tachypleisin I	3.2-6.4	12.8	1.6-3.2	6.4

Cecropins were originally isolated from the Cecropia moth; these are a family of antimicrobial peptides which lyse bacterial cell membranes – both Gram positive and Gram negative. The structures of Cecropins (Figure 1-3) show a multitude of alpha-helices, which form channels at low concentration but pores at higher concentration (Silvestro, 2000).

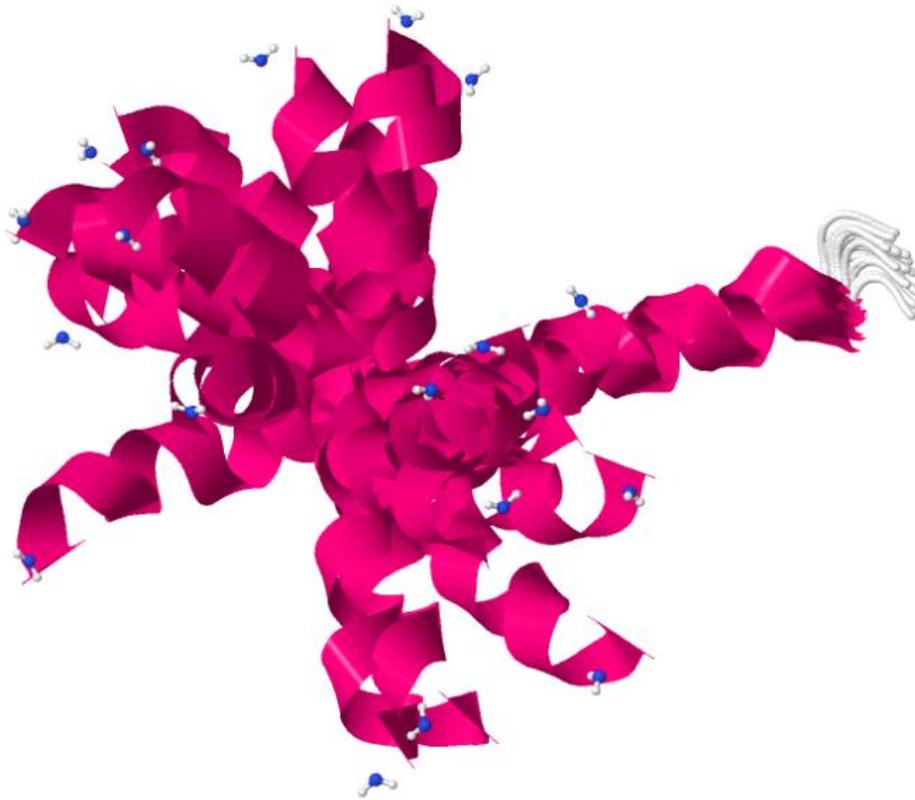


Figure 1-3 *Cecropin structure.*

Thionins are small proteins (5KDa) which exhibit toxicity to bacteria and fungi. Although the mechanism is not fully understood it is believed to be due to altering membrane and the production of channels (Han *et al.*, 1996).

Tachyplesin is a cationic peptide normally isolated from the horseshoe crab. It is active against both Gram positive and Gram negative bacteria, e.g. both *Salmonella typhimurium* and *Staphylococcus aureus* were inhibited by 6 µg/ml. It was suggested that its activity was due to its complexation with the lipopolysaccharide of the outer cell walls (Nakamura *et al.*, 1988).

Silver ion:

Silver ion (Ag⁺) is active against a large range of microbes at generally low concentrations. It has been used to disinfect drinking water (Berger *et al.*, 1976; Pyle *et al.*, 1992). At 8ppm, silver nitrate effectively inhibits *Pseudomonas aeruginosa* (Lambert and Pearson 2000), and caused a 3 log reduction of *Staph. aureus* in 10 minutes at 2 ppm (Lambert and Johnston 2000). In

combination with copper ion it has been shown to inactivate *Legionella pneumophila* (Lin *et al.*, 1996).

Three different dominant bacterial species; *Acinetobacter*, *Bacillus* and *Pantoea* were isolated from *Gerbera jamesonii* L. cv. Provence. The increase of population of these species was thought to be correlated with the stem break of gerbera cut flowers (Balestra *et al.*, 2005). Adding silver-nitrate to the vase water in this study reduced the number of bacteria found beneath the flower capitulum from 3.7×10^4 cfu g⁻¹ to 2.2×10^2 cfu g⁻¹ compared to using tap water only. The use of AgNO₃ in vase solutions delayed the primary stem break in gerbera to 14 days compared to 6 days when tap water only used (Figure 1-4).

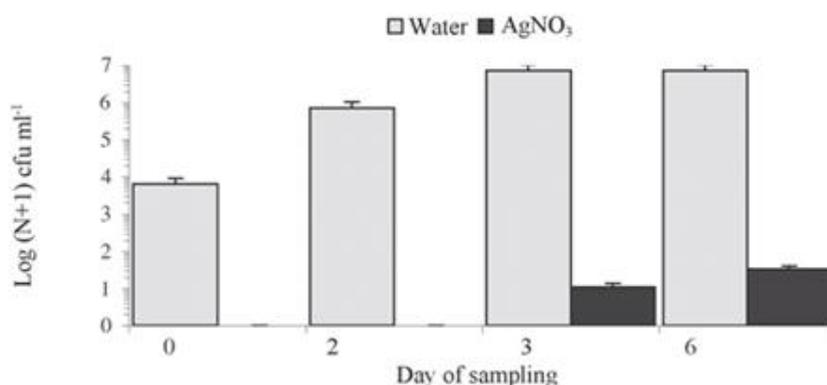


Figure 1-4 Cumulative bacteria populations in tap water and AgNO₃ solution (from Balestra *et al.*, 2005).

A new development with silver ion has been the rise of 'Nano-silver' and is currently a 'hot-research topic'. There is a fast growing industry in Nano-silver offering it as an effective antimicrobial, particularly for surfaces. Nano silver has been placed in products such as toothpastes, shampoos, bath towels and even baby-food bottles. Samsung have used Nano silver as a coating material in their domestic products such as fridges.

Nano-silver (NS) and its ability as antimicrobial was assessed and evaluated to control bacteria and extend the vase life of carnation cut flowers (Basiri *et al.*,

2011). The results showed that five concentrations of Nano-silver (5, 10, 20, 40 and 80 ppm) in 6% sucrose vase solutions has extended the vase life considerably and inhibited the growth of microorganisms in vase water.

The efficiency of silver nano particles (SNP) in combination with essential oils was evaluated as anti-microbial agents to control bacterial growth in vase water of gerbera cut flowers. A combination between essential oils; carvacrol (in concentration of 50 or 100 mg/L) and 1 or 2 mg/L SNP was found to be effective to extend the vase life of gerbera flowers from 8.3 to 16 days compared to controls (Solgi *et al.*, 2009). However, it should be noted that silver ion is a strong oxidising compound and many components of essential oils are easily oxidised to weak acids, known to inhibit microbial growth.

Other antimicrobials compounds:

Hojjati *et al.* (2007) used a variety of antimicrobials (8-hydroxyquinoline citrate (200, 300 & 400 ppm), aluminium sulphate (100, 150 & 200 ppm), cobalt chloride (200, 300 & 400 ppm), copper sulphate (100, 150 & 200 ppm), ethanol (2, 4 & 6%), aluminium sulphate and citric acid (150 ppm each)) in water with 2.5% sucrose to study the shelf-life of two cultivars of eustoma cut flowers (blue and cream).

Copper sulphate (100 ppm) and 8-hydroxyquinoline citrate (300 ppm) concentrations in sucrose were found to be the most effective among other concentrations used in prolonging the vase life and maintaining the quality of cut flowers (Hojjati *et al.*, 2007). This study also showed that the two cultivars of eustoma cut flower had different reactions to the chemical treatment as well as having different vase life. Using sugars (sucrose) alone without germicides present in vase solutions was not recommended because it helped bacterial growth and reduced the vase life.

A study on cut rose flowers found that when the numbers of bacteria exceed 10^6 cfu/g fresh weight the hydraulic conductance (the ease of water movement through pore spaces of plants) of the end segments of the stem (5 cm) was

reduced (van Doorn and Perik, 1990). However when hydroxyquinoline was added or when the pH was lowered to 3 by using buffer this did not occur. It was concluded that adding hydroxyquinoline or a buffer at pH 3 reduced the number of bacteria in basal end of flower stem and prevented vascular blockage.

The use of 2 or 10µl/l of chlorine dioxide (ClO₂) has extended the vase life of some selected cut flowers including rose, lilies and gerbera by 0.9-13.4 days. The effect of ClO₂ was apparently the reduction in the number of aerobic bacteria on the cut surfaces of the cut flower stems and in the vase water (Macnish *et al.*, 2008).

Using organic and natural materials to extend the vase life of cut flowers was explored recently as the demand for using less chemical preservatives has increased. The leaf extract of *Psidium guajava* (Guava) and *Piper betle* (a vine which belongs to the Piperaceae family (pepper), and is consumed as an addictive euphoria producing drug, i.e. bio-active) were evaluated and tested as antibacterials and antifungals to extend the vase life of two cultivars of carnation cut flowers (Carola and Pallas Orange).

These extracts showed similar effects on the quality of carnation cut flowers to the widely used artificial germicide 8-hydroxyquinoline citrate (Rahman *et al.*, 2012). In these experiments Sprite was used as a vase solution which provided the main source of energy to maintain the biochemical and physiological processes in the cut flowers. The sodium benzoate and carbon dioxide in the Sprite worked as ethylene inhibitors and the leaf extracts of *Psidium guajava* and *Piper betle* acted as antimicrobials.

pH as an inhibitor:

Microorganisms can grow in environments of high alkali (alkaliphiles) such as *Bacillus alkalophilus* (pH 10.5) and high acid (acidophiles) such as *Alicyclobacillus species* (pH 2). Internally, the pH of microbes is usually higher (if in an acid environment) or lower (if alkali). Figures 1-5 and 1-6 show the effect

of pH on the growth of two microorganisms; in the former the organism has a narrow range of pH over which to grow, whereas *Lactobacillus plantarum*, an effective spoilage organism of low pH foods, can grow over a much wider range (Lambert, 2010).

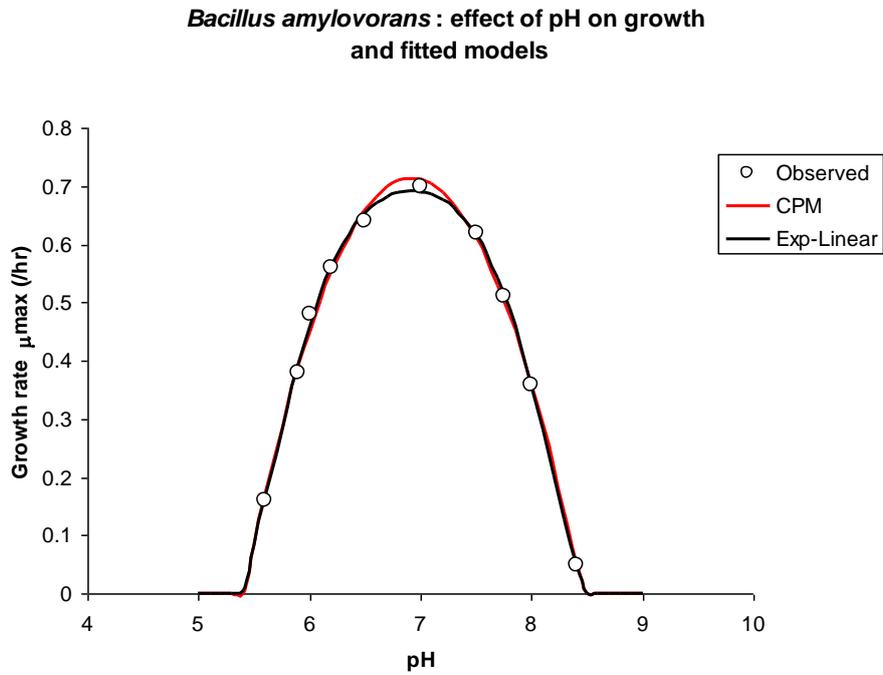


Figure 1-5 The effect of pH on growth and fitted models (from Lambert, 2010).

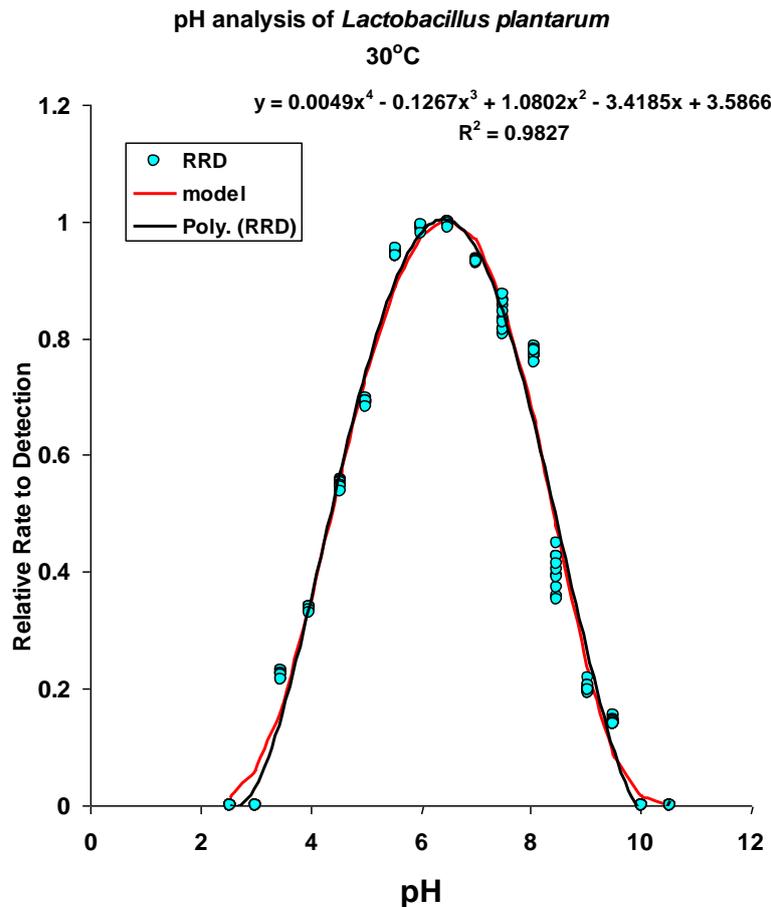


Figure 1-6 The pH analysis of *Lactobacillus plantarum* at 30°C (from Lambert, 2010).

The internal pH is actively maintained at a fixed level in order to maintain the proton-motive force used to drive ATP formation. If, for example, weak acids are introduced into an acidic environment, the internal pH of the microbes decreases due to the influx of the protonated form. Growth ceases depending on the concentration of the weak acid, its pKa and the external pH. The microbe can pump out the increase internal proton concentration using ATP and accumulate the anion of the weak acid. This results in the increase of the internal pH (Figure 1-7). If the internal pH returns to a normal value the organism can then grow. If, however, there is not enough ATP available then the internal pH cannot be increased to the minimum required for growth, and therefore the microbe becomes 'dormant' or dies (Lambert and Stratford 1999).

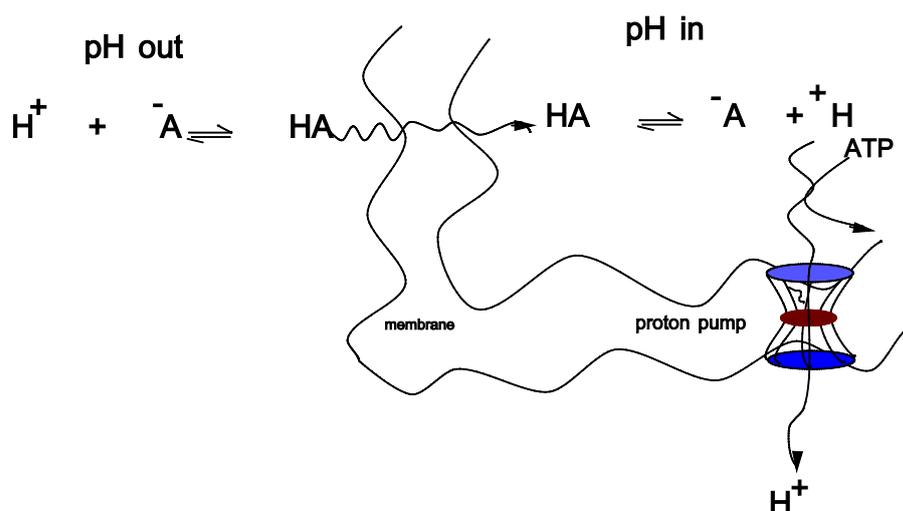


Figure 1-7 A schematic diagram of the microbial response to weak acid preservatives being added to an acidic environment (from Lambert and Stratford, 1999).

The pH of vase water therefore plays an important role in determining the growth or no growth (G/NG) of bacteria and fungi. To minimise the impact of fungi and yeast the pH should be as low as possible (approximately 3), but with pH over 4 the chance of bacteria to grow rapidly will increase (van Doorn, 1995).

Suitable pH for vase solution use to keep fresh cut flowers is 3.5-4.5. Acidic solutions have the ability to move up the stem more freely than alkaline or neutral solutions (da Silva, 2003). The chemical components in de-ionised, distilled or tap water are varied; such components can easily affect the quality of cut flowers (da Silva, 2003).

1.7 Flower food

Flower food is one of the flower care solutions which also include hydration solutions, anti-ethylene treatments and specialised care solutions. Each one of these care solutions is targeting one or more area of the production chain, from growers, wholesalers, retailers and final consumers. It is well known that flower food could double the vase life of cut flowers compared to using only tap water,

that is mainly due to the added sugars which are essential ingredients in providing nutrients to the plant, flower food may also contain antifungal and antibacterial components to control the microbial growth.

Cut flowers need a source of energy to keep the basic physiological activities of their tissues such as respiration. Adding sugars to the vase solutions is a well-known practice to extend vase life of cut flowers, the main benefits that the plant gets from added sugars are the increasing available energy for respiration and water relation improvement.

1.8 Beverage Microbiology

The activity of food spoilage organisms, in general, causes the visible or detectable changes of food's physical and sensorial properties (Loureiro and Querol, 1999). In acid drinks, with or without sugar, a rich gas production is an obvious sign of spoilage incidents; this may cause the packages to deform or blow up, it could also show other signs such as cloudiness, residue or pellicle formation. The taste and smell could also go off and replaced by a slight fermentation smell. Deterioration of food and drinks by spoilers may present other effects, more or less evident, according to the type of food (Table 3). Yeast growth is a major factor in the spoilage of such kind of foods and can be a very substantial economic problem. Despite the fact that the inhibition of spoilage yeasts such as *Zygosaccharomyces bailii* and *Saccharomyces cerevisiae* can be controlled by weak organic acid preservatives, often the required levels of such preservative are near or greater than the legal limits (Hazan *et al.*, 2004).

Table 3 Contamination and spoilage yeast recovered from foods and beverages. (From Loureiro and Malfeito-Ferreira, 2003).

Most frequent contaminants ^a (Deak and Beuchat, 1996)	Spoilage species (Pitt and Hocking, 1985)	Additional spoilage species (Tudor and Board, 1993)
<i>Saccharomyces cerevisiae</i>	<i>Brettanomyces intermedius</i> ^b	<i>Candida dattila</i>
<i>Debaryomyces hansenii</i>	<i>Candida holmii</i>	<i>Candida globosa</i>
<i>Pichia anomala</i>	<i>Candida krusei</i>	<i>Candida humicola</i>
<i>Pichia membranifaciens</i>	<i>Debaryomyces hansenii</i>	<i>Candida lactis-condensi</i>
<i>Rhodotorula glutinis</i>	<i>Kloeckera apiculata</i>	<i>Candida lipolytica</i>
<i>Rhodotorula mucilaginosa</i>	<i>Pichia membranifaciens</i>	<i>Candida parapsilosis</i>
<i>Torulaspota delbrueckii</i>	<i>Saccharomyces cerevisiae</i>	<i>Candida saké</i>
<i>Kluyveromyces marxianus</i>	<i>Schizosaccharomyces pombe</i>	<i>Candida versatilis</i>
<i>Issatchenkia orientalis</i>	<i>Zygosaccharomyces bailii</i>	<i>Candida zeylanoides</i>
<i>Zygosaccharomyces bailii</i>	<i>Zygosaccharomyces bisporus</i>	<i>Cryptococcus</i> spp.
<i>Candida parapsilosis</i>	<i>Zygosaccharomyces rouxii</i>	<i>Hansenula anomala</i>
<i>Zygosaccharomyces rouxii</i>		<i>Hansenula subpelliculosa</i>
<i>Candida guilliermondii</i>		<i>Kluyveromyces marxianus</i>
<i>Candida albidus</i>		<i>Pichia burtonii</i>
<i>Candida tropicalis</i>		<i>Pichia fermentans</i>
<i>Saccharomyces exiguus</i>		<i>Sporobolomyces roseus</i>
<i>Pichia fermentans</i>		<i>Torulaspota delbrueckii</i>
<i>Trichosporon pullulans</i>		<i>Trichosporon cutaneum</i>
<i>Hanseniaporea uvarum</i>		<i>Trycosporum pullulans</i>
<i>Candida zeylanoides</i>		

^a Species presented by decreasing level of occurrence.

^b Anamorph of *D. bruxellensis*.

The bacteria found in low pH beverages are heavily dependent on the pH itself. Low pH inhibits most bacteria, especially the pathogenic, but some acidophilic spp. can grow if other conditions are amenable. The most notable organisms are Acetic acid bacteria (pH 2.8 – 4.3), *Acetobacter*, *Acidiphilium*, *Gluconobacter*, *Acidomonas*, *Asaia*, *Gluconacetobacter*, *Kozakia Alicyclobacillus* (spp *acidoterrestris*, *acidocaldarius* & *pomorum*) and the Lactic acid bacteria. The most resistant moulds found to grow in beverages are shown in Table 4 which for each factor (sorbic acid etc) the numbers give the decreasing order of resistance (1 = highest) (Lambert, unpublished data).

Table 4 *Predominant resistant moulds in low pH beverages (Lambert, unpublished data).*

organism	sorbic	benzoic	SO ₂	DMDC	sugar	ferment	CO ₂
<i>Aspergillus niger</i>	5	2			5		
<i>Aspergillus phoenicis</i>	3			7	3		
<i>Aspergillus puniceus</i>		6					
<i>Circinomorcor circinelloides</i>			4	3		1	1
<i>Paecilomyces variotii</i>		4	3	4	4	2	2
<i>Pencillium decaturense</i>		1		6			
<i>Pencillium glabrum</i>	2	3		1	2		7
<i>Pencillium turbatum</i>				5	7		
<i>Penicillium chrysogenum</i>			6		6		6
<i>Penicillium corylophilum</i>	1	5	2	2	8		
<i>Penicillium spinulosum</i>	7						
<i>Penicillium steckii</i>	6			8	1		
<i>Trichoderma atroviride</i>	4		1			3	3
<i>Trichoderma koningii</i>						4	5
<i>Trichoderma longbranchiatum</i>			5				4

DMDC = Dimethyl Dicarbonate

From this a total score can be given to each organism and *P. glabrum* comes out as the most resistant *spp.* in acidic beverages.

The most resistant yeasts identified as being of serious problem were:

- *Zygosaccharomyces bailii*
- *Zygosaccharomyces bisporus*
- *Zygosaccharomyces rouxii*
- *Saccharomyces cerevisiae*
- *Dekkera bruxellensis*
- *Candida parapsilosis*

Of interest is the use of a system used in 'industrial forensic mycology' called the Davenport Scale which was introduced as a measure of the 'health' of a

beverages factory, Davenport grouped the spoilage organisms of low pH beverages into 3 groups.

Group 1 were all preservative resistant yeasts which will cause significant problems if contamination occurs, the Group 1 organisms are typically those yeasts identified above. Group 1 occur very rarely in a well -run, hygienic factory.

Group 2 contains all molds, opportunistic yeasts such as *Candida* and all spoilage bacteria. The presence of Group 2 organisms in product is usually an indication of some systems failure in processing/formulation. Group 2 are common organisms within a factory although at a low level, normally controlled by normal GMP.

Group 3 consists of hygiene indicator organisms such as *Rhodotorula*. These organisms will not cause spoilage in low pH beverages under any conditions, but do help to show the effectiveness of hygiene programs.

1.8.1 Predictive models

Many manufacturers have used predictive microbiology to reduce the risk of pathogen growth by expanding the knowledge base on these organisms. The same is almost certainly true for spoilage organisms, but these models are less well published by industry for obvious reasons. For yeast and mould spoilage, predictive mycology is still virtually terra incognita (Loureiro and Querol, 1999). In a recent International Conference on Predictive Modelling in Foods, a complaint was made [by Dantigny] that little time was given to fungal models – the reply was that few reliable models were available. A recent review by Dantigny *et al.* (2006) emphasised the gap between the bacterial (especially pathogen) models and those dealing with fungi: “In order to improve food quality and safety, there is a need for a tool allowing prediction of fungal development.”

As an example of a predictive model used in the beverages industry is the work of Professor D. W. Schaffner at Rutgers University (USA) sponsored by Kraft on the growth of a cocktail of *Aspergillus niger* and *Penicillium spinulosum*, both of

which are highly resistant to chemical preservatives. They modelled the effect of combined preservatives (sorbic and benzoic acids) in a typical beverage formulation [pH 2.8 to 3.8, sugar 8 to 16 Degree Brix ($^{\circ}$ Brix)] at 25 $^{\circ}$ C.

The results of the experiments showed that neither *A. niger* nor *P. spinulosum* grew in any samples at pH 2.8. At pH 3.3, mould was able to grow when the total preservative concentration was 325 ppm or less (one preservative at 100 ppm, and the other at 100 or 225 ppm), regardless of titratable acidity or $^{\circ}$ Brix. This was also true at pH 3.8, where mould grew in samples with 325 ppm combined preservatives or lower. No mould growth was observed at any other combination of preservatives at either pH 3.3 or 3.8. Figure 1-8 gives the probability of growth for combinations of K sorbate and Na benzoate at a specified pH, titratable acidity (0.25) and $^{\circ}$ Brix (12) after 8 weeks of observation (Battey *et al.*, 2001).

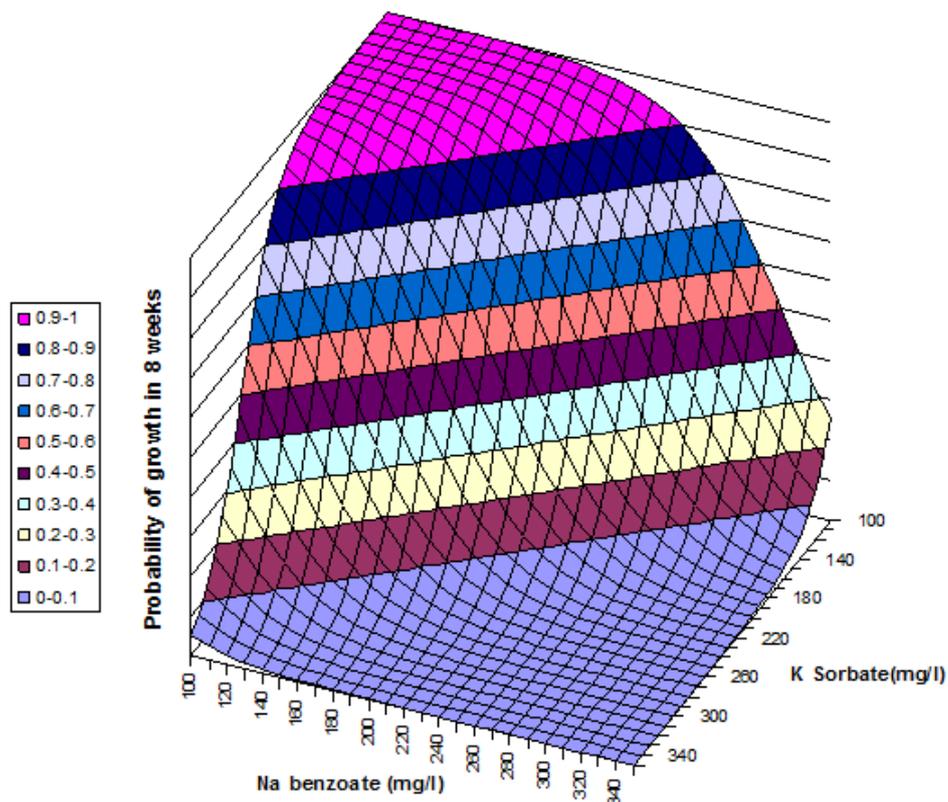


Figure 1-8 Effect of Na benzoate and K sorbate on the probability of growth of a yeast cocktail (*A. niger* and *P. spinulosum* at pH 3.3 after 8 weeks. (From Battey *et al.*, 2001)

1.8.2 Relevance of beverage microbiology and modelling to the cut flower food industry

Vase water containing flower food has a similar composition to a medium to low pH beverage. It has a high water activity, low pH (citric acid as an acidulant), presence of sugar(s) and additional preservation (e.g. hydroxyquinoline). The major difference is that the vase water is not a stable environment. Frequent top-ups with water change the environment, the vase is open to the air (in general) and the initial raw materials (i.e. the cut flowers) have a high degree of variability and are not sterile nor pasteurised before use! The ability to grow and the amount of growth of microorganisms in each environment must still be dictated by the physical characteristics of that environment, e.g. temperature, and pH.

The beverage industry has had a long history of modifying the environment to stop, slow down or eliminate the growth of microorganisms. Using predictive models to describe the effect that modifying the environment has on the growth has become a *force-major* in food preservation. Hence such studies can have an immediate impact on the analysis of the growth of microorganisms in vase water. For example the Figure 1-8 which shows the effect of combined sorbic and benzoic acids at pH 3.3 will be equally valid for an equivalent (or near equivalent) system used for cut flowers.

As previously mentioned, knowledge of that *Pseudomonas aeruginosa* is isolated from vase water when it is already known that the lower limit for growth is higher than the pH of vase water treated with certain flower food suggests immediately that the environment changes to allow greater growth. Studying the reason for this is the basis of this project.

1.9 Aims and Objectives

Background: cut flowers immersed in tap water without the benefit of the addition of cut flower food can quickly develop turbidity due to the growth of microorganisms. A multitude of different species can be obtained from such vase water including pathogenic organisms as well as spoilage or less harmful bacteria and fungi. However, in the presence of cut flower food these microorganisms can also be found. The initial composition of vase water with added flower food does not allow the growth of many of these organisms, e.g. *Pseudomonads*. Experiments conducted by Lambert on the growth/no growth profile of *Pseudomonas aeruginosa* and other *Pseudomonads* demonstrates that the vase water with cut flower food has a composition beyond the G/NG boundary of the organism, and in a constant environment cannot grow. The isolation of these microorganisms in high numbers suggests that the composition of the vase water with cut flower food changes with time, and these changes allow the growth of microorganisms previously inhibited.

Aims and objectives: The aims and objectives of this project are to investigate the influence of flower food on vase water quality and to correlate that with the flower appearance and microbial growth. The goal of this research is to provide Chrysal (the sponsor) and the industry with improved understanding of the treated vase water and how changes in the composition occur with time. From these studies we are aiming to be able to provide a deeper insight into where improvement can be made and therefore ultimately to provide “consumer satisfaction with every flower purchased”.

2 MATERIAL AND METHODOLOGY

2.1 Materials

2.1.1 Vase water

Two different types of water were used; standard water (SW) and reverse osmosis water (ROW). In each set of experiment the pH of vase water was controlled by adding cut flower food (FF), hydrochloric acid (HCl) or citric acid (CA) to standard water or reverse osmosis water.

2.1.1.1 Standard water (SW):

Standard water was prepared - according to the common formula used in the industry and was supplied by the sponsor Chrysal[®] - using the following steps:

- I. Solution (A): 19.84g of Magnesium Chloride Hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) (BDH Laboratory Supplies, Poole, BH15 1TD, England, UK) were added to 46.24g of Calcium Chloride Dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) (BDH Laboratory Supplies, Poole, BH15 1TD, England, UK) in a volumetric flask and then filled to 1000 ml with reverse osmosis water.
- II. Solution (B): 35.02g of Sodium Bicarbonate (NaHCO_3) (Aldrich Chemical Company, Inc., Craftsmen Chemistry, Milwaukee, WIS 53233, USA) were added in a volumetric flask and then filled to 1000 ml with reverse osmosis water.
- III. To prepare 1000 ml of standard water; 6 ml of solution A were added to 8 ml of solution B and then filled to 1000 ml of reverse osmosis water in a volumetric flask.

2.1.1.2 Reverse osmosis water (ROW):

Reverse osmosis water was obtained from the Cranfield Health Laboratory (Direct-Q3 UV water purification system, EMD Millipore Corporation, Billerica, MA, USA).

2.1.1.3 Cut flower food:

The cut flower food (Chrysal Clear Universal Liquid P4207) was used in all experiments and was supplied by Chrysal[®] International BV (Gooimeer 7, 1411 DD Naarden, Holland). The components of the flower food are (in descending order of percentage composition w/w): sugars, water, acidifier (citric acid) and additives (preservatives and salts). The dosage used was 10 ml per litre of vase water as recommended by the producer.

2.1.1.4 Hydrochloric acid:

1M of HCl acid was prepared by adding 50 ml of Hydrochloric acid solution (2M) (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG, UK) to 50 ml of reverse osmosis water in a 100 ml volumetric flask.

2.1.1.5 Citric acid:

200 ml of 0.1 M citric acid were prepared by placing 3.842g of citric acid (Sigma Chemical Co., P.O. Box 14508 St. Louis, MO 63178 USA) in 200 ml volumetric flask then filled to 200 ml mark with ROW.

2.1.2 Vases:

Transparent glass vases were used, each vase has approximately a capacity of 1.8 litres, a base diameter of about 14 centimetres and an opening top of about 9 centimetres (Figure 2-1). Each vase was marked and numbered according to the contents and treatment in each set of experiment.



Figure 2-1: *Example of glass vases used in the project.*

2.1.3 Flowers

The cut flowers used in the very first (i) and second (ii) set of experiments were bought from Sainsbury's and ASDA supermarkets respectively.

All cut flowers used in the rest of experiments (six in total) in this project were supplied by Finlays[®] (Sandy site, Bedfordshire, UK). Bouquets of flowers were used in each set of experiment rather than single flowers (Figure 2-2); each bouquet consists of 14-16 stems of the following cut flowers, shrubs and flowering plants:

- 2 stems of Rosa (variety 1).
- 2 stems of Rosa (variety 2)
- 2 stems of Gerbera (variety 1)

- 2 stems of Gerbera (variety 2)
- 2 stems of Chrysanthemum
- 2 stems of Lilium
- 2 stems of Salal
- 2 stems of Solidago



Figure 2-2: *Bouquet sample.*

Depending on the season there were some variations of bouquets composition. The following lists show the composition of each set, the date they have arrived in the supplier's site and the date of the experimental set:

- Experimental set 1 was started on 26th of March 2012:

- ❖ Rosa variety 1 (Red Calypso – FFUK – PO 254421 – arrived 25.03.2012)
 - ❖ Rosa variety 2 (Wild Thing – FFUK – PO 254421 – arrived 25.03.2012)
 - ❖ Gerbera variety 1 (Serena – FFBV, Grower Zuidplas – PO 251119 – arrived 24.03.2012)
 - ❖ Gerbera variety 2 (Mystique – FFBV, Grower Zuidplas – PO 251119 – arrived 24.03.2012)
 - ❖ Chrysanthemum (Romanov – FFSA – PO 255834 – arrived 26.03.2012)
 - ❖ Lilium (Oriental Lily Santander – FFUK, IBIS FARM – PO 254482 – arrived 26.03.2012)
 - ❖ Salal (Laurel – Cornwall Foliage – PO 251111 – arrived 24.03.2012)
 - ❖ Solidago (Solidago – Rivera – PO 255046 – arrived 23.03.2012)
- Experimental set 2 was started on the 16th of April 2012:
 - ❖ Rosa variety 1 (Red One – FFUK – PO 255439 – arrived 14.04.2012)
 - ❖ Rosa variety 2 (Wild Thing – FFUK – PO 257029 – arrived 15.04.2012)
 - ❖ Gerbera variety 1 (Serena – FFBV, Grower Zuidplas – PO 254204 – arrived 14.04.2012)
 - ❖ Gerbera variety 2 (Mystique – FFBV, Grower Zuidplas – PO 254204 – arrived 14.04.2012)
 - ❖ Chrysanthemum (Romanov – FFSA – PO 258024 – arrived 15.04.2012)
 - ❖ Lilium (Oriental Lily Cherbourg – FFUK, IBIS FARM – PO 257029 – arrived 15.04.2012)

- ❖ Salal (Rhodo – Cornwall Foliage – PO 256897 – arrived 14.04.2012)
- ❖ Solidago (Solidago – Agriver – PO 255985 – arrived 14.04.2012)
- Experimental set 3 was started on the 8th of May 2012
 - ❖ Rosa variety 1 (Red Calypso - FFUK - PO 258053 - arrived 05.05.2012)
 - ❖ Rosa variety 2 (Wild Thing - Shalimar - PO 257550 - arrived 07.05.2012)
 - ❖ Gerbera variety 1 (Red Explosion - FFBV, Grower Zuidplas - PO 259553 - arrived 08.05.2012)
 - ❖ Gerbera variety 2 (Orange Firestarter - FFBV, Grower Zuidplas - PO 259553 - arrived 08.05.2012)
 - ❖ Chrysanthemum (Zembla Cream - FFSA - PO 260089 - arrived 07.05.2012)
 - ❖ Lilium (Oriental Lily Red Velvet - FFUK, IBIS FARM - PO 258055 - arrived 07.05.2012)
 - ❖ Salal (Rhodo - Cornwall Foliage - PO 256897 - arrived 14.04.2012)
 - ❖ Solidago (Solidago - FFBV, Grower OZ Import B.V. - PO 258777 - arrived 05.05.2012)
- Experimental set 4 was started on 13th of June 2012:
 - ❖ Rosa variety 1 (Sweet Akito, Source: Finlays Fresh Produce, PO 262499, arrived 11.06.12)

- ❖ Rosa variety 2 (Furiosa, Source: Shalimar Flowers, PO 257581, arrived 11.06.12) Gerbera variety 1 (Orange Firestarter, Source: Finlay Flowers BV Zuidplas, PO 2625516, arrived 12.06.12)
- ❖ Gerbera variety 2 (Yellow Fabio, Source: Finlay Flowers BV Zuidplas, PO 2625516, arrived 12.06.12)
- ❖ Chrysanthemum (Reagan Splendid, Source: Finlays Horticulture South Africa, PO 263818, arrived 12.06.12)
- ❖ Salal (Salal, Source: Hilcrest UK, PO 261020, arrived 31.05.12) Solidago (Solidago, Source: Finlays Fresh Produce, PO 262499, arrived 11.06.12)
- Experimental set 5 was started on the 18th of July 2012:
 - ❖ Rosa variety 1 (Red Calupso, FFUK, PO 268687 arrived 17.07.2012)
 - ❖ Rosa variety 2 (Inka, FFUK, PO 267797, arrived 16.07.2012)
 - ❖ Gerbera variety 1 (Gremini Mundi, FFBV (LG Flowers), PO 268165, arrived 17.07.2012)
 - ❖ Gerbera variety 2 (Germini Bison, FFBV, PO 268166, arrived 18.07.2012)
 - ❖ Chrysanthemum (Expolis, FFSA, PO 268735, arrived 15.07.2012)
 - ❖ Salal (Pittos Verigated, Agriver UK, PO 269241, arrived 16.07.2012)
 - ❖ Solidago (Solidago, FFUK, PO 267797, arrived 16.07.2012)
- Experimental set 6 was started on 29th of August 2012:
 - ❖ Rosa variety 1 (Red Calypso, FFUK, PO 271974 arrived 27.08.2012)

- ❖ Rosa variety 2 (Fuschiana, FFUK, PO 271974 arrived 27.08.2012)
- ❖ Gerbera variety 1 (Gerbera Cerise, FFBV (Zuidplas), PO 274368, arrived 28.08.2012)
- ❖ Gerbera variety 2 (Gerbera Orange Firestarter, FFBV (Zuidplas), PO 274369, arrived 29.08.2012)
- ❖ Chrysanthemum (Zembla Cream, FFSA, PO 271748, arrived 28.08.2012)
- ❖ Salal (Rhodo, Forest Produce, PO 270223, arrived 26.08.2012)
- ❖ Solidago (Solidago, FFBV, PO 271974, arrived 27.08.2012)

2.1.4 Media

Different media were used to enumerate the microorganisms from vase water samples; media of various types were used for bacteria as well as media for fungi and yeasts. Tryptone Soya Agar – TSA (Oxoid Ltd., Basingstoke, Hampshire, England, UK) were used for general bacterial aerobic plate counts. For fungi and yeast general aerobic plate counts two different media were used; Dichloran Rose Bengal Chloramphenicol Agar – DRBCA (Oxoid Ltd., Basingstoke, Hampshire, England, UK) and Sabouraud Dextrose Agar – SDA (Oxoid Ltd., Basingstoke, Hampshire, England, UK). To suppress the growth of bacteria in both media (DRBCA and SDA) the antibiotic chloramphenicol was added to both media at concentration of 0.1 g/L (Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG, UK).

Selective media for isolation of different types of bacteria were also used, these include: Pseudomonas Isolation Agar (Fluka Analytical, Sigma-Aldrich Chemie, GmbH, CH-9471 Buchs) for the isolation of *Pseudomonas spp.*, and MacConkey Agar (Oxoid Ltd., Basingstoke, Hampshire, England, UK) for the isolation of Gram-negative bacteria

2.2 Methodology

Experiments summary:

A list of all the experiments conducted is summarised and given in appendix A. Validation study from previous project also included in appendix B.

Preliminary Experiments:

Experiment i:

- Vase 1, 2, 3 and 4: Flowers + SW + FF (Flowers were bought from Sainsbury's Supermarkets) [Top up: SW]

Measurements: OD, pH, Plate's count

Experiment ii:

- Vase 1, 2, 3, 4, 5, 6, 7, and 8: Flowers + SW + FF (Flowers were bought from ASDA Supermarkets) [Top up: SW]
- Vase 7: SW (control)
- Vase 8: SW+FF (control)

Measurements: Vases weight, OD, pH, Plate's count, HPLC

Experimental Sets:

Experiment 1:

- Vase 1 and 2: Flowers + SW + FF [Top up: SW+FF]
- Vase 3: Flowers + ROW + FF [Top up: ROW+FF]
- Vase 4: Flowers + ROW [Top up: ROW]
- Vase 5: Flowers + SW [Top up: SW]
- Vase 6: ROW + FF (control)
- Vase 7: SW (control)

Measurements: OD, pH, Plate's count, vases weight, HPLC

Experiment 2:

- Vase 1 and 2: Flowers + SW +FF [Top up: SW+FF]
- Vase 3 and 4: Flowers + SW +FF [Top up: SW]
- Vase 5: Flowers + SW [Top up: SW]
- Vase 6: SW + FF (control)

Measurements: OD, pH, Plate's count, vases weight, HPLC, LC/MS

Experiment 3:

- Vase 1 and 2: Flowers + ROW + FF [Top up: ROW+FF]

- Vase 3 and 4: Flowers + SW + FF [Top up: SW+FF]
- Vase 5: ROW + FF (control)
- 5 conical flasks of individuals flowers: roes, gerbera, chrysanthemum, lilium, salal and solidago

Measurements: OD, pH, Plate's count, vases weight, HPLC, plate's count for initial vase water.

Experiment 4:

- Vase 1 and 2: Flowers + SW +FF [Top up: SW+50%FF]
- Vase 3 and 4: Flowers + SW +FF [Top up: SW+25%FF]
- Vase 5: Flowers + SW +FF [Top up: SW]

Measurements: pH, Plate's count, vases weight

Experiment 5:

- Vase 1,2 and 3: Flowers + SW + FF (pH reduced to 3 by adding HCl) [Top up: SW+FF at pH 3]
- Vase 4,5 and 6: Flowers + ROW + FF [Top up: ROW+FF]
- Vase 7: SW + FF (control) [pH reduced to 3 by adding HCl]
- Vase 8: ROW + FF (control)

Measurements: pH, Plate's count, vases weight, plate's count for the basal part of stems.

Experiment 6:

- Vase 1 and 2: Flowers + SW + FF [Top up: SW+FF]
- Vase 3 and 4: Flowers + ROW + FF [Top up: ROW+FF]
- Vase 5: Flowers + SW + FF (pH reduced to 3.3 by adding citric acid) [Top up: SW+FF at pH 3.3]

Measurements: pH, Plate's count, vases weight

In each set of experiment and prior to the arrival of the flowers from the supplier site (Finlays, Sandy - Bedfordshire) clean and marked vases with the correct amount of solution were prepared. The vases started with 1 litre of solution plus 10 ml of cut flower food, top up with more solution occurred when the level of vase solution had reached or fallen just below 200 ml mark.

The flowers were prepared upon arrival within 1 hour into the designated vases. The preparations included: checking the right number of stems in each bouquet,

removing and replacing any broken stems with the extra ones supplied, cut and remove 2-3 cm of the basal end of each stem and removal of any dead leaves.

All vases were kept in a control environment room in the Cranfield Health laboratory 2nd floor (temperature: 20°C ±1, humidity: 40-60%, lights: 10-12 hours/day). Measurements of vases weights, pH, turbidity (optical density) and aerobic plate counts (bacteria and fungi) were done approximately every day during the period of the each experiment set which lasted 10-14 days. A sample of 10-15 ml of vase water was taken approximately every day in clean plastic containers to carry out the above measurements and also to prepare filtered sample (3 ml) to be frozen and later used to run HPLC and LC/MS analysis. The remaining of the samples would be marked and kept frozen in -20°C to be used later if needed.

The pH measurements were done by using HANNA pH metre, HI 8519 N (HANNA Instruments, UK).

Optical density (OD) was measured by using spectrophotometer with wave length of 600 nm (M350 Double Beam U.V. Visible Spectrophotometer).

Incubation period for aerobic plate counts was 2-3 days for bacteria at 30°C and 7-10 days for fungi and yeast at 25°C. Two replicate plates from 3 different dilutions for each vase were plated by spreading 100 µl of the sample over the entire surface of the plate using a sterile bent-glass spreader, and then the average numbers of colonies (CFU) were calculated - after incubation - to obtain the initial inoculum number per millilitre of vase solution. The log₁₀ CFU per millilitre was also calculated.

2.2.1 Standard water experiment

The following sets of experiments were done using the standard water as vase solution with the presence of cut flowers:

- Standard water, top up with standard water

- Standard water + cut flower food, top up with standard water
- Standard water + cut flower food, top up with standard water + cut flower food
- Standard water + cut flower food, top up with standard water + 50% of the dose of cut flower food
- Standard water + cut flower food, top up with standard water + 25% of the dose of cut flower food

2.2.2 Reverse osmosis water experiment

The following sets of experiments were carried out using the reverse osmosis water as vase solution with the presence of cut flowers:

- Reverse osmosis water, top up with reverse osmosis water
- Reverse osmosis water + cut flower food, top up with reverse osmosis water + cut flower food

2.2.3 HPLC experiment

High-performance liquid chromatography (HPLC) was used to determine the sugar content in vase water (Agilent 1200 series, Agilent, Berkshire – UK). The following steps were used to carry out the experiment:

- Preparation of mixed calibration standards: concentration of 0.05, 0.25, 0.5, 1.25 and 2.5 mg/ml glucose, fructose and sucrose were prepared and dispensed in 1.0 ml aliquots of each standard into HPLC vials, labelled and stored at -40°C until required.
- Preparation of samples: 2-3 ml of vase water from each vase were filtered using 0.2 µm filter, dispensed into sterile plastic vials and stored at -20°C until required. 0.5 ml from each filtered sample was dispensed into HPLC vials ready for analysis.

- HPLC parameters: two different columns were used for the stationary phase; 1- Rezex RCM-Monosaccharide Ca⁺ (8%), size 300 × 7.80 mm (S/N 427381-2, Part No OOH-0130-KO), 2- Rezex RCM-Monosaccharide Ca²⁺ (8%), size 300 × 7.80 mm (S/N 542870-21, Part No OOH-0130-KO). HPLC grade water was used as a mobile phase. The injection volume for the standards and samples was set to 20 µl and the column oven temperature was set to 80°C to allow equilibration before starting analysis.

2.2.4 Accurate-mass Q-TOF LC/MS experiment

The Agilent 6540 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS system is designed to provide high quality data and advanced analytical capabilities for profiling, identifying, characterizing, and quantifying low molecular-weight compounds and biomolecules. The machine is capable of providing rapid analyses in the following areas:

- Combinatorial chemistry target compound analysis
- Compound profiling (e.g., bioavailability)
- Natural products screening
- Biomarker discovery
- Impurity profiling
- Metabolomics
- Protein/peptide identification and characterization

Thus, it was considered that the machine could provide evidence of changes in the temporal profile of vase water, by providing metabolic profiles. By analysing the changes in these profiles it would be possible to understand the changes occurring during the vase life. The analysis of the products obtained uses a library of compounds to identify them through an accurate mass determination.



Model used: 6540 UHD Accurate-Mass Q-TOF LC/MS (Agilent Technologies, Santa Clara, CA, USA). Column: ZORBAX Eclipse Plus[®] C18, rapid resolution HD 2.1mm×150mm 1.8 Micron (Agilent Technologies – USA). Mobile phase A: 0.1% formic acid in water, mobile phase B: 0.1% formic acid in Acetonitrile.

Figure 2-3 *Agilent 6540 Q-TOF LC/MS integrates Ultra High Definition TOF technology, Agilent Jet Stream technology, and MassHunter Workstation data mining tools for sensitive, high resolution, accurate-mass MS and MS/MS analyses.*

3 RESULTS

In this section the following abbreviations will be used:

- F: Cut Flowers
- FF: Flower Food
- ROW: Reverse Osmosis Water
- SW: Standard Water
- (ROW+FF+F, ROW+FF): Vase started with reverse osmosis water + flower food + flowers and topped up with reverse osmosis water + flower food
- (ROW+FF+F, ROW): as above but topped up with ROW
- (ROW+F, ROW): vase started with ROW + F and topped up with ROW
- (SW+FF+F, SW+FF): Vase started with standard water + flower food + flowers and topped up with standard water + flower food
- (SW+FF+F, SW): as above but topped up SW
- (SW+F, SW): vase started with SW + F and topped up with SW

3.1 Sugar analysis

Samples of vase solution were analysed using HPLC to determine the sugar content (glucose, fructose and sucrose). Retention times and elution profiles using known standards for each sugar are shown in Figure 3-1.

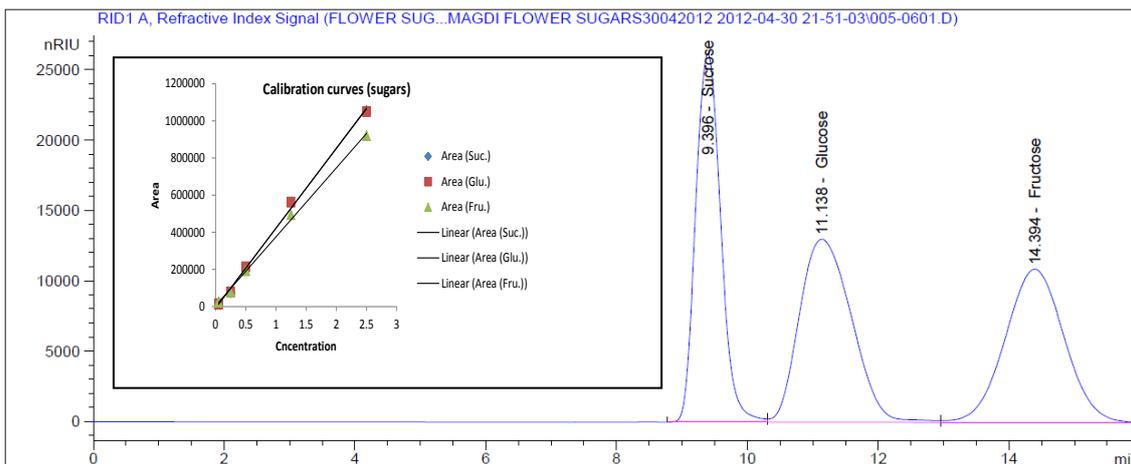


Figure 3-1 The elution for sucrose, glucose and fructose standards (with embedded calibration curves): retention times were 9.4, 11.14 and 13.39 minutes respectively.

Results from samples taken from vase water were calculated against calibration curves of sucrose, glucose and fructose external standards using a standard linear equation ($y = mx + b$), from which the final equation: (Concentration (x) = [Area (y) ± b] / [m]) was derived to get the final concentration of sugars in each sample.

Glucose and fructose sugars were found in higher quantity than sucrose, the source of these sugars was the flower food added to the vase solution. The results showed that the amount of fructose and glucose sugars in the recommended dose of flower food (10 ml per litre of vase water) was between 3.5-3.9 mg/ml. These results are in line with the concentration of glucose and fructose known to be in the flower food (3.75 g each per 10ml of flower food).

Two different columns were used to analyse sugars in vase water by HPLC, initial results obtained from the first column (Rezex RCM – Monosaccharide Ca^+) were found to be hampered by the sensitivity of the column to the pH of the vase water and as found later due to the possible complex nature of the sugars in the flower food. These results had shown inconsistent concentration during the vase life when topping up with standard water was used in the presence of cut flowers (Figure 3-3) or in the case of controls containing only standard water and flower food when constant concentration was expected

(Figure 3-2). It was also noticed that the glucose and fructose concentrations were approximately 1:1 and this consistent result was obtained from both columns.

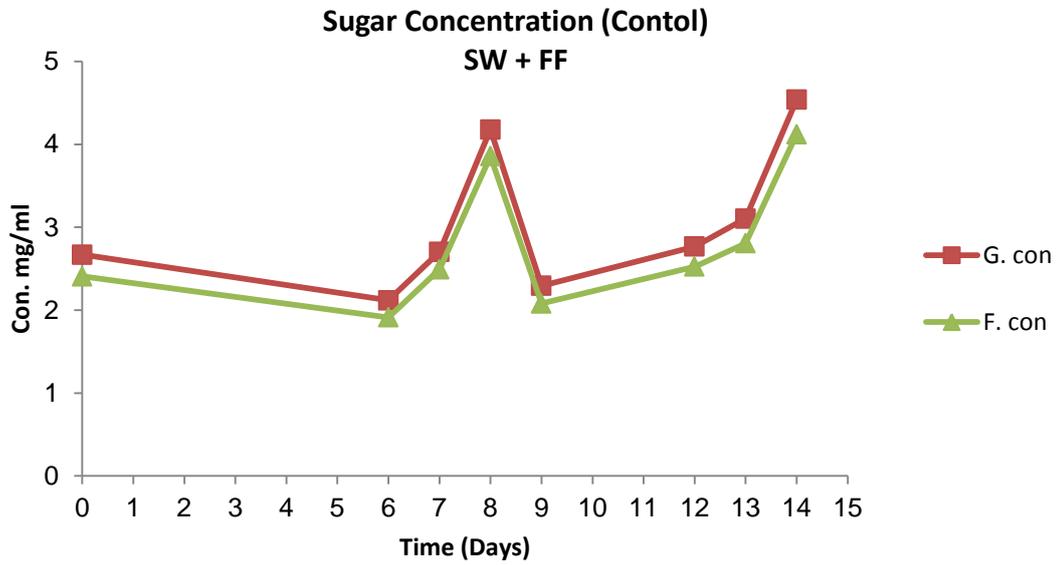


Figure 3-2 *Glucose and Fructose concentration in control vase solution containing standard water and flower food.*

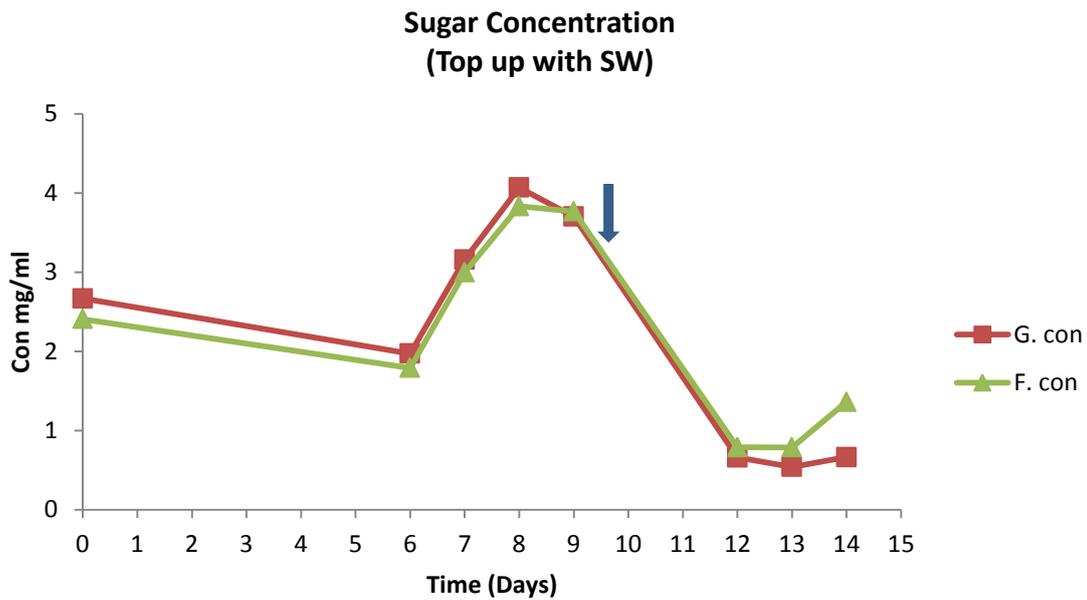


Figure 3-3 *Glucose and Fructose concentration of vase solution which contained standard water and flower food then topped up with standard water (blue arrow shows the topping up day).*

The concentration of sugars in each vase during the vase life depended on the topping up solution; when the flower food (FF) was used to top up the concentration remained relatively constant, but when topped up with standard water (SW) or reverse osmosis water (ROW) alone the concentration fell to its minimum as the sugar was diluted out (Figure 3-4 and 3-5).

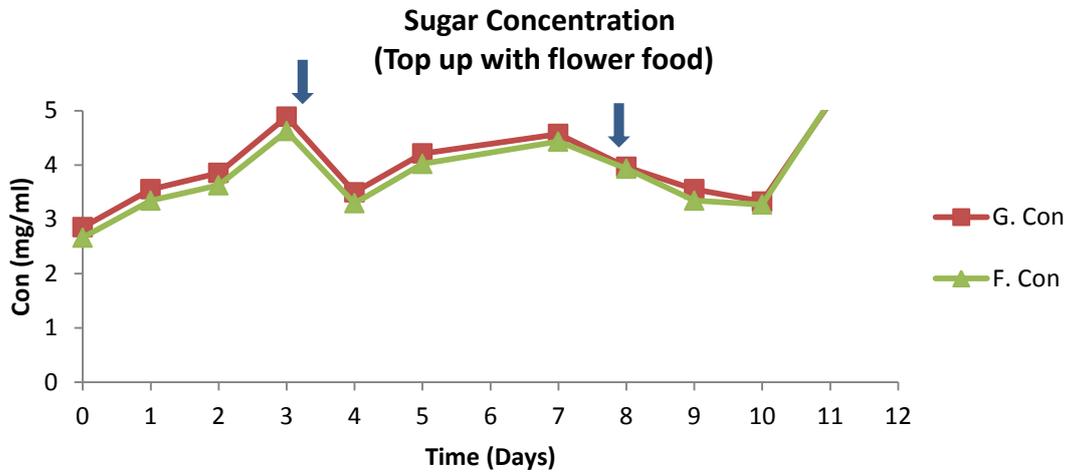


Figure 3-4 Glucose and Fructose concentration in vase solution started with standard water and flower food and topped up with standard water and flower food (blue arrows show the top up days).

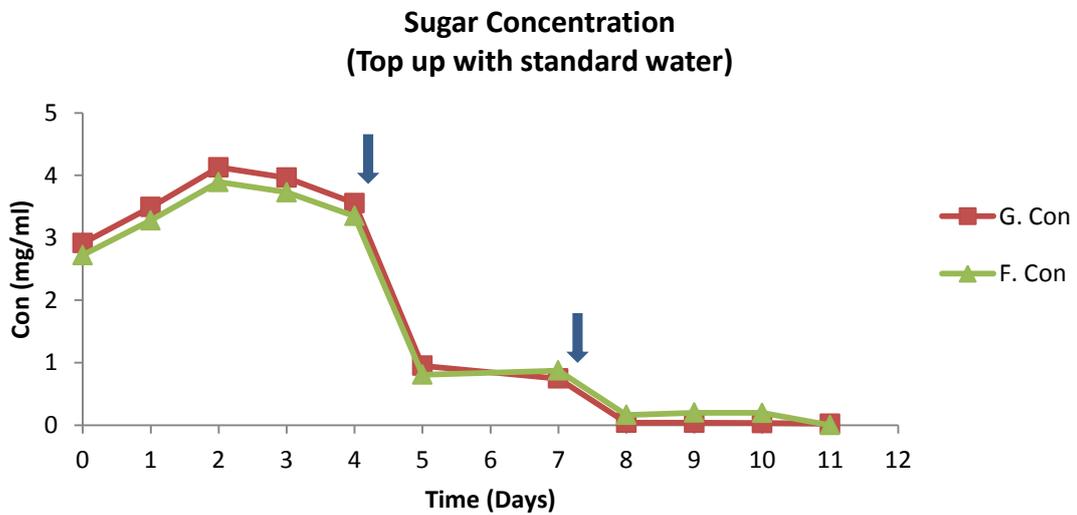


Figure 3-5 Glucose and Fructose concentration of vase solution which was started using standard water and flower food then topped up with standard water (blue arrows show the top up days).

3.2 Changes in pH

The initial pH of vase solution used in each experiment depended upon the type of water used (ROW or SW). SW had a pH between 7.7 and 8.2, whereas ROW had a pH of 5.4 – 5.6. Addition of the recommended amount of flower food resulted in a decrease of pH in both cases to between 4.4 – 4.6 for SW and 2.9 – 3.1 for ROW, (Figure 3-6).

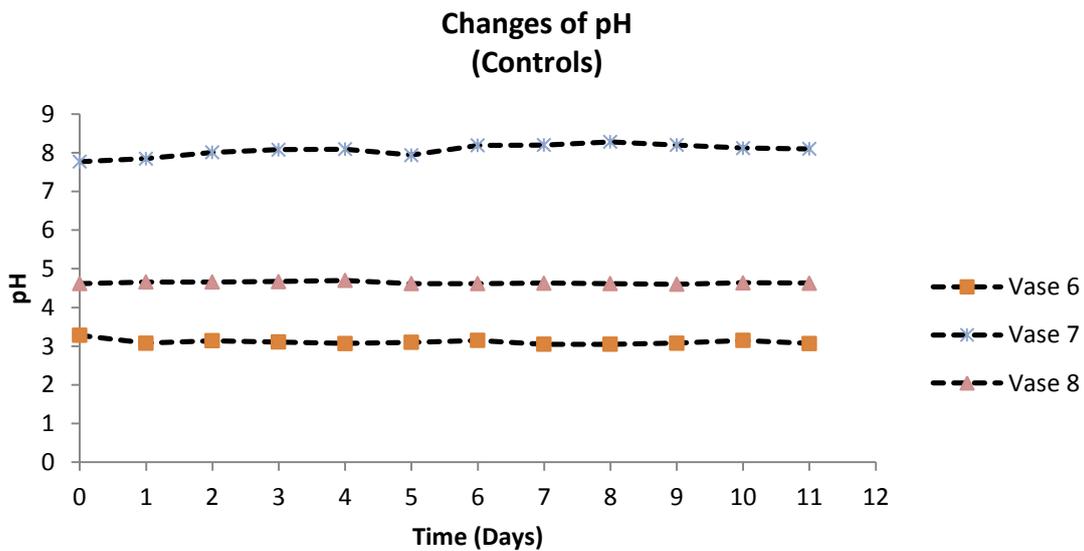


Figure 3-6 The pH of different vase solutions without cut flowers (controls); reverse osmosis water with flower food (Vase 6), standard water (Vase 7) and standard water with flower food (Vase 8).

It is interesting to note that ROW with flower food has a pH below the minimum pH for growth for the majority of pathogenic as well as spoilage bacteria, whereas SW with flower food is just above the minimum pH for the majority of pathogenic and spoilage organisms.

3.2.1 Standard water

Using SW alone as a vase solution (with flower present) the pH remained at a high value (6.5-7.8) for the duration of the experiment. A small reduction

occurred presumably due to CO₂ absorption, as when the solution was topped up with SW a slight rise occurred. When flower food (FF) was added to the SW, although the pH had a lower constant value for the first few days, on topping up with SW it increased to around 6.5. The pH dropped back to approximately 4.5 after about 3 days, but then increased and remained at 6.5 on a further top up with SW. When SW with flower food and flowers was topped up with SW + FF, then the pH remained close to the initial value of 4.5 (Figure 3-7).

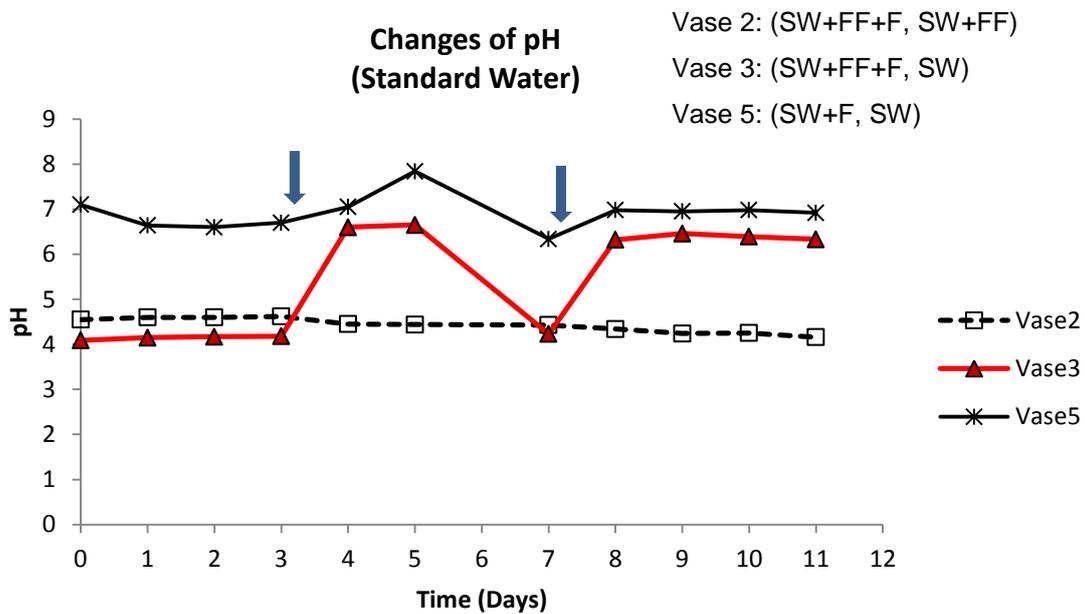


Figure 3-7 The pH changes of standard water (SW) as a vase solution ± flower food (FF) + cut flowers (F) when using different top up regimes. (Top up days marked with blue arrows).

Using smaller bouquets had allowed one topping up at later stage of vase life compared to earlier and more than one topping up when using larger bouquets, however the same behaviour of pH was noticed in all vases using SW with FF and topped up with SW only (Figure 3-8).

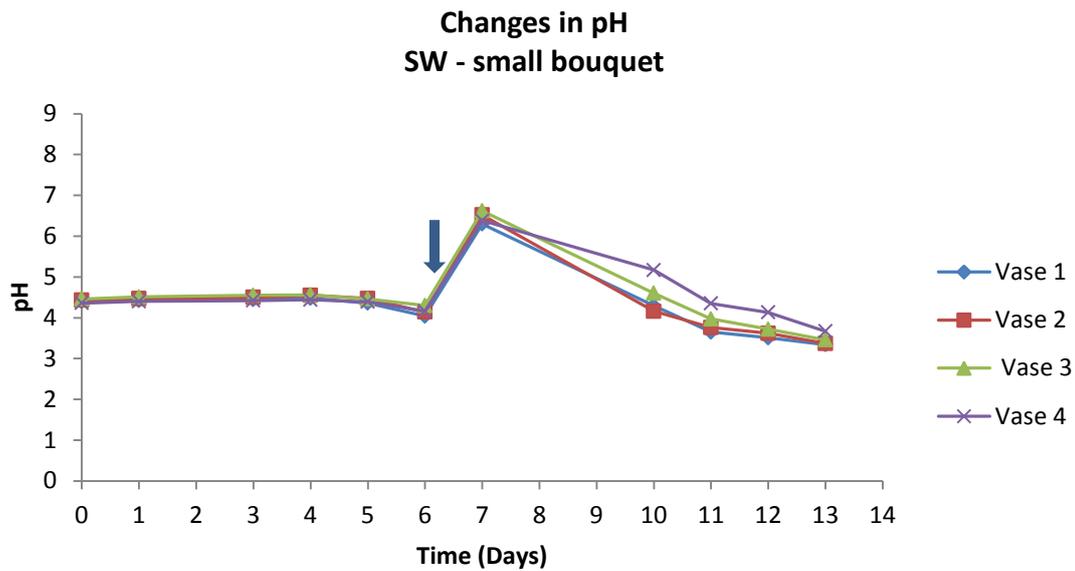


Figure 3-8 The pH changes of standard water (SW) as a vase solution + flower food (FF) + cut flowers (F) when using SW for top up. (Top up day marked with blue arrow).

3.2.2 Reverse osmosis water

Using ROW alone as a vase solution (with flowers present, (+F)), the pH increased from its initial value of 5.5 to over 7. Topping up with only ROW showed small variation in pH between 6.5 and 7.5. ROW with added flower food (FF) had an initial low, but constant, pH value. However, when topped up with ROW only the pH increased to over 7 and then decreased back to pH 4 over a period of few days. Addition of more ROW gave a general increase in pH only. However, ROW with flower food topped up with ROW and FF had a constant pH of around 3.2 for the duration of the experiment (Figure 3-9).

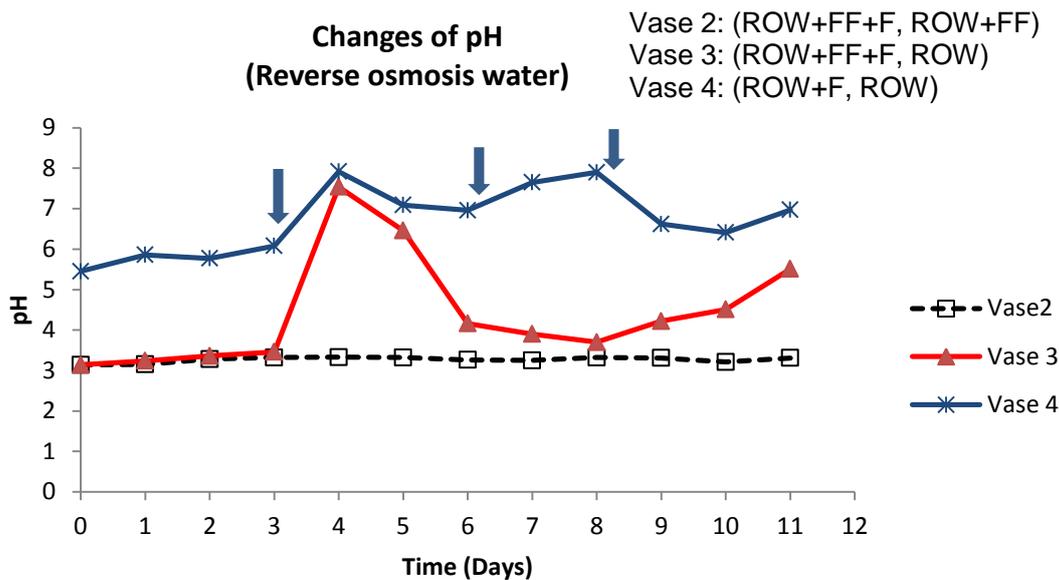


Figure 3-9 The pH changes of reverse osmosis water (ROW) as a vase solution ± flower food (FF) + cut flowers (F) when using different top up regimes. (Top up days marked with blue arrows).

3.2.3 Flower food concentration

Using different concentrations of flower food (FF) for top up solution showed no differences on the effect of pH changes between the various concentrations. Half (50%) and quarter (25%) of the recommended dose (10ml/litre) was used to top up vase solutions had already started with standard water and a full dose of flower food. The pH increased slightly after top up but decreased later on to reach the same initial level (Figure 3-10).

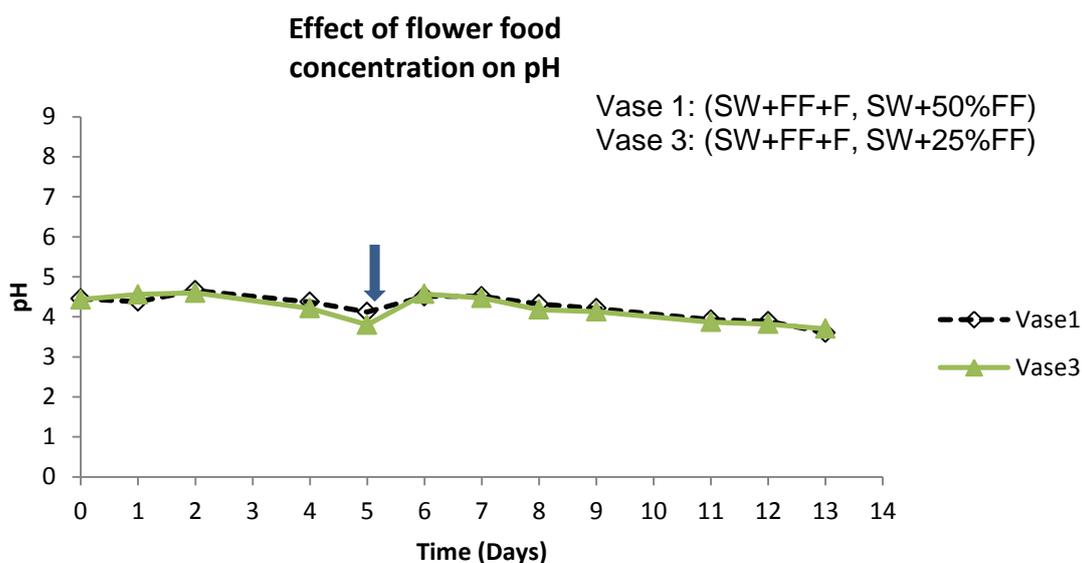


Figure 3-10 *The effect of flower food (FF) concentration on the pH when used in the top up solution (Top up day marked with blue arrow).*

3.3 Microbial Growth

3.3.1 Preliminary studies

Media other than TSA were used occasionally in the beginning of the project such as Pseudomonas Isolation Agar (PIA) and MacConkey Agar. Sampling technique used at the time was not perfect (due to the heterogeneity nature of vase water); therefore some data was lost due to either too much diluted samples were taken resulting in no count (0), or not enough dilution was carried out resulting in too many colonies which couldn't be counted (U.C.), the result in both cases was missing data as shown in Table 5 and 6.

Table 5 *Bacterial population (cfu/ml) from different vases using MacConkey agar for Gram negative bacteria. Mixed bouquets were used in all 8 vases and started with SW+FF+F and topped up with SW.*

Day	V1	V2	V3	V4	V5	V6	V7	V8
5	0	0	1000	2000	35000	0	0	0
6	0	0	0	0	46000	0	0	0
7	0	0	20000	0	470000	0	0	0
8	0	0	10000	0	1010000	0	0	0
12	1300	900	2200	7700	U.C	3600	16400	0
13	19600	19100	53200	9900	U.C	6800	41100	100

V= Vase, U.C= Uncountable Colonies.

Table 6 *Bacterial population (cfu/ml) from different vases using Pseudomonas isolation agar. Mixed bouquets were used in all 8 vases and started with SW+FF+F and topped up with SW.*

Day	V1	V2	V3	V4	V5	V6	V7	V8
5	3000	0	1000	3000	29000	17000	0	0
6	0	0	0	0	28000	0	1000	0
7	0	0	40000	0	350000	10000	0	0
8	0	0	80000	0	890000	0	0	0
12	100	300	21100	15600	U.C	3200	5100	0
13	6800	18700	41000	3700	U.C	1300	29000	100

V= Vase, U.C= Uncountable Colonies

3.3.2 Bacteria

The aerobic plate count for bacteria was calculated after 24-48 hours incubation at 30°C on TSA, the incubation period was extended sometimes up to 4-5 days when vase solutions had low pH (3).

Aerobic plate count was used in all experiments. Preparation of samples, media and incubation time were explained in Section 2. Samples were taken from different combination of vase water (ROW, SW, ROW + FF and SW + FF) with the presence of flowers and in the case of controls without flowers.

The results showed some variation between the different treatments and the different combinations of vase solution. The number of microorganisms (bacteria or fungi) depended greatly on the composition of vase solution at the beginning of the experiment and at topping up time.

3.3.2.1 Standard water

Using flower food (FF) at a dose of 10 ml/litre with standard water (SW) showed a delay of up to 3 days before the number of bacteria started to increase. The effect of topping up on the increase of the bacterial numbers was also noticed (Figure 3-11). This effect was generally reduced when topped up with standard water and flower food. The maximum numbers of bacteria was slightly less than that found when topped up with only standard water (Figure 3-12).

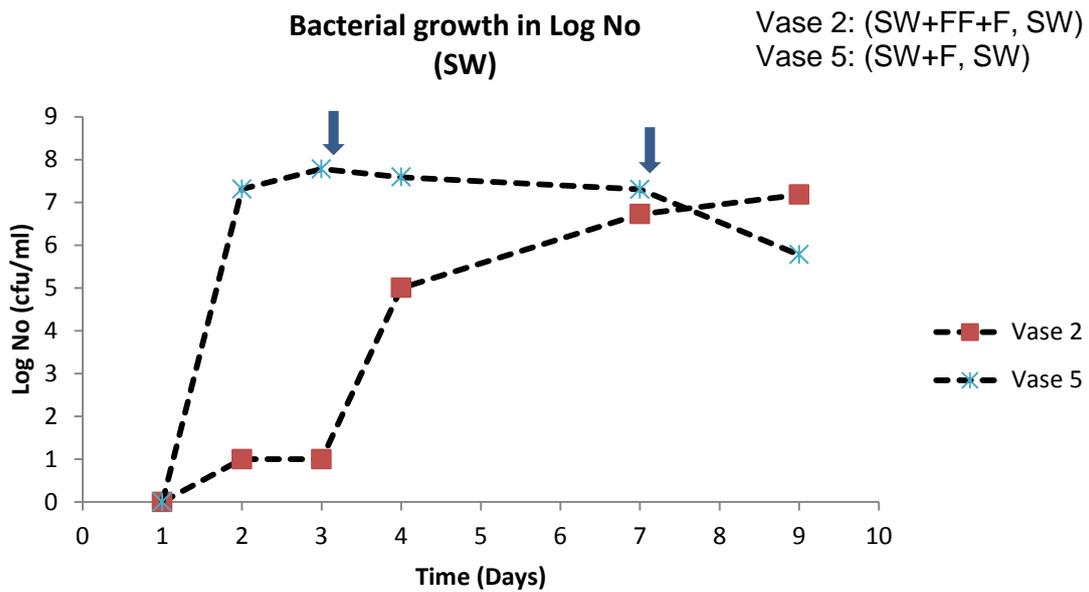


Figure 3-11 Log No for bacteria on TSA when using standard water (SW) ± flower food (FF) and top up with SW, no samples were taken on day 0, (top up days marked with blue arrows).

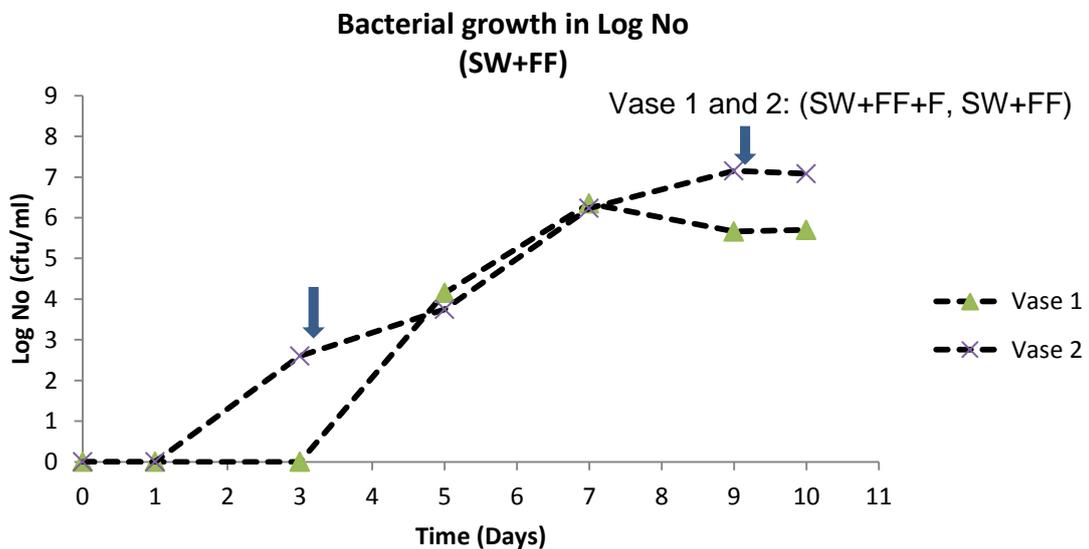


Figure 3-12: Log No for bacteria on TSA when using standard water (SW) + flower food (FF) and top up with SW + FF, (top up days marked with blue arrows).

When the pH of standard water was reduced to around 3 by adding drops of (1M) Hydrochloric Acid (HCl) and the recommended dose of flower food, the delay of the bacterial growth was also noticed, but after topping up the numbers of bacteria increased and remained the same at around 6-7 Logs (Figure 3-13). The same effect was noticed when Citric Acid (CA) was added to standard water with flower food to reduce the pH to around 3.3 for the duration of vase life. Figure 3-14 shows the effect of adding citric acid to SW + FF compared to just SW + FF.

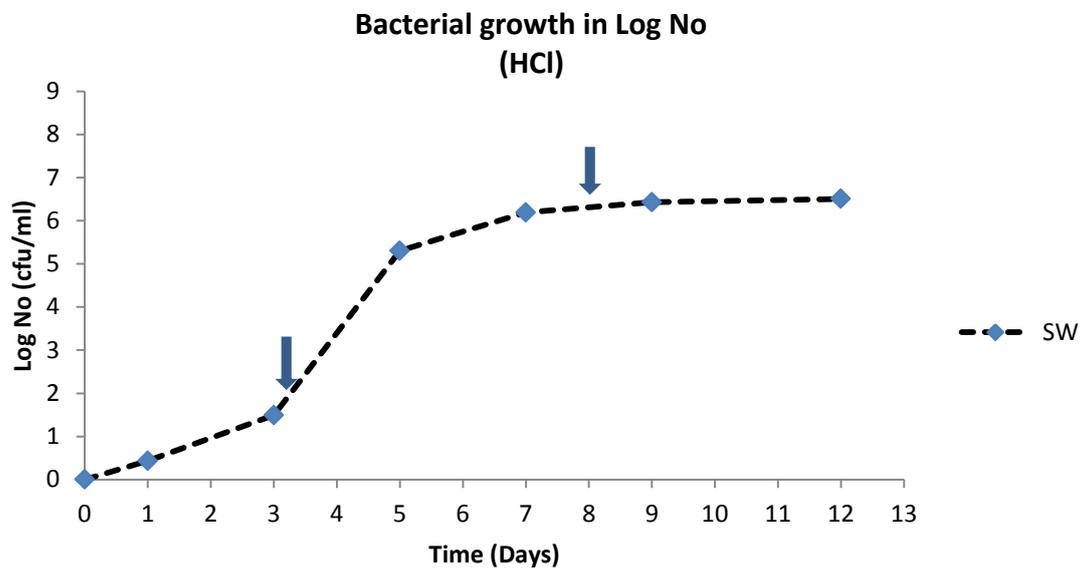


Figure 3-13: Log No for bacteria on TSA when using standard water (SW) + flower food (FF) + HCl (1M) and top up with SW + FF + HCl (top up day marked with blue arrow).

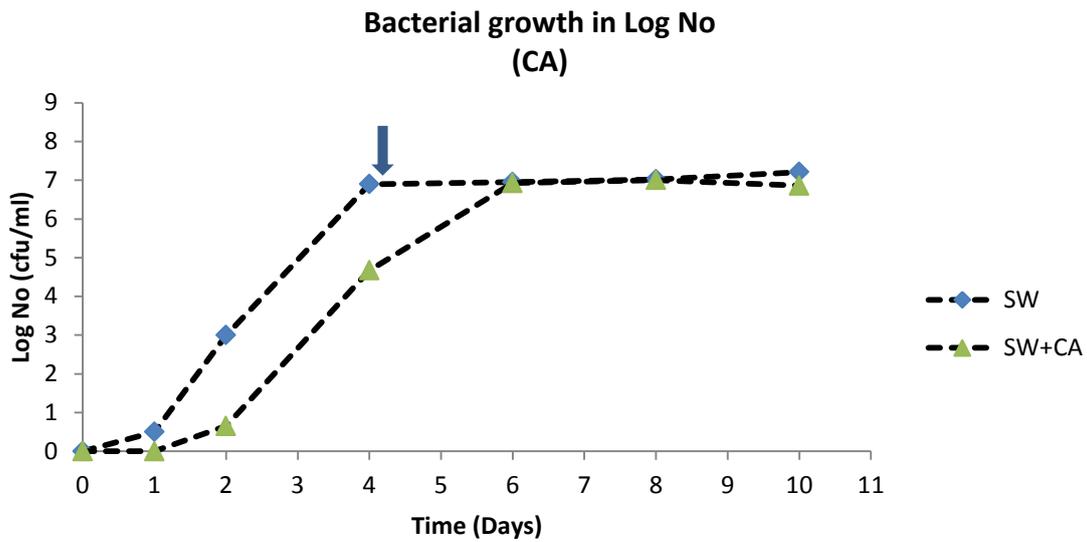


Figure 3-14 Log No for bacteria on TSA when using standard water (SW) + flower food (FF) ± citric acid (CA) and top up with SW + FF ± CA (top up day marked with blue arrow).

3.3.2.2 Reverse osmosis water

The use of reverse osmosis water (ROW) with added flower food helped to reduce the pH of vase solution to around 3; this controlled, to an extent, the growth of some bacteria. Topping up with ROW without flower food showed an increase of bacterial growth whether the vase solution started with added flower food or not (Figure 3-15).

The use of flower food from the start and at topping up showed a significant control of bacterial growth in some experiments (Figure 3-16). The results showed a delay in microbial growth up to 6 days and a maximum bacterial number of less than 3 logs. However with some other experiments it showed less control (Figure 3-17).

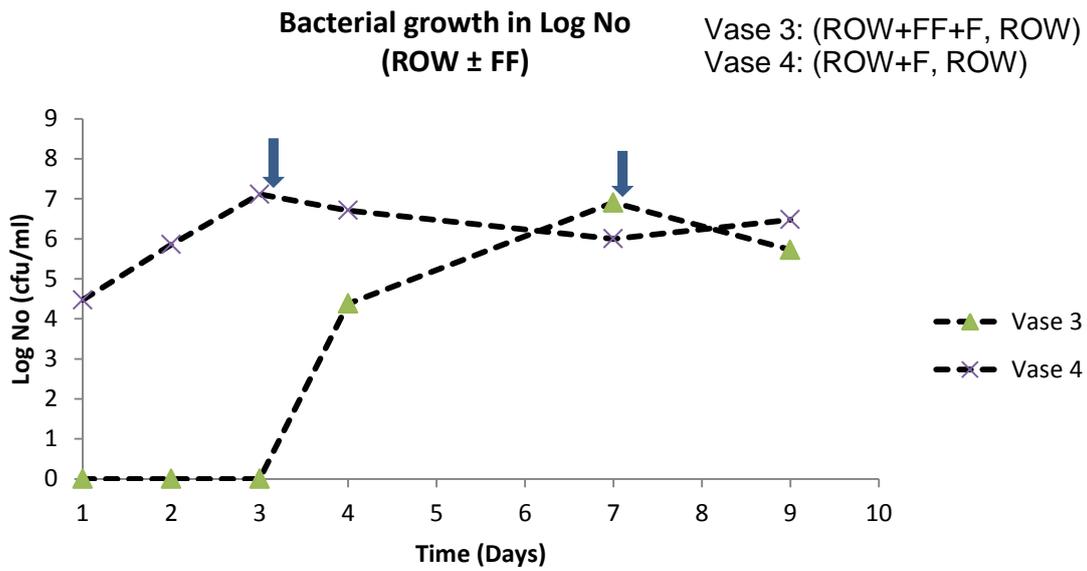


Figure 3-15 Log No for bacteria on TSA when using reverse osmosis water (ROW) ± flower food (FF) and top up with ROW, no samples were taken on day 0, (top up days marked with blue arrows).

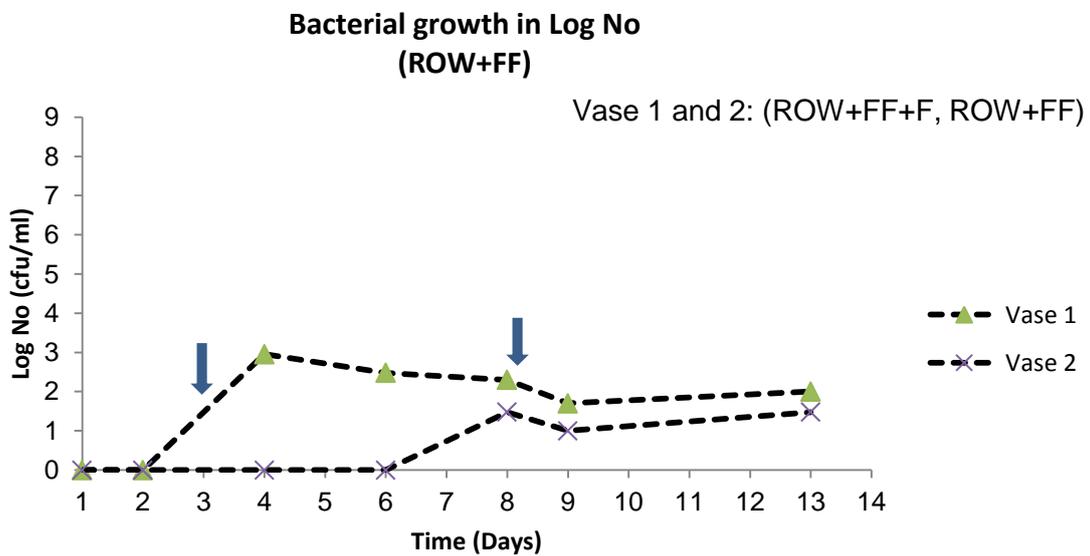


Figure 3-16 Log No for bacteria on TSA when using reverse osmosis water (ROW) + flower food (FF) and top up with ROW + FF (top up days marked with blue arrows).

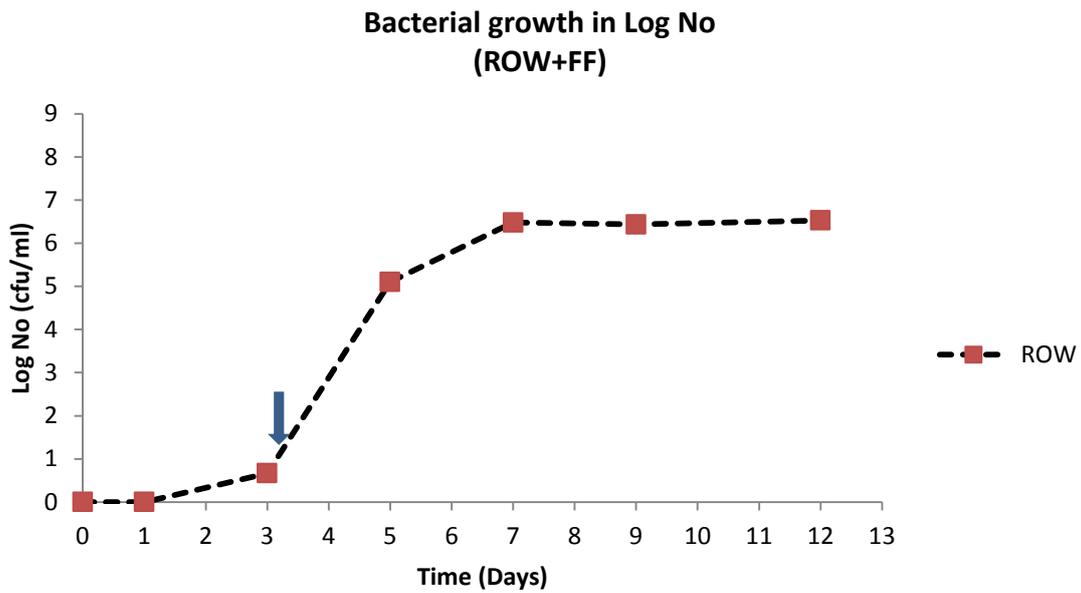


Figure 3-17 *Log No for bacteria on TSA when using reverse osmosis water (ROW) + flower food (FF) and top up with ROW + FF (top up day marked with blue arrow).*

3.3.2.3 Flower food concentration

The use of standard water with different concentrations of flower food at topping up did not show significant improvement in controlling bacterial growth. The results were similar to those where the full recommended dose was used. Figure 3-18 shows the effect of two different concentrations of flower food (25% and 50%) on bacterial growth during 13 days of vase life.

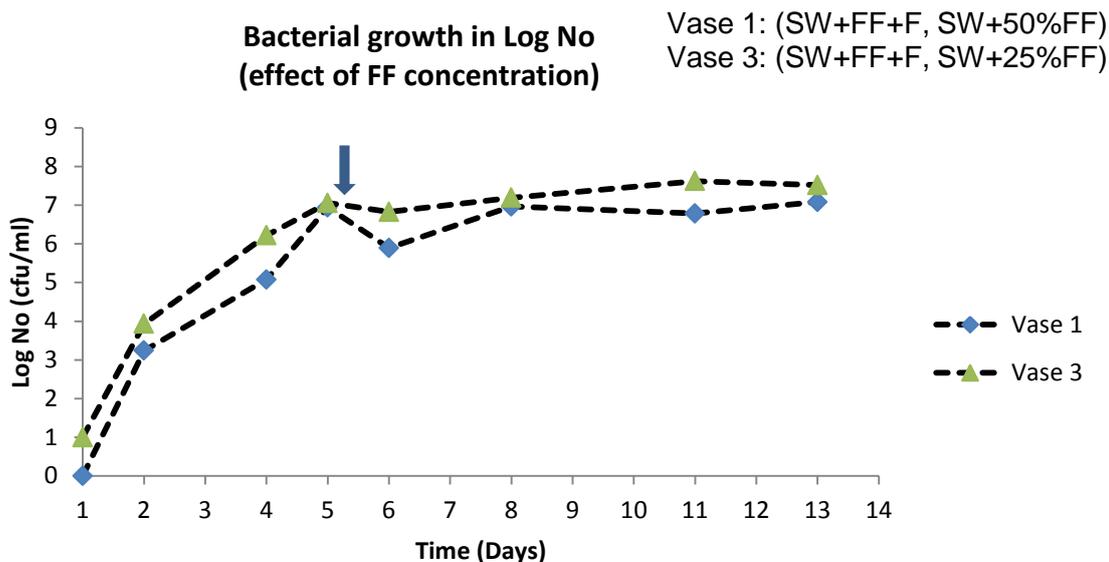


Figure 3-18 Effect of flower food concentration on bacterial growth on TSA when using standard water (SW) + flower food (FF) and top up with SW + 50% or 25% FF (Top up day marked with blue arrow).

3.3.3 Filamentous fungi and yeasts

Two different media were used; Dichloran Rose Bengal Chloramphenicol Agar (DRBCA) and Sabouraud Dextrose Agar (SDA), chloramphenicol (0.005%) were added in both media to inhibit bacterial growth. The incubation period for filamentous fungi and yeasts aerobic plate counts was 7-10 days at 25°C. It was observed in the majority of experiments that the number of yeasts colonies counted was more than the filamentous fungi colonies.

3.3.3.1 Standard water

The use of flower food with standard water showed a delay on fungal growth compared with using only standard water (Figure 3-19). Data from day 5 onward (vase 5) is not shown in Figure 3-19 due to an error when choosing the right serial dilution to plate out. The effect of using flower food from the start in the vase solution and at top up times is also shown in Figure 3-20; the maximum Log No was reduced compared to when topping up with only standard water was used.

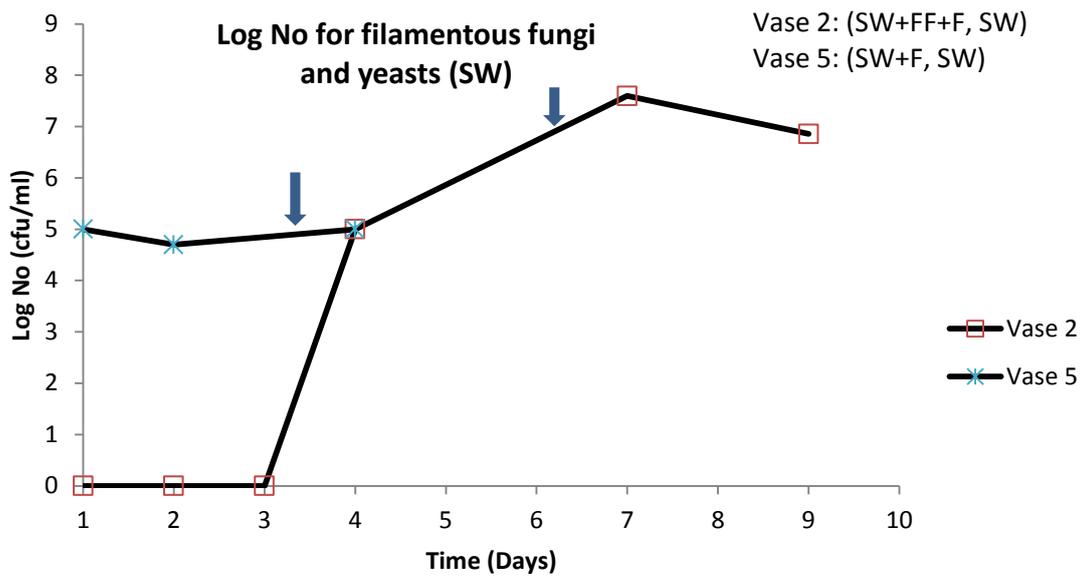


Figure 3-19 Log No for filamentous fungi and yeasts on DRBCA when using standard water (SW) ± flower food (FF) and top up with SW (top up days marked with blue arrows).

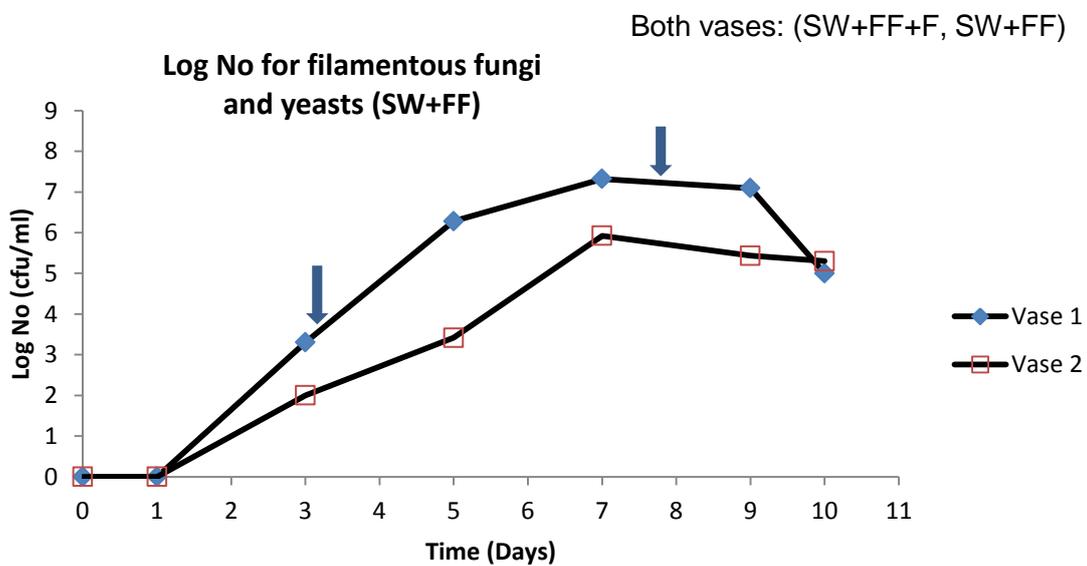


Figure 3-20 Log No for filamentous fungi and yeasts on DRBCA when using standard water (SW) + flower food (FF) and top up with SW + FF (top up days marked with blue arrows).

The pH of standard water with added flower food was reduced to 3 by adding 2.8 – 3.8 ml of 1M HCl acid and to 3.3 by adding 39 – 40 ml of citric acid. The effect of adding HCl acid on the total numbers of fungi and yeast is shown in Figure 3 -21 below. The effect of adding citric acid is not shown due to unanticipated contamination during the incubation of the plates.

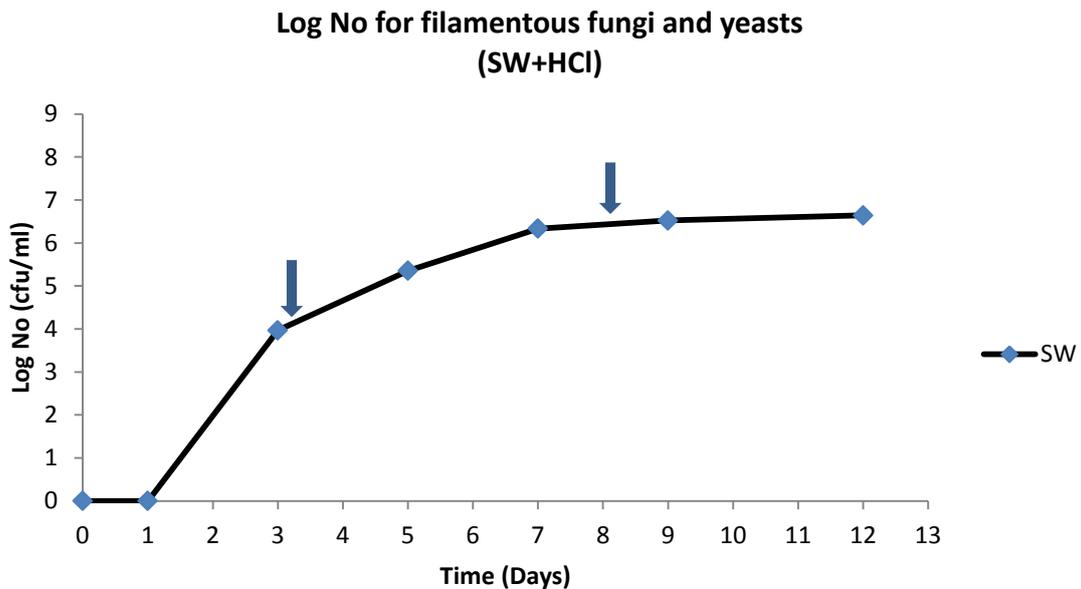


Figure 3-21 Log No for filamentous fungi and yeasts on DRBCA when using standard water (SW) + flower food (FF) + HCl (1M) and top up with SW + FF + HCl (top up days marked with blue arrows).

3.3.3.2 Reverse osmosis water

The vase solution of reverse osmosis water with flower food has a low pH of 3. The impact of low pH on controlling the numbers of filamentous fungi and yeasts was observed when flower food was used at the beginning and at topping up (Figure 3-22). The numbers of fungi were also controlled in the first 3 days of vase life when flower food was used at the beginning but not at top up. On the other hand, when flower food was not used at the beginning or at top up the numbers of fungi was relatively high initially and remained high during the rest of vase life (Figure 3-23). The use of flower food at the beginning and at top up showed less control of fungi growth in some other experiments (Figure 3-24). The reason for this is not fully understood.

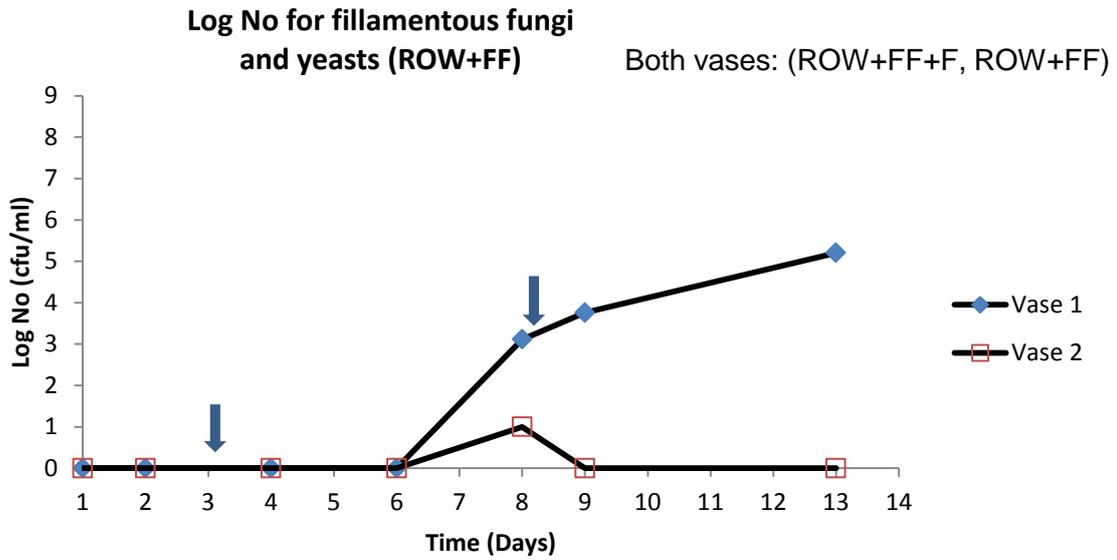


Figure 3-22 Log No for filamentous fungi and yeasts on DRBCA when using reverse osmosis water (ROW) + flower food (FF) and top up with ROW + FF (top up days marked with blue arrows).

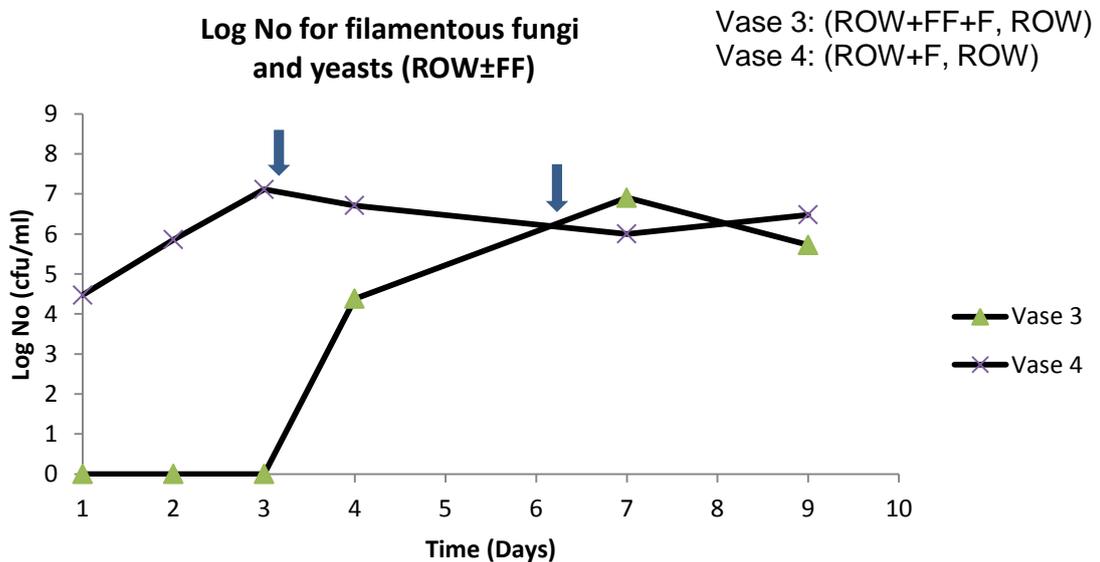


Figure 3-23 Log No for filamentous fungi and yeasts on DRBCA when using reverse osmosis water (ROW) ± flower food (FF) and top up with ROW (top up days marked with blue arrows).

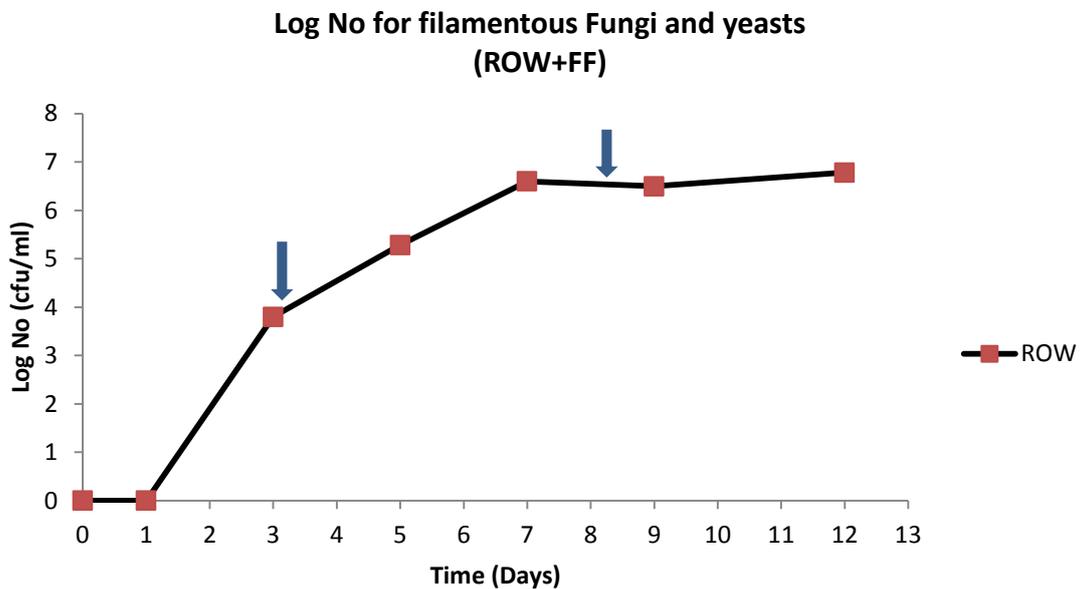


Figure 3-24 Log No for filamentous fungi and yeasts on DRBCA when using reverse osmosis water (ROW) + flower food (FF) and top up with ROW + FF (top up days marked with blue arrows).

3.4 Effect of pH and sugar concentration on microbial population

The effect of pH and sugar concentration (fructose and glucose) on the microorganisms' numbers during the vase life was observed when different vase solutions and different top up systems were used.

3.4.1 Standard water

Using standard water with flower food as a vase solution maintained the pH value between 4.2 and 4.6, the sugar concentration of fructose and glucose in such solution was between 3.5 – 3.9 mg/ml. It was noticed these values remained in place during the first few days before top up of the vase solution. The change in these values depended on the top up solution; when standard water and flower food was used to top up they remained within the mentioned range (Figure 3-25), but when standard water alone was used the concentration

of sugar dropped and the value of pH increased (Figure 3-26). On the other hand the numbers of microorganisms increased in line with the increase of pH at top up points and also when flower food was not used.

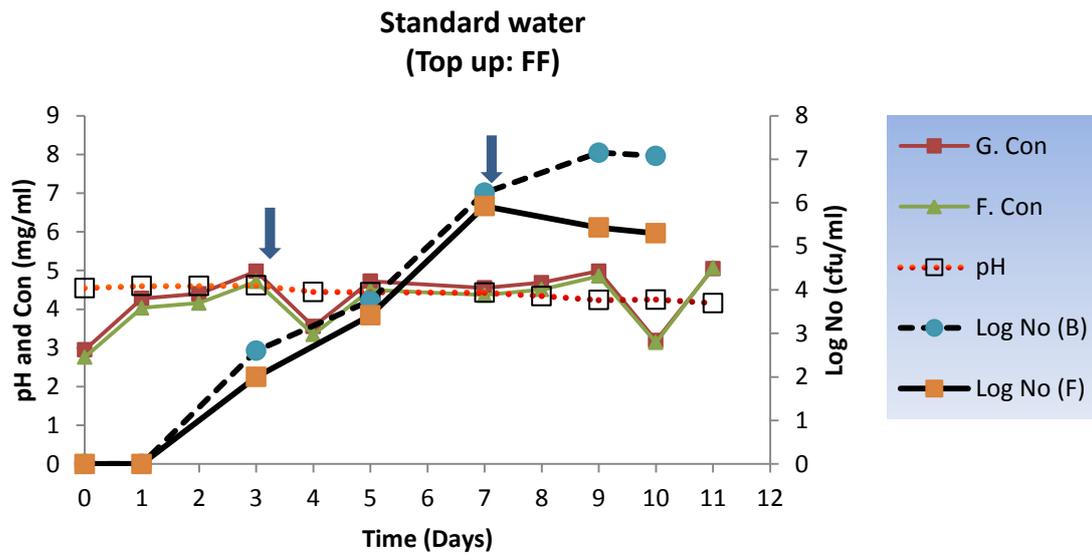


Figure 3-25 Standard water: The effect of pH and sugar concentration on microbial population in (TSA and DRCBA) when top up with flower food (FF). [G.Con=Glucose Concentration, F. Con= Fructose Concentration, B=Bacteria, F=Fungi, Blue arrows=Top up days]

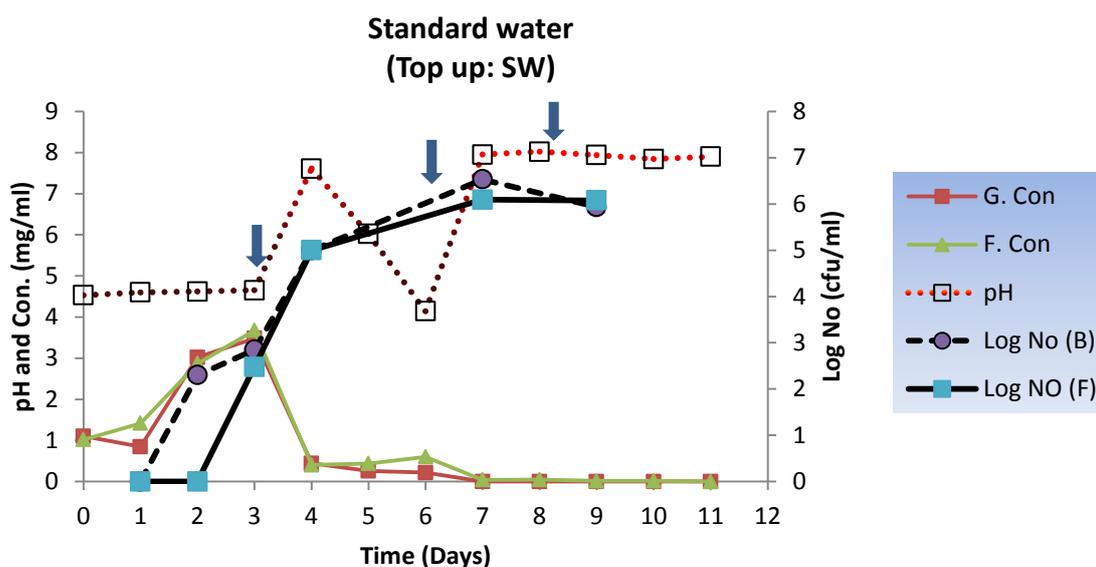


Figure 3-26 Standard water: The effect of pH and sugar concentration on microbial population (in TSA and DRCBA) when top up with standard water (SW). [G.Con=Glucose Concentration, F. Con= Fructose Concentration, B=Bacteria, F=Fungi, Blue arrows=Top up days]

3.4.2 Reverse osmosis water

The pH of reverse osmosis water with added flower food was around 3 and the sugar concentration (fructose and glucose) was the same as mentioned above in 3.4.1 (3.5 – 3.9 mg/ml). The values of pH and sugar concentration remained unchanged in the first few days of vase life and after top up points when flower food was used (Figure 3-27). When reverse osmosis water was used alone without the flower food the pH values were increased and the sugar concentration values decreased (Figure 3-28). The populations of microorganisms were controlled up to the point of top up, but have increased afterwards in the case of reverse osmosis water top ups and remained controlled in the case of adding flower food at top up.

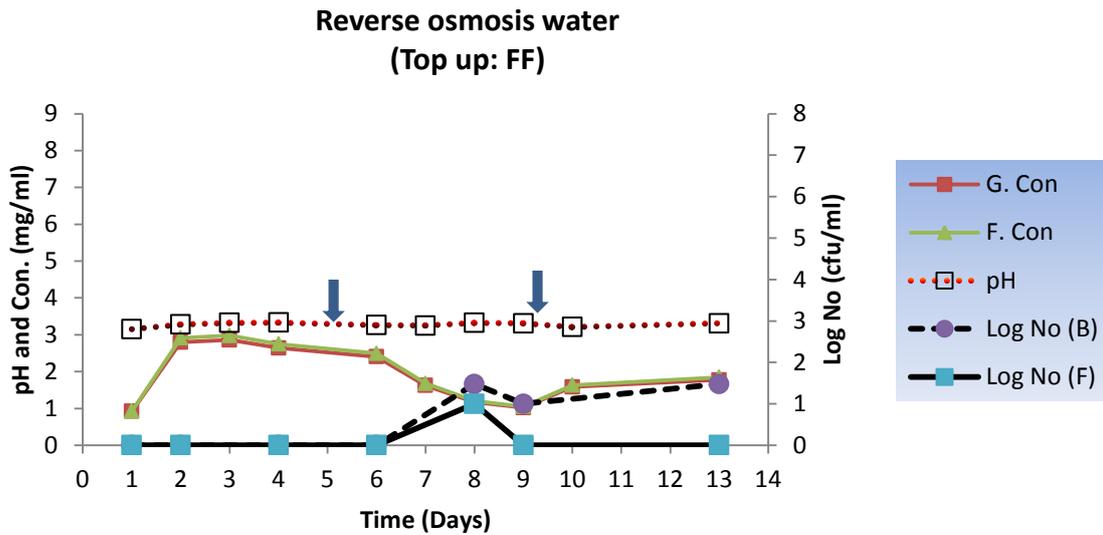


Figure 3-27 Reverse osmosis water: The effect of pH and sugar concentration on microbial population (in TSA and DRCBA) when top up with flower food (FF). [G.Con=Glucose Concentration, F. Con= Fructose Concentration, B=Bacteria, F=Fungi, Blue arrows=Top up days]

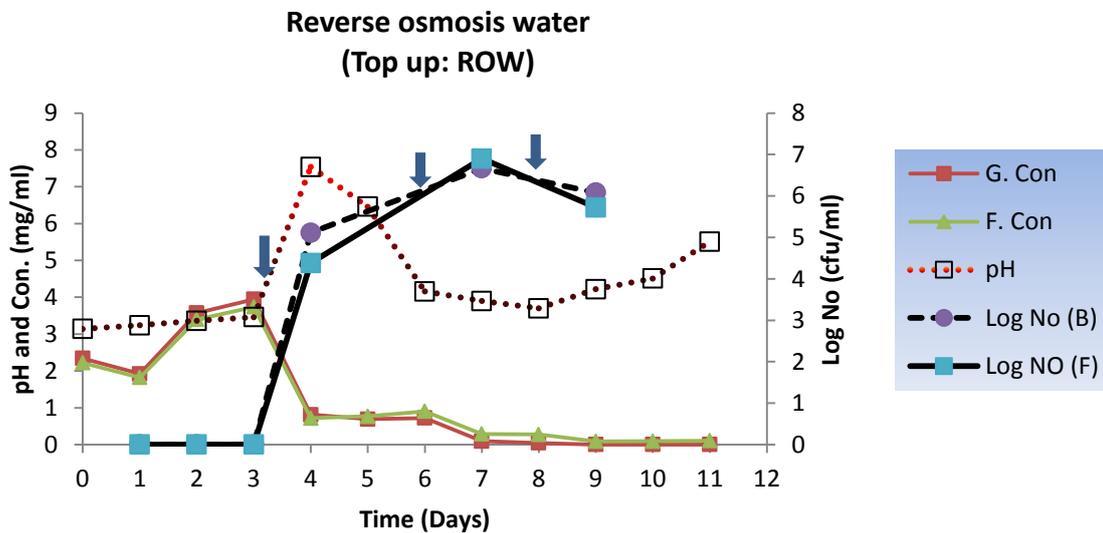


Figure 3-28 Reverse osmosis water: The effect of pH and sugar concentration on microbial population (in TSA and DRCBA) when top up with reverse osmosis water (ROW). [G.Con=Glucose Concentration, F. Con= Fructose Concentration, B=Bacteria, F=Fungi, Blue arrows=Top up days]

3.5 Water uptake

The daily water uptake by the cut flowers from vase solutions was measured by subtracting the vase weight on each day from the vase weight of the previous day taking into consideration the evaporation rate, the vase and the flowers weight.

The rate of water uptake was slightly higher when ROW was used compared to SW, for equivalent bouquets, however the difference between water uptake rate before and after top up was slightly lower in the latter, this is suggesting that the flowers were still able to uptake water and the complete blockage to the xylem did not occur.

When the water volume data are analysed such that the total water usage was plotted against the number of days, it was clear that there were only small differences between vases which had flower food added and those that did not (Figure 3-29), or those which either had standard water or had reverse osmosis water (Figure 3-30), for a given style of bouquet. Also results showed that water uptake rate was almost the same when using different types of water with low pH (Figure 3-31) or when using standard water with added citric acid (Figure 3-32).

The total water used was analysed using a quadratic regression analysis. The curvature observed as the VL increased was well modelled by this equation. From the data given in the Tables below, the initial rate of water uptake was very similar in most cases, given that the absolute value would be dependent on actual flower make-up of the vase. The statistical analysis suggests that whatever was happening in the vase water (pH, microbes, treatments) did not affect very much the total water uptake during the vase life. This is contrary to what was expected, given the available literature on the subject.

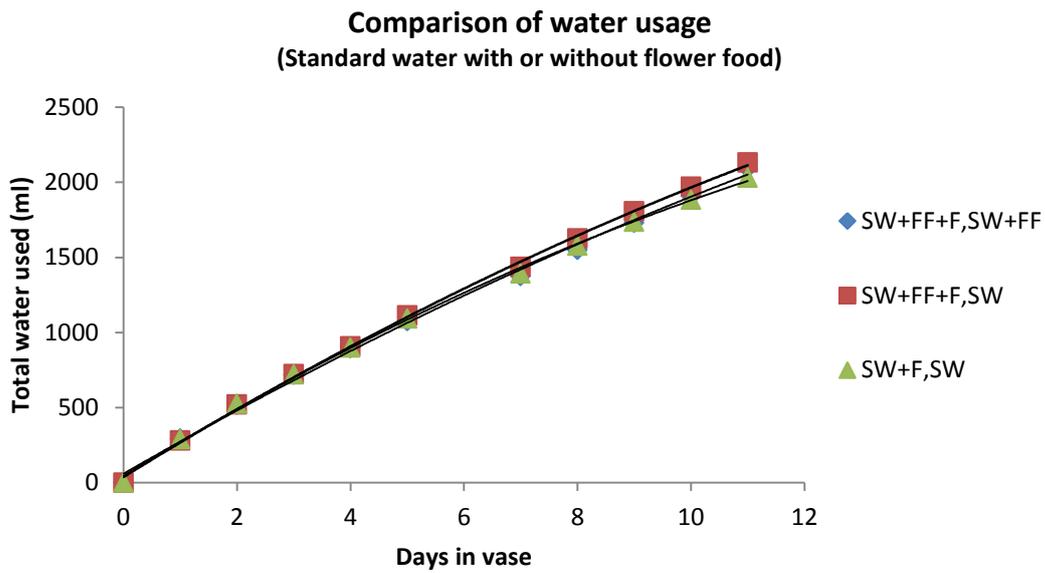


Figure 3-29 Water uptake over the experimental period for vases with standard water with or without flower food, topped up with an equivalent environment.

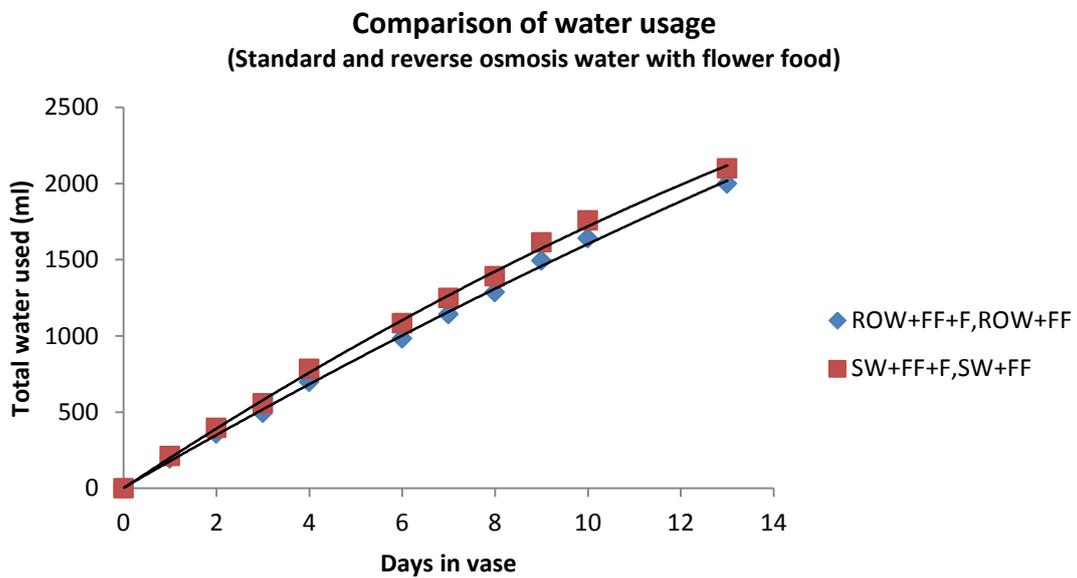


Figure 3-30 Water uptake over the experimental period for vases with standard and reverse osmosis water with added flower food, topped up with an equivalent environment.

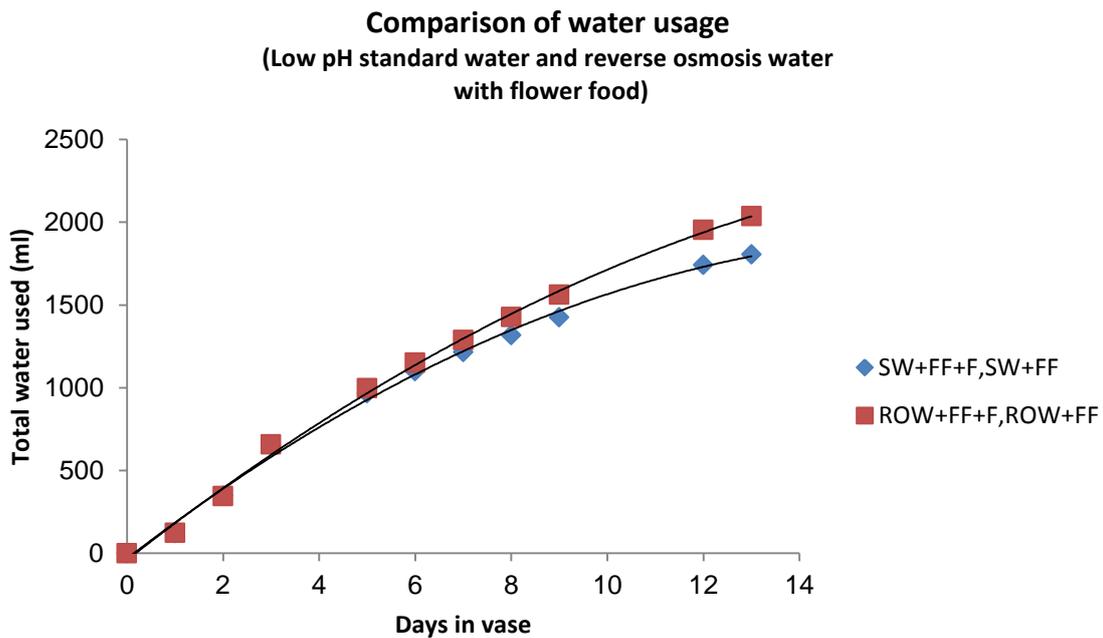


Figure 3-31 Water uptake over the experimental period for vases with standard and reverse osmosis water with added flower food at low pH (3), topped up with an equivalent environment.

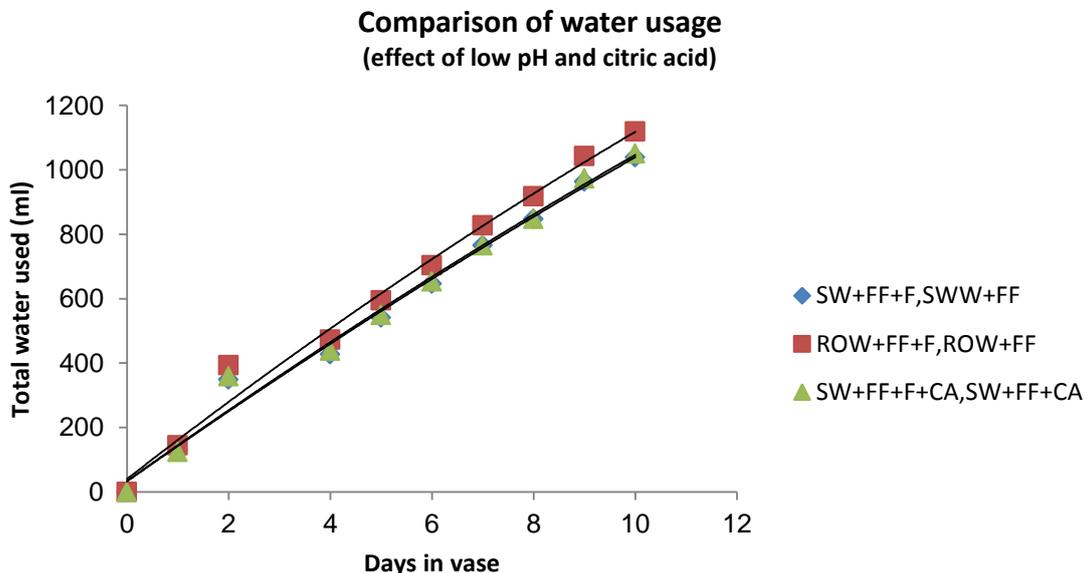


Figure 3-32 Water uptake over the experimental period for vases with standard water (with or without added citric acid) and reverse osmosis water with added flower food, topped up with an equivalent environment.

Table 7 Statistical analysis values of the total water uptake for different vases of experiment 2 (comparison between SW with or without FF).

		Experiment 2		
Vase treatment	Parameter	Value	Lower 95.0%	Upper 95.0%
SW+FF+F,SW+FF	Intercept	59.86	-8.24	127.96
	Day ²	-3.29	-5.94	-0.65
	Day	217.39	187.18	247.61
SW+FF+F,SW	Intercept	38.10	-5.13	81.34
	Day ²	-4.06	-5.74	-2.38
	Day	233.35	214.16	252.53
SW+F,SW	Intercept	44.08	-4.19	92.36
	Day ²	-4.94	-6.81	-3.06
	Day	233.01	211.59	254.43

Table 8 Statistical analysis values of the total water uptake for different vases of experiment 3 (comparison between SW and ROW).

		Experiment 3		
Vase treatment	Parameter	Value	Lower 95.0%	Upper 95.0%
ROW+FF+F,ROW+FF	Intercept	3.22	-38.81	45.25
	Day ²	-1.65	-2.87	-0.43
	Day	176.54	160.86	192.22
SW+FF+F,SW+FF	Intercept	0.87	-45.10	46.83
	Day ²	-2.96	-4.29	-1.63
	Day	201.42	184.27	218.56

Table 9 Statistical analysis values of the total water uptake for different vases of experiment 5 (comparison between SW and ROW at low pH).

	Experiment 5			
Vase treatment	Parameter	Value	Lower 95.0%	Upper 95.0%
ROW+FF+F,ROW+FF (at pH 3)	Intercept	-40.73	-107.80	26.35
	Day ²	-5.23	-7.04	-3.43
	Day	227.77	203.50	252.04
SW+FF+F,SW+FF (at pH 3)	Intercept	-32.00	-107.59	43.58
	Day ²	-6.42	-8.46	-4.39
	Day	223.95	196.59	251.30

Table 10 Statistical analysis values of the total water uptake for different vases of experiment 6 (comparison between SW, ROW and SW with added citric acid).

	Experiment 6			
Vase treatment	Parameter	Value	Lower 95.0%	Upper 95.0%
SW+FF+F,SW+FF	Intercept	32.59	-45.89	111.07
	Day ²	-1.02	-4.61	2.56
	Day	110.93	74.18	147.68
ROW+FF+F,ROW+FF	Intercept	40.11	-50.12	130.33
	Day ²	-1.49	-5.61	2.63
	Day	122.73	80.48	164.98
SW+FF+F+CA,SW+FF+CA	Intercept	33.75	-48.92	116.41
	Day ²	-1.09	-4.86	2.69
	Day	112.20	73.49	150.92

3.6 Turbidity and Optical Density of Vase Water

The measurement of vase water turbidity was done by using M350 Double Beam U.V. Visible Spectrophotometer with wave length of 600 nm. Results obtained at first did not appear to show a correlation between the visible turbidity in vases and microbial counts with readings from the spectrophotometer. However, this was in part due to the criterion for visible turbidity being much greater than that need for a plate count. In general a count of greater than 1×10^6 cfu/ml is required to have a detectable OD above the background. In many of the experiments carried out, especially with ROW with FF, the numbers did not reach the criteria required. Using longer cuvettes i.e. having a path length greater than 1cm would improve sensitivity, or using a smaller wavelength. Results from the preliminary study (Figures 3-33 and 3-35) showed an increase in OD, until the top-up point. Thereafter OD values were relatively stable. The reduction in pH observed suggest the growth of acidophiles, whose growth was arrested after top up.

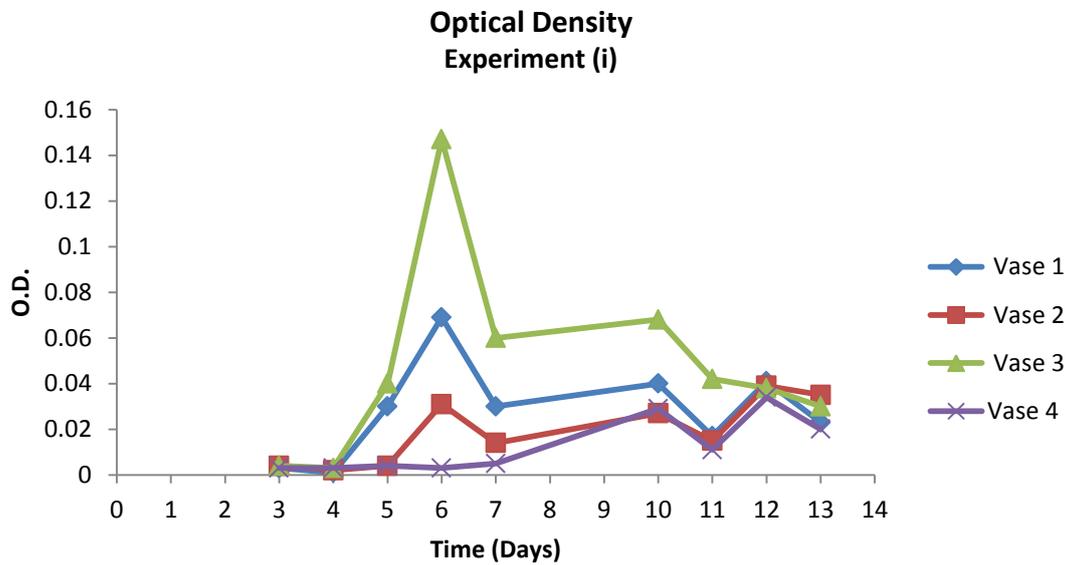


Figure 3-33 Optical density for different vases from Experiment (i), all vases contained SW+FF+F and topped up with SW on day 7.

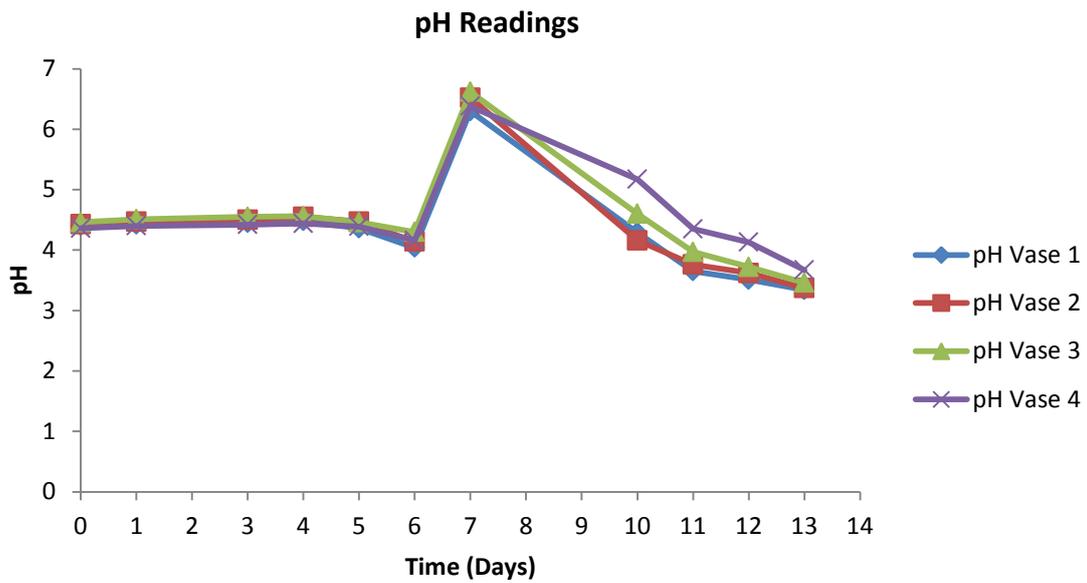


Figure 3-34 pH reading for the same experiment in Figure 3-33.

The use of flower food has a clear effect on bacterial numbers and subsequently the optical density as shown in vases 4 and 5 in Figure 3-36, which have shown higher bacterial numbers from day 2 onwards compared to vases 1, 2 and 3. On the other hand these two vases have also shown higher optical densities over the same period up to day 9 (Figure 3-35). After day 9 there were sudden changes in OD probably due to the heterogeneity of the vase water.

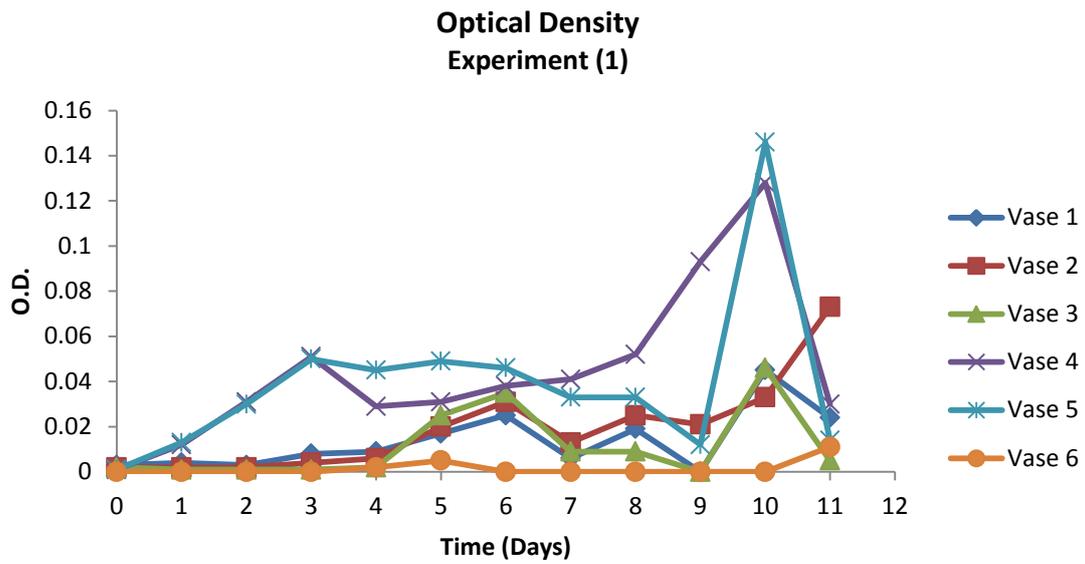


Figure 3-35 Optical Density for different vases (Experiment 1), vase 1 and 2: SW+FF+F, SW+FF; vase 3: ROW+FF+F, ROW+FF; vase 4: ROW+F, ROW; vase5: SW+F, SW and vase 6: ROW+FF (control). All vases (except control) were topped up on days 3, 6 and 9.

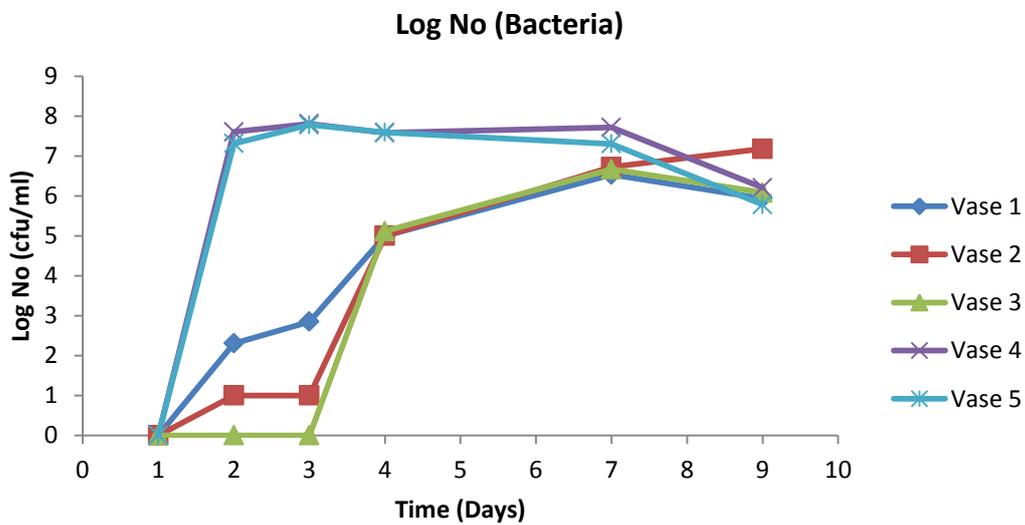


Figure 3-36 Bacterial Log No (cfu/ml) on TSA for the same experiment in Figure 3-35.

3.7 ACCURATE-MASS Q-TOF LC/MS

The use of the Q-TOF LC/MS system to analyse vase water failed to show the large concentrations of glucose and fructose known to be in the flower food. It was then determined that the Q-TOF profiles for the sugars, showed them to be a complex mixture of polysaccharides (Table 11). It was hypothesised that these are condensation polymers of glucose and fructose produced in the low pH of the concentrated flower food. Different profiles of the vase waters were observed over the course of 2 different experiments using the profiling system of the Q-TOF LC/MS. When FF was used to top up, the profiling did not show significant changes apart from one unidentified peak (compound) appeared up to day 4 (Figure 3-37 A and B) and disappeared after that up to the end of the experiment (Figure 3-37 C and D). however when topping up with only SW the profiling showed similarity to the one shown in Figure 3-37 only at the start of vase life, but after topping up it showed irregularity probably due to the complex nature of vase water containing different salts coming from SW and organic exudate coming from cut flowers and/or microorganism (Figure 3-38).

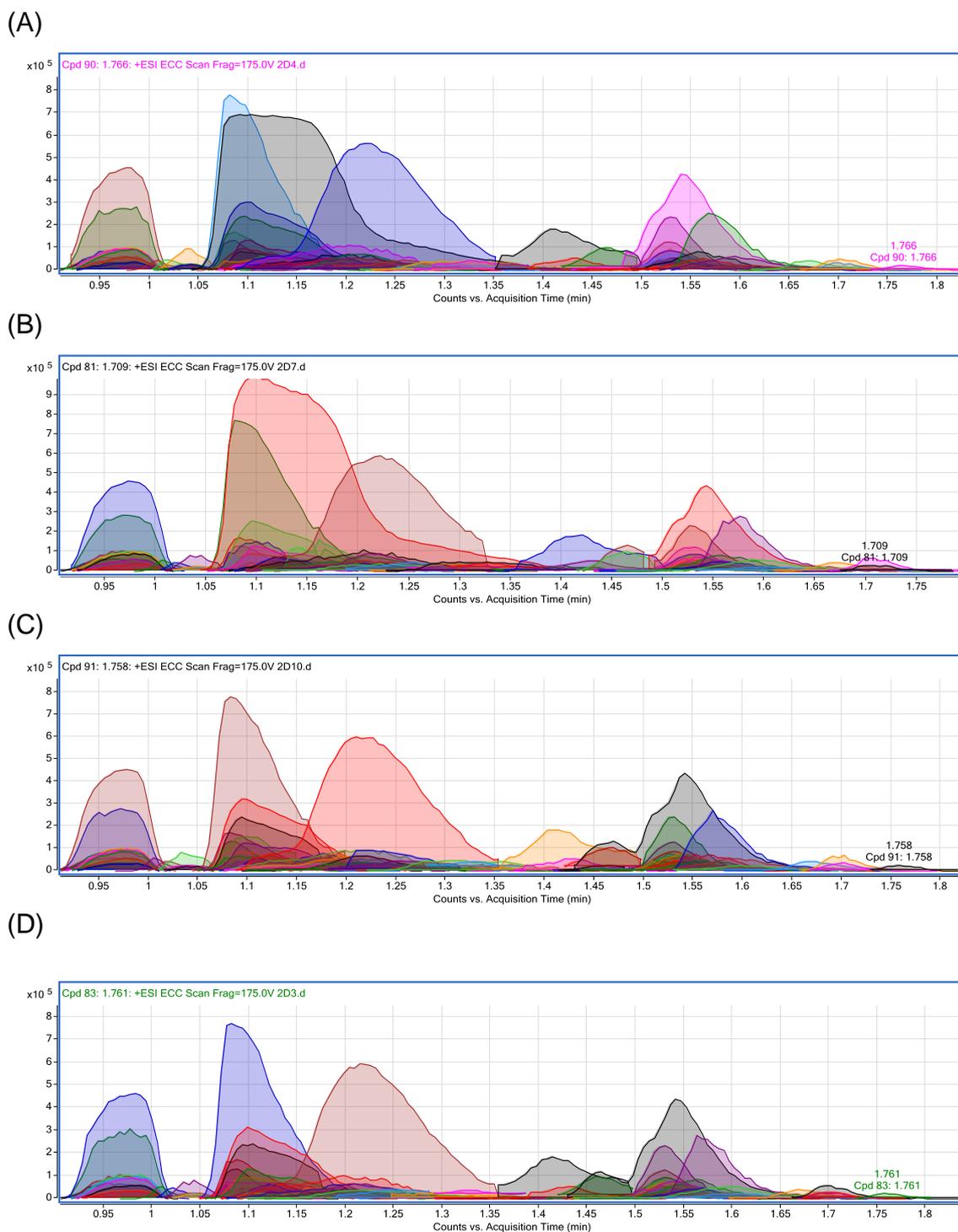
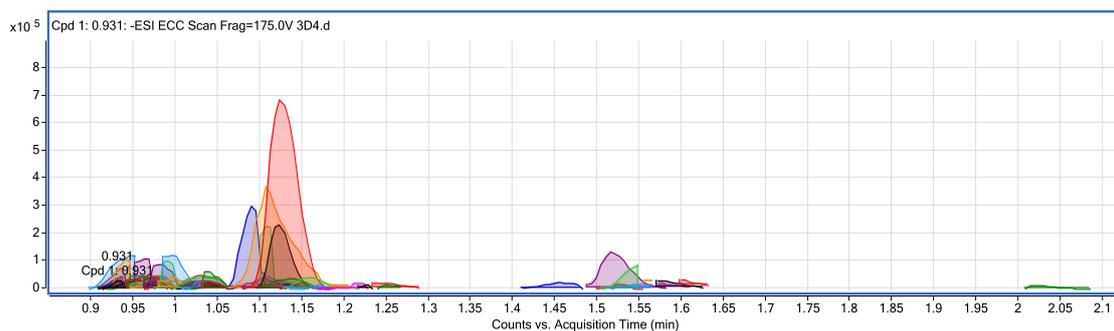
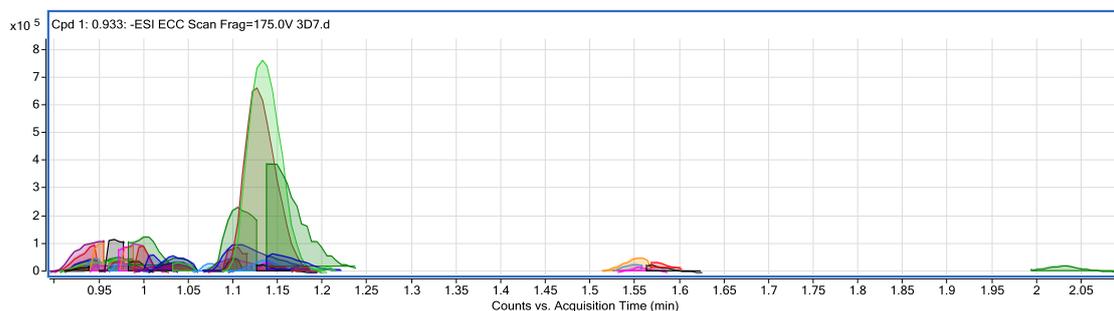


Figure 3-37 Compounds profile for vase 2 (SW+F+FF, SW+FF) at A: Day 3 (before first top up), B: Day 4 (after first top up), C: Day 7 (after second top up) and D: Day 10 (last day of the experiment).

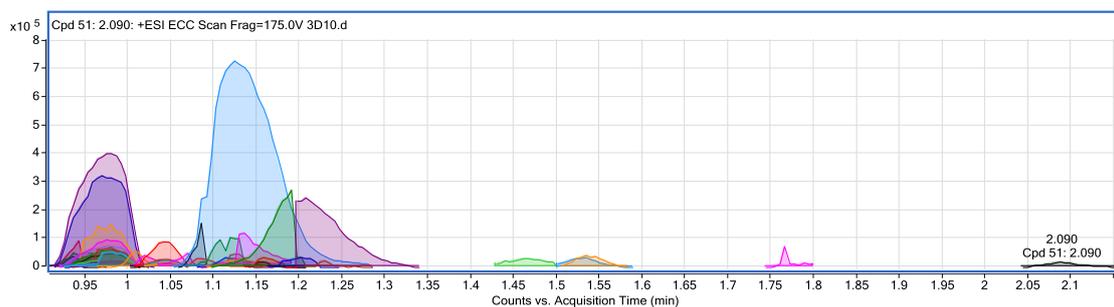
(A)



(B)



(C)



(D)

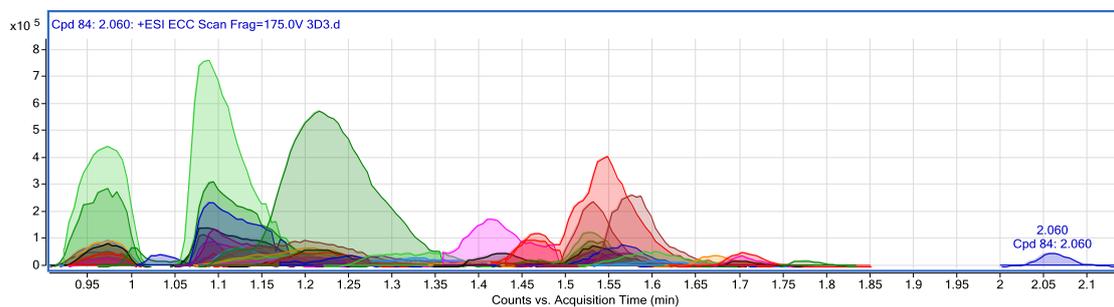


Figure 3-38 Compounds profile for vase 3 (SW+F+FF, SW) at A: Day 3 (before first top up), B: Day 4 (after first top up), C: Day 7 (after second top up) and D: Day 10 (last day of the experiment).

A list of compounds was obtained from (Merlin) database shown in Table 11. Samples were taken from 2 different vases; vase 2 (SW+F+FF, SW+FF) and vase 3 (SW+F+FF, SW).

Table 11 List of compounds obtained from Merlin database for two different vases.

Day	Compounds	
	V2 (SW+F+FF, SW+FF)	V3 (SW+F+FF, SW)
3	1,3-Dimethyluric acid, Dihydroxyacetone (glycerone), Sucrose, Quinic acid, Trp Cys Ala, Edetate, D-glucose, 2-Furoic acid, Dehydroascorbic acid, 2,3-Dioxogulonic acid, 2-Hydroxyadipic acid, Ketoconazole Metabolite, Amylose.	Gluconic acid, Beta-lactic acid, Metoclopramide N4-sulfate, Chlorogenic acid, Dihydroxyacetone (glycerone), Sucrose, Quinic acid, 2-Furoic acid, Dehydroascorbic acid, 2,3-Dioxogulonic acid, 2-Hydroxyadipic acid, Ketoconazole Metabolite, Amylose.
4	Theobromine, Metoclopramide N4-sulfate, Galactonic acid, Chlorogenic acid, 1,3-Dimethyluric acid, Dihydroxyacetone (glycerone), Sucrose, Quinic acid, Trp Cys Ala, D-glucose, 2-Furoic acid, Dehydroascorbic acid, 2,3-Dioxogulonic acid, 2-Hydroxyadipic acid, Ketoconazole Metabolite, Amylose.	Gluconic acid, Dihydroxyacetone (glycerone), Quinic acid, 2,3-Dioxogulonic acid.
6	Dihydroxyacetone (glycerone), Sucrose, Quinic acid, Edetate, D-glucose, 2-Furoic acid, Dehydroascorbic acid, 2,3-Dioxogulonic acid, 2-Hydroxyadipic acid, Ketoconazole Metabolite, Amylose.	Hydroxypyruvic acid, N-Monodesmethylidiltiazem, Gluconic acid, Reserpic acid, Chlorogenic acid, Iduronic acid, 2-Furoic acid, 2,3-Dioxogulonic acid, Ketoconazole Metabolite.
7	Galactonic acid, Chlorogenic acid, Dihydroxyacetone (glycerone), Sucrose, 2-Furoic acid, Dehydroascorbic acid, 2,3-Dioxogulonic acid, 2-Hydroxyadipic acid, Ketoconazole Metabolite, Amylose.	2,3-Dioxogulonic acid.
10	1,3-Dimethyluric acid, Chlorogenic acid, Edetate, 2-Furoic acid, Dehydroascorbic acid, 2,3-Dioxogulonic acid, 2-Hydroxyadipic acid, Ketoconazole Metabolite, Amylose.	None

3.8 Flowers appearance and quality

Photographs of flowers were taken approximately every day during the course of each set of experiment. Examples of these photographs at the start, middle, and end of some experiments are shown in Table 12 and Table 13.

The use of flower food with standard or reverse osmosis water has clearly improved the flowers quality and appearance during vase life, noticeably when the pH dropped to around 3 as shown in the first and third row of Table 13 (marked with red line borders). However the quality and appearance of cut flowers improved partially when flower food was used with standard water at top up with higher pH (4-4.5) as shown in the first and third row of Table 12 (marked with green line borders). Using standard or reverse osmosis water alone at top up did not improve the flowers quality as shown in the rest of the photographs in Table 12 and 13.

Discolouration of stems and leafs was noticed when low pH vase water was used, also white spots on the flowers were reported as shown in Figure 3-39.



Figure 3-39 *Discolouration of stems and flowers in low pH vase water.*

Table 12 Photographs of flowers at the beginning, middle and end of different experiments using standard water with or without flower food (at high pH).

Vase Treatment	Start	Middle	End
SW+F+FF, SW+FF			
SW+F+FF, SW			
SW+F+FF, SW+50%FF			
SW+F, SW			

Table 13 Photographs of flowers at the beginning, middle and end of different experiments using standard and reverse osmosis water with or without flower food (at low pH).

Vase Treatment	Start	Middle	End
ROW+F+FF, ROW+FF			
ROW+F, ROW			
SW+F+FF pH 3, SW+FF pH3			
SW+F+FF pH 3.3 CA, SW+FF pH 3.3 CA			

4 DISCUSSION

The laboratory set up used in this project follows from the publication of Reid and Kofranek (1980), who attempted to standardise the conditions used in the study of vase life and vase quality; a temperature of 18 – 22°C, with 60 to 70% RH, with a given level of light (10-12 hours per day). These conditions were matched in the laboratory, although CO₂ levels, air velocity and ethylene concentrations were not studied. Despite these omissions the standardisation carried out meant we had good control of the overall experimental set-ups undertaken.

The work done in our standardised laboratory clearly showed a link between the initial pH of the vase water, the type of top-up-water and the growth of microorganisms and therefore the onset of turbidity. The water uptake rate was, however, not correlated with these (unless a very low pH (approx. 3.0) was used).

Standard water (SW) is buffered hard water (Mg and Ca ions) carbonate system, with an initial pH of approximately 4.5. This is a recommended base solution for vase quality trials. This environment allows common bacteria as well as moulds such as *Botrytis cinerea* to grow quickly, especially in the nutrient rich and warm environment of the vase water. Initially, low levels of bacteria were present but this increased dramatically on topping up with more water, replacing that which had been respired or evaporated off. The bacteria obtained from vase water with higher pH were primarily *Pseudomonads* and have come from the growing environment of the flowers and reach the vase water from the flowers themselves (Kates et al., 1991). Topping up with SW diluted the existing sugars but increased the pH substantially, allowing faster microbial growth. Interestingly the increase in pH through dilution favours the growth of bacteria and inhibits the growth of moulds.

Topping up the vase water with flower food (FF) maintains the initial environment. However, if microbial growth is already initiated then it will continue, topping up with FF simply maintains the growing environment. Hence

the microbial ecology will follow the basis of which organisms are present on the original flowers (i.e. pre and post-harvest contamination) and which are capable of growing in the given environmental conditions. Certainly molds can grow, but bacteria suited to moderately low pH can also compete (e.g. *Enterobacteriaceae* and *Pseudomonads*).

This work was concerned with the vase life and quality of cut flowers; the length of the vase life can be defined as the time to the loss of the quality of the ornamental appearance. As discussed by van Doorn in several publications, it is generally regarded that the length of vase life is a consequence of water stress and/or infection. That the visual appearance becomes anathema to the customer also influences the actual vase quality: customers will have different tolerances to the visual appearance of senescent flowers. In a recent review [Fanourakis *et al* \(2013\)](#) suggest that a fundamental analysis of pre and post-harvest conditions be instigated, suggesting this is needed because of the interaction between genotype and the growing environment coupled to the phenotype and the post-harvest situation. However, this seems like an appeal to review the entire field rather than analyse the current theories and assumptions on which the length of vase life (and therefore the industry) is based.

One thing is clear, however, vase quality is a multivariate problem. There is no one measure of vase quality. The terminating criteria are generally subjective (e.g. wilting head, discolouration or the level of *Botrytis* blooms on the stems) but other criteria to which a value can be attached such as microbial population level, weight loss or significant changes in water uptake can also be used. It is worth considering a multivariate statistical approach to the determination of vase life and vase quality, such as principal component analysis (PCA) or Neural network analysis.

The blockage of cut flower stem by microorganisms or other factors has been widely reported in the literature ([Put and Jansen, 1989](#); [Put, 1990](#); [van Doorn and de Witte, 1995](#); [van Doorn and Cruz, 2000](#); [Loubaud and van Doorn, 2004](#); [van Meeteren *et al.*, 2006](#)). This is considered a major VL terminating criterion.

As the bacteria form plugs in the xylem vessels the reduction of water uptake leads to increased stress and senescence. De Witte and van Doorn (1988) stated that different bacterial species decreased the water uptake in a similar way, hence it was hypothesised that the population density was the governing factor for the water uptake reduction and not what species was present. In this report we have shown that this is not necessarily the case; when water uptake by the cut flowers was measured the results showed little influence by the presence of microorganisms or flower food, even when the population density was very high.

The results also showed that the water uptake rate was independent of the pH, the water type (SW or ROW) or whether flower food was used or not (see Figures 3-29 and 3-30). The analyses of the uptake rates suggest that the uptake gradually diminishes with time (the data were fitted to a decreasing quadratic rather than a simple linear regression fit). The idea that when water uptake becomes negative is an indicator of vase quality is partially borne out by the results. As time increase the curvature increases, but in no cases was a stationary value observed. Only at a pH of 3 did the curvature significantly approaching a stationary value after 12 days (Figure 3-31). In contrast, however, at pH 3.3 when citric acid was added the uptake rate was almost linear for the whole of the duration of the experiment (Figure 3-32).

It is important to reiterate that these results appear to be at odds with the literature, which states that water uptake rates are dependent on the level of bacteria (or microbes in general) present in the vase water.

4.1 Sugar analysis

In this project we have shown that the concentration of sugars in each vase during the vase life depended on the topping up solution; when topped up with standard water or reverse osmosis water alone the concentration fell to its minimum as the sugar was diluted out, but when the flower food was used to top up the concentration remained relatively constant. Hence the idea that the microorganisms present use up the available sugars is seen to be false.

Although the optical density analysis was flawed – the solutions were too ‘clear’ for an effective reading using 1cm cuvettes- there was a good correlation with the microbial numbers obtained from plate counts (when counts exceeded the detection criterion of approx. 1×10^6 cfu/ml). From the optical density analysis it appeared that when the vase water was topped up, there was a major reduction in the growth of the bacteria, which suggest the sudden reduction in the sugar concentration (to about 10%) had induced a lag as well as reducing absolute numbers. Further top ups with only ROW or SW appear to reduce the sugar levels available to the microbes, but only marginally affect the overall numbers. When topped up with flower food although numbers were diluted by a factor of a log, there was little difference between the amount of subsequent growth observed (see Fig 3-25), which shows no real change in the growth rate on topping up.

Although the presence of sugars in the vase water is reported to play a major role in providing nutrient for the cut flowers as well as microorganisms (Raven *et al.*, 2003, Pun and Ichimura, 2003, vanDoorn and Han, 2011, Arrom and Munné-Bosch, 2012) it clearly did not affect the rate of water uptake; when topped up with a full, half or quarter dose of flower food, or simply SW or ROW, water uptake was unchanged.

4.2 Changes in vase water quality during vase life

Water type, pH and microbial growth:

The pH and microbial growth in vase solutions were affected by the type of water used at the start and at topping up.

Reverse osmosis water (ROW):

- a. ROW at the start and at topping up: the pH initial value was 5.5 then increased to over 7 within 4 days, after top up had another increase to around 7.5. Microbial growth started high from day one and remained high through the rest of vase life.

- b. ROW with FF at the start and at topping up: ROW with added FF had an initial low, but constant, pH value of around 3.2 for the duration of vase life. Microbial growth was inhibited in the first 2-3 days and remained low the rest of vase life in most experiments.
- c. ROW with FF at the start and only ROW at topping up: the initial pH was around 3.2, when topped up with ROW only the pH increased to over 7 and then decreased back to pH 4 over a period of a few days. Microbial growth was inhibited in the first 2-3 days but increased after topping up.

In contrast; reverse osmosis water has a lower pH and less buffering capacity. In the presence of FF it has a pH of approximately 3, below the limit of most common bacteria associated with pathogenesis or spoilage, e.g. *E. coli* or pseudomonads. However, the environment is capable of sustaining acidophilic organisms such as *lactobacilli* as well as yeasts and moulds. Again, if microbial growth is initiated then even if the environment is topped up with FF it will continue. Often low pH will mean slow growth – but this is not always the case.

Standard water (SW):

- a. SW at the start and at topping up: pH started and remained at high level (7.1-7.8) for the duration of the vase life. Microbial growth started as early as day one and reached its maximum level at day 2-3 and remained high for the rest of vase life.
- b. SW with FF at the start and at topping up: the pH remained close to the initial value of 4.5 for the duration of the vase life. Microbial growth was inhibited in the first 2-3 days but increased after topping up and remained relatively high for the rest of shelf life.
- c. SW with FF at the start and only SW at topping up: although the pH had a lower constant value for the first few days, on topping up with SW it increased to around 6.5. The pH dropped back to approximately 4.5, but then increased and remained at around 6.5 on a further top up with SW.

microbial growth started low at the first 2-3 days, but increased after topping up and stayed high for the rest of vase life.

In contrast, if the standard water is topped up with flower food, then growth, if already started, will continue – this is because the top up water is providing the same environment as the vase water – there is no added antimicrobial activity. Even although the sugar concentration is substantially reduced when topping up with only SW, this makes little difference to the rate of microbial growth – i.e. it is the shunt in pH which is by far the major effect stimulating the growth.

4.3 Recommendations

The results described herein showed that reverse osmosis water with flower food has a pH lower than the pH suitable for the growth of the majority of pathogenic and spoilage microorganisms. However the standard practice in the cut flower industry and household is to use tap water with added flower food, this type of vase water has a pH similar to standard water with flower food as we have shown in our results, this pH is just above the minimum pH suitable for the growth of the majority of pathogenic and spoilage microorganisms. The sudden change in pH when topping up with tap water or standard water surely allows the already existing organisms in the vase water to grow rapidly and contribute to the blockage of xylem of cut flowers. From these observations few points need to be considered through the entire chain of cut flowers industry:

- The need to standardise the vase water where cut flowers are kept whether at wholesale points or retailers to ensure that the suitable pH is maintained before reaching the final customer and also to minimise the risk from the initial contamination.
- Supply the final customer at home with the right dose of flower food to be sufficient at the start and at topping up points, also to consider the type of tap water in the geographical area when producing flower food, this will ensure that the right pH is maintained during the vase life.

- Fresh clean water should be observed at all time through the chain prior reaching the final customer. Special attention to cleanness and disinfection of containers and vases where cut flowers are kept.
- [Vercesi et al \(1997\)](#) described the inhibition of *B. cinerea* with tartaric and malic acids. The pH that the investigations were conducted was not given. [Hosen et al \(2010\)](#) showed that at pH 4.5, growth was prolific, once sporulation occurred. Whereas at pH 6.5, growth was much less (1/5 as fast). The optimum temperature for growth was 20°C. In vases plant debris was a source of *B. cinerea* ([Fanourakis et al, 2013](#)). Addition of weak acid preservatives such as benzoic acid or sorbic acid could control or prevent the growth of such acidophilics, whilst allowing a pH compatible with the flowers to be maintained. Studies done by [Lambert](#) have shown that the minimum pH for growth of the acidophilic bacterium *Lactobacillus plantarum* increase from below 3 to 4 on addition of sorbic and benzoic acid mixtures.

5 CONCLUSIONS

In this project it has been found that the quality of the water is dependent on several factors;

1. Standard water versus reverse osmosis water in conjunction with flower food.
2. Frequency of top-up and with what type of water.
3. Initial contamination level.
4. Initial microbial species present.
5. Whether initiation of growth occurs early or is retarded by the environment.

The results in this report showed that the quality and type of water used has played a major influential role in both the pH and microbial content thereafter of vase solutions. For example if reverse osmosis water (ROW) is used with flower food the initial pH is lower than the pH minimum for almost all common pathogens and the majority of common spoilage organisms, but if growth is initiated due to the presence of microbes capable of growth in the low pH environment, then growth will continue regardless of topping up solution. In the other hand topping up with ROW with flower food maintains the low pH environment.

The use of flower food with standard water (SW) reduces the pH of vase water but not sufficiently enough to inhibit the growth of common pathogens or spoilage organisms. Also adding weak acid preservatives such as benzoic acid or sorbic acid could control or prevent the microbial growth, whilst allowing a pH compatible with the flowers to be maintained.

Sugar concentration levels in vase water have no subsequent effect on the growth or otherwise of the microbes even when diluted with top up water, however the water uptake by the flowers is little affected by the presence of flower food or the microbial population.

Possible strategies which can be used:

1. Top up with flower food as a standard methodology.
2. Addition of weak acid preservatives-
 - a. Generally Recognised As Safe (GRAS) listed, e.g. benzoic, sorbic, hydroxybenzoates.
 - b. Non-GRAS listed.
3. Better buffering system for the flower food to maintain low pH (at or below 4). Nb/ Weak acids allow the minimum pH for growth to be moved to a higher pH value.
4. Use of softened water (e.g. Brita Filtered).

Future work:

More research work needs to be carried out in looking at and adopting a hurdle technology similar to the one used in the beverage industry when producing flower food.

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Appendix A List of all experiments carried out during the research period

		Experiment Number							
		i	ii	1	2	3	4	5	6
Vase	1	SW+F+FF, SW	SW+F+FF, SW	SW+F+FF, SW+FF	SW+F+FF, SW+FF	ROW+F+FF, ROW+FF	SW+F+FF, SW+50%FF	SW+F+FF pH 3, SW+FF pH3	SW+F+FF, SW+FF
	2	SW+F+FF, SW	SW+F+FF, SW	SW+F+FF, SW+FF	SW+F+FF, SW+FF	ROW+F+FF, ROW+FF	SW+F+FF, SW+50%FF	SW+F+FF pH 3, SW+FF pH3	SW+F+FF, SW+FF
	3	SW+F+FF, SW	SW+F+FF, SW	ROW+F+FF, ROW+FF	SW+F+FF, SW	SW+F+FF, SW+FF	SW+F+FF, SW+25%FF	SW+F+FF pH 3, SW+FF pH3	ROW+F+FF,ROW+F F
	4	SW+F+FF, SW	SW+F+FF, SW	ROW+F,RO W	SW+F+FF, SW	SW+F+FF, SW+FF	SW+F+FF, SW+25%FF	ROW+F+FF, ROW+FF	ROW+F+FF,ROW+F F
	5		SW+F+FF, SW	SW+F,SW	SW+F,SW	ROW+FF	SW+F+FF, SW	ROW+F+FF, ROW+FF	SW+F+FF pH 3.3 CA, SW+FF pH 3.3 CA
	6		SW+F+FF, SW	ROW+FF	SW+FF			ROW+F+FF, ROW+FF	SW+F+FF pH 3.3 CA, SW+FF pH 3.3 CA
	7		SW	SW				SW+FF pH3	
	8		SW+FF					ROW +FF	
Analysis		pH	pH	pH	pH	pH	pH	pH	pH
		Plate Counts	Plate Counts	Plate Counts	Plate Counts	Plate Counts	Plate Counts	Plate Counts	Plate Counts
		OD	OD	OD	OD	OD			
			HPLC	HPLC	HPLC	HPLC			
			Weight	Weight	Weight	Weight	Weight	Weight	Weight
					LC/MS				
					Initial Values		Stems		

Code (water type + flowers + flower food, top up with water type), e.g. ROW+F+FF,ROW+FF means that the vase had flowers added with flower food at the standard dose, all done with reverse osmosis water, top up water was ROW with flower food (at the standard concentration) added.SW - standard water; ROW –reverse osmosis water; F- flowers in vase; FF- flower food added. Analyses performed: pH; plate counts – Total aerobic, pseudomonads, Gram negative, mould and yeasts; HPLC – sugar analysis performed; LCMS use of the liquid chromatography, Mass-spec Q-TOF; Weight – analysis of water uptake.

Appendix B Validation study from the thesis of a previous master degree project in Cranfield University

Culture and inoculums preparation: From a previously prepared and stored slope of the pure culture (*L. monocytogenes* L- 252), a portion was removed with a sterile loop, transferred into a conical flask as explained in the culture preparation section.

Two of the resulting pelletized cultures were taken and the spent broth was discarded. One of the pellets was resuspended in approx. 2 ml of TSB and then added to the other. The other two centrifuge tubes were retained for any accidental contamination, error, etc. of the experiment. 1ml of the resuspended culture was transferred into 9ml of TSB in a universal tube and mixed thoroughly; 1ml of this suspension was added to 9ml TSB in a universal tube, which then labelled as Zero dilution – it will be used as the standard for both serial dilutions. The optical density at 600nm of the zero dilution was obtained, using cuvette filled with TSB as the blank.

Serial dilutions: Two sets of dilutions were prepared: a decimal dilution and a half-fold dilution sequence. For the ten-fold, 9 universal tubes were prepared with 9 ml TSB each; for the half-fold, 9 universal tubes with 5 ml TSB were prepared. For the 10-fold dilution: 1 ml from the Zero dilution was taken and added to the first tube containing 9 ml TSB (labelled as 10f-1), and vortexed. 1 ml was taken from the 10f-1 dilution and added to the second 9 ml of TSB (labelled as 10f-2) and so on. For the half-fold dilution: 5 ml from the Zero dilution were taken and added to the first tube containing 5 ml TSB (labelled as $\frac{1}{2}f$ -1) and vortexed. 5 ml were taken from the $\frac{1}{2}f$ -1 and added to the next tube with 5 ml of TSB (labelled as $\frac{1}{2}f$ -2) vortexed and so on.

Plating and colonies counting: From the tubes labelled -5 and -6 decimal dilution, 0.1 ml of each was transferred and spread onto previously prepared tryptone soya agar (TSA) plates in triplicate and incubated at 30°C for 1-2 days. Plates with <300 cfu were counted and the approximate log number of the initial

(zero dilution) culture were calculated. The following calculation is an example of this method:

- Plates counts for -6 dilution: 102, 123 and 107 colonies
- Average counts: 111 colonies
- Due to the plating dilution the number of colonies will be multiply by 10 (111x10)
- To get the approximate colonies number in the Zero dilution multiply by 10^6 (6 serial dilutions from -6 to 0)
- The initial inoculum is: 1.11×10^9 cfu.