Cape Peninsula University of Technology

EFFECT OF ULTRAVIOLET TREATMENT ON SHELF LIFE, VARIOUS SPOILAGE MICROORGANISMS AND THE PHYSICOCHEMICAL CHARACTERISTICS OF ROOIBOS ICED TEA

by

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DECLARATION

I, Moneah Mmabatho Monyethabeng, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed Date

ABSTRACT

Rooibos iced tea (RIT), as one of the products of Rooibos is fast becoming very popular as a beverage in society due to the benefits of the phenolic compounds that are associated with this herbal tea. Some of the commercially available products have been found to contain, if any, lower contents of the major phenolic compounds, namely aspalathin and its oxidation products, iso-orientin and orientin. Their presence is considered as indicators of a good quality product. The purpose of this study was to investigate the effect of ultraviolet-C (UV-C) light as an alternative treatment to heat treatment on the shelf life, pH, phenolic composition, colour and microorganisms associated with Rooibos.

Two formulations of RIT were used in order to determine the efficacy of the UV-C on the shelf life whilst three formulations were used for the physicochemical analysis. Only one formulation was used for inoculation with three spoilage bacteria, yeast and mould spoilage microorganisms namely; *Escherichia coli* K12, *Staphylococcus aureus*, *Salmonella* sp., *Saccharomyces cerevisiae* and *Cladosporium* sp. The UV-C dosages of 0, 918, 1 836, 2 754 and 3 672 $J.l^{-1}$ were used to treat the RIT using a pilot-scale UV-C system with a turbulent flow at a constant flow rate of 4000 $l.hr^{-1}$. A log count of 4 $\log_{10}$ was considered the limit for the spoilage growth since it is the average $\log_{10}$ afternormal pasteurisation.

The use of UV-C treatment was found to have significantly ($p<0.05$) extended the shelf life of RIT. The microbiological counts of RIT following treatment with all UV-C dosages were recorded to be below the chosen 4 $\log_{10}$ microbiological limit. The difference between the effect of the UV-C dosages was not significant. As such the optimum UV-C dosage for the shelf life extension was selected as 3 672 $J.l^{-1}$ since it delayed the growth of the microorganisms the most. Although the pH of the RITs had shown a slight decrease by the end of the shelf life, no significant difference was recorded. Physicochemical analysis of the RIT, which included colour and phenolic compounds analysis was only conducted on day 1 of the shelf life. The use of UV-C treatment had no significant effect on the three major components of RIT, aspalathin, iso-orientin and orientin in the three formulations. There was also no statistical difference between all three formulations and UV-C dosages, indicating that the various compositions of the RIT formulations did not affect the effectivity of the UV-C treatment. UV-C resulted in a perceivable colour difference ($\Delta E^*>1$) effect on the overall colour difference of the RIT in formulations A, B, and C.

All the spoilage microorganisms were significantly reduced by UV-C dosage to less than 4 $\log_{10}$ except the *Cladosporium* sp. The *S. cerevisiae* was the most sensitive microorganism whilst *Cladosporium* sp. was the most resistant. The effect of UV-C on the

spoilage microorganism followed the sequence: *S. cerevisiae*>*Salmonella* sp.>*S. aureus*>*E. coli* K12>*Cladosporium* sp. This study indicated that microbiological reduction was achieved as a function of increasing UV-C dosage. In order to achieve the highest $\log_{10}$ reduction, the highest UV-C dosage of 3 672 $J.l^{-1}$ may be used. However, the dosage may need to be increased in order to achieve the desired results in the treatment of *Cladosporium* sp.

It can thus be concluded from the above investigations that UV-C dosage treatment of 3 672 $J.l^{-1}$ is optimum in the non-thermal treatment of RIT.

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I lovingly dedicate this thesis to my family for their continuous support. My mom, **Maphela**, with this I honour your wish to study further, your zeal has inspired me throughout. My loving husband, **Ishmael** you have always been my source of strength and courage. Thank you for your full support and patience. Most importantly, all praises to **God** for leading and guiding me all the way, I love You **Lord**.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscript where each chapter is an individual entity and some repetitions between chapters have, therefore, been unavoidable.

CHAPTER ONE
INTRODUCTION

1.1 Background

Rooibos (*Aspalathus linearis*) tea has been noted as one amongst others, a success story of South Africa. It has gained popularity internationally due to its health properties (Nel *et al*., 2007). This tea has been enjoyed as an herbal tea (Joubert *et al*., 2008). Various branded products have since been developed due to the popularity of ready-to-drink iced tea prepared from the herbal, "fermented" Rooibos. The health-enhancing properties of Rooibos have been mainly focused on, where its antioxidant activity, anti-cancer properties and anti-diabetic effects has received much attention (Joubert & De Beer, 2011). Aspalathin is the major phenolic compound found only in Rooibos to date. Aspalathin is however susceptible to oxidation, which occurs during fermentation, as a processing step of this herbal tea. During this reaction, the aspalathin is converted to its two main oxidation products, iso-orientin and orientin (Joubert, 1996).

Tea is rich in nutrients and multiple functional phytochemicals including polyphenols and is susceptible to microbial contamination. Thus finding a suitable method to inactivate the microorganisms is necessary for tea beverage products. However, the loss of sensory and nutritive qualities during heat processing has limited the development of tea in the drink market (Wang *et al*., 2000). In addition, some researchers observed that tea polyphenols (TP's) are sensitive to heat processing (Kinugasa & Takeo, 1990). Tea constituents and high ambient temperatures, where it is grown, also make tea a good medium for the growth of many saprophytic fungi (Keegel, 1956). World-wide very little information relating to the mycoflora and mycotoxins of tea and the associated quality problems is available (Cloete & Kotze, 1990).

Thermal pasteurization is a well-known method applied in tea manufacturing for the main purpose of microbial inactivation. A study conducted by Joubert *et al*. (2009) showed that simulated normal-temperature sterilisation (NTS at 121°C/15 min) and high temperature sterilisation (HTS at 135°C/4 min), but not necessarily pasteurization (93°C/30 min) significantly reduced the aspalathin, iso-orientin and orientin contents of different RIT formulations.

The demand for food that has been minimally processed has influenced the efforts for the development of alternative non-thermal processes for the purpose of microbial inactivation (Gayán *et al*., 2013). Hence, the investigation of these non-thermal technologies that deliver unchanged flavours in foods being one of today's major objectives (Guerrero-Beltrán & Barbosa-Cánovas, 2005). Non-thermal technologies are being used in food processing as an alternative to thermal pasteurization. These include

amongst others, pulsed electric field (PEF), high pressure processing (HPP), pulsed light (PL), ultrasound (UL) and ultraviolet (UV) light. These alternative technologies can deliver food products without hazardous microorganisms and enzymes that may reduce the nutritional and sensory characteristics of foods (Butz & Tauscher, 2002).

Ultraviolet treatment is amongst others a non-thermal technology of interest (Butz & Tauscher, 2002). Ultraviolet processing involves the use of light from the ultraviolet region of the electromagnetic spectrum for the purpose of disinfection. The wavelength of UV processing ranges from 400 to 100 nm (Diffey, 2002). The UV spectrum is further subdivided into three regions, UV-A, UV-B and UV-C. The third region, UV-C, which ranges from 200 to 280 nm is termed the germicidal range since it effectively inactivates bacteria and viruses and is used for microbial inactivation in foods systems (Bintis *et al*., 2000). Ultraviolet-C inactivates microorganisms by interrupting the DNA replication through causing cross-linking between nearby pyrimidine nucleoside bases (thymine and cytosine) within the same strand of DNA. Ultraviolet-C can inactivate most microorganisms, including bacterial spores (Hijnen *et al*., 2006) without generating chemical residues, uses no heat and is of low cost compared to other methods such as pasteurisation (Guerrero-Beltrán & Barbosa-Cánovas, 2004).

Literature suggests that the non-thermal treatment of tea beverages for microbial inactivation has no effect on the quality properties of tea and effectively inhibits the growth of spoilage microorganisms. This was demonstrated by Zhao *et al*. (2008; 2009) during the investigation of the effect of Pulsed Electric Field (PEF) on the microbial inactivation and physical-chemical properties of green tea. In addition, UV-C has been found to have no negative effect on the physical and chemical properties of beverages. The aim of this study was to investigate the effect of UV-C treatment on RIT beverage, as a non-thermal technology and an alternative to thermal processing.

1.2 Statement of research problem

Rooibos as an herbal tea has proven to have health benefits, which are associated with its phenolic composition. Excessive heat application has been found to have detrimental effects on the phenolic compounds of RIT while some commercial products were to contain very low contents of the major phenolic compounds. The use of UV-C as a non-thermal treatment has been proven to extend the shelf life of different kinds of food product while preserving their physicochemical components. It is therefore important to investigate the effect of UV-C on RIT.

1.3 Broad objective

The main objective of this study was therefore to investigate the efficacy of UV-C treatment as an alternative method to heat treatment of RIT beverage.

1.3.1 Specific objectives of the research

The specific objectives of the study included:

1.3.1.1 Determining if the use of UV-C treatment will significantly extend the shelf life of RIT, and the optimum dosage thereof.

1.3.1.2 Establishing the effect that various UV-C dosages will have on the physicochemical properties of the RIT.

1.3.1.3 Evaluate the effect of various UV-C dosages on different Rooibos-associated spoilage microorganisms.

1.4 Significance of the research

Termed one of South Africa's success stories, Rooibos as a beverage receives recognition from more than 37 countries in the world. As a beverage it is often enjoyed as RIT. It has been proven to contain nutritional characteristics that can help reduce the risk of most life style and chronic diseases. This is mainly attributed to its phenolic compounds. It is caffeine-free and contains low levels of tannin. Rooibos is found to be the only source of the unique major phenolic compound, aspalathin and its oxidative products, iso-orientin and orientin. The presence of these three compounds in Rooibos products is an indication of a good quality product.

Rooibos tea is being explored by researchers and manufacturers. Sterilisation has been found to have a significant detrimental effect on the phenolic compounds and colour of RIT. However pasteurisation did not detrimentally affect these components. As such it is of particular interest to investigate the effect of UV-C, a non-thermal treatment as an alternative to thermal treatment on the RIT.

The use of UV-C has been reported to not have any detrimental effect on physicochemical components of certain food products. This technology is non-thermal and easy to use and requires low maintenance. The use of this method in RIT may also assist in the current electricity challenges being faced by South Africa to reduce electrical consumption. In addition, the preferred non-use of preservatives in food products may be achieved through application of this technology.

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CHAPTER TWO
LITERATURE REVIEW

2.1 Tea and herbal teas

Tea, produced from *Camellia sinensis* leaves, has been consumed as a beverage in many countries for a very long time, and interest in it is growing today since scientific reports indicate that tea could be beneficial to health and may prevent chronic diseases (Dufresne & Farnworth, 2000; Sharangi, 2009; Pan *et al*., 2013). Prepared by water infusion of dried leaves (Del Rio *et al*., 2010), tea is amongst the most popular drinks globally (Khan & Mukhtar, 2008), only second to water. Three quarters of the world's population consume tea (Sinija *et al*., 2007).

As a beverage, tea's history dates back to 2700 BC by Chinese during the time of Emperor Shen Nung. However, it was first mentioned to be recorded in the old Chinese workbook Erh, around 350 BC. The tradition of tea drinking was introduced to Japan around the 6th century from China. Only the privileged community used tea, then and it was available for everyone only around 700 years ago. It then was later introduced to Indonesia, followed by Holland via the then Dutch Colonials. Tea was cultivated in India and then exported to England, where it gained popularity (Weisburger, 1997).

Camellia sinensis tea is mainly consumed as green, black, or Oolong in various areas of the globe. About 78% of the tea produced in the world is black (Li *et al*., 2013) which is mostly famous in the Western countries. The non-fermented green tea is about 20%, very famously consumed by people of the Asian countries, while 2% Oolong tea, which is semi-fermented, is commonly consumed in Southern China (Khan & Mukhtar, 2007).

There are three types of recognised herbal teas in South Africa; Rooibos, Honeybush and bush tea popularly known as Zulu tea. Primarily, the latter is generally used as a household food product in rural areas while it is informally traded in the urban areas (Rampedi & Olivier, 2005). Although there are different plants used as herbal teas in South Africa, only two Cape fynbos plants, *Aspalathus linearis* commonly known as Rooibos, and different species of *Cyclopia*, commonly known as Honeybush, have been commercially successful. The bush tea from the plant *Athrixia phylicoides* was seen as another native South African herbal tea with great commercial potential according to Rampedi & Olivier (2005). The use of Rooibos and Honeybush ranges from medicinal to non-medicinal whereby herbal tea is drunk for enjoyment (Joubert *et al*., 2008).

2.1.1 Tea and herbal tea products

As cited by Sinija *et al.* (2007) tea in the last century has been processed to be represented in many different ways ranging from loose tea to blended teas, to packet teas,

to tea bags and ultimately instant teas including flavoured ones (Boriah, 1997). In order to meet the application needs of the food industry, tea extracts can be processed in many different physical forms including instant tea (dry powders), concentrated drinks or just pure catechins (Wang *et al*., 2000). Pure catechins may be employed as a marketing tool and improve the appeal of various products (Wang *et al*., 2000). Tea is also produced and consumed as a ready-to-drink (RTD) beverage which is known as iced tea.

Ready-to-drink iced tea was introduced for the first time at the St. Louis World's Fair in 1904 during a hot weather spell (Weisburger, 1997). Iced tea drinking remains commonly an American practice. In the United States, almost 75% of the tea is drunk in cold form, and the practice and consumption has spread to other parts of the world such as Europe, Canada and Asia where it continues to increase (Weisburger, 1997; Tanaka *et al*., 2011). Except for the United Kingdom, tea consumption as a hot or cold beverage is equally preferred in the Western countries (Del Rio *et al*., 2010). In spring and summer, the RTD version shows significant preference as it is easily available and this is enhanced by its availability in different flavours, some fortified with vitamins while others have sweeteners as sugar replacers.

The quality in terms of polyphenol content among different RTD tea products varies (Seeram *et al*., 2008), mostly based on the preparation method of the tea leaves as preferred by the brand and the technological treatment undertaken to ensure safety of the product. Polyphenols are those antioxidants that include of phenolic ring in the chemical structure (Pietta, 2000).

2.2 Antioxidants

Increased life expectancy is generally well perceived however, according to Duthie *et al*. (2003) it results in an increase in the prevalence of illnesses that affect people in adulthood stages. Cancer, being one of the most life-threatening diseases where tissues are primarily damaged by free radicals has prompted the investigation of the functions of free radicals in diseases such as these (Higdon & Frei, 2003). Thus, nutrients and compounds that have the ability to reduce or eliminate free radicals from food and the human body are given specific attention. Antioxidants are such compounds and therefore are extensively studied.

Antioxidants are defined as substances that when present in foods or in the body at low concentrations compared with that of oxidizable substrates markedly delay or prevent the oxidation of the substrate or target molecule (Gutteridge & Halliwell, 2010, Tirzitis & Bartosz, 2010). Food-grade antioxidants have been used by food manufacturers for preventing food quality deterioration and maintaining their nutritive values (Shahidi, 1997). Included in the natural antioxidants, derived from dietary sources, are phenolic and

polyphenolic compounds, vitamins, enzymes, chelators, carotenoids and carnosine. The mechanism by which these antioxidants are involved in the control of food autoxidation and rancidity prevention may be different. However, their presence in the live plants may be for the sake of protecting plant tissues from injury damage. In addition to the benefits of consuming natural plant foods, they have in some ways been attributed to the presence of antioxidants within the plant, which are associated with reducing the risks of cancer, cardiovascular disease and cataracts, amongst other degenerative diseases of aging (Shahidi, 1997; Kaur & Kapoor, 2001; Pokorný, 2007).

In recent years, plant origin antioxidants have been extensively studied. Plants have various antioxidants contained within them which account for various activities. Spices and herbs including teas and their oleoresins, oils and oilseeds, grains, cereals and protein hydrolysates have also been extensively studied (Shahidi, 1997; Pokorný, 2007).

2.2.1 Natural antioxidants vs. synthetic antioxidants

There is a general trend towards replacing the use of synthetic antioxidants in food processing with natural antioxidants or with ingredients that naturally possess antioxidant activity (Pokorný, 1991). The research on the synthetic antioxidants has concentrated on their potential negative influence on health. Some of the advantages of natural antioxidants include acceptance by consumers, as they are considered to be safe and not a “chemical”. Natural antioxidants are “generally recognised as safe” and there are no safety tests required by legislation for a component of food that falls under this category. Natural antioxidants can be labelled as flavourings, can positively affect sensory properties and can act as preservation agents (Pokorný, 2007).

Pokorný (2007) summarised the major sources of natural antioxidants in the human diet, which among others include cereals, vegetables, oilseeds, legumes and beverages such as tea, coffee, beer, red wine and fruit juices.

2.2.2 Tea and herbal tea antioxidants (polyphenols)

Tea contains a very high content of polyphenols and is one of the naturally-containing polyphenolic products (Wanasundara & Shahidi, 1998). The tea plant kingdom is a rich source of flavonoids which serve as effective antioxidants. The slight astringent, bitter taste of green tea is attributed to polyphenols (Gupta *et al*., 2008). Tea polyphenols act as antioxidants *in vitro* by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions (Lo *et al*., 2006).

Tea extracts may even be used to replace artificial antioxidants in products such as meat (Bañón *et al*., 2007). The manufacturers of toothpastes, mouthwashes, breath fresheners and chewing gums have begun to incorporate tea extracts in their formulations

(Miki *et al*., 1991). According to by Viljoen (2008) the antibacterial effect of tea catechins has given rise to the claims “slows tooth decay” and “freshens breath” (Sanaka, 1991) including their deodorising effect (Yusada, 1992). Tea extracts are also being used increasingly in skin and hair care products such as shampoos, moisturising creams, perfumes and sunscreens (Alexis *et al*., 1999). The tea extracts presumably have a soothing effect on the skin and protect it from the action of free radicals (Wang *et al*., 2000).

Green tea and fermented black tea have been claimed to have biological, anti-cancer and pharmacological effects (Yang & Wang, 1993). Polyphenols are especially found within brewed tea along with many other compounds and a number of studies indicate that polyphenolic compounds that are found in tea are capable of reducing the risk of many diseases (Yang & Wang, 1993; Mukhtar & Ahmad, 1999, 2000).

There is growing interest in the antioxidants found in tea and herbal teas. One such plant of particular interest is Rooibos herbal tea due to its high antioxidant content.

2.3 Rooibos tea (*Aspalathus linearis*)

Rooibos tea is termed one of Africa’s success stories and is endemic to South Africa. Due to its health properties, this indigenous plant occurring in the mountains of the Cedarberg and its surrounding areas has gained international popularity (Nel *et al*., 2007). In the South-Western and Southern areas of the Western Cape, Rooibos is a part of the Fynbos vegetation. Rooibos tea has since become one of South Africa’s most exclusive export goods following re-entry into the world market. While consumers from various countries in the world are becoming attracted by Rooibos as an herbal tea, it is generally enjoyed in South Africa (Joubert & De Beer, 2011).

Rooibos tea is commonly used as an herbal tea in its fermented form in the food industry. This form has also found popular application in the production of RTD iced teas and yoghurt (Joubert *et al*., 2010). Since fermentation results in the leaves of the tea being red/orange in colour, this form of Rooibos is termed red tea, which resulted in the Afrikaans name “Rooibos” meaning “redbush”. The unfermented/un-oxidised type, which is usually called green Rooibos, is distinguished by its green colour that results in a tan/yellow infusion with a mild “green” taste (Erickson, 2003; Joubert *et al*., 2008). Figure 2.1 shows the fermented Rooibos tea leaves.

Rooibos tea is traditionally used in South Africa for, amongst other reasons, the alleviation of infantile colic, asthma, allergies and dermatological issues. However, it is currently popular in the herbal tea market (Joubert *et al*., 2008). Rooibos tea is commonly used in bathing babies and children suffering from skin allergies like eczema (Joubert *et al*., 2008). Anecdotal evidence suggests that Rooibos reduces tension of the nervous

Figure 2.1: Photographic representation of fermented Rooibos tea

system, improves appetite and assists with peaceful sleep (Morton, 1983). It is also reported to reduce indigestion, heartburn and nausea (Van Wyk *et al*., 1997). Rooibos tea contains no caffeine and also has health-promoting characteristics.

In summer the Khoi used Rooibos tea which they harvested from the mountain as a beverage (Morton, 1983). The use of Rooibos tea, as an herbal tea began at the start of the 20th century, where it was consumed strong as a hot brew with sugar and milk. Preparation involved boiling of the leaves together with the stems in water and then maintaining at low heat. Depending on the concentration and taste, more leaves or water were added following each serving (Joubert *et al*., 2008). In today's modernised lives with convenience as an important aspect and priority, tea bags instead of loose leaf tea are used for preparing the infusion: 2 g (one tea bag) of tea in water that has been freshly boiled for about 2 to 5 minutes to allow the release of the flavour and colour, and then it is served hot, with or without milk and sugar is added based on taste (Joubert *et al*., 2008). Infused Rooibos tea is defined by a characteristic flavour described as a combination of honey, woody and herbal-floral notes with a slightly sweet taste and delicate astringency (Koch *et al*., 2012). Rooibos tea has also found use in baking and cooking. Particularly in soups, marinades, sauces, stews and cakes, Rooibos tea is used to replace the liquid component at the same time adding to the flavour and nutrition. The availability of Rooibos in its unfermented form has led to the production of aspalathin-enriched extracts, contributing to the pathways for the production of aspalathin enhanced products (Joubert *et al*., 2010).

Ready-to-drink iced tea that is prepared from fermented Rooibos has became popular which has led to a number of branded products both internationally and locally (Joubert *et al*., 2009). This has seen a growing market for Rooibos iced teas in the recent years (Joubert *et al*., 2010).

2.3.1 Industry

Rooibos tea was first marketed as "Eleven O' Clock" in 1904 by an immigrant from Russia, Benjamin Ginsberg. He was a pioneer within the area and a descendant of a family who had been in the tea industry in Europe for centuries (Joubert *et al*., 2008). He began his trade by buying the tea from the people of the Cedarberg Mountains and selling it in other regions. This was the beginning of a productive new industry. It was only in 1930 that its agricultural value was realised (Joubert *et al*., 2008). The demand for Rooibos was triggered by the shortage of Oriental tea during World War II. However, following the war due to the availability of cheaper coffee and Oriental teas in convenient formulations, the Rooibos tea market collapsed (Joubert *et al*., 2008). The production of Rooibos was left uneconomical following the decreased demand, production overruns and low and

inconsistent quality. In order to regulate the marketing and ensure the grading of quality pertaining to all Rooibos tea traded within South Africa, The Rooibos Tea Control Board was established (Joubert, 1994).

According to Joubert *et al*. (2008), 524 tons of Rooibos were first exported in 1955. Increased international market demand resulted in increased exports where 750 tons were exported in 1993 and 7200 tons in 2007. In 2000 two and a half times more Rooibos was exported than in 1999 meanwhile a continual growth is being noted. Approximately 70% of bulk Rooibos is exported via Clanwilliam-based Rooibos Ltd., a partnership of private growers and processors and a small and large farmers cooperative in the region (Erickson, 2003). Other Rooibos tea marketers in South Africa include Khoisan, Cape Natural Tea Products, Coetzee & Coetzee and Carmien Tea.

In 2007, the total Rooibos production inclusive of unfermented Rooibos was more than 14 000 tons where Germany was the leading market internationally with 53%, followed by the Netherlands, UK, Japan and USA at 11, 7, 6 and 5%, respectively (Joubert *et al*., 2008). Almost all of the two main community producers' harvest reaches the international market, with approximately 30% of the country's harvest which is exported (Wilson, 2005).

After successful entrance into the European market, Rooibos that has been certified by Fair Trade was first introduced to the United States in 2005, where 26854 pounds was purchased by US consumers. Sales then increased to volumes of 44 788 pounds in 2006. Six companies were licensed by USA Fairtrade for Rooibos tea distribution by 2006 in the US (Raymonds & Ngcwangu, 2010).

The Perishable Products Export Control Board (PPECB), a South African company, ensures the phytosanitary inspection and certification of all Rooibos products to be exported, making certain that the products are free of bacteria and impurities. Different organisations internationally, like Ecocert and Lacon from Germany, monitor organic Rooibos and provide certification. Rooibos is graded according to colour, flavour and cut length. The highest grade is labelled "supergrade".

2.3.2 Rooibos tea products

Both the unfermented and fermented Rooibos are available in plain or flavoured, loose or in tea bags, organic and conventionally grown (Erickson, 2003). The first non-beverage use of Rooibos extracts was in skin care products (Joubert *et al*., 2008). There is a variety of topical products from pharmacies that have Rooibos among the main ingredients. According to Joubert & De Beer (2011) extracts rich in aspalathin are produced using unfermented or green Rooibos plant materials and are used by the cosmetic and

neutraceutical industry (Otto *et al*., 2003). Among these products are skin cream, soap and shampoo.

2.3.3 Botany

Indigenous to South African Western Cape Mountains, Rooibos is a legume that is a fynbos shrub (Van der Bank *et al*., 1995). There are over 270 species of the genus *Aspalathus* (Dahlgren, 1988). *Aspalathus linearis* which is commercially cultivated for tea has needle-like leaves, while different wild forms each have its typical morphology and geographic distribution. Some types of Rooibos tea plants are horizontal or prostate with a height of less than 30 cm and the other types (Rocklands type) grow erect and can have a height of up to 2 m. The size, density of branching, short shoots development, size of the leaves, and flowering time of the biotypes are considerably different (Dahlgren, 1968).

Rooibos has adapted to coarse, nutrient-poor, acidic soil and hot, dry summers (Muofhe & Dakora, 1999). In addition to a network of roots just below the soil surface, the plant has a long tap root that reaches as deep as 2 m and helps the plant find moisture during summer drought (Erickson, 2003). As a legume, Rooibos contains nodules of nitrogen-fixing bacteria on its roots; this characteristic helps the plant survive in the poor Cedarberg soils and minimises the need for fertilising commercial crops with nitrogen (Muofhe & Dakora, 1999). Attempts to grow this plant elsewhere in South Africa or the world has not been successful. Rooibos tea needs a Mediterranean-type climate. It does not occur below 450 m above sea level and flourishes only up to an altitude of about 900 m. It is sensitive to frost and snow when very young, but mature plants are adapted to cold winters and summers. The optimum amount of rain is 380 to 635 mm per year and is mainly in the winter, with occasional showers in early summer and late autumn (Morton, 1983).

2.3.4 Cultivation and harvesting

The first attempts at Rooibos cultivation were in the early 1930s, but it was only after the World War II that commercial cultivation started in earnest. Seedlings are used to propagate the plants, leading to large phenological and general variation in the cultivated plants (Joubert *et al*., 2008). Planting of seedlings takes place between July and August and approximately 8 months after planting, the plants are topped to a height of 30 cm to stimulate branching.

The first harvest takes place during the second summer, but full production is only realised after 3 years. The lifespan of the plants is severely limited by die-back problems (Joubert & Schulz, 2006). This tea, like other teas is still harvested by manually cutting with a sickle. Rooibos shrubs are harvested between December and March, in other

words between summer and early autumn, when the climate lends itself to optimal tea production (Morton, 1983). The top half of the plant is cut to about 30 cm from the ground at harvest time (Erickson, 2003). It is crucial to ensure some healthy leaves are left on the plant after harvesting, otherwise the plant will not survive. In the second year of harvesting, one should not harvest below the height harvested the year before. Thereafter it is important to harvest slightly higher each year so that new growth can come from the previous seasons' wood. No flowers should be present on the shrubs during harvesting as these impart an unpleasant flavour to the tea (Joubert & Schulz, 2006).

2.4 Chemical composition of Rooibos tea

Rooibos tea does not contain caffeine (Rabe *et al*., 1994) and is considered a low tannin beverage, especially when compared to black teas (Joubert *et al*., 2008). The tannin content of the leaf of fermented Rooibos is 3.2% to 4.4% (Joubert *et al*., 2008).

Some antioxidants are referred to as polyphenols since they contain a phenolic ring with more than one hydroxyl group in their chemical structure. Laboratory studies have established that Rooibos tea contains polyphenols that include flavonoids, such as flavones, flavanones and flavonols as well as phenolic acids that are effective free radical scavengers (Joubert *et al*., 2008). The polyphenols that have been identified in Rooibos tea include the monomeric flavonoids aspalathin and nothofagin. Aspalathin and nothofagin are similar in their chemical structures but, differ in their antioxidant activity. Rooibos tea also contains several other C-C linked β-D-glucopyranosides, i.e. the flavones orientin and iso-orientin (Koeppen & Roux, 1965a), vitexin and isovitexin (Rabe *et al*., 1994), and the flavanones, dihydro-orientin and dihydro-iso-orientin (Bramati *et al*., 2002) and hemiphlorin (Shimamura *et al*., 2006). Other flavones found to be present in Rooibos include chrysoeriol, luteolin andluteolin-7-*O*-glucoside. The flavonols present in Rooibos are quercetin and its *O*-linked glycosides, quercetin-3-*O*-robinobioside, hyperoside, iso-quercitrin and rutin (Rabe *et al*., 1994; Bramati *et al*., 2002; Shimamura *et al*., 2006). According to Rabe *et al*. (1994), a number of phenolic acids were isolated from fermented Rooibos namely: caffeic acid, *p*-hydroxy benzoic acid, protochatechuic acid, vanillic acid, *p*-coumaric acid, syringic acid and ferulic acid, which also have antioxidant activity. Phenolic acids, like flavonoids, are polyphenol substances found in fruits, vegetables, and grains. Other compounds that are reported to be present in Rooibos are lignans, coumarin and esculetin (Rabe *et al*., 1994; Shimamura *et al*., 2006; Krafczyk & Glomb, 2008).

Aspalathin and nothofagin are found to be present in high amounts in unfermented Rooibos tea (Joubert, 1996; Bramati *et al*., 2002). Unfermented Rooibos contains between 3.8 and 9.7% aspalathin and between 0.2 and 2.4% nothofagin (Joubert & Schultz, 2006). During the process of fermentation, however, the aspalathin and

nothofagin oxidise to other substances (Joubert, 1996). Aspalathin is unique to Rooibos and is one of the major compounds and therefore will be focused on to a great extent.

Since 1966, Rooibos has been the only known natural source of aspalathin (Koeppen & Roux, 1966), which is the major antioxidant in this tea. Aspalathin is a β-dihydrochalcone with significant antioxidant (Joubert *et al*., 2004; Joubert *et al*., 2005) and antimutagenic activity (Snijman *et al*., 2007). It is one of the major constituents of the water extract of Rooibos tea (Bramati *et al*., 2002), despite its extensive decrease during fermentation. Aspalathin in fermented Rooibos is present at average levels of between 0.02 and 1.16% (Joubert & Schultz, 2006). This antioxidant is susceptible to oxidation, especially under the conditions prevalent during processing of the plant material required for the development of the characteristic sweet flavour and red-brown colour of the traditional herbal tea (Joubert, 1996). According to by Joubert *et al*. (2008), a photochemical conversion of aspalathin to dihydro-iso-orientin and dihydro-orientin was shown by Koeppen & Roux (1965b) in an ethanolic solution that had been exposed to sunlight and oxygen. This dihydro-iso-orientin was preferentially formed with traces of dihydro-orientin. Its concentration increased with time, and was considered to be a result of the conversion of dihydro-iso-orientin. The same findings of the oxidative conversion of aspalathin were confirmed by Marais *et al*. (2000). This was conducted in the presence of light and heat at 30°C, resulting in a mixture of the diastereomeric flavones, (*S*)- and (*R*)-eriodictyol-6-*C*-β-D-glucopyranoside.

2.4.1 Antioxidant activity of Rooibos polyphenols

The antioxidant activity of plant extracts and teas is of great significance since antioxidants have the ability to scavenge free radicals resulting in the protection of cells in the human body against oxidative damage.

Part of the popularity of Rooibos tea is its health-promoting properties, of which antioxidant activity has been the main focus (Joubert *et al*., 2008). Aspalathin has been proven to show effective antioxidant activity in a variety of test systems (Von Gadow *et al*., 1997). In 1997, Von Gadow *et al*. conducted a study to compare the antioxidant activity of aspalathin with that of other plant phenols of Rooibos tea, α-tycopherol, butylated hydroxytoluene (BHT) and butylated hydroxyanisole(BHA). The study was conducted using two methods being: the *β*-carotene bleaching method and the 1,1–diphenyl-2-2-picrylhydrazyl (DPPH) radical scavenging method. According to the *β*-carotene bleaching method, the antioxidant activity of all compounds decreased in the order of BHT > luteolin > BHA > α-tocopherol > quercetin > aspalathin > vanillic acid > ferulic acid > vitexin > isoquercitrin > syringic acid >*p*-hydroxybenzoic acid >*p*-coumaric acid > rutin > (+)-catechin > protocatechuic acid > caffeic acid. Aspalathin was found to have a relatively

high antioxidant activity. This might have been due to the 2', 4', 6'–hydroxyl groups of the A-ring, as well as the keto-enol equilibrium (Rabe *et al*., 1994). The results showed that although aspalathin was more potent than all the phenolic acids, it was less effective than all the reference standards BHT, BHA and α-tocopherol.

The antioxidant activity of all compounds as determined by the DPPH radical scavenging method decreased in the order of caffeic acid > quercetin > (+)-catechin > isoquercitrin > aspalathin > rutin > luteolin > protocatechuic acid > α-tocopherol > syringic acid > BHA > ferulic acid > BHT > vanillic acid >*p*-hydroxybenzoic acid >*p*-coumaric acid > vitexin. Aspalathin was found to be a more potent DPPH radical scavenger than all the reference standards and all the phenolic acids except for caffeic acid (Von Gadow *et al*., 1997).

Snijman *et al*. (2009) investigated the antioxidant activity of Rooibos flavonoids, including the dihydrochalchones aspalathin and nothofagin and their corresponding flavones glycosides using the 2.2'-azinobis (3-ethylbenzothiazoline 6-sulfonic acid) radical cation, metal chelating, and Fe(II)-induced microsomal lipid peroxidation essays where EGCG and Trolox were used as reference standards. Aspalathin and epigallocatechin gallate (EGCG) were found to be the most potent radical scavengers, followed by quercetin and nothofagin. Isovitexin and vitexin were the least effective radical scavengers. The most effective inhibitors of lipid peroxidation were quercetin and EGCG whilst aspalathin and catechin showed similar potencies. However, nothofagin was found to be almost as ineffective as its flavone glycoside analogues. Figure 2.2 A and B represent the chemical structures of the three major compounds found in Rooibos tea, aspalathin, iso-orientin and orientin, respectively.

2. 5 Processing and production of Rooibos tea

The green leaves and stems are either bruised and fermented or immediately dried to prevent oxidation (Joubert *et al*., 2008). This latter form of Rooibos was developed in order to maximise the antioxidant levels in response to recent interest in health benefits associated with the antioxidants found in green teas. The unfermented type is distinguished by its very mild "green" taste reminiscent of green tea, yet without the astringency (Erickson, 2003).

Previously the Khoi chopped the shoots of the plant with the use of an axe and crushed them with a mallet. The crushed shoots and bruised pieces were then fermented in the hollows of stone reefs and then sun-dried. Today this process is used as the basis for the manufacturing of the fermented Rooibos tea (Joubert *et al*., 2008). Despite the alterations in the processing techniques of Rooibos tea introduced by the Board through the years, the processing of Rooibos is still not standardised and controlled.

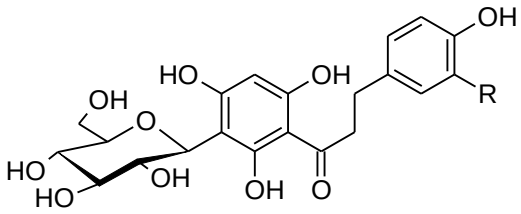

Figure 2.2 A: Major dihydrochalcones in Rooibos [Aspalathin: R = OH; Nothofagin: R = H]

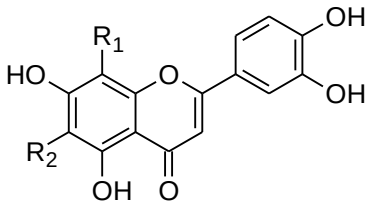

Figure 2.2 B: Major flavones in Rooibos [Orientin: R_1 = β-D-glucopyranoside, R_2 = H; Iso-orientin: R_1 = H, R_2 = β-D-glucopyranoside]

The basic traditional process which is done in the open is still used with some variations (Joubert, 1994). The processing of Rooibos tea is comprised of the following steps: cutting, bruising, addition of water, aeration, fermentation followed by sun-drying and sieving, then steam pasteurisation of the dried product before packaging (Joubert *et al*., 1998).

The following current processing of Rooibos tea is according to Joubert *et al*. (1994). The Rooibos in bundles is first cut into lengths of 3 to 4 mm with the use of a cutting machine. Next the cut tea leaves are fermented on a cement drying yard. The heap is then run over by means of a tractor a few times for the sole purpose of bruising. This process is done in the late afternoon in order to allow for the fermentation to occur during the night which in turn allows for drying to take place the following day. In order to accelerate the fermentation process, the heap is first wetted with water followed by bruising. The heap is aerated by turning with a rotavator up to two times during the night. This is done in order to allow for oxidation to take place. The temperature of the heap will naturally rise and increase during the fermentation process and is preferred to be between 38 and 42°C. The recommended temperature of the heap is however 35°C and at night it is covered with jute bags for temperature maintenance since low temperatures occur. Depending on the climate conditions, plant composition and the amount of added water, fermentation can last from 8 to 24 hours (h) and on average between 12 to 18 h (Joubert, 1994). As soon as the characteristic sweet, honey-like scent and red-brown colour of the tea have developed fully, the heap is then spread into a thin layer using a mechanical spreader in order to dry in the sun. To prevent over-fermentation, the tea should be dried as soon as possible. Sieving of the dried tea and steam pasteurisation to ensure a product of high microbial quality is done before the tea is packed (Joubert & Schultz, 2006).

Unfermented Rooibos is produced by either drying of the shredded plant material without delay under vacuum and/or drying of whole shoots to critical moisture content before shredding or steaming. Mostly unfermented Rooibos is produced by spreading the shredded plant material in a thin layer in the sun for quick drying. This leads to the loss of the aspalathin content, and if not properly dried, slow browning may take place (Joubert *et al*., 2008).

Other forms of Rooibos processing include the production of aqueous extracts and extract powders from the fermented product (Joubert & Schultz, 2006). The development of instant Rooibos aimed at providing consumers with a more convenient form of the tea was first researched in the early 1980s (Joubert, 1984; Joubert, 1988). However, this method of powder extraction was found to have an effect on the flavour whereby it was found that preference was rather given to tea that has been freshly brewed (Joubert *et al*., 2008).

For the production of the Rooibos tea extract, the tea is extracted using water. The water extract is then spray-dried into a fine hygroscopic powder with maltodextrin which contains no other additives, preservatives, or colourants (Afriplex (Pty) Ltd). Clarification which entailed the removal of the precipitate that formed upon concentration and cooling markedly decreased the polyphenol content of the dried extract (Joubert, 1990). Higher temperatures, higher water-to-leaf ratios, and longer extraction times favour extraction of soluble solids and polyphenols from fermented Rooibos. Figure 2.3 represents the process and material flow chart for Rooibos tea extract powder manufacture. Predominantly, the powdered aqueous extract forms the basis of the RTD RIT.

2.5.1 Effect of processing on Rooibos tea

There are questions that surround the quantity and quality of antioxidants in foods, including their bioavailability (Katan & De Roos, 2004). The flavonoid content of foods are also affected by a number of factors that include environmental factors (Høgedal & Mølgaad, 2000; Celiktas *et al*., 2007), processing techniques i.e. thermal processing (Torregrosa *et al*., 2006) and storage (Manzocco *et al*., 1998).

The stability of phenolic compounds in Rooibos tea, most importantly aspalathin and the flavones iso-orientin and orientin, is greatly influenced by some of the processing stages during the production of the tea (Joubert *et al*., 2009). The polyphenolic content and composition of unfermented Rooibos differs from that of the fermented Rooibos (Joubert, 1996). Von Gadow *et al*. (1997b) found that the antioxidant capacity of aqueous extracts prepared from processed Rooibos was found to be less than that of extracts prepared from unprocessed Rooibos, although the effect of the different processing stages was not investigated.

The oxidation process, fermentation, which is essential for development of the characteristic Rooibos flavour and colour, conversely has a great detrimental effect on the aspalathin content of the product (Joubert, 1996), resulting in the formation of its flavones, iso-orientin and orientin (Krafczyk & Glomb, 2008).

Thermal processing is one of the methods, amongst many others, used in the preservation of food and extends the shelf life, and most importantly inactivates microorganisms (Torregrosa *et al*., 2006). This technology is greatly used in the tea industry, mainly to inactivate microorganisms. The effect of heat on Rooibos iced tea was studied by Joubert *et al*. (2009). Part of the study included the investigation of the effects of processes involved during the production of the Rooibos tea extract. The effect of the spray drying, normal temperature sterilisation (NTS) at 121°C for 15 min and high temperature sterilisation (HTS) at 135°C for 4 min was recorded. According to the results recorded, NTS and HTS reduced the aspalathin, iso-orientin and orientin content while

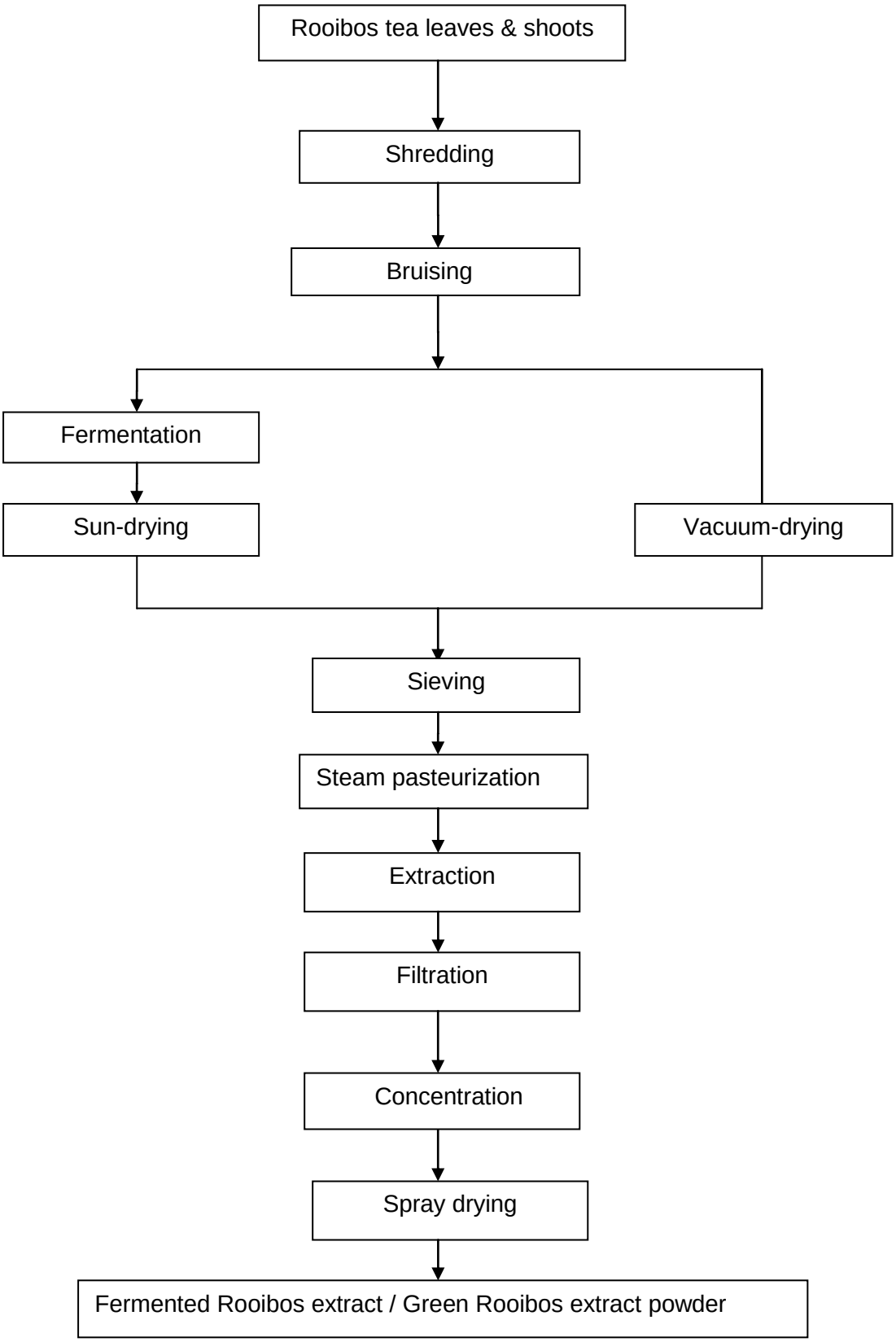

Figure 2.3: Schematic flow diagram representing the processing of Rooibos tea extract

spray-dying was found not to have significantly reduced the three compounds. The loss of aspalathin due to heat was found to be greater than that of iso-orientin and orientin. In the same study, sterilisation as a heat treatment method essential for the RTD fermented Rooibos with an extended shelf life was found to have a detrimental effect on the retention of aspalathin. Between 10 and 78% of loss were seen, which greatly depended on the severity of the heat treatment and the presence of other food ingredients, such as ascorbic acid and citric acid. Unfermented Rooibos extract is thus an alternative basic ingredient for the production of RTD iced tea due to its high levels of aspalathin content.

2.6 Food Spoilage in general

The spoilage of food is a complicated process where microbiological and biochemical interaction may occur (Huis in't Veld, 1996; Kong & Singh, 2011), resulting in excessive amounts of foods lost even with modern day preservation techniques (Gram *et al*., 2002). To the food industry food spoilage is a critical problem as it results in food being deemed unfit for consumption by humans (Loureiro, 2000). It is characterised by any alterations in the food product which consequently makes it unacceptable for consumption in terms of its sensory properties to the consumer (Nychas & Panagou, 2011). The changes may include physical damage (may be shown by visible microbial growth or slime appearance), chemical alterations, or off-odours and off-flavours which result from microbial growth and product metabolism (Gram *et al*., 2002). The primary factors associated with food spoilage are associated with the intrinsic properties of the food. These may include substrate, light and oxygen sensitivity, endogenous enzymes and cross contamination during harvesting or slaughter, pH, water activity (α_w), atmosphere and processing together with temperature abuse (Huis in't Veld, 1996, Gram *et al*., 2002; Nychas & Panagou, 2011). Food spoilage is often a result of microbial activity, which is by far the most common cause (Loureiro, 2000; Gram *et al*., 2002) and the microorganisms that propagate in the product have a high dependence on the contamination of the ingredients or the product including the environmental conditions such as the product composition and storage conditions (Loureiro, 2000). It is of great advantage to know the microorganisms present in the product that cause spoilage and understand the source of contamination so as to aid in the ability to design sufficient spoilage preventative measures (Loureiro, 2000). The potential of a microorganism to cause spoilage is in the ability of the pure culture of the microorganisms to produce the metabolites associated with the spoilage of a specific food product (Gram *et al*., 2002). According to Bobby & Wimpenny (1992), the spoilage of food by microorganisms is a process that involves the growth of microorganisms to 10^7 to 10^9 cfu.g^{-1} where these microorganisms interact and exert influence on each other's growth.

Every particular food product has its own distinctive microflora that is uniquely identified with it whether during manufacturing or storage. This is a result of the microorganisms in the raw materials, processing, preservation and storage conditions (Gram *et al*., 2002). These specific and distinctive microorganisms of a certain food product are termed specific spoilage organisms (SSO).

Food and beverages are considered to be low in pH (i.e. acidic) when they have a pH range of between 3.7 and 4.6 and are highly acidic if the pH is lower than 3.7 (Smit *et al*., 2011). Microorganisms can sometimes have a beneficial effect on food, such as in the case of fermented food products. However, in soft drinks microorganisms are never beneficial with the exception of Kombucha tea which is fermented by yeasts, moulds and acetic acid bacteria (Stratford & Capell, 2003). Contained within beverages and fruit juices are water, organic acids, sugars, vitamins and trace elements which all serve as an excellent environment for microbial spoilage, which is predominantly caused by yeasts, moulds and acidic microorganisms (Vasavada, 2003). However, some soft drinks have deficient nutrients that suppress microbial growth (Stratford & Capell, 2003). According to Stratford & Capell (2003), the spoilage of soft drinks may be due to bacteria, yeasts or moulds.

2.6.1 Bacterial spoilage of foods and beverages

Nutrients in foods serve not only for human needs, but are also vital for the growth and survival of bacteria. Thus, if food is not consumed or preserved upon harvest, it is more prone to spoilage. Bacterial spoilage is inherent in foods (Zottola, 2003). Numerous Gram-negative bacteria that are rod shaped may grow rapidly at cold temperatures and cause food spoilage. Such bacteria include: *Pseudomonas*, *Aeromonas*, *Photobacterium*, *Shewanella* and *Vibrio* spp (Huis in't Veld, 1996). The spoilage occurs through enzymes production resulting in flavour and odour defects, production of slime and the formation of visible colonies that may be pigmented (Huis in't Veld, 1996). Most Gram-negative bacteria are however eliminated through heat or pasteurisation.

Spoilage bacteria in soft drinks are categorised into three main groups namely: spore-formers, lactic and acetic acid bacteria (Stratford & Capell, 2003). The most common bacterial species that cause spoilage in soft drinks and fruit juices include *Acetobacter*, *Alicyclobacillus*, *Bacillus*, *Clostridium*, *Gluconobacter*, *Lactobacillus*, *Leuconostoc*, *Saccharobacter*, *Zymomonas* and *Zymobacter* (Vasavada, 2003). Since most soft drinks are pasteurised, acidophilic spore-forming bacteria are usually easily eliminated in fruit juice products (Stratford & Capell, 2003). However, the strictly aerobic *Acetobacter* and *Gluconobacter* are notorious for causing spoilage in fruit concentrates, apple cider and soft drinks (Vasavada, 2003). *Alicyclobacillus* is a microorganism that is

problematic in the fruit juice industry due to its heat-resistant, acidophilic, spore-forming characteristics (Gram *et al*., 2002; Vasavada, 2003; Smit *et al*., 2011).

2.6.2 Spoilage of foods and beverages by yeasts

According to Loureiro (2000), Fleet in 1992 attributed the increase of yeast spoilage in the recent years due to the decreased preservative doses and preservation processes that are milder, which is required for higher food quality standards. Loureiro & Querol (1999) cited Thomas (1993) that the spoilage of food and drinks caused by yeasts had been seen as being responsible for significant economic losses and thus yeast spoilage has an increased significance in food technology.

According to authors Pitt and Hocking (1985), as cited by Loureiro & Querol (1999) there are about ten species of yeast that are considered responsible for food spoilage, including: *Dekkera bruxellensis*, *Issatchenkia orientalis*, *Debaryomyces hansenii*, *Kloeckera apiculata*, *Pichia membranifaciens*, *Zygosaccharomyces bailii*, *Zygosaccharomyces bisporus*, *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii*, *Schizosaccharomyces pombe* and *Candida holmii* (*Saccharomyces exiguus*). These yeasts are found in food products that were produced and packaged in a manner corresponding to normal good manufacturing practice standards.

Yeasts unlike bacteria, are commonly resistant to extreme conditions and are thus commonly found in products with low pH values and those that contain preservatives which prevent the growth of bacteria (Loureiro, 2000). Yeast spoilage in foods can be seen by changes in the physical or sensorial properties as a result of their activity. Most commonly, the changes are seen to take place in drinks that contain acids, sugar or no sugar and are thus characterised by excessive gas production which may blow or deform the packages, cause cloudiness, sediment formation, off-tastes and off-flavours giving a pronounced slight fermentation smell resulting from alcohol, carbon dioxide and esters (Loureiro & Querol, 1999). Yeast growth in excess of 10 cells per millilitre results in a visible cloud or haze while some produce proteolytic or pectolytic enzymes than are capable of destroying the natural hazes of fruit juices. When yeasts cause spoilage in a food product, it implies that they were initially present as potential contaminants where the conditions of the environment were more advantageous for growth of yeasts than bacteria and moulds (Loureiro & Querol, 1999).

2.6.3 Spoilage of foods and beverages by moulds

Moulds are eukaryotic, multicellular organisms that are filamentous and widely distributed in nature. They can grow to a macroscopic size visible to the eye and commonly contaminate agricultural materials, feed, foods, and beverages (Bullerman, 2003; Ottaviani

& Ottaviani, 2003). Due to the pellicular structure of moulds, they behave differently compared to bacteria during preservation and laboratory analytical methods. Moulds can control ionic homeostasis, pertaining to a_w and pH, and are also not sensitive to the antibiotics that are used to suppress growth of bacteria. Many moulds have the ability to grow and survive in a wide range of pH, from 2 to 8 (Ottaviani & Ottaviani, 2003). Moulds have greater tolerance to relatively harsh environments and are capable of adapting to more severe stresses. The optimum growth temperatures of moulds range between 25 and 35°C, although they can grow at a wide variety of temperatures. However, some mould species have the ability to grow at low temperatures such as 0 to 5°C whilst a few others can grow just below freezing point. Only a few can grow at high temperatures exceeding 60°C (Bullerman, 2003).

Moulds are very much capable of converting nutrients into cell material and mycelial biomass where the by-products, carbohydrates and lipids are stored. During the growth of the mould, particularly at slow rates when conditions are suitable, the carbohydrates and lipids may be converted to alcohols, organic acids and complex heterocyclic biochemical products (secondary metabolites). These secondary metabolites may accumulate in the substrate and cause off-flavours and other problems including toxicity (Bullerman, 2003).

Most moulds have the ability to cause the spoilage of soft drinks. A few of the most common ones include: *Aspergillus niger* and *A. ochreceus*, *Byssoclamys nivea*, *B. fulva*, and *Phialophora mustea*. This spoilage of soft drinks by moulds is usually visual and obvious. Given more oxygen via loose seals, large headspace or oxygen-permeable packaging, moulds tend to form pellicles and may sporulate. The growth of mould in citrus fruit juice can also eliminate clouds. The result of mould spoilage in soft drink may result in the formation of taints and musty flavours (Stratford & Capell, 2003).

Most mould growths are restricted, and at times inhibited due to the addition of legally permitted concentrations of preservatives (propionic, sorbic and benzoic acids) between 0.1 and 0.2% (Ottaviani & Ottaviani, 2003). Pasteurisation of fruit juices can range from 10 to 15 min at 70 to 75°C, but may however not eliminate all moulds (Stratford & Capell, 2003). Moulds also have less resistance to heat and are destructed easily when a food product with a_w above 0.93 is treated at pasteurisation temperatures (Ottaviani & Ottaviani, 2003). However, heat resistant moulds are seen in high salt or sugar environments since they are enhanced by these factors. Heat resistant ascospores or sclerotia are produced by a few moulds. Such species include *Byssochlamys*, *Talaromyces* and *Neosartorya* and some can resist internal temperatures of 80 to 90°C in canned fruits (Ottaviani & Ottaviani, 2003; Stratford & Capell, 2003).

2.6.4 Microbiology of Rooibos tea

A study revealed the microbiological contamination of Rooibos tea by *Escherichia coli* type 1 and *Salmonella* which was attributed to the practice of open air processing during the final stage of drying (Du Plessis & Roos, 1987). Following the open air sun-drying process for 8 to 24 h of the Rooibos tea, samples were collected and analysed. The results showed high counts of coliforms that included *E. coli* type 1 and *Salmonella* spp. in processed Rooibos tea. The rapid increase of these coliforms in 24 h indicated that they were probably involved in the fermentation process. The recovery of *Salmonella* serotypes from fermentation heaps indicated that they multiplied rapidly during the fermentation process. Steam treatment of the tea effectively reduced the bacterial counts (Joubert, 1994).

In another study by Swanepoel (1987) various serovars of *Salmonella* were isolated from untreated Rooibos. A large number of these serovars were identified which were suspected to be of a variety of origins including; rodents, staff hands, contaminated water, birds, insects, reptiles or even fertilisers. *Salmonella* can be found in almost all animals and as such the contaminations could have occurred during the different processing stages.

According to Afriplex (Pty) Ltd, a private company that produces Rooibos tea extracts, the following microorganisms may be present in Rooibos: yeast and moulds, *Lactobacillus*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* and therefore microbial specifications have been set for the quality of the produced product.

Microbiological specification of Rooibos dried tea extract manufactured by Afriplex (Pty) Ltd.:

Total viable aerobic count	<2 000 CFU.g^{-1}
Yeast and Mould	<100 CFU.g^{-1}
Lactobacillus	<100 CFU.g^{-1}
Thermophillic acidophilic bacteria	absent.g^{-1}
Escherichia coli	absent.g^{-1}
Staphylococcus aureus	absent.g^{-1}
Salmonella	absent.g^{-1}

The following microbiological specifications of Rooibos tea are set by the South African National Department of Health (Anon, 1997):

Rooibos tea at the point of sale shall comply with the following microbiological specifications:

(a) for Rooibos tea in bulk, the total viable colony count shall not exceed 75 000 CFU.g^{-1};

(b) for Rooibos tea packed in retail packaging, the total viable colony count shall not exceed 150 000 CFU.g^{-1}, and
(c) *Escherichia coli* shall not exceed 20 CFU.g^{-1}; and
(d) *Salmonella* organism shall be absent in a sample of 25 grams of the product.

Export Standards and Requirements for Rooibos and Rooibos mixtures as according to the South African Department of Agriculture, Forestry and Fisheries (Anon, 1990):

a) Shall be free from *Salmonella* organisms;
b) May have a total bacterial count of not more than 30 0000 CFU.g^{-1}
c) Shall be free from *E. coli* organisms.

Europe, as a country that imports herbal teas, has its own specifications that the importer must comply with. Table 2.1 represents the specifications for trade in herbal tea infusion raw materials as recommended by the European Herbal Infusions Association (EHIA, 2011).

2.7 Preservation

Safe food production involves scrutinising raw materials that are received within the food chain, inhibiting growth of microorganisms and reducing or inactivating the microbial load through processing and preventing contamination after processing (Lado & Yousef, 2002). Food preservation is composed of the application of knowledge based on science through a variety of available technologies and processes, to prevent the deterioration and spoilage of food products and extend their shelf life, while assuring a product free of pathogenic microorganisms (Sancho-Madriz, 2003). This is also defined as an action or method meant to maintain the shelf life of foods at a desired or acceptable levels of priorities or nature for their greatest benefits (Rahman, 2007). The shelf life of food is considered as the time it takes for the product to deteriorate or decline to an undesirable point (Sancho-Madriz, 2003) where it is no longer fit for human consumption as it may pose a health hazard. The deterioration of foods can result in the quality attributes being varied, such as appearance, flavour, texture, colour and other sensory properties (Sancho-Madriz, 2003; Rahman, 2007). The quality of the food is also affected during deterioration. The spoilage and deterioration of food can mainly be caused by physical, biological, chemical, microbiological, biochemical and mechanical factors (Sancho-Madriz, 2003). According to Rahman (2007), food preservation's main purposes are to therefore overcome inadequate agricultural planning, produce products of added value, and allow various diets (Rahman, 1999).

Table 2.1: European Herbal Infusions Association's recommended microbiological specification for dry herbal infusions (EHIA, 2011)

Microbiological identification	Specification	Method
Aerobic plate count	≤10^7 / g	ISO 4833: 2003
Yeasts	≤10^5 / g	ISO 21527-2: 2003
Moulds	≤10^5 / g	ISO 21527-2: 2003
E. coli	≤10^3 / g	ISO 16649-1: 2001
Salmonella	absent in 25 g	ISO 6579: 2002 Amd.1: (2007)

Today consumers require food that are tasty, safe, healthy, organic, natural, and fresh (Koutchma *et al*., 2009), yet less processed with a longer shelf life (Brul & Coote, 1999). Consumers are gradually becoming knowledgeable about health benefits together with the risks which are associated with food consumption. As a way to meet consumer expectations, the food industry is dedicating a considerable amount of resources and expertise to the manufacturing of safe and healthy food products (Lado & Yousef, 2002). Olasupo *et al*. (2003) cited that consumers also require food that is fresher, contains no additives and with a natural taste and at the same time maintain microbial safety (Gould, 1996). Therefore, of importance to the food industry, is the use of natural antimicrobial compounds such as organic acids and aromatic compounds (Nazer *et al*., 2005).

According to Gould (1989, 1995), based on the mode of action, the major food preservation procedures can be categorised into three groups (Rahman, 2007). These categories are listed as (1) slowing down or inhibiting chemical deterioration and microbial growth, (2) directly inactivating bacteria, yeasts, mould, or enzymes, and (3) avoiding recontamination before and after processing.

Figure 2.4 represents the “major food preservation techniques” or methods from the above mentioned categories. Only techniques that are applicable in the tea industry will be discussed.

2.7.1 Microbial inhibition - adding preservatives (chemical preservation)

Chemical usage in food is a very popular preservation method (Rahman, 2007). Chemical preservatives are defined by the Food and Drug Administration as “any chemical that, when added to food, tends to prevent or retard deterioration, but does not include common salt, sugars, vinegars, spices or oils extracted from spices, substances added to food by direct exposure thereof to wood smoke or chemicals applied for their respective insecticidal or herbicidal properties (Barbosa-Cánovas *et al*., 1998). Various chemicals are added to food for the main purpose of preservation by controlling the pH, for example decreasing the pH below 5 (Gram *et al*., 2002), antimicrobial activity and antioxidant activity, and provision of food functionality (Rahman, 2007). Chemical preservatives are subject to undergo a review process including data on toxicological aspects prior to their approval (Sancho-Madriz, 2003). Consequently, the development of antimicrobials as food additives is receiving more interest (Olasupo *et al*., 2003).

Some of the legally permitted chemical substances used to preserve food are organic acids and esters, including sulphites, nitrites, acids such as acetic, citric, lactic, sorbic and benzoic acid, sodium diacetate, sodium benzoate (which eliminates Gram-negative microorganisms) and sodium propionate as well as methyl, ethyl and propyl paraben (Gram *et al*., 2002; Rahman, 2007). Acids such as acetic, citric, propionic, malic,

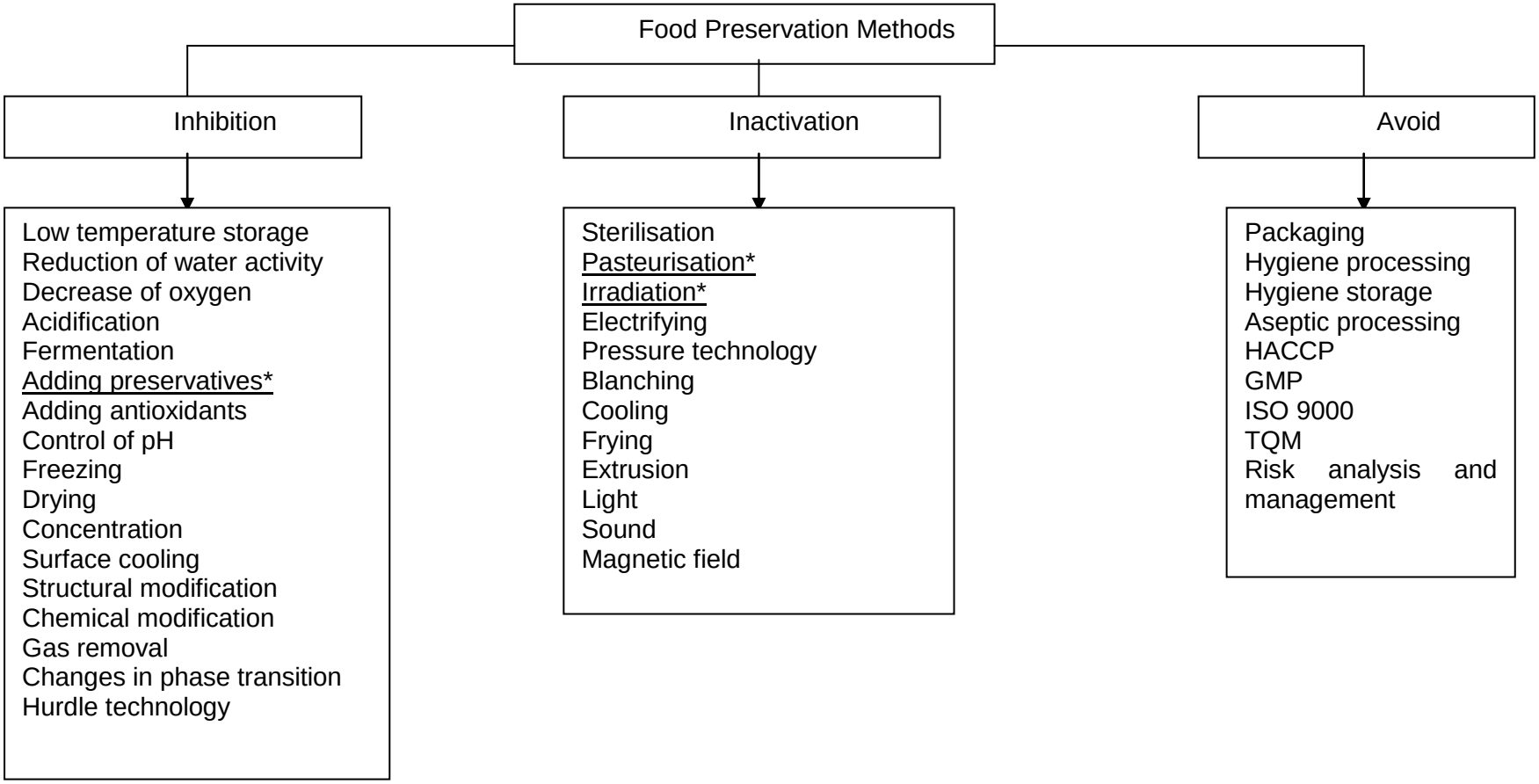

Figure 2.4: Major food preservation techniques (Adopted from Gould, 1989)

fumaric and tartaric acid are mainly used as acidulants, but they may also inhibit the growth of moulds (Sancho-Madriz, 2003). Organic acids are also added to foods as antimicrobials. These acids can also act as chelating agents and therefore inhibit lipid oxidation, control pectin gel formation, inhibit browning and aid in sucrose inversion (Barbosa-Cánovas *et al*., 1998). Acids can either be found naturally within the food products or added as part of the formulation (Nazer *et al*., 2005).

The growth of microorganisms is inhibited or inactivated by organic acids through affecting one or more of the following targets: cell wall, cell membrane, metabolic enzymes, protein synthesis system or genetic material (Barbosa-Cánovas *et al*., 1998). At lower pH values, chemical preservatives are optimum in inhibiting activity as the uncharged, undissociated state of the molecule is freely permeable across the plasma membrane and is thus able to enter the cell (Brul & Coote, 1999). The effect is also associated with the concentration of the acid in its undissociated form, and is the one that is mainly responsible for the antimicrobial effect of the acid (Dziezak, 2003). Inside the cell wall, as the higher pH value is encountered, the molecule dissociates, whereby it results in the decrease of the charged anions and protons which are not capable of crossing the plasma membrane. Ultimately the molecule of the preservative is diffused into the cell to a point where it reaches equilibrium according to the gradient of the pH across the membrane, resulting in the anion and proton accumulating within the cell (Brul & Coote, 1999). Another vital factor during antimicrobial action is the nature of the acid. At the same pH, weak acids are found to be more effective in controlling microbial growth (Dziezak, 2003). Weak acids are also lipophilic and thus are able to penetrate the membrane. Therefore, the main effects are to lower the pH of the cytoplasm, while undissociated acids may have certain effects on the metabolism which results in amplifying the weak acid effect (Rahman, 2007). Organic acids are considered to be antimicrobials with more effective activity than inorganic acids (Barbosa-Cánovas *et al*., 1998).

The actual inhibitory activity of weak acids against yeasts has been proposed to be probably the result of the generation of a stress response that is energetically expensive. This stress attempts to restore homeostasis, resulting in decreased available energy growth pools including other vital metabolic actions (Bracey *et al*., 1998).

Barbosa-Cánovas *et al*. (1998) noted citric acid as being the most used organic acid within the food industry. It was also stated as accounting for more than 60% of all consumed acidulants. It is also used as a standard when analysing the effect of other acids (Dziezak, 2003). It is natively found in the tissues of plants and animals and most profusely in citrus foods such as lemon (4 to 8%), grapefruit (1.2 to 2.1%), tangerine (0.9 to 1.2%) and orange (0.6 to 1.0%). Citric acid is available in a variety of forms, with the liquid form most convenient for incorporation into food systems. It is also available in granulated

form (Dziezak, 2003). Amongst others, foods preserved with citric acid include ice cream, sherbets, beverages, salad dressings, fruit preserves, jams, and jellies (Barbosa-Cánovas *et al*., 1998). It is normally added to non-alcoholic beverages to complement the flavours of the fruit, add to the bitterness, act as a preservative, chelate metal ions and control the pH of media so as to obtain the sweetness properties that are desired (Dziezak, 2003). The main advantages of citric acid include high solubility, flavour-enhancing effects, the ability to produce a "burst" of tartness, powerful chelating properties and widest buffer range of the food grade acids. Its ability to strongly chelate metal ions allows antioxidants to function more properly in retarding lipid oxidation (Barbosa-Cánovas *et al*., 1998).

2.7.2 Microbial inactivation

There are two categories of microbial inactivation methods namely; thermal and non-thermal techniques. Thermal pasteurisation uses heat for the inactivation of enzymes and heat-sensitive microorganisms for extending the shelf life in foods (Pereira & Vicente, 2010; Mendonca & Potter, 2012), whilst non-thermal methods does not use heat as part of the process. Due the detrimental effect that heat has on food, new technologies such as non-thermal treatments are being investigated and also gaining interest in the food industry. This is mainly because they have been found to not affect the quality properties of food (Pardo & Zufia, 2012). In recent years, non-thermal technologies have attained significance as methods for replacing or complementing the traditional thermal food processing methods (Vega-Mercado *et al*., 1997; Pardo & Zufia, 2012). These technologies, by offering value-added products, new market opportunities and added safety margins consequently meet industry needs (Morris *et al*., 2007). The success of the new processing technologies highly depends on knowledge and understanding of the microbiological composition and physiology during post treatment (Lado & Yousef, 2002).

2.8 Thermal treatment

Thermal pasteurisation implies the application of heat for the conversion and/or preservation of food (Morris *et al*., 2007). This technique which is sometimes referred to as heat treatment is traditionally applied to pasteurise or sterilise food (Lado & Yousef, 2002) and dominates the food industry (Morris *et al*., 2007). Among other thermal processing methods, using heat includes pasteurisation, sterilisation, evaporation and drying. The mentioned methods remain commonly used in the food industry with the focus of ensuring microbiological safety for food products (Pereira & Vicente, 2010). Thermal pasteurisation and sterilisation are primarily used and preferred by the food industry due to their effectiveness and safe product record. Household applications of thermal pasteurisation include processing procedures such as cooking and blanching (Butz &

Tauscher, 2002). The traditional techniques of heating fundamentally rely on heat generation outside the product to be treated, by fuel combustion or by electric resistive heater, and its transmission into the product via conduction and convection mechanisms (Pereira & Vicente, 2010).

Modern food technology deals with further development of traditional methods, e.g. high temperature short time (HTST) pasteurisation, ultra-high temperature (UHT) sterilisation or vacuum cooking (Butz & Tauscher, 2002) which allow the optimisation of thermal processing for maximum efficacy against microbial contaminants and minimum deterioration of food quality (Lado & Yousef, 2002). Thermal pasteurisation generally, however, causes undesirable changes in food that compromise the sensory qualities such as flavour, colour and texture including the nutritional attributes (Morris *et al*., 2007; Mosqueda-Melgar *et al*., 2012).

Steam pasteurisation is the common heat treatment applied to Rooibos tea leaves for ensuring a product of high microbial quality (Joubert *et al*., 2008). This is achieved at a temperature of 93°C. An investigation by Joubert *et al*. (2009) revealed that the heat processing (sterilisation) of RTD iced tea formulations at different temperatures had a detrimental effect on the content of the three major compounds and antioxidants, aspalathin, iso-orientin and orientin. Similarly, aspalathin was significantly reduced by steam pasteurization in Rooibos infusions as compared to unpasteurized Rooibos (Koch *et al*., 2013). It is therefore imperative to find other alternative methods of microbial inactivation that will not have any destructive effects on the overall quality of the Rooibos iced tea. As already mentioned the detrimental effect that thermal technologies have on food is undesirable and in this regard a new field of study will therefore be focused on the investigation of effects of non-thermal microbial inactivation methods.

2.9 Non-thermal treatment

With consumers increasingly perceiving fresh food as healthier than heat-treated foods, the food industry is currently looking for alternative methods that will still maintain the quality attributes together with the safety and shelf life of the food (Ahvenainen, 1996; Mosqueda-Melgar *et al*., 2012). In addition, according to Pereira & Vicente (2010), consumer demands for food that is more convenient has seen an exponential growth in the past 25 years, including faster production rate needs, value-added quality and extended shelf life.

The investigation for non-thermal technologies that deliver unchanged flavours in foods is one of industry's major objectives today (Butz & Tauscher, 2002; Guerrero-Beltrán & Barbosa-Cánovas, 2005; Noci *et al*., 2008). Non-thermal processes are used in the manufacturing of food as an alternative to thermal treatment. These include amongst

others, pulsed electric field (PEF), high pressure processing (HPP), pulsed light (PL), ultrasound and UV light. These alternative techniques may deliver microbiologically safe food products, without enzymes which may detriment the nutritional and organoleptic properties of foods (Butz & Tauscher, 2002).

The increase in demand for nutritious fresh foods with great taste and acceptable shelf life by consumers has prompted more research interest in non-thermal microbial inactivation techniques in the last decade (Devlieghere *et al*., 2004). Food scientists, producers and consumers show interest in these processing techniques since they have minimal or no effect on the sensorial and nutritional characteristics of the foods and also extend their shelf life through the inhibition or complete destruction of microorganisms (Morris *et al*., 2007). Knowledge and proper estimation on the effect of new preservation technology on pathogenic and spoilage foodborne microorganisms can result in successful evaluation of such technologies (Lado & Yousef, 2002).

The physicochemical and nutritional characteristics of blueberry juice following treatment with high pressure processing were investigated by Barba *et al*. (2013). The results showed no significant difference between the untreated and treated in terms of the ascorbic acid, total phenolic and total anthocyanin contents. Similarly, the significant differences were noted for the colour and antioxidant capacity. An initial microbial load of approximately 10^4 cfu.ml^{-1} inoculated in the juice resulted in a concentration less than 10 cfu.ml^{-1}.

A study by Muñoz *et al*. (2012) was conducted on the effect of pulsed light (PL) on the inactivation of *E. coli* and the quality attributes of apple juice. The apple juice treated with PL resulted in significantly reduced *E. coli* concentrations as compared to the non-treated one. The energy levels of the PL had no significant effect on the quality properties measured, except the colour of the juice.

With the purpose of finding an alternative, non-thermal technology for the inactivation of microorganisms in green tea, Zhao *et al*. (2008) investigated the effect of pulsed electric field (PEF) on microbial inactivation of pathogens *S. aureus* and *E. coli* and physicochemical properties of green tea. Samples of green tea extract which were inoculated with *S. aureus* and *E. coli* were subjected to electric field strengths of 18.1, 27.4 and 38.4 kV.cm^{-1} at treatment times of 40, 80, 120, 160 and 200 µs. After treatment with 38.4 kV.cm^{-1} for 160 and 200 µs, the inactivation of *S. aureus* reached a log reduction of 5.6 whilst *E. coli* was 4.9 $\log_{10}$. Pulsed electric field (PEF) treatment had no significant effect on the colour and the polyphenol content.

Ultraviolet light is also a non-thermal technology that has received a lot of attention from researchers and the food industry. The use of this processing method to inactivate microorganisms is of particular interest due to the positive outcomes of many studies.

2.10 Ultraviolet light – UV-C

Ultraviolet light is also used in the inactivation of microorganisms in liquid foods. It is being viewed as a potentially effective method of microbial inactivation. Literature suggests that this technology has less or no detrimental effect on the quality of food.

Ultraviolet light entails only a minor component of the electromagnetic spectrum that is inclusive of infrared radiation, visible light, X-rays and gamma (ɣ) radiation (Diffey, 2002). Ultraviolet processing uses light from the ultraviolet region of the electromagnetic spectrum for disinfection (U.S.F.D.A., 2000). The wavelength characterises the properties of any spectrum region (Diffey, 2002). The wavelength for UV treatment ranges from 100 to 400 nm. The UV spectrum is further subdivided into three regions (Diffey, 2002). The regions are divided as UV-A (315-400 nm) which is primarily responsible for human skin alterations leading to darkening or tanning, UV-B which causes burning of the skin and consequently leads to skin cancer, as well as UV-C termed the germicidal range as it is effective in the inactivation bacteria, viruses, moulds and yeasts (Diffey, 2002). Ultraviolet light may be produced by either heating an object to the temperature of the radiant, such as in solar UV or running electric power through a gas, where vaporised mercury is usually used. The latter, is a process for artificial production of UV radiation (Diffey, 2002).

The germicidal characteristics of UV-C primarily occur via DNA mutation which is prompted by UV light absorption of the DNA molecule (Tran & Farid, 2004). The optimum DNA UV-C absorption efficacy is between 250 and 280 nm. A physical shifting occurs as a result of the absorbed UV light rendering fragmentation of the DNA bonds, delaying the reproduction or eventually leading to cell death (Guerrero-Beltrán & Barbosa-Cánovas, 2004). This generally means that the germicidal effect of UV-C occurs at the nucleic acid level. Due to the UV-C treatment, a cross-linking occurs between the thymine and cytosine of the same DNA strand (Wright *et al*., 2000). Ultimately, the transcription and replication of the DNA become blocked which compromises the functionality if the cell resulting in cell death. The effect of DNA cross-linking is relative to the amount of UV-C light treatment. Production of DNA mutations within the injured organism my also occur as a result of UV-C treatment (Sastry *et al*., 2000).

The effect of UV-C treatment on microorganisms may vary from one species to another, may be dependent on the strain, growth stage, growth media (Wright *et al*., 2000), density of the microorganisms including other properties such as the food type and its composition. During disinfection, large microorganisms such as fungi are found to be more resistant, however, higher dosage levels should be considered when using UV-C treatment (Guerrero-Beltrán & Barbosa-Cánovas, 2004). Ultraviolet-C is reported to have germicidal effects against microorganisms such as viruses, protozoa, bacteria, yeasts moulds and algae (Bintsis *et al*., 2000).

2.10.1 UV-C application

The UV-C light acts as a physical method for microbial inactivation (Guerrero-Beltrán & Barbosa-Cánovas, 2004). UV-C treatment, as a disinfection method is conducted at low temperatures which is why it is regarded as a non-thermal process (Tran & Farid, 2004). With precaution, UV-C is user-friendly and inactivates a wide variety of microorganisms (Bintsis *et al*., 2000). Low intensity - long periods (time) or high intensity - short time UV-C can be used to obtain microbial reduction (Bachmann, 1975). UV-C treatment as a disinfection technology has for many years been used in the pharmaceutical, electronic and aquaculture industries. Ultraviolet light has been used in water disinfection since 1985 and also replaced some of the conventional chlorination processes in European countries (Gibbs, 2000). UV-C has also been applied in disinfection of surfaces (Sizer & Balasubramaniam, 1999).

Today however interest in using ultraviolet light for the preservation of foods is growing. The electromagnetic spectrum of UV-C can be used in the disinfection of liquid foods. For treatment of water and different foods, such as juices and surface disinfection, a wavelength of 254 nm is applied (Guerrero-Beltrán & Barbosa-Cánovas, 2004). The required dosage levels of UV disinfection may vary due to the extensive organism variety including strains. This is according to the optimum required effects for every food product.

As a result of the various UV-C light penetration capacities through different physical mediums, bacteria that are suspended in liquids are found to be less sensitive to UV-C as opposed to that suspended in air (Bintsis *et al*., 2000). As cited by Guerrero-Beltrán & Barbosa-Cánovas (2005), the penetration effect of UV-C light is found to be very short in liquids other than clear water (Shama, 1999). In juices, the UV-C light penetration is dependent on the liquid type, its UV-C absorptivity, soluble solids and suspended matter within the liquid. The more the soluble solids in the liquid, the less the penetration depth of the UV-C light. The penetration of UV-C light into juices is about 1 mm for absorption of 90% of the light (Sizer & Balasubramaniam, 1999). Therefore, in liquid processing, a turbulent flow is recommended (Guerrero-Beltrán & Barbosa-Cánovas, 2004). The penetration effect of UV-C light depends on the type of liquid, its UV-C absorptivity, soluble solids in the liquid and suspended matter in the liquid. The larger the amount of soluble solids, the less the depth of the penetration of the UV-C light in the liquid. The incidence of the light on the microbial load may also be blocked by large suspended particles (Bintsis *et al*., 2000).

In order to ensure adequate treatment of the food product, the doses of UV-C should be exposed to the entire food system equally. There are some important factors that may affect the effectiveness of the UV-C which include the product transmissivity, the reactor's geometric arrangement, the power, wavelength and physical arrangement of the

UV-C source(s), the flow profile of the product, as well as the light path length. UV-C may also be used as a hurdle in conjunction with other alternative processing technologies, and different potent oxidising agents like ozone and hydrogen peroxide.

2.10.2 Microbial inactivation by UV-C

UV-C light has been previously used to inactivate microorganisms on surfaces such as solid foods and in clear liquids such as water (Guerrero-Beltrán & Barbosa-Cánovas, 2004), however over the years its success in other concentrated liquid foods has been demonstrated. A vast number of investigations have been carried out to determine the efficacy of UV-C to inactivate various microorganisms in liquid systems of different consistencies.

Surface UV-C effect on microorganisms inoculated in liquid egg white was investigated by Unluturk *et al*. (2010). The liquid egg was inoculated with *E. coli* K12, *E. coli* O157:H7 and *Listeria inocua* and then subjected to a 26.44 $mJ.cm^{-2}$ dose level of UV-C resulting in a log_{10} reduction of 0.896, 1.403 and 0.960, respectively. Although a reduction was seen on the initial microbial load, it was concluded that this novel technology would be best applied as a pre-process method or alternatively a method applied together with heat treatment. This study has shown that the non-pathogenic *E. coli* K12 was more sensitive to UV-C as opposed to the pathogenic *E. coli* O157:H7 thereby indicating that different *E. coli* strains exhibit different UV sensitivities.

In 2008, Keyser *et al.* investigated the effect that UV-C had on various fruit juices and arrived at the conclusion that its application was successful. This study has also shown, like many others (Tran & Farid, 2004; Guerrero-Beltrán & Barbosa-Cánovas, 2005; Guerrero-Beltrán *et al*., 2008; Walkling-Ribeiro *et al*., 2008) that the consistency of the liquid affects the ability of the UV-C light to penetrate and inactivate some microorganisms. Fruit juices with high amounts of suspensions such as guava and orange juice needed higher UV-C dosage levels due to its high turbidity. Clear apple juice however, required lower dosages. Table 2.2 below shows the summarised results of the study.

The reduction of *Listeria monocytogenes* by UV-C in goat milk was investigated by Matak *et al*. (2005). The results indicated that a >5 log_{10} microbial reduction could be attained by using UV-C in the inoculated milk.

Amongst other advantages of UV-C are its non-chemical residue production effects, non-by-product and non-radiation production. The process is easy, dry and cold with low maintenance requirements. It is also cost effective since it does not use energy as a mode of treatment. It extends the foods' shelf life while preserving the organoleptic properties (Guerrero-Beltrán & Barbosa-Cánovas, 2004).

2.11 Conclusion

Consumers are now aware of the health risks associated with some food additives such as chemical preservatives and with the rise of the health market they now prefer food products without any chemical additives. Another issue that poses a threat to the food industry is the deterioration of the food quality properties due to the microbial inactivation methods that are mostly used, in particular thermal treatment. This technique that constitutes the use of heat has been found to have a detrimental effect on food's sensory and chemical and physical properties.

Research and industry have introduced non-thermal microbial inactivation methods that are gaining popularity. These technologies had no effect or very little on the quality properties of foods and beverages. UV-C is one such a technology and its effect on Rooibos iced tea needs to be investigated.

Rooibos RTD iced tea is one of the many popular soft drink beverages for which the market continues to grow with new formulations and flavours. Currently the mode of preservation used in RIT is pasteurisation with the addition of antimicrobials (citric acid) to prolong the shelf life.

Table 2.2: The log microbial counts of the aerobic plate count (APC) (cfu.ml^{-1}) and yeast and moulds (YM) present in the various fruit juices after UV (J.l^{-1}) treatment as adopted from Keyser *et al*. (2008)

Fruit juice	Log_{10} microbial counts at various UV dosages (J.l^{-1})							
UV dosages	0	230	459	689	918	1148	1377	1607
Apple juice								
APC	3.50	0	0	N/A	0	N/A	0	N/A
YM	2.99	0	0	N/A	0	N/A	0	N/A
Guava-and-pineapple juice								
APC	4.48	2.59	2.34	N/A	2.07	N/A	1.17	N/A
YM	4.48	2.86	2.25	N/A	0	N/A	0	N/A
Orange juice 2								
APC	2.30	2.07	2.07	N/A	1.85	N/A	1.41	N/A
YM	1.49	1.43	1.35	N/A	1.41	N/A	1.91	N/A
Orange juice 1								
APC	1.60	N/A	N/A	1.30	N/A	3.30	N/A	1.30
YM	2.2	N/A	N/A	3.41	N/A	2.81	N/A	1.90

N/A: no treatment had been applied at specified UV-C dosage.

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CHAPTER THREE
EFFECT OF ULTRAVIOLET TREATMENT ON THE SHELFLIFE AND PHYSICOCHEMICAL CHARACTERISTICS OF ROOIBOS ICED TEA

3.1 Abstract

The effect of ultraviolet-C (UV-C), as an alternative process to thermal treatment on shelf life, pH, colour and phenolic content in terms of aspalathin, iso-orientin and orientin was investigated. UV-C dosages of 918, 1 836, 2 754 and 3 672 $J.l^{-1}$ using a turbulent flow unit were applied to three RIT formulations at a constant flow rate of 4000 $l.hr^{-1}$. A control sample that was not subjected to UV-C treatment was also analysed.

All UV-C dosages resulted in log_{10} counts of below the specified limit of 4 log_{10} in APC and YM after 39 and 37 days in formulation A and B, respectively. No coliforms were detected in both formulations. UV-C did not cause any significant change ($p>0.05$) to the pH of both formulations. The aspalathin, iso-orientin and orientin contents were not affected by the UV-C. However, the colour of both RIT formulations was affected by the UV-C treatment.

Based on the results obtained, the use of UV-C treatment in RIT processing is promising as an alternative to thermal treatment. The optimum UV-C dosage of 3 672 $J.l^{-1}$ may be effectively used in extending the shelf life whilst preserving the phenolic compounds and maintaining the pH. The treatment of RIT with UV-C resulted in a perceivable colour difference which was acceptable, almost unacceptable and minimum for formulations A, B and C, respectively.

3.2 Introduction

Deterioration of beverages occurs gradually during storage and might be a result of chemical, physical and microbial activity in the product. This rate of deterioration depends on the product composition, initial level of contamination and the degree of processing such as heating and the storage conditions (McMeekin & Ross, 1996; Kong & Singh, 2011).

As cited by García-García *et al*. (2008) shelf life is defined by the United Kingdom Institute of Food Science and Technology (IFST) as “the period of time during which the food product remains safe, retain its desired sensory, chemical and microbiological characteristics and comply with any label declaration of nutrition data when stored under the recommended conditions” (IFST, 1993). Shelf life studies may include the assessment of several analytical and sensory properties (Pedro & Ferreira, 2006; Amézquita *et al*., 2011). To meet consumers’ expectations for high quality products, shelf

life studies ought to be conducted on the food products (Pedro & Ferreira, 2006). The appropriate shelf life depends on the manufacturer who makes the product and can be to their benefit by marketing safe products and by preserving their brand from quality loss (García-García *et al*., 2008; Amézquita *et al*., 2011).

According to Joubert *et al*. (2010) there had been a growth in the ready-to-drink (RTD) iced tea market, including products containing extracts of the herbal tea, Rooibos. Fermented Rooibos extract powder commonly forms the basis of RIT (Joubert *et al*., 2009). Rooibos tea is an excellent source of unique polyphenolic compounds (Joubert & De Beer, 2011). The dihydrochalcone aspalathin is the major compound found in this herbal tea and has been shown to exhibit effective antioxidant activity in various tests (Von Gadow *et al*., 1997). According to Schulz *et al*. (2003) it accounts for approximately 43% of the total antioxidant capacity of aqueous extracts of unfermented Rooibos. The oxidation process used for the production of fermented Rooibos typically reduces the content of the major compound, aspalathin (Joubert, 1996; Von Gadow *et al*., 1997; Standley *et al*., 2001) resulting in the retention of less than 7% (Joubert, 1996). The loss of this compound led researchers to investigate the use of unfermented Rooibos tea as a source material for production of RIT with high aspalathin content (Joubert *et al*., 2005). As cited by Joubert *et al*. (2008), amongst other compounds present in Rooibos are the C-C linked flavone glycosides orientin and iso-orientin (Krafczyk & Glomb, 2008) which result from the oxidation of the aspalathin via the intermediary flavones, dihydroiso-orientin and dihydro-orientin (Koeppen & Roux, 1965a). The three compounds, aspalathin, iso-orientin and orientin are indicators of product quality in Rooibos products (Viljoen, 2008).

Thermal technologies are most widely applied to food products to eliminate microorganisms and extend their shelf life. On the other hand these processes have a detrimental effect on certain components of the food, reducing the vitamin content and other nutrients as well as the sensory and physical properties making them less attractive in terms of colour and texture (Falguera *et al*., 2011). A study conducted by Joubert *et al*. (2009) has indicated that NTS at 121°C for 15 min and HTS at 135°C for 4 min had a significant ($p<0.05$) reduction on the aspalathin, iso-orientin and orientin contents of different RIT formulations. Meanwhile, Koch *et al*. (2013) found a similar effect on aspalathin from steam pasteurisation of rooibos tea leaves at ±96°C for 6 sec. In both studies, the colour of the products was significantly ($p<0.05$) affected by the pasteurisation processes where results by Koch et al (2013) indicated a reduction in the total colour and Joubert et al. (2009) found an increase in the browning of RIT which contained Rooibos, de-ionized water and citric acid. Another study revealed a similar significant decrease of the majority or phenolic compounds in a “cup-of-tea” strength of Rooibos infusion

(Stanimirova *et al*., 2013). In the study by Koch *et al*. (2013) steam pasteurisation of Rooibos tea infusions resulted in a significant change of the sensory profile. It therefore remains imperative to seek a microbial inactivation method that will not have a negative effect on the quality of RIT which will also preserve or extend its shelf life.

Ultraviolet processing comprises the use of light from the ultraviolet region of the electromagnetic spectrum for the purposes of disinfection (U.S. F.D.A., 2000). This is a physical method of preservation and according to Koutchma (2009), has a positive consumer image and is of interest to the food industry as it is a non-thermal method of processing. It is easy to use and eliminate most types of microorganisms (Bintsis, 2000). As cited by Guerrero-Beltrán & Barbosa-Canovás (2005) the effect of UV-C on microorganisms depends mainly on the density and type of microorganisms, liquid type, UV-C absorptivity of the liquid, suspended and soluble solids in the liquid (Shama, 1999; Bintsis *et al*., 2000) and also the colour of the liquid (Guerrero-Beltrán *et al*., 2009). The UV-C technology has been studied for microbial inactivation in various liquids including milk (Smith *et al*., 2002; Matak *et al*., 2005), liquid egg white (Unluturk *et al*., 2010), juice (Yeom *et al*., 2000; Guerrero-Beltrán & Barbosa-Canovás, 2005; Keyser *et al*., 2008; Walkling-Ribeiro *et al*., 2008) and wine (Fredericks *et al*., 2011). The use of UV-C has been proven to be effective in the elimination of microorganisms and to a greater extent has minimal or no effect on the physicochemical attributes of foods. Its use in the rapidly growing RIT industry has not been investigated as a potentially effective preservation and microbial inactivation method. Therefore, the aim of this study was to investigate the effect of UV-C on the shelf life, and physicochemical properties of unfermented RIT.

3.3 Materials and methods

3.3.1 Preparation of the RIT formulations

Three formulations of RIT were prepared using municipal water. Table 3.1 shows the ingredients and quantities used to produce the three RIT formulations. Formulation A consisted of a combination of unfermented Rooibos extract powder (Afriplex, Paarl, South Africa) and municipal water. Food grade sucrose (Selati, South Africa) and citric acid (Kimix, South Africa) were added to the basic mixture of formulation A in order to prepare formulation B. For the preparation of formulation C, the food grade sucrose was added to the basic solution of formulation A.

Figure 3.1 shows an image of the pilot scale UV-C system used in the study. The water (27.5 l) was filled in the pilot-scale UV-C system barrel and exposed to a UV-C dosage of 918 $J.l^{-1}$ at a constant flow rate of 4000 $l.hr^{-1}$ to ensure microbiological destruction of any microorganisms that might be present. The elimination of

Table 3.1: Ingredients used in the three RIT formulations

Formulation	Ingredients	Weight (g)	Volume (l)
A	Unfermented Rooibos extract powder	48.13	-
	Water	-	27.5
B	Unfermented Rooibos extract powder	48.13	-
	Sucrose	1 650	-
	Citric acid	33	-
	Municipal water	-	27.5
C	Unfermented Rooibos extract powder	48.13	-
	Sucrose	1 650	-
	Water	-	27.5

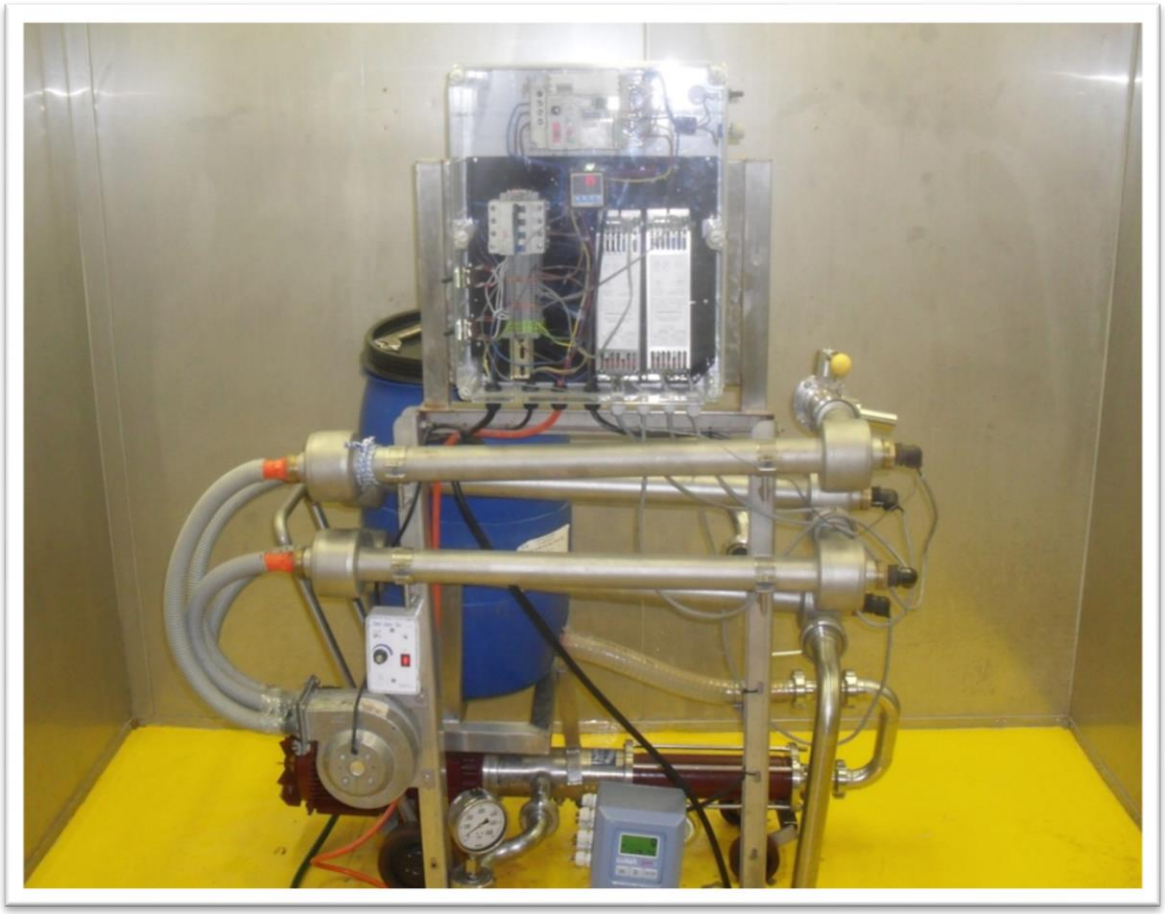

Figure 3.1: Pilot-scale UV-C reactor system used in this study

microorganisms from the used water was verified by analysing the water for aerobic plate count (APC) using plate count agar (PCA) and coliforms using violet red bile agar (VRBA).

To prepare the mixture, the Rooibos powder extract was manually reconstituted with the UV-C treated water (approximately 1000 ml) in a 2000 ml sterile Schott bottle until completely dissolved and subsequently transferred to the UV-C system barrel. This process was carried out thrice. The RIT solution was then allowed to mix completely with the rest of the water in the drum by running the system without exposure to UV-C light for 2 to 3 minutes. This served as formulation A

For the preparation of formulation B, the sucrose and citric acid were reconstituted in 2000 ml of UV-C treated water in a sterile Schott bottle and then consecutively added to the Rooibos solution and allowed to mix properly by running the system without UV-C light exposure for 2 to 3 minutes. The same process was repeated for preparation of Formulation C, however, the citric acid was not added, only sucrose.

3.3.2 Cleaning of the pilot-scale UV-C reactor system

The UV-C unit used for this study was similar to the unit used by Fredericks *et al*. (2011). Unlike the previously used system, the current unit was fitted with 4 cylindrical low pressure mercury lamps, which emit 100 watts (W) power each placed in quartz sleeves. The cleaning procedure was modified, where instead of 1% alkaline solution and 0.5% Perasan solution, caustic Trisol 2000 and P3-oxonia active containing 2-10% peracetic acid and 20-60% hydrogen peroxide were used respectively. Trisol 2000 was used at 0.5% at 65°C for ±30 min, followed by rinsing with ±25°C water for 10 min. P3-oxonia was added to the final rinse water at 1% at ±25°C for ±15 min.

3.3.3 UV-C treatment of RIT and collection of samples

Directly after mixing and prior to the actual UV-C treatment of each formulation (A, B, C) 11 x 50 ml samples were drawn from the outlet nozzle of the UV-C system, as controls. Table 3.2 shows the UV-C dosages of 918, 1 836, 2 754, 3 672 $J.l^{-1}$ with corresponding exposure times of 4.1, 8.3, 12.4 and 16.5 min, respectively that were then applied to the RIT formulations. After each UV-C dosage 11 x 50 ml samples were collected and stored at refrigeration temperature of 4°C for shelf life analysis. Only two formulations were used for shelf life analysis. One 50 ml sample from each treatment and from the control samples was retained and decanted into 10 x 2 ml eppendorf tubes for high performance liquid chromatography (HPLC) analysis. The samples that were microbiologically analysed were also used for colour determination. The above mentioned procedures were performed in triplicate and average results were recorded.

Table 3.2: The comparison between UV-C dosages as $J.l^{-1}$ and $mJ.cm^{-2}$ and contact time for a 27.5 l of sample volume in a pilot-scale UV-C system using four UV-C lamps (Adapted from Keyser *et al*., 2008)

Dose per volume ($J.l^{-1}$)	Dose per area ($mJ.cm^{-2}$)	Contact time for 27.5 l of RIT (min)
0	0	0
918	936	4.1
1 836	1 872	8.3
2 754	2 808	12.4
3 672	3 744	16.5

3.3.4 Microbiological shelf life analysis

Each 50 ml control and UV-C treated sample for each treatment of formulations A and B, was taken from 4°C and firstly thoroughly vortexed to evenly distribute and mix the sediment of the iced tea. An aliquot of 1 ml of each sample was aseptically transferred to 9 ml quarter strength Ringers' solution. Serial dilutions were then prepared from 1 x 10^{0} to 1 x 10^{-11}, depending on the spoilage rate based on previous results. Each dilution was thoroughly vortexed before 1 ml was aseptically transferred to a petri dish. Pour plates were prepared using selective media in order to determine the number of microorganisms in colony forming units (cfu.ml^{-1}). This procedure was repeated and followed for the shelf life duration and time intervals for each formulation as pre-determined during the trials. Table 3.3 indicates the shelf life day interval and duration for formulations A and B.

Enumeration of APC, coliforms and YM was carried out. All selective media used for the microbiological analysis was supplied by Merck (Biolab). Plate count agar was used for the enumeration of APC and the petri dishes were incubated at 37°C for 48 h. Rose Bengal chloramphenicol agar was used to enumerate YM and the plates incubated at 25°C for 5 days. Violet Red Bile agar was used to determine the presence of presumptive coliforms after incubation at 37°C for 48 h. All experiments were done in triplicate and the average of the results was analysed. A log count of 4 $\log_{10}$ was considered the upper limit for the spoilage growth. This was chosen since it is the average $\log_{10}$ after normal pasteurisation.

3.3.5 pH determination of formulations A and B

The pH of all the samples was measured using a pH meter (Martini, Romania). The pH meter was standardised using buffer reference standards of pH 4 and 7. The pH of each treated and untreated sample was determined immediately after every microbial analysis.

3.3.6 HPLC quantification of the phenolic content of formulations A, B and C RIT

The quantification of aspalathin, iso-orientin and orientin was performed by HPLC with diode-array detection (DAD) as described by Joubert *et al*. (2009). The apparatus used was an Agilent 1200 system (Agilent, Santa Clara, CA, USA) comprising a quaternary pump (model G1311A), autosampler (model G1329A), column thermostat (model G1316A) and diode-array detector (model G1315A). Chemstation software for LC 3D systems (Agilent, Rev. B.02.01-[244]) was used to control the system and record data. Separation was performed at 37°C on a Zorbax Eclipse SB-C18, 5 µm, 100×4.6 mm column (Agilent) protected with a SB-C18 guard column (5 µ). The chromatograms were recorded at 288 nm for aspalathin and 350 nm for iso-orientin and orientin. Table 3.4 shows the gradient profile for separation.

Table 3.3: Shelf life time interval for sampling of formulations A and B

Formulation	Time interval (days)
A	1, 3, 6, 9, 11, 13, 15, 19, 27, 35, 39
B	1, 5, 10, 15, 17, 19, 23, 27, 29, 33, 37

Table 3.4: The gradient profile for HPLC analysis

Time (min)	2% acetic acid (%)	Acetonitrile (%)
0	90.0	10.0
2	90.0	10.0
19	85.2	14.8
22	80.8	19.2
25	0.0	100.0
30	0.0	100.0
34	90.0	10.0
40	90.0	10.0

The UV-C treated RIT formulations samples and respective controls were prepared for HPLC analysis by mixing 1 ml iced tea with 100 µl of 10% ascorbic acid. All samples were filtered before HPLC analysis using Millex-HV 0.45 µm syringe tip filters (Microsep).

3.3.7 Colour measurement of formulations A, B and C

Colour determination was conducted only on the day that each formulation was prepared and treated in order to avoid biased results due to the influence that may arise from microbial growth. The colour of the samples was measured using the Hunter-Lab colourflex (Poretech, South Africa). The instrument was calibrated using the black and white tiles provided. The colour was expressed in Hunter Lab units, L* (lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). The samples were illuminated with a D65-artificial daylight 10^{o} standard angle. The samples were filled into the sample cup, cleared of any bubbles and then fitted with a white tile which rests on a rubber band in the cup to ensure a consistent volume for all samples and then covered for measurement. Four measurements were performed at different angles of 90˚ after dispersing all particles throughout the cup, and the average results were then recorded for each sample. The colour difference was then calculated and expressed as Δ*E** using the following equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}.$$

The +L* refers to the lightness and -L darkness, +a* is the redness whilst -a* is the greenness and +b* is the yellowness whilst -b* is the blueness. The results of the two UV-C treated RIT formulations were then compared to those of the respective controls.

3.3.8 Statistical analysis

All analyses were carried out in triplicate and results were expressed as the average. A repeated measure of analysis of variance (ANOVA) was performed, using general linear modes. Probability levels of 5% or less was considered significant. The levels obtained from the ANOVA Greenhouse-Geisser WS were carried out using statistical package for the social sciences (SPSS) version 21.

3.4 Results and discussion

3.4.1 Microbiological analysis - shelf life

3.4.1.1 Formulations A and B - APC

Formulation A of RIT was treated with UV-C with the aim of determining its shelf life through analysing for APC, coliforms and YM. Different UV-C dosages as outlined in the materials and methods were applied at a constant flow rate of 4000 $l.hr^{-1}$.

Figure 3.2 A represents the detection of APC in Formulation A of RIT. The untreated RIT showed APC detection of 0.37 $\log_{10}$ cfu.ml^{-1} on day 1 which decreased to 0.06 $\log_{10}$ cfu.ml^{-1} on day 3. The count then increased by 0.23 $\log_{10}$ on day 6. A further increase to 1.01 $\log_{10}$ cfu.ml^{-1} was detected on day 9 which increased to 1.54 $\log_{10}$ on day 11 but slightly decreased to 1.51 $\log_{10}$ on day 13. By day 15 a log count of 2.63 $\log_{10}$ was reached and had almost doubled on day 19 to 4.43 $\log_{10}$. A slight increase of 0.07 $\log_{10}$ was seen on day 27. On day 35, the log count increased to 5.43 but slightly decreased to 5.19 $\log_{10}$ on day 39.

Treatment of formulation A with minimum UV-C treatment of 918 J.l^{-1} showed an initial 0.31 $\log_{10}$ detection of APC on day 11. However on day 13 no APCs were detected. A log count of 1.04 was seen on day 15 which decreased to 0.35 $\log_{10}$ on day 19 but increased again to 1.76 $\log_{10}$ on day 27. On day 35, 2.16 $\log_{10}$ of APC was recorded followed by 3.55 $\log_{10}$ on day 39.

Formulation A treated with 1 836 J.l^{-1} dosage of UV-C indicated initial detection of 0.51 $\log_{10}$ of APC on day 9. No APCs were detected on day 11 whilst on day 13, 0.35 $\log_{10}$ was recorded. No further detection of APC was seen thereafter until day 35 when 1.16 $\log_{10}$ was recorded which subsequently increased to a log count 1.41 on day 39.

UV-C dosages of 2754 J.l^{-1} and 3 672 J.l^{-1} which were applied to formulation A managed to successfully delay the growth of APCs over the shelf life study period but detection of 0.94 and 1.16 $\log_{10}$ respectively were seen on day 39.

Formulation B of RIT treated with different dosages of UV-C for the determination of its shelf life. Figure 3.2 B represents the microbiological growth during the shelf life period. The growth of APC in untreated RIT was gradual over the duration of the shelf life study with an initial detection of 0.22 $\log_{10}$ count on day 5 and a maximum of 5.19 $\log_{10}$ on day 37. A log count of approximately 1 was reached on day 15. Twenty five days later 2.52 $\log_{10}$ was reached which was followed by a rapid growth count of 4 $\log_{10}$ on day 27 and 5.02 $\log_{10}$ on day 29 where an increase of approximately 1 $\log_{10}$ on day 33 and 5.19 $\log_{10}$ on day 37.

The treatment of RIT with a minimum UV-C dosage of 918 J.l^{-1} showed a delay in APC growth by 15 days where an initial detection of 0.13 $\log_{10}$ was recorded. However, the initial log count was reduced by 0.07 to 0.06 $\log_{10}$ 2 days later. A gradual increase was then observed where 0.80 $\log_{10}$ was recorded on day 19. By day 27 a log count of 2.12 was noted. The increase was then gradual and a log count of 3.01 was only seen on day 33 which the decreased to 2.91 on day 37.

The UV-C treatment of RIT at a dosage of 1836 J.l^{-1} only began to show growth of APC on day 23 with a log count of 0.65. Approximately 1 $\log_{10}$ count was reached on day 27. Growth of APC's increased gradually and only reached 2.09 $\log_{10}$ on day 37.

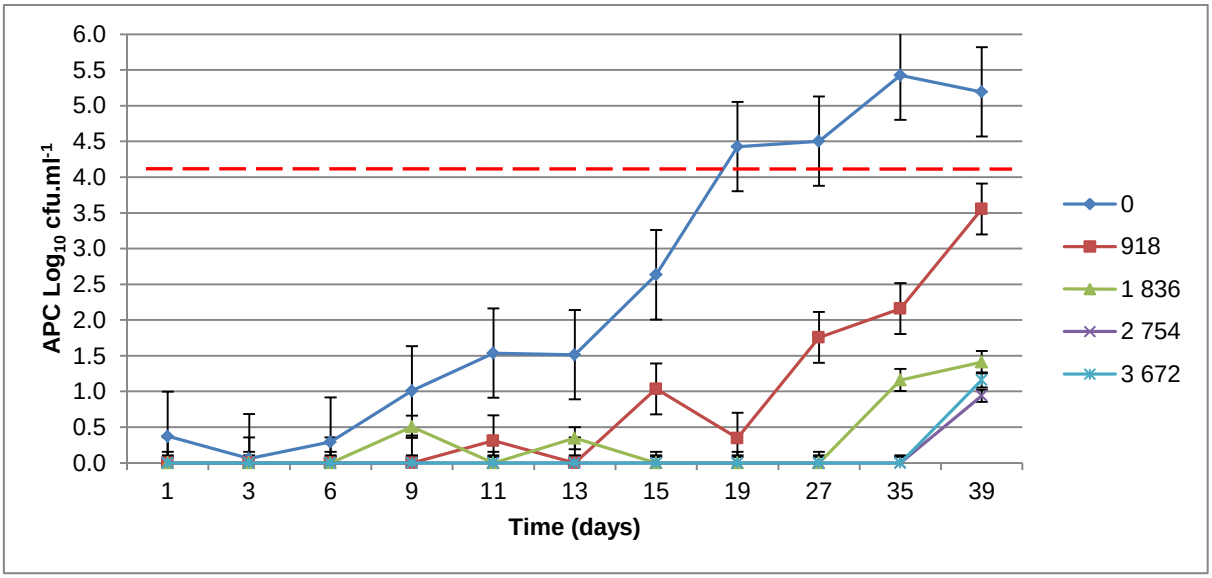

Figure 3.2 A: Total APC counts in untreated and UV-C ($J.l^{-1}$) treated RIT - formulation A during storage at 4°C. The red dashed line indicates the upper acceptable limit of 4 $\log_{10}$ $cfu.ml^{-1}$

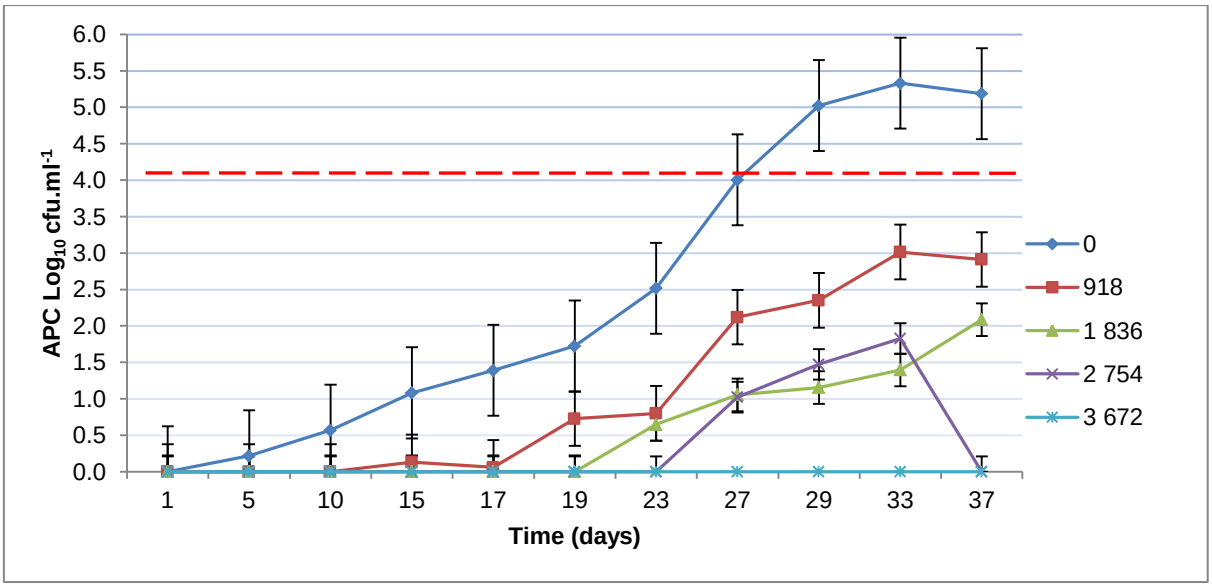

Figure 3.2 B: Total APC counts in untreated and UV-C ($J.l^{-1}$) treated RIT - formulation B during storage at 4°C. The red dashed line indicates the upper acceptable limit of 4 $\log_{10}$ $cfu.ml^{-1}$

The effect of UV-C 2 754 $J.l^{-1}$ showed gradual growth with an initial log count detection of 1.02 on day 27 followed by 1.47 on day 29 and 1.83 on day 33. There was a sudden drop to 0 in APC on day 37. The highest UV-C dosage of 3672 $J.l^{-1}$ successfully preserved the RIT until the end of the shelf life study with no APC detected.

3.4.1.2 Formulations A and B - YM

Figure 3.3 A represents the YM results obtained in formulation A. The first detection of YM in untreated formulation A was seen on day 3 with a log count of 0.10. However, day 6 showed no detection of YM whilst 1.25 log_{10} was recorded for day 9. The growth of YM gradually increased to 1.49, 1.61 and 2.84 log_{10} respectively on days 11, 13 and 15. On day 19, 4.11 log_{10} YM was recorded which was followed by a gradual increase to 5.22, 5.76 and 5.95 log_{10} on days 27, 35 and 39 respectively.

Formulation A RIT treated with lowest UV-C dosage of 918 $J.l^{-1}$ only began to show growth of YM on day 11 with 0.28 log_{10} which slightly increased to 0.33 log_{10} on day 13. On day 15 the growth of the YM had increased to 1.08 log_{10} but decreased to 0.84 log_{10} on day 19. The log count was then recorded at 1.89 on day 27 which further increased to 2.95 and 3.55 log_{10} on day 35 and 39 respectively.

Treatment with 1 836 $J.l^{-1}$ UV-C delayed the growth of YM up to day 13 where 0.35 log_{10} was detected. This growth gradually increased to 0.46 and 0.52 log_{10} on day 15 and 19 respectively. There was no detection of YM on day 27. On day 35 a log count of 1.16 was recorded and 1.49 on day 39. The detection of YM following treatment with UV-C dosage of 2 754 $J.l^{-1}$ was first seen on day 27 with a log count of 1.16. Day 35 showed no presence of YM in the sample but on day 39 1.26 log_{10} was recorded.

The highest UV-C dosage of 3 672 $J.l^{-1}$ showed the first detection of YM on day 13 with 0.22 log_{10} but none was detected on day 15. However, presence of 0.77 log_{10} was recorded on day 19 but, was again followed by non-detection on days 27 and 35. Once again, a detection of 1.16 was seen on day 39.

Figure 3.3 B shows the YM growth for formulation B RIT. The YM showed a growth pattern that was very similar to that of APC which was observed for this shelf life study. Analysis of YM in untreated RIT showed an initial detection of 0.23 log_{10} on day 5. On day 10 0.61 log_{10} was recorded. By day 15, over 1 log_{10} load of YM was noted where 1.27 log_{10} was recorded, followed by 1.65 log_{10} on day 17 and 1.84 log_{10} on day 19. A log count of 2.46 was detected on day 23 and almost double on day 27 where 4.10 log_{10} was recorded. Over 5 log_{10} YM load was recorded on day 29. The gradual growth ended with 5.63 log_{10} on day 37.

Treatment of RIT with UV-C dosage of 918 $J.l^{-1}$ began to show detection of YM on day 15 with 0.89 log_{10}. No YM were recorded on day 17, however on day 19 1.03 log_{10}

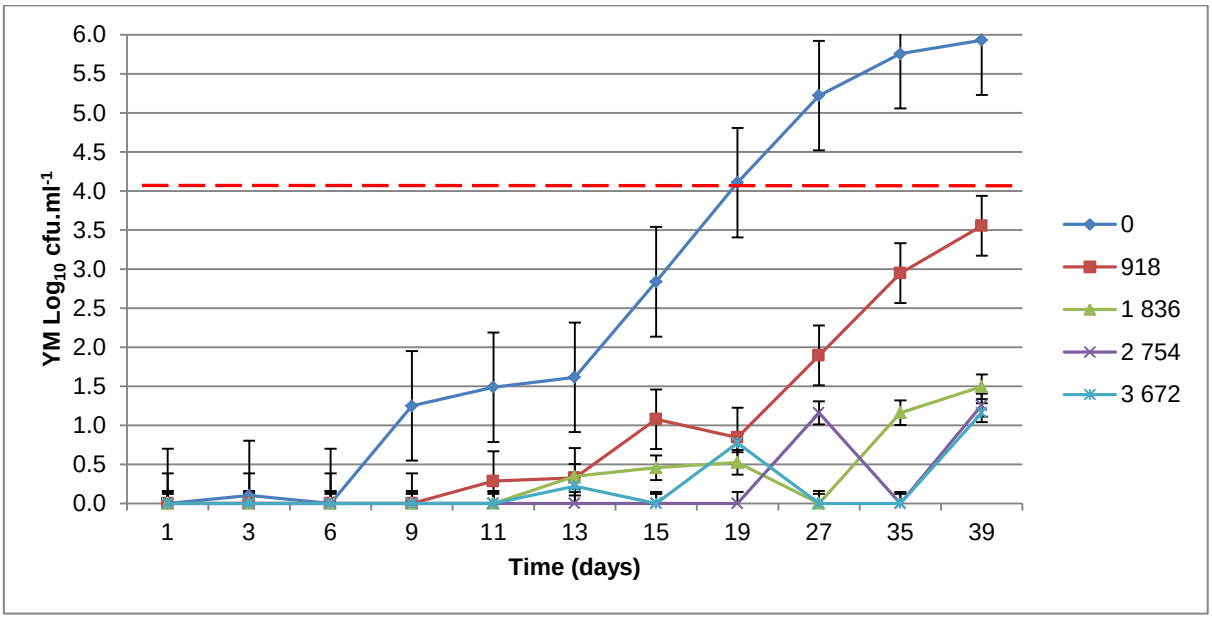

Figure 3.3 A: Total YM counts in untreated and UV-C ($J.l^{-1}$) treated RIT - formulation A during storage at 4°C. The red dashed line indicates the upper acceptable limit of 4 log_{10} $cfu.ml^{-1}$

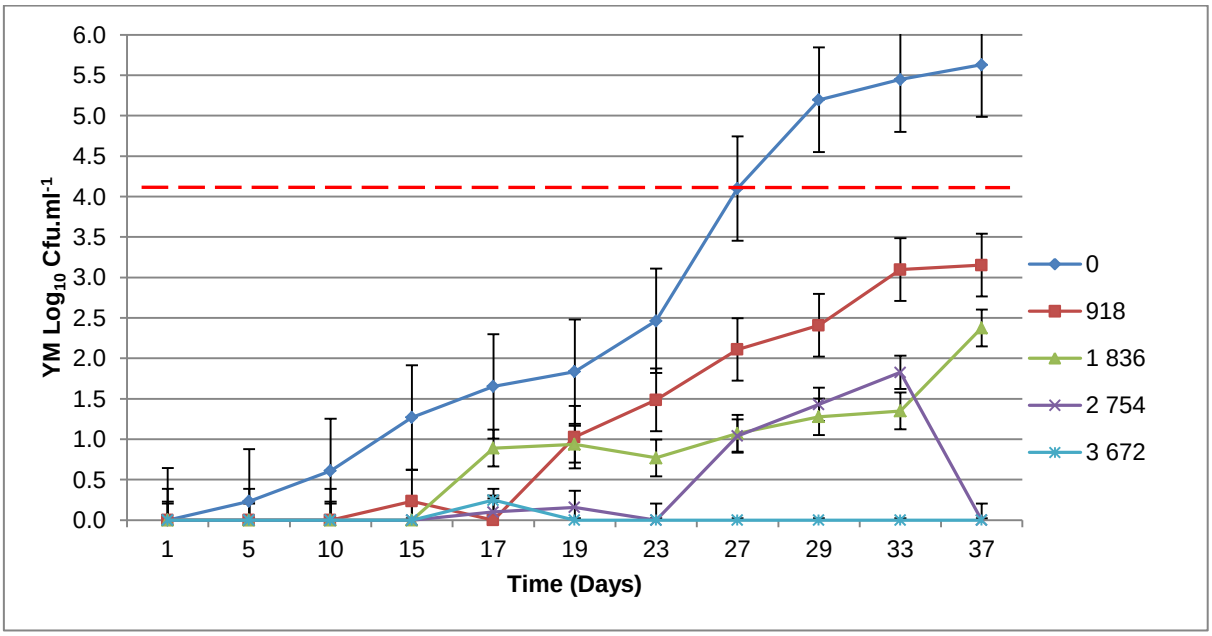

Figure 3.3 B: Total YM counts in untreated and UV-C ($J.l^{-1}$) treated RIT - formulation B during storage at 4°C. The red dashed line indicates the upper acceptable limit of 4 log_{10} $cfu.ml^{-1}$

was detected which grew gradually reaching 2.11 $\log_{10}$ on day 27. Over 3 $\log_{10}$ was detected on day 33 and by the end of the study, on day 37, 3.15 $\log_{10}$ YM load was recorded.

Unlike APC's, the initial detection of YM in RIT treated with 1 836 $J.l^{-1}$ was seen on day 17 with a log count of 0.89. A slight increase was seen on day 19 where0.94 was recorded, however a slight decrease of 0.17 was recorded on day 23. From day 27 there was a gradual growth with started with 1.07 $\log_{10}$, followed by 1.28 $\log_{10}$ for day 29 and 1.35 $\log_{10}$ for day 33. A sudden increase of YM to 2.38 $\log_{10}$ was seen on day 37 which was the last day of the shelf life study. UV-C treatment of RIT with 2 754 $J.l^{-1}$ resulted in 0.10 $\log_{10}$ initial detection of YM on day 17. This was followed by an increase of 0.06 $\log_{10}$ on day 19. However 0 $cfu.ml^{-1}$ was detected on day 23. A recording of 1.04 $\log_{10}$ was taken on day 27, followed by 1.43 $\log_{10}$ on day 29 and 1.83 on day 33. Like APC, nothing was detected on day 37.

UV-C treatment of highest dosage, 3 672 $J.l^{-1}$ successfully preserved the RIT, but 0.25 $\log_{10}$ of YM was detected only on day 17.

3.4.1.3 Formulations A and B – coliforms

Coliforms were not detected throughout the shelf life study of both formulation A and B of the untreated and UV-C treated RIT. This may indicate that there was no initial presence of coliforms in the RIT.

Statistical analysis of both formulations indicated that there was a significant difference in the effect of UV-C on the RIT shelf life ($p<0.05$) (Table 3.5). In formulation A, no significant difference was noted between the untreated and UV-C treated RIT APC counts on days 3, 9, 13, 35 and 39. The same statistical results were recorded for YM on days 3, 13 and 39. Untreated RIT was however found to be significantly different ($p<0.05$) for all UV-C dosage treatment on days 1, 6, 13, 15, 19 and 27, while no significant differences were recorded between the various UV-C dosages. This same pattern was recorded for YM on days 9, 11 and 19. On days 15, 27 and 35 untreated RIT was not significantly different to UV-C dosage treatment of 918 $J.l^{-1}$, but was significantly different ($p<0.05$) to 1 836, 2 754 and 3 672 $J.l^{-1}$ in terms of YM. Overall, there was a significant difference ($p<0.05$) on the effect of the UV-C on the shelf life of the formulation A RIT.

In formulation B, there was no significant difference for APC on days 1, 5, 10, 15, 17, 19, 23, 27 and 33. On day 29 there was no significant difference between the untreated RIT and UV-C treatment dosages of 918, 1 836 and 2 754 $J.l^{-1}$, but a significantly difference ($p<0.05$) at UV-C 3 672 $J.l^{-1}$. However on day 37, the untreated RIT was significantly different ($p<0.05$) to UV-C dosage treatment of 2 754 and 3 672 $J.l^{-1}$.

The same was recorded for YM. The overall statistical analysis indicated a significant effect of UV-C on the shelf life of formulation B RIT.

Table 3.5 indicates the pairwise comparison between the control and the various UV-C dosages. The results indicate various interactions between the UV-C dosages and the control. For APC in formulation A, the untreated RIT was found to be significantly different ($p<0.05$) to all UV-C treated RIT samples. No significant difference was noted between RIT treated with UV-C dosages of 918 and 1 836 $J.l^{-1}$, but UV-C 918 $J.l^{-1}$ was significantly different ($p<0.05$) to 2 754 and 3 672 $J.l^{-1}$. There was no significant difference between UV-C dosages 1 836, 2 754 and 3 672 $J.l^{-1}$. The same was observed for the YM, with slight differences where no significant difference was recorded between all UV-C dosage treatments. The statistical comparison of Formulation B was however different. There was no significant difference between the untreated RIT and all the UV-C dosages with the exception of the highest UV-C dosage of 3 672 $J.l^{-1}$ for APC. Similarly, there was no significant difference between all the UV-C dosages. The YM was completely different as there was no significant difference ($p>0.05$) between the untreated RIT and all the UV-C dosages.

A log count of 4 $\log_{10}$ was considered the upper limit for the spoilage growth. This was chosen since it is the average $\log_{10}$ during normal pasteurisation. The growth of APC and YM in untreated RIT for both formulations was found to be more than 4 $\log_{10}$ where approximate 5 $\log_{10}$ for APC and YM were recorded on the last day of the shelf life study. Contrarily, all UV-C treated samples were below the specified limit.

Aerobic plate counts and YM have been studied extensively in UV-C treatment investigations serving as the basis of shelf life determination (Palgan *et al*., 2011; Corrales *et al*., 2012; Feng *et al*., 2013; Pala & Toklucu, 2013; Müller *et al*., 2014; Santhirasegaram *et al*., 2015). Based on the results obtained in this study, UV-C had a significant effect on the shelf life of RIT. This effect was similarly noted by various other studies (Escalona *et al*., 2010; Manzocco *et al*., 2011; Torkamani & Niakousari, 2011, Darvishi *et al*., 2012; Cote *et al*., 2013; Pala & Toklucu, 2013; De Souza & Fernandez, 2014) in different food products. The effect varies depending on the dosage of the UV-C that was applied and the type of food used as a medium. The shelf life of liquid egg products stored for 8 weeks at 4°C was found to be prolonged by UV-C after microbial analysis (De Souza & Fernandez, 2014). The egg white was treated with UV-C dosage of 9.8 $kJ.l^{-1}$, the egg yolk with 63 $kJ.l^{-1}$ and 18 $kJ.l^{-1}$ for the whole egg. Amongst other microorganisms, the total aerobic mesophilic counts (TAM) showed a detection of <2 $\log_{10}$ on the last day of analyses in egg white and total aerobic psychotrophic counts (TAP) of 2.2 ± 0.2 $\log_{10}$. Enterobacteriaceae and YM showed no increase throughout the study and overall, the

Table 3.5: Overall pairwise comparison of the control and various UV-C dosages of formulations A and B

UV-C dosage ($J.l^{-1}$)	UV-C dosage ($J.l^{-1}$)	*p*-value formulation A		*p*-value formulation B	
		APC	YM	APC	YM
0	918	0.000^{a}	0.002^{a}	0.445^{a}	0.904^{a}
	1 836	0.000^{ab}	0.000^{ab}	0.097^{ab}	0.324^{a}
	2 754	0.000^{b}	0.000^{ab}	0.058^{ab}	0.134^{a}
	3 672	0.000^{b}	0.000^{ab}	0.019^{ab}	0.053^{a}
918	0	0.000^{a}	0.002^{a}	0.445^{a}	0.904^{a}
	1 836	0.246^{b}	0.416^{b}	1.000^{a}	1.000^{a}
	2 754	0.036^{a}	0.169^{b}	1.000^{a}	1.000^{a}
	3 672	0.042^{a}	0.145^{b}	0.918^{a}	1.000^{a}
1 836	0	0.000^{a}	0.000^{a}	0.097^{a}	0.324^{a}
	918	0.246^{b}	0.416^{b}	1.000^{a}	1.000^{a}
	2 754	1.000^{b}	1.000^{b}	1.000^{a}	1.000^{a}
	3 672	1.000^{b}	1.000^{b}	1.000^{a}	1.000^{a}
2 754	0	0.000^{a}	0.000^{a}	0.058^{a}	0.134^{a}
	918	0.036^{a}	0.169^{b}	1.000^{a}	1.000^{a}
	1836	1.000^{b}	1.000^{b}	1.000^{a}	1.000^{a}
	3 672	1.000^{b}	1.000^{b}	1.000^{a}	1.000^{a}
3 672	0	0.000^{a}	0.000^{a}	0.019^{b}	0.053^{a}
	918	0.042^{a}	0.145^{b}	0.918^{ab}	1.000^{a}
	1 836	1.000^{b}	1.000^{b}	1.000^{ab}	1.000^{a}
	2 754	1.000^{b}	1.000^{b}	1.000^{ab}	1.000^{a}

$^{a-b}$ means with different superscripts in the same column differ significantly ($p<0.05$)

critical counts that determine the end of shelf life were not reached, thus UV-C was effective. In whole egg, TAM was 1.8 ± 0.2 $\log_{10}$, while in egg yolk 2.7 ± 0.1 $\log_{10}$ for TAP was detected. Darvishi *et al*. (2012) found that the shelf life (in terms of yeasts) of fresh strawberry stored at -1 to -5°C was extended by 3 days after UV-C treatment with dosages of 0.25 and 0.50 $kJ.m^{-2}$. No mould growth was detected in the UV-C treated strawberries. The highest UV-C dosage of 0.50 $kJ.m^{-2}$ was found to be the most efficient. The researchers therefore concluded that the dosage, as a factor, has significant effect on microbial growth (Darvishi *et al*., 2012).

As expected, like in the latter study, the higher the UV-C dosage the longer it takes before the RIT began to show growth of microorganisms, thus higher UV-C dosages result in a longer shelf life. This was also the effect in a study by Feng *et al*. (2013), where the shelf life of UV-C on watermelon juice was investigated. The results showed that APC was reduced by increasing the intensity of the dosage. On the other hand YM showed no increase in the number of inactivated counts. The same phenomenon was noted in the current study where higher UV-C dosages yielded lower microbiological counts and vice versa, but the YM was different as it showed the same growth pattern as APC. The results of YM in strawberries and tomatoes following low and high intensity UV-C showed no statistical difference (Cote *et al*., 2013). Only 12% of strawberries stored at 10°C, for both high and low intensities, showed fungal growth in comparison to 68% of the controls. Similarly, after 9 days at 20°C, 5% of tomatoes were spoiled when compared to 23% of the controls.

The highest microbial population that was detected even after lowest UV-C dosage 918 $J.l^{-1}$ still remained below the microbial spoilage limit of 4 $\log_{10}$, thus indicating that UV-C was effective in extending the shelf life of RIT and similar to Cote *et al*. (2013) there was no significant difference between the lowest and highest UV-C dosages in formulation A YM and both APC and YM in formulation B. Although the final $\log_{10}$ counts varied per UV-C dosage, statistically there was however no significant difference in the effect of the different UV-C dosages on the last day of the shelf life in formulation A. A similar effect was observed by Escalona *et al*. (2010) where the effect of UV-C dosage of 2.4 $kJ.m^{-2}$ was found to have no significant difference to that of 12 or 24 $kJ.m^{-2}$ on spinach leaves. Notably, in formulation A, there was no significant difference between the untreated RIT and only UV-C dosage treatments of 918 and 1 836 $J.l^{-1}$, and there was no significant differences noted between all the UV-C dosages. It is imperative to note that although the results showed no statistical differences, microbiologically there is a difference which cannot be dismissed.

The growth of YM was slightly higher than that of APC at UV-C dosages of 918 and 1 836 $J.l^{-1}$ reaching the highest log counts of 3.15, 2.38 and 2.91, 2.09 respectively for

formulations A and B on the last day of the shelf life study. After UV-C treatments at dosages of 1 836, 2 754 and 3 672 $J.l^{-1}$ the YM growth in both formulations was noted to be inconsistent or not gradual. However, formulation B RIT treated with UV-C dosage of 1 836 $J.l^{-1}$ was slightly different. The inconsistency and slightly higher growths may have been due to the resistance of yeasts and moulds to UV-C (Tran & Farid, 2004). According to Palgan *et al*. (2011), the resistance of yeasts and moulds may be attributed to their DNA structures which contain less pyrimidine bases, but more thymine bases, when compared to bacteria. Furthermore, unlike bacteria which consist of a polysaccharide peptidoglycan, yeasts have a β (1 → 3) glucan which may contribute to the high resistance. The percentage in YM inactivation in Chokanan mango juice was found to be less than APC, an indication that YM exhibit some form of resistance to UV-C (Santhirasegaram *et al*., 2015). The authors attributed this to the cell wall thickness and size of the microorganism.

Although slight differences were noted, it is also most important to note the similar pattern in growth of both the APC and YM. This could be indicative that the spoilage of the RIT may have mainly been through YM as the moulds are known for their ability to survive at low pH and high sugar concentration (Cabib *et al*., 2001). However, at the same time, formulation A contained no sugar or citric acid. As such the spoilage over the shelf life period could be associated with the spoilage organisms of Rooibos tea which may likely consist more of YM. As mentioned before, the microbiology of RIT is currently not regulated. However, Afriplex has listed the following as potential spoilage microorganisms associated with Rooibos: YM, Lactobacillus, Thermoacidophilic bacteria, *E. coli*, *S. aureus* and *Salmonella*.

The combination of UV-C with chemical treatment on various microorganisms has been investigated by various researchers in different food systems. However, there are no publications on shelf life studies with regards to RIT. Although citric acid is usually used as an acidulant and not necessarily a preservative, it has been found to inhibit the growth of certain spoilage organisms such as moulds (Sancho-Madriz, 2003). The effect of the citric acid added in this study was not specifically analysed. At the same time, the data of the two formulations could not be statistically compared due to the fact that the analysis was carried out on different days. However, trends on the common days 15, 19 and 27 showed that formulation A untreated samples, in terms of APC and YM counts were higher than those of formulation B. This may have been due to the antimicrobial effect of the citric acid. Following UV-C treatment, the APC counts of formulation B on days 15 and 19 and YM counts on day 15 were found to be slightly lower than those of formulation A. Contrarily, on day 27 the APC counts of formulation B were higher than those of formulation A. A similar pattern was noted for YM on days 19 and 27. These

conflicting results may however suggest that citric acid did not have an effect on the shelf life of the RIT. In addition, it may be assumed that if the citric acid plays any antimicrobial effect on the RIT, the results may suggest that the effectiveness is reduced by time and may be affected by increasing UV-C dosages. At the same time, it must be mentioned that the yeast and moulds were not separated during colony counting and as such the effect of citric acid on moulds may not be completely dismissed due to its reported antimicrobial effect on moulds.

In another study, clover sprouts was inoculated with *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes* were treated with a combination of fumaric acid and UV-C which resulted in a significant decrease of the microorganisms (Kim *et al*., 2009). The combination treatment when compared to single treatments of UV-C and fumaric acid respectively, was found to be most effective. Ultraviolet light in conjunction with preservatives dimethyl dicarbonate, hydrogen peroxide and potassium sorbate added after UV application, were found to have resulted in greater *E. coli* reductions than when preservatives were added before UV treatment (Quicho, 2005). In the latter study, it is however very important to note that preservatives were used as opposed to the acidulant in the current investigation and that of Kim *et al*. (2009).

The same study was conducted on an additional formulation of RIT which consisted of Rooibos solution and sugar only, without citric acid. The data was however not published due to fact that it could not be statistically analysed because of extremely varying microbiological $\log_{10}$ counts.

In both formulations A and B, there was an overall marked significance effect of the UV-C on the shelf life of the RIT. This was also supported by the fact that in both formulations, the UV-C treated RIT did not reach the microbiological limit of 4 $\log_{10}$ in both the APC and YM. Although there may have been no significant differences noted between some of the UV-C dosages, microbiological results may not be disregarded based on the statistical data. It would be advisable to select the optimum UV-C dosage based on the microbiological results and as such the highest UV-C dosage of 3 672 $J.l^{-1}$ will be most effective in the extension of the shelf life of the RIT.

3.4.2 pH of formulations A and B

Figure 3.4 A represents the pH values of the untreated and UV-C treated formulation A samples. The pH values were found to follow the same trend where the initial values ranged from 5.53 of the untreated RIT to an average of 5.23 for the UV-C treated RIT. On day 15 there was a slight decrease to pH 4.93 in the untreated RIT. Although another increase to 5.13 was observed only on day 35, by day 39 it had dropped to 4.79. The

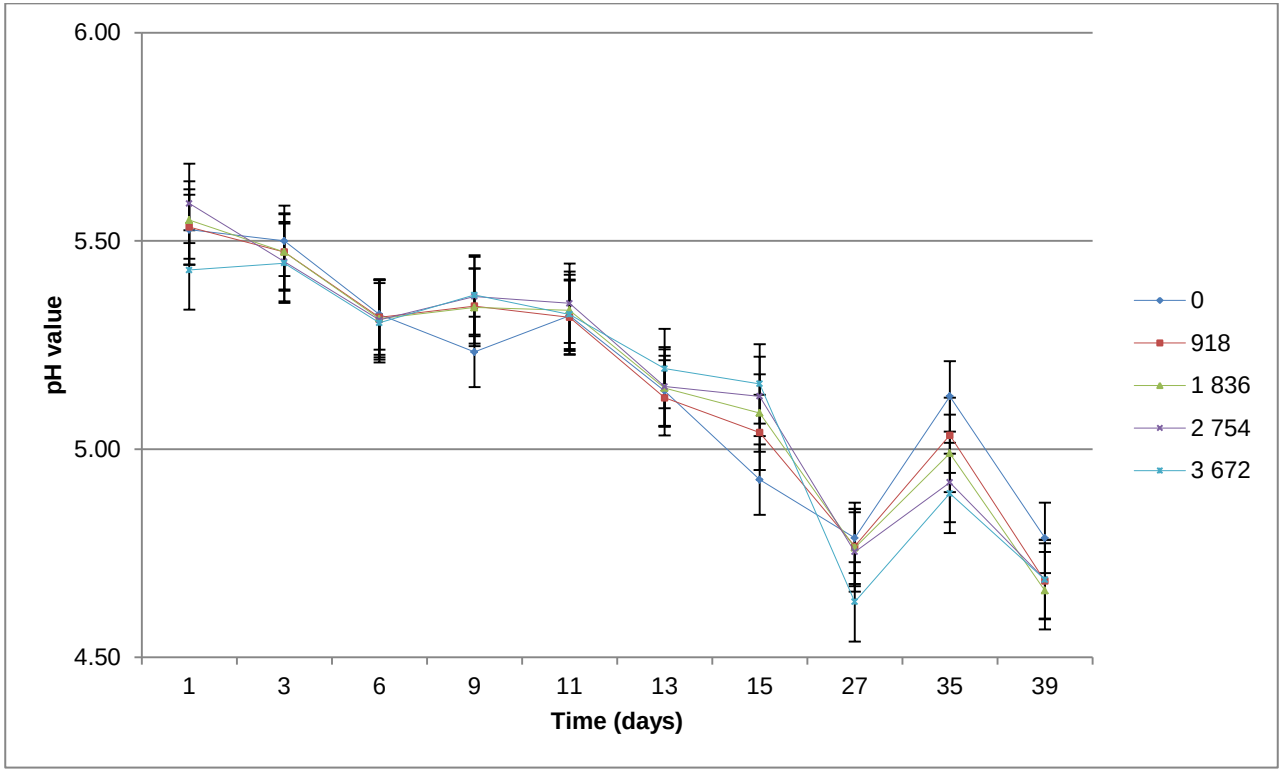

Figure 3.4 A: Illustration of pH in untreated and UV-C ($J.l^{-1}$) treated formulation A of RIT over the shelf life duration of 39 days

same pattern was observed for RIT treated with 918 $J.l^{-1}$ UV-C where the first decrease in pH was seen on day 15 to 4.94, followed by a slight increase to 5.03 on day 35 and ultimately a final pH of 4.68 was recorded on day 39. A small variation was observed for RIT treated with UV-C dosages of 1 836, 2 754 and 3 672 $J.l^{-1}$. The decrease in pH was found to be 4.76 on day 27 and 4.90 and 4.63 on day 19 for the above mentioned UV-C dosage treatments, respectively. It was maintained in this region until the last day where it was recorded at 4.66, 4.69 and 4.69, respectively. The decrease in pH may be an indication that there was acid production during the storage. Overall, a slight decrease in pH was observed for both the untreated and UV-C treated RIT during storage. There was significant difference ($p<0.05$) in the pH over the duration of the shelf life study. However, according to statistical analysis of the results recorded, there was no significant difference ($p>0.05$) in the pH of the untreated and UV-C treated RIT samples of formulation A. Feng *et al*. (2013) found different results for untreated and UV-C treated watermelon juice where the untreated juice showed a decrease in pH after day 25 of the 37 days storage period whilst significant increases for the UV-C treated juice were noted after the same time. The decrease in pH was attributed to a faster growth of the aerobic bacteria and YM.

Figure 3.4 B shows the graphical representation of formulation B pH results. The pH values of the untreated RIT started at 3.00 on day 1 which decreased to 2.95 on day 5. The values fluctuated between day 5 and day 23 with 2.93 as the lowest value and 2.98 as the highest. A slight increase was observed between days 27 and 29 with pH of 3.03 and 3.05, respectively. However, another decrease was seen on day 33 to pH 3.00 and further to 2.89 on the last day. For the UV-C treated samples, the initial pH values were 2.99, 2.96, 2.95 and 2.92 for dosage treatments of 918, 1 836, 2 754 and 3 672 $J.l^{-1}$, respectively. These values only increased to ranges above 3.00 on day 27 and 29 with 3.03 as the highest, but however decreased to 2.89 for 918 $J.l^{-1}$ and 2.90 for other UV-C treatments. Even with the changes mentioned above, there was no significant difference ($p>0.05$) in pH of the untreated and UV-C treated RIT of formulation B during the storage period. It was however noted that there was a significant difference ($p<0.05$) on the pH over the shelf life period. Although there was increasing growth of microorganism, particularly for untreated and UV-C (918 $J.l^{-1}$) treated RIT, there was no significant acid production that would decrease the pH values.

It was noted in both formulations that UV-C treatment had no significant effect on the pH of the RIT. Several studies which evaluated the effect of UV-C in different beverages (Pala & Toklucu, 2013; Corrales *et al.*, 2012; De Souza & Fernández, 2011; Tran & Farid, 2004) also reported similar findings.

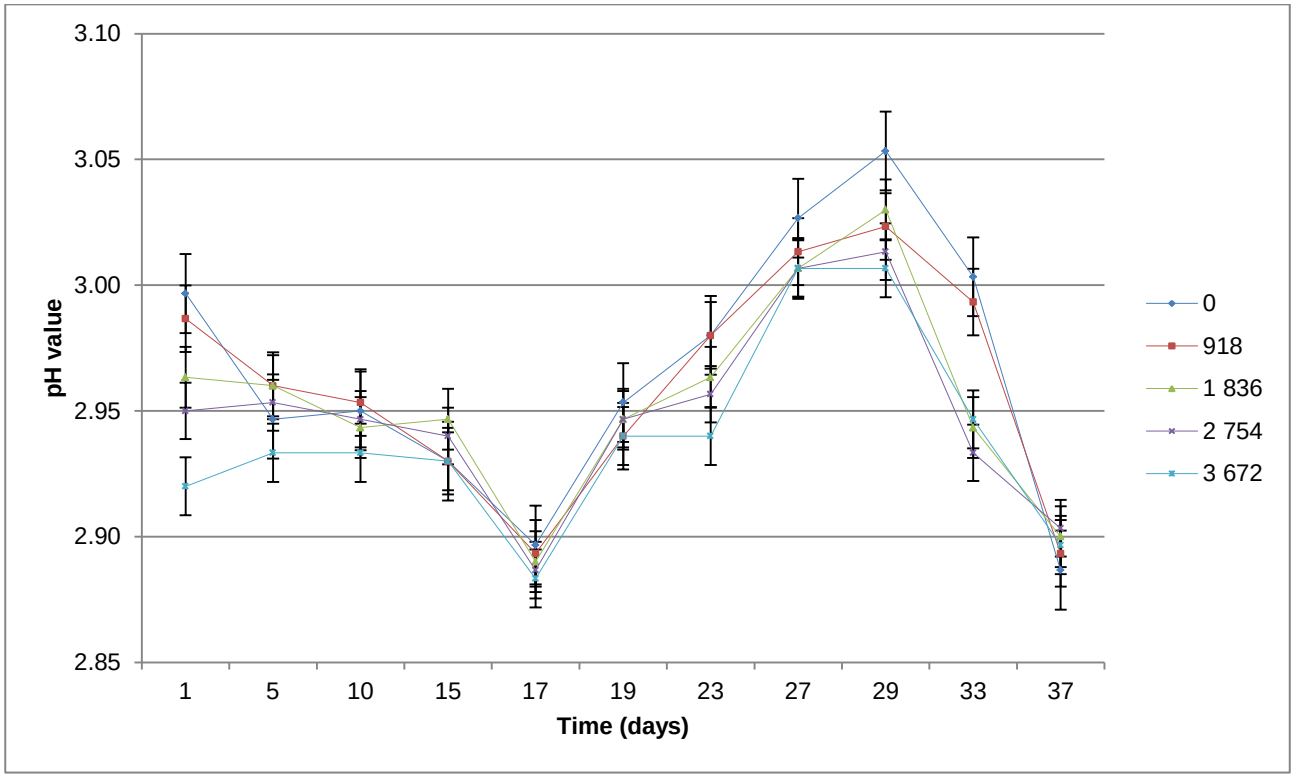

Figure 3.4 B: Illustration of pH in untreated and UV-C ($J.l^{-1}$) treated formulation B of RIT over the shelf life period of 37 days

3.4.3 Phenolic compounds of formulations A, B and C

The effect of UV-C on the major phenolic compounds of RIT was analysed using HPLC. The compounds analysed included aspalathin, iso-orientin and orientin. Different formulations, A, B and C were evaluated.

Figure 3.5 A shows the graphical representation of the results for formulation A. Table 3.6 A shows the statistical results for formulation A. The initial concentration of the aspalathin before UV-C treatment was 35.57 mg.l^{-1}. This was slightly reduced by 2.58 mg.l^{-1} following UV-C treatment of 918 J.l^{-1}. A slight increase of 1.28 mg.l^{-1} was noted after UV-C treatment of 1 836 J.l^{-1} followed a further slight increase of 1.15 and increase of 0.28 mg.l^{-1} after dosage treatments of 2 754 and 3 672 J.l^{-1}, respectively.

Fluctuating variations were also noted for the iso-orientin content of the RIT. Prior to UV-C treatment, the concentration was recorded as 19.12 mg.l^{-1} for iso-orientin. The minimum UV-C treatment of 918 J.l^{-1} resulted in a slight decrease of 0.93 mg.l^{-1}, followed by increase of 0.73 and 1.22 mg.l^{-1} after dosage treatments of 1 836 and 2 754 J.l^{-1}, respectively. The highest UV-C dosage of 3 672 J.l^{-1} showed a very slight decrease to 19.92 mg.l^{-1}. The same trend was noted for evaluation of the orientin. The initial concentration of the untreated RIT was 19.50 mg.l^{-1}. A decrease of 0.96 mg.l^{-1} was seen following the minimum UV-C treatment of 918 J.l^{-1}. A gradual increase of 0.87 and 1.20 mg.l^{-1} followed after UV-C treatments of 1 836 and 2 754 J.l^{-1}, respectively. Similar to the iso-orientin, a slight decrease of 0.41 mg.l^{-1} was recorded following UV-C treatment with the highest dosage of 3 672 J.l^{-1}.

Figure 3.5 B illustrates the results of phenolic contents for formulation B. The aspalathin content of formulation B was recorded as 37.85 mg.l^{-1} before UV-C treatment. A slight decrease of 1.34 mg.l^{-1} after minimum UV-C treatment of 918 J.l^{-1} was followed by an increase to 37.09 mg.l^{-1} after dosage treatment of 1 836 J.l^{-1}. A slight decrease of 1.54 mg.l^{-1} was noted after UV-C treatment of 2 754 J.l^{-1}. An increase of 0.68 mg.l^{-1} was recorded after treatment with the highest UV-C dosage of 3 672 J.l^{-1}. The iso-orientin content before UV-C treatment was initially recorded as 17.92 mg.l^{-1}. Following the minimum treatment of 918 J.l^{-1}, the content slightly decreased to 17.46 mg.l^{-1}. However, this slightly increased after treatment with UV-C dosage of 1 836 J.l^{-1}. Another slight decrease of 0.51 mg.l^{-1} was noted after UV-C treatment of 2 754 J.l^{-1}, whilst the highest UV-C dosage treatment of 3 672 J.l^{-1} resulted in a slight increase to 17.95 mg.l^{-1}. The orientin content showed slight variations between UV-C treatments. The initial orientin content prior to UV-C treatment was recorded to be 20.05 mg.l^{-1}. The minimum UV-C dosage of 918 J.l^{-1} resulted in a slight decrease to 19.51 mg.l^{-1}. A slight increase of 0.56 mg.l^{-1} was recorded following treatment with 1 836 J.l-1. A slight decrease of 0.56 and an

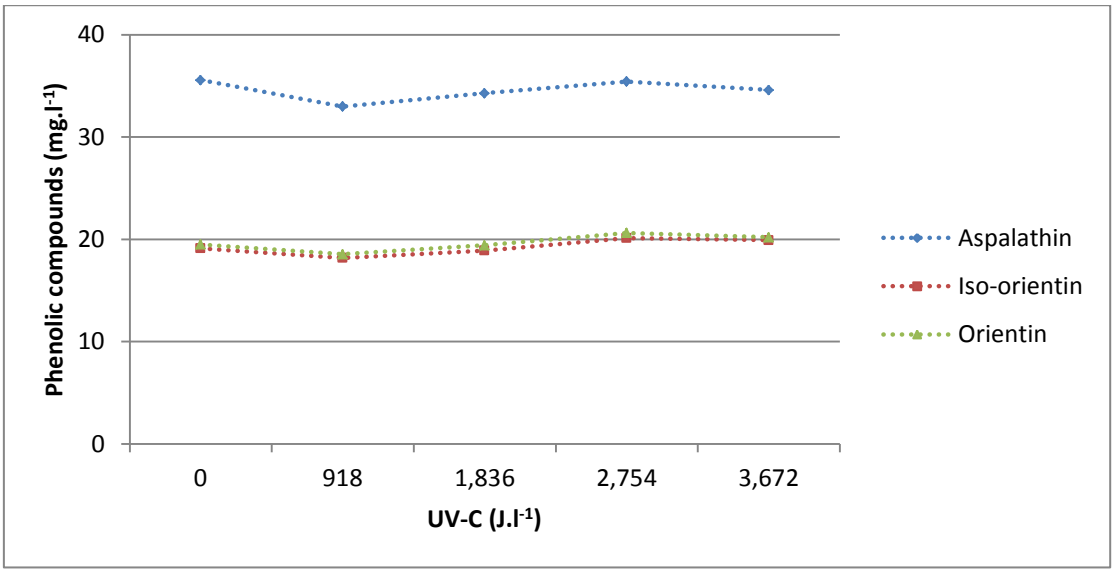

Figure 3.5 A: Effect of UV-C on aspalathin, iso-orientin and orientin content in RIT formulation A

Table 3.6 A: Aspalathin, iso-orientin and orientin content of untreated and UV-C treated RIT in formulation A

UV-C dosage ($J.l^{-1}$)	Phenolic compounds ($mg.l^{-1}$) (Average mean ± SD)		
	Aspalathin	Iso-orientin	Orientin
0	35.57 ± 2.89[a]	19.12 ± 0.31[a]	19.50 ± 0.47[a]
918	32.99 ± 6.58[a]	18.19 ± 1.86[a]	18.54 ± 2.25[a]
1 836	34.27 ± 2.00[a]	18.91 ± 0.89[a]	19.42 ± 0.60[a]
2 754	35.42 ± 3.34[a]	20.13 ± 1.62[a]	20.62 ± 1.46[a]
3 672	34.60 ± 2.26[a]	19.92 ± 0.11[a]	20.21 ± 0.13[a]

[a] means with different superscripts in the same column differ significantly ($p<0.05$)

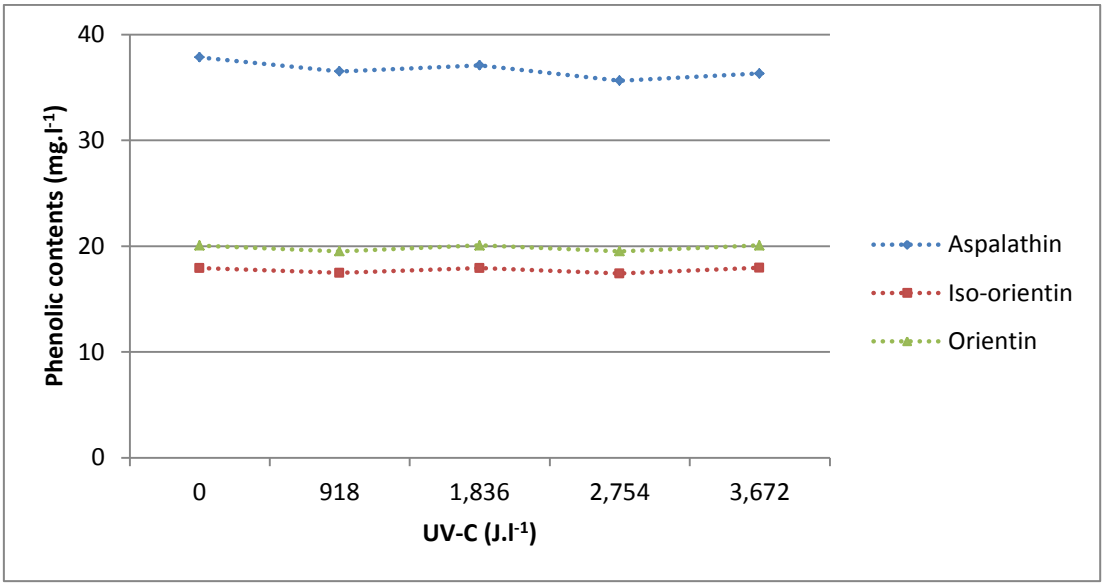

Figure 3.5 B: Effect of UV-C on aspalathin, iso-orientin and orientin content in RIT formulation B

increase of 0.56 mg.l^{-1} were recorded following treatment with UV-C dosages of 2 754 and 3 672 J.l^{-1}, respectively.

Table 3.6 B shows the statistical results of the phenolic compound contents in formulations B. Based on the three major phenolic compounds of the RIT, UV-C treatment had no significant ($p>0.05$) effect in both formulations. There are no published studies on the effect of UV-C on the phenolic composition of RIT currently. Various studies have shown different effects of UV-C on total antioxidants and/or phenolic compounds in foods. Whilst some have shown no effect on the antioxidant content, some have shown that UV-C can have a negative effect on these compounds. On the other hand, UV-C has been found to have an enhancing effect on the antioxidant content of some food products.

The treatment of watermelon juice with various UV-C dosages resulted in no significant difference in the lycopene and total phenolic content when compared to the untreated juice (Feng *et al*., 2013). Pala and Toklucu (2013) also recorded no significant differences in the total phenols and antioxidant capacity of orange juice following UV-C treatment with 12.03, 24.06, 36.09 and 48.12 kJ.l^{-1}. The same effect was also reported by Corrales *et al*. (2012) in the UV-C treatment of fresh tiger nuts' milk beverage. It may have been expected that increasing UV-C dosages would result in a decrease in the phenolic contents of the RIT, but this was not the case. This was contrary to the results that were observed in the former study by Corrales *et al*. (2012).

Contrary to the aforementioned studies and the current study, UV-C has been reported to result in a decrease in the antioxidant properties of juices. In a study by Shamsudin *et al*. (2014) UV was found to result in a significant decrease of the phenolic content in pineapple juice where repetitive UV (UV-UV) was applied. Similarly, antioxidant capacity of Nam Dok Mai Si Thong mangoes was significantly decreased after UV-C treatment of 4.93 kJ.m^{-2} (Safitri *et al*., 2015). Increasing UV-C dosages resulted in a significant decrease in the phenolic compounds of coconut milk, while the antioxidant activity was not affected (Ochoa-Velasco *et al*., 2014). The preservation of phenolic compounds and antioxidants may be attributed to the presence of solids within a liquid product. Although not specifically analysed, this phenomenon may have been proven in the current study. The precipitate that was noted in the RIT samples was not removed prior to analysis, instead the vials were vortexed in order to evenly distribute the constituents. The suspended solids may have played a protective role on the phenolic compounds against transmission of the UV-C. The higher the soluble solids contents, the lower the UV-C penetration.

Table 3.6 B: Aspalathin, iso-orientin and orientin content of untreated and UV-C treated RIT in formulation B

UV-C dosage ($J.l^{-1}$)	Phenolic compounds ($mg.l^{-1}$) (Average mean ± SD)		
	Aspalathin	Iso-orientin	Orientin
0	37.85 ± 1.81[a]	17.92 ± 0.32[a]	20.05 ± 0.52[a]
918	36.51 ± 1.84[a]	17.46 ± 0.82[a]	19.51 ± 0.83[a]
1 836	37.09 ± 1.02[a]	17.92 ± 0.19[a]	20.07 ± 0.36[a]
2 754	35.64 ± 0.73[a]	17.41 ± 0.33[a]	19.51 ± 1.81[a]
3 672	36.32 ± 0.68[a]	17.95 ± 0.16[a]	20.07 ± 0.26[a]

[a] means with different superscripts in the same column differ significantly ($p<0.05$)

Sometimes UV-C enhances some components of food products by increasing the contents. This effect has been reported amongst others by Pataro *et al*. (2015) where the treatment of fresh tomatoes with UV-C resulted in enhancement of the total carotenoids, lycopene, phenolics and antioxidant activity. The same phenomenon was discovered in apples by Kondo (2015).

Some researchers have compared the effect of UV-C on quality and nutritional properties of food to that of thermal treatment. The total polyphenol content of mango juice was found to have decreased by 38% following thermal pasteurisation. Contrarily, UV-C had significantly increased the polyphenol content of the juice (Santhirasegaram *et al*., 2015). The authors cited that the enhancement was attributed to the increased enzyme (phenylalamine ammonia lyase) activity caused by UV-C which consequently contributes to the activation of the phenolic biosynthesis pathway, leading to the increase of the phenolic compounds (Alothman *et al*., 2009). Ochoa-Velasco *et al*. (2014) also compared the effect on heat treatment and UV-C on coconut milk. As already mentioned, a decrease in the phenolic compounds was reported, but no significant difference ($p>0.05$) was noted on the antioxidant activity following increasing UV-C dosage treatment. Thermal treatment was however found not to have had any detrimental effect on the phenolic content and antioxidant activity of the milk. Pala & Toklucu (2013) reported that there was no significant difference in between UV-C and heat treatment of orange juice when compared to the control.

In 2009, Joubert *et al.* investigated the effect of thermal treatment on the aspalathin, iso-orientin and orientin content of RIT. The findings showed that following NTS at 121°C/15 min and HTS at 135°C/4 min the aspalathin, iso-orientin and orientin were significantly reduced. The loss was however lesser in the iso-orientin and orientin in comparison to the aspalathin. The findings were reported to possibly be attributed to structural difference of the compounds, where under oxidative conditions, the C-3 chain of the aspalathin would be subject to ring closure. As such there is an expectation of accelerated conversion due to heat. On the other hand, pasteurisation (93°C/30 min), which is normally used by industry for the treatment of RIT did not greatly affect the levels of the contents of the three compounds. Instead an increase in the aspalathin content was noted. Though no evidence is available, this increase was reported to possibly be due to the release of aspalathin upon interaction with other compounds (i.e proteins) in the Rooibos medium during heating.

To further understand the effect that UV-C may have on the phenolic compounds of the RIT, an additional formulation of the RIT was analysed. The formulation consisted of Rooibos extract + water + sugar, and was termed formulation C. Figure 3.5 C shows the effect of UV-C on phenolic compounds RIT formulation C. Table 3.6 C outlines the

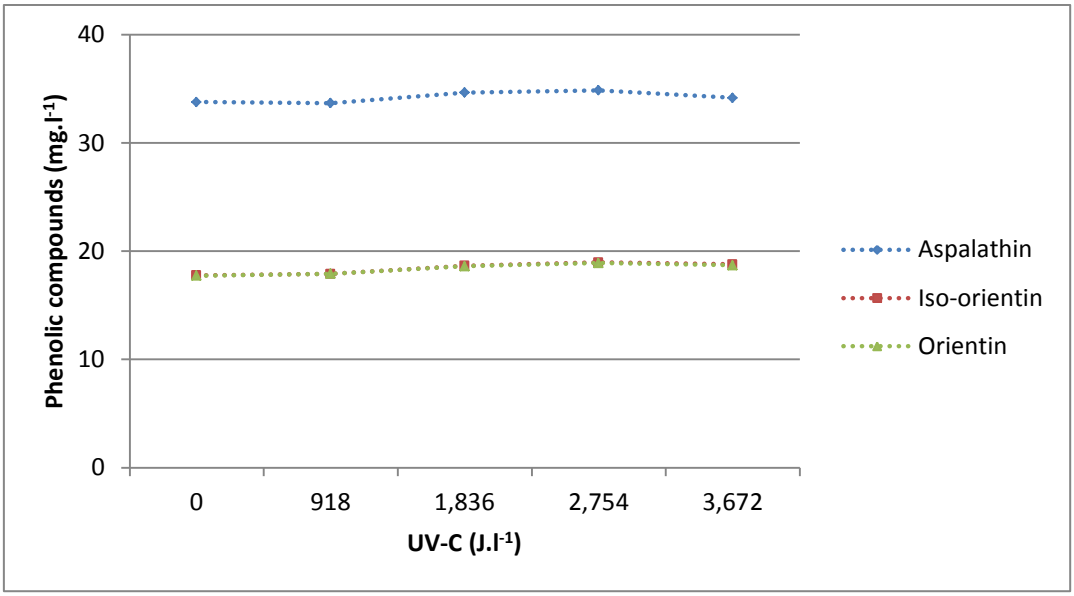

Figure 3.5 C: Effect of UV-C on aspalathin, iso-orientin and orientin content in RIT formulation C

Table 3.6 C: Aspalathin, iso-orientin and orientin content of untreated and UV-C treated RIT in formulation C

UV-C dosage (J.l^{-1})	Phenolic compounds (mg.l^{-1}) (Average mean ± SD)		
	Aspalathin	Iso-orientin	Orientin
0	33.77 ± 1.34^{a}	17.76 ± 0.68^{a}	17.74 ± 0.65^{a}
918	33.67 ± 1.50^{a}	17.89 ± 0.89^{a}	17.92 ± 0.82^{a}
1 836	34.64 ± 1.53^{a}	18.65 ± 0.88^{a}	18.61 ± 0.88^{a}
2 754	34.84 ± 1.17^{a}	18.96 ± 0.47^{a}	18.90 ± 0.50^{a}
3 672	34.15 ± 1.28^{a}	18.79 ± 0.59^{a}	18.69 ± 0.57^{a}

^{a} means with different superscripts in the same column differ significantly ($p<0.05$)

statistical results of the various UV-C dosages on the phenolic compounds. Like formulations A and B, the UV-C dosages did not show consistent results on the three phenolic compounds. Most importantly no significant difference was noted for the three compounds following UV-C treatments. Although no significant differences were recorded, slight increases in the three components were noted. However, the increases were not gradual and thus were not as a function of increasing UV-C dosage.

Slight variations were noted in the overall concentrations of aspalathin where formulation B contents were slightly higher than those of formulations A and C. The increased content may be attributed to the addition of citric acid. There are no published studies on the effect of combined UV-C and chemical treatment on phenolic compounds of food products. Joubert *et al.* (2009) reported that heat treated RIT with citric acid was found to have had lesser decrease in the aspalathin content when compared to the basic formulation of Rooibos tea extract, water and sucrose. It was reported that phenolic compounds are more heat stable at lower pH. This could be attributed to the slightly higher aspalathin content of formulation B in the current study, however the aspalathin content of the untreated RIT in formulation B was evidently higher than the two formulations. A statistical comparison of the three formulations indicated no significant difference ($p>0.05$).

It can thus be concluded that UV-C has no significant effect on the aspalathin, iso-orientin and orientin of RIT irrespective of the composition.

3.4.4 Colour measurement of formulations A, B and C

The colour of the untreated and UV-C treated RIT was investigated. This was done only on the first day in order to avoid interference of the results by microbial growth. The +L* refers to the lightness and -L darkness, +a* is the redness whilst -a* is the greenness and +b* is the yellowness whilst -b* is the blueness. The total colour difference is expressed as ΔE*, which is the change in the L*, a* and b* values calculated by the equation: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$. This serves as an indication as to what extent the colour change occurred. Table 3.7 shows the different ΔE* values that can be obtained and thus the index classification shows the region at which the results can be concluded. As cited by Murevanhema (2012), just noticeable colour differences are defined by ΔE*=1, which are perceivable by a trained colour observer. Between 4 and 8, the differences are deemed acceptable (Sharma, 2004). In addition, colour difference classification adopted from Corrales *et al*. (2012) has been used to describe all obtained results.

Table 3.8 A shows the results of untreated and UV-C treated formulation A RIT. An increase in the UV-C dosage resulted in a decrease in the L* (-) value which indicated a shift towards the darkness. Contrarily, the a* and b* values showed increases after UV-

Table 3.7: ΔE* difference classification (adopted from Corrales *et al*, 2012)

ΔE*	colour classification
0.00 - 2	minimum
2.0 - 3.0	acceptable
3.0 – 5.0	almost unacceptable
> 5.0	unacceptable

Table 3.8 A: Colour (L*, a*, b*) values and total colour change (ΔE*) of untreated and UV-C treated formulation A of RIT

UV-C Dosage ($J.l^{-1}$)	L*	a*	b*	Δ*E**	Colour change
0	44.38 ± 0.14^{a}	21.62 ± 0.41^{a}	49.95 ± 2.13^{a}	0.00	NA
918	44.02 ± 0.25^{a}	22.02 ± 0.70^{ab}	52.13 ± 1.22^{a}	2.52 ± 2.84^{a}	acceptable
1 836	43.25 ± 0.16^{b}	22.64 ± 0.26^{b}	51.10 ± 1.10^{a}	2.85 ± 1.97^{a}	acceptable
2 754	42.93 ± 0.24^{bc}	22.49 ± 0.39^{b}	50.35 ± 1.99^{a}	3.24 ± 1.39^{a}	almost unacceptable
3 672	42.55 ± 0.28^{c}	23.40 ± 0.42^{c}	51.94 ± 1.01^{a}	3.46 ± 0.69^{a}	almost unacceptable

[a-c] means with different superscripts in the same column differ significantly ($p<0.05$)

C treatments which showed a shift towards the redness and yellowness, respectively. The changes in the L* value were gradual and decreased as the UV-C dosages increased. The recorded results indicated degrees of statistical variety in terms of the L* and a* values following UV-C treatment. For the L* value, there was no significant difference ($p>0.05$) between the untreated RIT and UV-C dosage 918 $J.l^{-1}$ and between 1 836 and 2 754 $J.l^{-1}$ and lastly between 2 754 and 3 672 $J.l^{-1}$. Contrarily, the changes recorded for the a* and b* components were not gradual, indicating that the changes were not as a function of increasing UV-C dosage. Significant differences were noted between the a* value between the untreated RIT and UV-C dosages of 1 836, 2 754 and 3 672 $J.l^{-1}$. No significant differences were noted between the untreated RIT and 918 $J.l^{-1}$ and between 918, 1 836 and 2 754 $J.l^{-1}$ UV-C treatments. However treatment of the RIT by UV-C 2 754 $J.l^{-1}$ was significantly different to all other UV-C treated RIT. No significant difference ($p>0.05$) was noted in the b* values.

Although the latter changes were observed, the overall colour change can only be expressed by ΔE*. Following UV-C dosages of 918, 1 836, 2 754 and 3 672 $J.l^{-1}$, ΔE* results were 2.52, 2.85, 3.24 and 3.46, respectively. According to the ΔE* colour classification, following UV-C treatment of 918 and 1 836$J.l^{-1}$, the colour change was “acceptable”. After treatment with 2 754 and 3 672 $J.l^{-1}$, the change was “almost unacceptable”. These results increased gradually and showed that the colour difference was as a result of increasing UV-C dosage. Although an increase in ΔE* was noted after treatment with every UV-C dosage, no significant differences ($p>0.05$) were noted on the effect of the UV-C dosages.

Table 3.8 B represents the results of formulation B RIT. The L* value was found to be (-) after all UV-C dosages, indicating a shift towards the darkness. On the contrary, overall UV-C treatment resulted in an increase in a* where it was (+) indicating a shift towards the redness. The b* value showed a decrease and a shift towards the blueness after overall UV-C treatment. No significant difference ($p>0.05$) was noted between the untreated and the various UV-C treated RIT samples for the L*, a* and b* values. The results of ΔE* for formulation B were recorded to be 3.66, 3.03, 3.79 and 3.72 after UV-C dosages of 918, 1 836, 2 754 and 3 672 $J.l^{-1}$, respectively. The colour difference classification was recorded as “almost unacceptable” after all UV-C dosages. Unlike formulation A, the ΔE* changes were not gradual indicating that the changes were not dependent on the increasing UV-C dosages. There was no significant difference ($p>0.05$) in the ΔE* of the various UV-C dosages.

According to Falguera *et al*. (2011), light has been reported to have detrimental effect on the stability of natural pigments. This was true for all formulations of the RIT treated with UV-C in the current study and was indicated by a change in ΔE* which was

Table 3.8 B: Colour (L*, a*, b*) values and total colour change (ΔE*) of untreated and UV-C treated formulation B of RIT

UV Dosage ($J.l^{-1}$)	L*	a*	b*	Δ*E**	Colour change
0	42.97 ± 1.35[a]	17.75 ± 0.49[a]	44.95 ± 4.43[a]	0.00	NA
918	42.33 ± 0.90[a]	18.10 ± 0.55[a]	46.83 ± 0.65[a]	3.66 ± 2.29[a]	almost unacceptable
1 836	41.78 ± 1.11[a]	18.25 ± 0.74[a]	45.78 ± 1.92[a]	3.03 ± 0.19[a]	almost unacceptable
2 754	41.64 ± 0.76[a]	18.40 ± 1.21[a]	44.34 ± 0.99[a]	3.79 ± 2.05[a]	almost unacceptable
3 672	41.60 ± 0.51[a]	17.68 ± 1.00[a]	44.54 ± 2.24[a]	3.72 ± 1.48[a]	almost unacceptable

[a] means with different superscripts in the same column differ significantly ($p<0.05$)

perceivable. Research by Guerrero-Beltrán and Barbosa-Cánovas (2005) showed that UV-C significantly affected the colour of apple juice even at the lowest treatment time. After treatment for 5 and 30 min, the colour difference was found to be ΔE*=2.58 and ΔE*=4.16, respectively (Guerrero-Beltrán & Barbosa-Cánovas, 2005). The results indicated that an increase in the UV-C treatment time results in an increase in ΔE* which effects greater colour changes. The total colour difference of grape, cranberry and grapefruit juice was found to have increased as the UV-C treatment time increased (Guerrero-Beltrán *et al*., 2009). Static and dynamic UV-C were used to treat liquid egg white (LEW), liquid egg yellow (LEY) and liquid whole egg (LWE) at dosages of 0.612 and 3.645 $J.cm^{-2}$, respectively for both treatments. Results indicated an increase in ΔE* as a function of increasing UV-C in both static and dynamic UV-C with the exception of LWE under static UV-C (De Souza & Fernandez, 2011). In the current study this phenomenon was also observed in formulation A alone. Similarly, Feng *et al*. (2013) found the lowest UV-C dosage of 2.7 $J.ml^{-1}$ had a lower colour difference of ΔE*=0.31 in watermelon juice when compared to ΔE*=0.90 found after treatment with 37.5 $J.ml^{-1}$.

The ΔE* results for formulation B were slightly higher than those of formulation A. However, there was no significant difference ($p>0.05$) between the two formulations. The slightly higher ΔE* results of formulation B may be attributed to the addition of citric acid. However, in order to confirm the afore-mentioned statement, results of the two formulations were compared to formulation C which contained sugar but no citric acid.

Table 3.8 C represents the colour results for formulation C. The results of the L* value, like in the other two formulations indicated a shift towards the darkness (-L*) after overall UV-C treatment. The same was observed for the b* value but not the a* value. Significant differences ($p<0.05$) were noted between the UV-C dosages for the L* and b* values, but not for the a* values. The effect of UV-C on the overall colour difference ΔE* was recorded as “minimum” after treatment with all UV-C dosages. The values of ΔE* in formulation C were found to be less than formulation A and B. Similar to formulation A and B, there was no significant difference between the ΔE* results of the various UV-C dosages. Furthermore, there was no significant effect in the ΔE* results of the three formulations. The changes in the total colour difference of formulation C were, however not as a function of increasing UV-C dosage. The same effect was found by Corrales *et al*. (2012). Tiger nuts’ milk beverage treated with UV-C dosages of 0.14, 0.28, 0.71 and 1.41 $J.cm^{-2}$ resulted in ΔE* values of 0.36, 0.23, 0.99 and 0.97, respectively.

As already mentioned, the effect of the colour difference between the different UV-C dosages was not significant. Similar results were recorded by Ochoa-Velasco *et al*. (2014) where no significant differences were noted after various UV-C dosage treatment of coconut milk.

Table 3.8 C: Colour (L*, a*, b*) values and total colour change (ΔE*) of untreated and UV-C treated formulation C of RIT

UV Dosage ($J.l^{-1}$)	L*	a*	b*	ΔE*	Colour change
0	38.70 ± 0.22^{a}	16.48 ± 0.21^{a}	23.81 ± 0.15^{a}	0.00	NA
918	38.62 ± 0.29^{a}	17.27 ± 0.70^{a}	23.29 ± 0.45^{ab}	1.24 ± 0.42^{a}	minimum
1 836	38.38 ± 0.32^{ab}	16.59 ± 0.66^{a}	23.24 ± 0.44^{ab}	1.00 ± 0.43^{a}	minimum
2 754	37.76 ± 0.18^{bc}	17.33 ± 0.61^{a}	22.95 ± 0.47^{ab}	1.69 ± 0.64^{a}	minimum
3 672	37.91 ± 0.43^{bc}	17.07 ± 1.01^{a}	23.36 ± 0.16^{b}	1.56 ± 0.32^{a}	minimum

$^{a-c}$ means with different superscripts in the same column differ significantly ($p<0.05$)

The decrease in the L* value of all three formulations makes the colour of the RIT darker and may be greatly attributed to the colour change that occurred. Under ambient temperatures (Viljoen, 2008), aspalathin in a solution has been reported to show slow conversion which results in some brown indefinable substances (Koeppen & Roux 1965b, 1966). This may contribute to the darkness of the RIT and the colour differences, as expressed by the results of all formulations.

Heat treatment (NTS and HTS) of various unfermented RIT formulations resulted in increased browning (absorbance). Pasteurisation also increased absorbance however at a lower rate. The increase in the absorbance was attributed to the high aspalathin content of the unfermented RIT (Viljoen, 2008). The same may be attributed to the increased darkness (-L) of the three formulations in the current study since unfermented Rooibos extract was also used.

The results obtained in this study showed a perceivable colour change after all UV-C dosages in all RIT formulations. The best results were achieved in formulation C as the over colour difference was minimum. Although the colour difference classification showed different values for the different UV-C dosages, there was no significant difference between all the dosages. As such the optimum UV-C treatment that may be applied in the treatment of RIT is 3 672 $J.l^{-1}$, since it delays the spoilage of RIT. Furthermore, there was no significant difference in the ΔE* results of the three formulations.

3.5 Conclusion

The investigation of UV-C on the shelf life of two RIT formulations stored at 4°C has been found to be successful. The UV-C treated RIT in comparison to the untreated (control) RIT was found to have not reached the microbiological limit of 4 $\log_{10}$. This was the case even in the lowest UV-C treatment of 918 $J.l^{-1}$. The results of this study have indicated that the increase in the UV-C dosage results in an increase in the shelf life by delaying the growth of microorganisms.

Yeast and mould have been reported to be the main spoilage organisms for low acid (pH) and high sugar containing products. The RIT of formulation B consisted of high sugar concentration (60 $g.l^{-1}$) and a low pH of 3.00. However, formulation A was different since it contained no sugar and no citric acid where the pH was higher at approximately 5.00. In the current study, the most particular interest was the pattern of microbial growth for both APC and YM which was noted to be extremely similar. This may support the aforementioned statement that YM are mostly responsible for spoilage in such media. However, due to the same observation made in formulation A, the spoilage may also be attributed to the Rooibos extract since the water used was microbiologically analysed and

no microorganisms were detected. Due to the resistant nature of the YM, it may be required that higher UV-C dosages be applied for an even longer extended shelf life of RIT. There was no growth of coliforms observed during the shelf life duration in both formulations.

The difference between the two formulations was the addition of citric acid and sugar in formulation B. It cannot be ascertained that the citric acid had any effect on the microbiological population of the RIT as this was not specifically analysed. However, the untreated RIT of formulation B showed lower microbiological detections in comparison to formulation A. Day 27 of formulation B showed that the APC and YM counts of formulation B were higher than formulation A. This may serve as an indication that the effect on the shelf life was mainly due to UV-C and not the citric acid. A decrease in the pH of both formulations was noted at the end of the shelf life, which may be attributed the growth of bacteria resulting in acid production. The decrease was however found to not be significant.

The phenolic composition of RIT is of particular interest as it is indicative of its antioxidant effect, with aspalathin being the most important antioxidant in Rooibos. The various UV-C treatments applied on the RIT were found not to have any detrimental effect on the phenolic content of aspalathin and its oxidative products, iso-orientin and orientin. This was in agreement with research that UV-C does not affect the phenolic contents of food products. The phenolic contents of aspalathin and orientin in formulation B were noted to be slightly higher than in formulation A. This may be assumed to the result of the added citric acid, but was however not confirmed. Research has indicated that sterilisation has a negative effect on the phenolic content of RIT. On the other hand, pasteurisation which is applied commercially in the microbiological treatment of RIT was found to not have a negative effect on these three compounds (Joubert *et al*., 2009). This was similar with UV-C. However, since UV-C has been found to be economic as it uses less power, it would be of benefit to employ the non-thermal treatment.

The colour of the RIT in all three formulations following UV-C treatment was found to be perceivable ($\Delta E^*>1$), where the RIT was noted to be slightly darker when compared to the untreated RIT. The least colour differences were noted in formulation C. Unlike in formulation A, the effect of the UV-C on the colour of the RIT could however not be attributed to the increase in dosage in formulation B and C. Statistical analysis showed that there was no significant difference between the UV-C dosages and the three formulations.

Although no statistical difference was noted between UV-C 1 836, 2 754 and 3 672 $J.l^{-1}$ in formulation A and between all UV-C dosages in formulation B, it is imperative to take into account the microbiological results. Based on the microbiological effect of UV-C

on the shelf life of RIT, the optimum UV-C dosage that can be used is 3 672 $J.l^{-1}$ for all formulations since it delayed the growth of microorganisms. The chosen optimum UV-C dosages for the two formulations also, like other dosage treatments, did not have any effect on the pH and the phenolic contents. All UV-C dosages applied resulted in a perceivable effect on all three formulations of the RIT.

The effect of UV-C on the phenolic contents, aspalathin, iso-orientin and orientin and the colour were only conducted on samples drawn on the first day. The effect of these components over the duration of the shelf life is imperative in order to give an overall indication of the UV-C effect on the final product. Furthermore research into the total antioxidant activity of UV-C treated RIT will assist in ascertaining the benefits of consuming the RIT. Since unfermented Rooibos contains higher concentrations of phenolic compounds in comparison to fermented Rooibos, it would be beneficial to the lower premium market to investigate the effect of UV-C on fermented RIT. Taste is of utmost importance in the food industry and thus it also recommended that sensory evaluation be conducted on the UV-C treated RIT.

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CHAPTER FOUR
EFFECT OF UV-C TREATMENT ON THE MICROBIAL INACTIVATION OF ROOIBOS ICED TEA

4.1 Abstract

UV-C has been investigated for its effectiveness in inactivating various food spoilage microorganisms that included bacteria, viruses, yeasts and moulds. Usually thermal treatment is used to inactive spoilage microorganisms in beverages such as RIT. The aim of this study was to investigate the effect of UV-C on *Escherichia coli* K12, *Staphylococcus aureus*, *Salmonella* sp., *Saccharomyces cerevisiae* and *Cladosporium* sp. individually inoculated in RIT, as an alternative to thermal treatment.

UV-C dosages of 0, 918, 1 836, 2 754 and 3 672 $J.l^{-1}$ were used to treat the RIT using a pilot-scale UV-C system with a turbulent flow. *Saccharomyces cerevisiae* was successfully eliminated by a UV-C dosage of 2 754 $J.l^{-1}$, with the 4 $\log_{10}$ reduction achieved after 1 836 $J.l^{-1}$. There was no significant difference between *Salmonella* sp. and *E. coli* K12 and the microorganisms showed an approximate 4 $\log_{10}$ reduction following UV-C dosage of 1 836 and 3 672 $J.l^{-1}$, respectively. An approximate 4 $\log_{10}$ reduction for *S. aureus* was only recorded after the highest UV-C dosage treatment of 2 754 $J.l^{-1}$. *Cladosporium* sp. showed the most resistance amongst all microorganisms. The effect of UV-C on different microorganisms varied and yeasts were found to be the most sensitive to this technology when compared to bacteria and moulds.

The use of the SurePure UV-C turbulent reactor could be used for the inactivation of RIT-associated microorganisms as an alternative to heat treatment.

4.2 Introduction

Spoilage of food and beverages results from microbial activity of a wide range of microorganisms. The microbial flora that colonizes a particular food or beverage is highly dependent on the characteristics of the product, its processing and storage (Huis in't Veld, 1996). Ready-to-drink beverages have high water activity contents that allow for microbial growth (Battey *et al*., 2001). In addition, the spoilage of soft-drinks may be a result of yeasts, moulds or bacteria. The most predominant spoilage is that of yeasts and moulds favoured by the low pH and high sugar contents (Aneja *et al*., 2014). Vegetative bacterial pathogens such as *Salmonella* spp and Verocytotoxin *Escherichia coli* and most bacteria are also accountable for this spoilage (Stratford & Capell, 2003). The growth of yeasts in excess of 1 x 10^5 $cells.ml^{-1}$ results in a visible cloud or haze (Loureiro & Querol, 1999; Stratford & Capell, 2003). However, in other instances spoilage by yeasts cannot be

easily identified, mainly in fermented foods or drinks where the produced metabolites contribute to the flavour and aroma of the products (Loureiro & Querol, 1999). Adding to visible spoilage, moulds are also able to spoil foods through the formation of mycotoxins (Huis in't Veld, 1996; Aneja *et al*., 2014). The most significant spoilage yeasts are highly fermentative and produce CO_2 that increases the pressure of the packaging which may lead to distorting or bursting (Stratford & Capell, 2003; Kregiel, 2015). To maintain a good quality soft-drink beverage acceptable to consumers, methods that suppress or delay the growth of spoilage microorganisms are therefore essential for the product.

To date, pasteurisation remains the most effective process to eliminate the presence of microorganisms in soft drinks (Turtoi, 2014). Vegetative yeasts and mould cells are heat-sensitive and almost all are destructed after 10 min exposure to 60°C (Stratford & Capell, 2003). In fruit juices, which are heavily contaminated with yeasts and moulds, pasteurisation ranges from 10 to 15 min at 70-75°C, however this does not eliminate the heat resistant forms of moulds such as ascospores (Stratford & Capell, 2003). As cited by Battey *et al*. (2001), hurdle combinations such as sugar content, chemical preservatives and pH is another process that has been adopted in the food industry which prevents the growth of most microorganisms in RTD beverages (Leistner, 1995). Thermal treatments also have a negative impact on particular components and sensory features of various foods that make them less attractive in terms of colour and textural properties (Falguera *et al*., 2011; Turtoi, 2014). It thereby remains imperative to find an alternative technology that does not have any detrimental effects on the food properties, yet is effective in microbial inactivation.

Ultraviolet light has the ability to eliminate the microbial flora without compromising the sensory quality of the food (Falguera *et al*., 2011). The use of this non-thermal technology as a physical preservation method is of interest to the food industry (Koutchma *et al*., 2009). A lot of recent studies have shown that the use of this technology holds promise in food processing as alternative to thermal technology for liquid food such as fresh juices, soft drinks and beverages (Müller *et al*., 2014; Kaya *et al*., 2015; Rizzotti *et al*., 2015; Usaga *et al*., 2015).

The UV wavelength for processing ranges from 100 to 400 nm. These wavelengths are further subdivided into three regions namely UV-A, UV-B and UV-C (Diffey, 2002). Ultraviolet-A ranges between 320 - 400 nm and is generally responsible for the causing changes in the human skin. The range for UV-B is 280 - 320nm and usually causes skin cancer (Guerrero-Beltrán & Barbosa-Cánovas, 2004). The UV range with a germicidal effect is UV-C which ranges from 200 - 280 nm (U.S. F.D.A., 2000). This is used for the disinfection of surfaces, water and various food products (Bintsis *et al*., 2000), being effective in the inactivation of bacteria and viruses, protozoa, moulds,

yeasts and algae (U.S. F.D.A., 2000, Guerrero-Beltran & Barbosa-Cánovas, 2005). The UV-C light effect on microorganisms occurs at the DNA level where both the replication and transcription of the nucleic acid is affected (Guerrero-Beltran *et al*., 2008).

The success of UV-C treatment in liquids is determined by the characteristics of the liquid being treated. The presence of colour compounds, type of liquid, UV-C absorptivity organic solutes and suspended matter in liquid foods results in low transmission of the UV light which in turn lowers the performance efficiency of the UV pasteurisation process (Guerrero-Beltran & Barbosa-Cánovas, 2005; Koutchma *et al*., 2009). Non-thermal UV-C treatment has been used successfully in the pasteurisation of liquid foods amongst others, such as milk and fruit juices and wine (Matak *et al*., 2005; Keyser *et al*., 2008; Fredericks *et al*., 2011).

Various types of UV-C equipment with different flow reactors have been extensively studied in the investigation of liquid foods. These include the laminar, Tailor-Couette, Dean and the turbulent flow reactors. The flow reactor is the most important aspect of the equipment as, if designed correctly, can reduce high UV-C absorbance interference and viscosity which are characterised by other liquid foods, thus enhancing the inactivation of microorganisms (Koutchma, 2009; Orlowska *et al*., 2014). Turbulent flow reactors (SurePure) have been investigated by various researchers and its effectiveness has been proven (Keyser *et al*., 2008; Fredericks *et al*., 2011).

The effect of UV-C treatment on microorganisms may vary from one species to another. The effect may depend on the strain, growth media, stage of growth (Wright *et al*., 2000), density of the microorganisms and other characteristics, such as the type and composition of the food (Guerrero-Beltran & Barbosa-Cánovas, 2005). Fungi and yeasts are large microorganisms and tend to be more resistant during UV-C treatment (Guerrero-Beltran & Barbosa-Cánovas, 2005). The use of UV-C treatment in beverages continues to prove its success and preserves the nutritional and sensory characteristics of the food products. The aim of this study was therefore to investigate the effect of UV-C on different microorganisms that include bacteria, yeast and mould individually inoculated in RIT beverage, using the SurePure turbulator reactor system.

4.3 Materials and methods

4.3.1 Cultivation of the cultures

Table 4.1 shows the cultures used together with the broth and selective media used for each microorganism. Pure cultures of *E. coli* K12 and *Salmonella* sp. were obtained from the Department of Biotechnology, University of the Western Cape. The *E. coli* K12 cells were maintained on VRBA and grown in tryptic soy broth (TSB) prior to inoculation at 37°C for 24 h. *Salmonella* sp. was maintained on xylose deoxycholate (XLD) agar and

Table 4.1: Selective media used for the enumeration of the different microorganisms inoculated in the RIT

Microorganism	Broth	Selective media	Incubation temperature
E. coli K12	TSB	VRBA	37°C/48 h
S. aureus	TSB	MSA	37°C/24 h
Salmonella sp.	BPW	XLD	37°C/24 h
S. cerevisiae	TSB	RBCA	25°C/5 days
Cladosporium sp.	S dH_20	RBCA	25°C/5 days

TSB: Tryptic Soy Broth
VRBA: Violet Red Bile Agar
MSA: Mannitol Salt Agar
BPW: Buffered Peptone Water
XLD: Xylose Deoxycholate
RBCA: Rose Bengal Chloramphenicol Agar
S dH_20: Sterile distilled water
E. coli: *Escherichia coli*
S. aureus: *Staphylococcus aureus*
sp.: *species*
S. cerevisiae: *Saccharomyces cerevisiae*

grown in buffered peptone water at 37°C for 24 h. *Staphylococcus aureus* obtained from the Department of Food Technology, Cape Peninsula University of Technology, was maintained on mannitol salt agar (MSA) and was grown in TSB at 37°C for 24 h. The mould, *Cladosporium* sp. that was used in this study was isolated from previously prepared RIT allowed to stand in an ambient temperature for 30 days. The *Cladosporium* sp. was maintained on rose bengal chloramphenicol agar (RBCA) and grown in sterile distilled water at 37°C for 24 h. *Saccharomyces cerevisiae* was cultivated from food grade yeast (Anchor Yeast, South Africa) and grown in TSB at 37°C for 24 h. All selective media and broth used in this study were obtained from Biolab (Merck, South Africa). All organisms used in this study were non-pathogenic. The inocula of all organisms were cultured to a desired final concentration of between 6 x 10^6 and 9 x 10^6 cfu.ml^{-1} using the pour plate method.

4.3.2 Cleaning of the pilot-scale UV-C reactor system

The UV-C reactor system used for this study was supplied by SurePure (Milnerton, South Africa). This system is similar to the unit used by Fredericks *et al*. (2011) and Keyser *et al*. (2008). Figure 4.1 shows the image of the UV-C reactor system. Unlike the previously used system, the current unit used was fitted with 4 cylindrical low pressure mercury lamps which emit 100 watts (W) power each placed in quartz sleeves. The product was pumped through the reactor at a constant flow rate of 4000 l.hr.$^{-1}$ which results in the desired Reynolds value (Re) of 7500 subsequently resulting in a turbulent flow.

The cleaning procedure, cleaning in place (CIP) was modified, where caustic Trisol 2000 and P3-oxonia active containing 2-10% peracetic acid and 20-60% hydrogen peroxide, were used, respectively. Trisol 2000 was used at 0.5% at 65°C for ±30 min, followed by rinsing with ±25°C water for 10 min. P3-oxonia was added to the final rinse water at 1 % at ±25°C for ±15 min.

4.3.3 Preparation of the RIT

Unfermented Rooibos extract powder (Afriplex, Paarl, South Africa) and food grade sucrose (Selati, South Africa) was used for the preparation of the RIT. Only one formulation was prepared for the inoculation study. Table 4.2 shows the quantified ingredients used in the preparation of the RIT.

Municipal water was used as the solvent for the preparation of the RIT. A 27.5 l of water was used to fill the UV system drum (27.5 l) followed by exposure of the tap water to a UV-C dosage of 918 J.l^{-1} at a constant flow rate of 4 000 l.hr^{-1} to ensure microbiological destruction of any microorganisms that might be present. The elimination of microorganisms from the used water was verified by analyzing the water for total viable

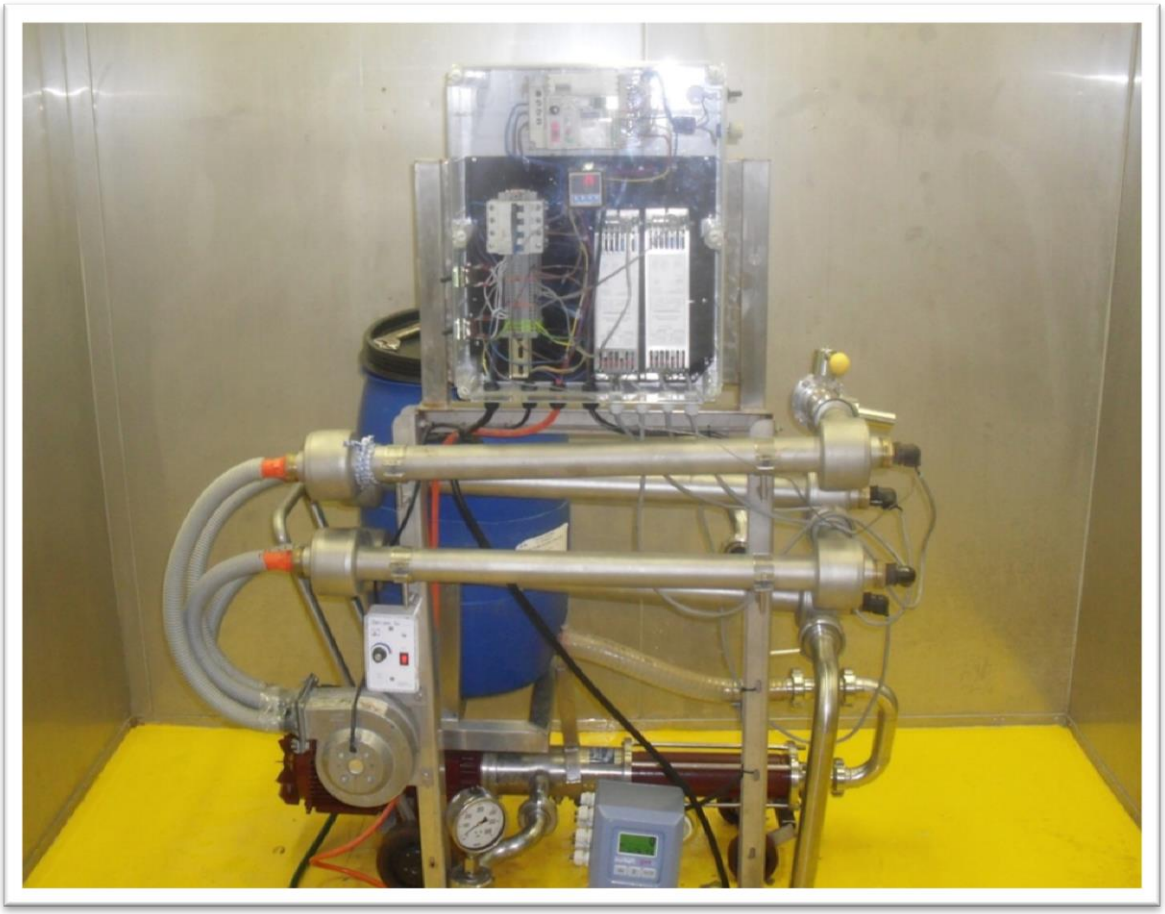

Figure 4.1: Pilot-scale UV-C reactor system used in this study

Table 4.2: The constituents of the RIT used for the inoculation experiment

Ingredients	Weight (g)	Volume (l)
Unfermented Rooibos extract powder	48.13	-
Sucrose	1 650	-
Municipal water	-	27.5

counts (TVC) using PCA and coliforms using VRBA.

To prepare the mixture, the Rooibos powder extract and sucrose were reconstituted with approximately 1000 ml of the UV-C treated water in a 2 litre sterile Schott bottle until completely dissolved and subsequently transferred to the UV-C system drum. The RIT solution was then allowed to mix completely with the rest of the water in the drum by running the system without exposure to UV-C light for 2 to 3 minutes.

4.3.4 Inoculation, UV-C application and sample collection

Three bacteria, *E. coli* K12*, S. aureus* and *Salmonella* sp*.*, one yeast *S. cerevisiae* and one mould, *Cladosporium* sp*.* were individually inoculated into untreated RIT at different times. Duplicate 50 ml samples of the inoculated RIT that were not subjected to UV-C, were drawn from the outlet nozzle of the UV system, as controls. Table 4.3 shows the UV-C dosages with the corresponding exposure times that were then applied to the inoculated RIT, followed by sample collection of 50 ml after each single treatment. The samples were then analysed immediately.

4.3.5 Microbiological analysis of the inoculated RIT and the control samples

Each 50 ml sample was thoroughly vortexed to evenly distribute the cells in the RIT. A 1 ml aliquot of each sample was pipetted into 9 ml quarter strength Ringers' solution. Serial dilutions of 1×10^0 to 1×10^6 were prepared. The solution was thoroughly vortexed and aseptically pour plated using the selective media in order to determine the number of cells in colony forming units ($cfu.ml^{-1}$) after incubation, for every UV-C treatment.

The same analysis conducted on the individually inoculated RIT samples were also carried out for the control samples during each experiment.

The legal microbiological limit for fruit juices following UV-C treatment is achieving a minimum of 5 $\log_{10}$ (U.S. F.D.A., 2000). Due to the lack of microbiological regulation of RIT, the highest limit was chosen to be 4 $\log_{10}$ as it was the average log count of the inocula and the average log reduction for pasteurisation.

4.3.6 Statistical analysis

All experiments were conducted in the triplicate. The data were subjected to analysis of variance using general linear models in NCSS version 2. The values obtained from the Tukey-Kramer multiple-comparison test were used. Probability levels of 5% ($p<0.05$) were considered to be significant.

Table 4.3: The comparison between UV-C dosages as $J.l^{-1}$ and $mJ.cm^{-2}$ and contact time for a 27.5 l of sample volume in a pilot-scale UV-C system using four UV-C lamps (Adapted from Keyser *et al*., 2008)

Dose per volume ($J.l^{-1}$)	Dose per area ($mJ.cm^{-2}$)	Contact time (min) for 27.5 l of RIT
0	0	0
918	936	4.1
1 836	1 872	8.3
2 754	2 808	12.4
3 672	3 744	16.5

4.4 Results and discussion

The effect of UV-C to inactivate three bacteria, *E. coli* K12, *S. aureus* and *Salmonella* sp., a yeast, *S. cerevisiae* and a mould, *Cladosporium* sp. inoculated in unfermented RIT was investigated. Each inoculum had a microbial count of approximately 3.87-5.87 $\log_{10}$ prior to inoculation. The RIT which was used for the inoculation was prepared without citric acid to eliminate the possibility of the microorganisms being inactivated by the preservative. The RIT was subjected to UV-C dosages of 918, 1 836, 2 754 and 3 672 $J.l^{-1}$ at a constant flow rate of 4 000 $l.hr^{-1}$.

Figure 4.2 represents the effect of the different UV-C dosages on the individual microorganisms. The initial microbial concentrations of the inoculated RIT samples varied from 3.87 $\log_{10}$ for *Cladosporium* sp., 4.50, 5.19, 5.49 and 5.87 $\log_{10}$ for *S. aureus*, *S. cerevisiae*, *Salmonella* sp. and *E. coli* K12, respectively (*Cladosporium* sp.<*S. aureus*<*S. cerevisiae*<*Salmonella* sp.<*E. coli* K12). All UV-C dosages applied to the RIT significantly ($p<0.05$) reduced all microorganisms, with the exception of *Cladosporium* sp.. *Saccharomyces cerevisiae* was the only microorganism that was completely eliminated. This was accomplished after UV-C treatment dosage of 2 754 $J.l^{-1}$. The sequence of microbial reduction was expressed as *S. cerevisiae*>*Salmonella* sp.>*S. aureus*>*E. coli* K12>*Cladosporium sp.* This clearly indicates that various microorganisms showed various UV-C sensitivities.

Table 4.4 represents the statistical analysis of the various concentrations of the untreated and UV-C treated RIT. Statistically, UV-C treatment has varying effects on different microorganisms, although no significant differences were observed on the various microorganisms following inoculation. There was a significant ($p<0.05$) difference in the initial RIT concentration of other microorganisms, however the different UV-C dosages had varying effects on the microorganisms where treatment after the different UV-C dosages resulted in no significant difference amongst some microorganisms.

Figure 4.3 represents the results of *E*. *coli* K12. The *E. coli* had an initial count of 5.87 $\log_{10}$ prior to UV-C treatment. A log reduction of 3.16 was observed following a minimum UV-C dose of 918 $J.l^{-1}$. Log reductions of 3.85 and 3.87 were observed after UV-C exposure of 1 836 and 2 754 $J.l^{-1}$ respectively. The highest UV-C dosage of 3 672 $J.l^{-1}$ resulted in the highest log reduction of 4.37. However, the dosage treatment of 3 672 $J.l^{-1}$ was found to have no significant difference ($p>0.05$) when compared to UV-C dosages of 1 836 and 2 754 $J.l^{-1}$. Table 4.5 shows Effect of different UV-C dosages on *E. coli* K12. The desired 4 $\log_{10}$ reduction was noted after treatment with a UV-C dosage of 3 672 $J.l^{-1}$.

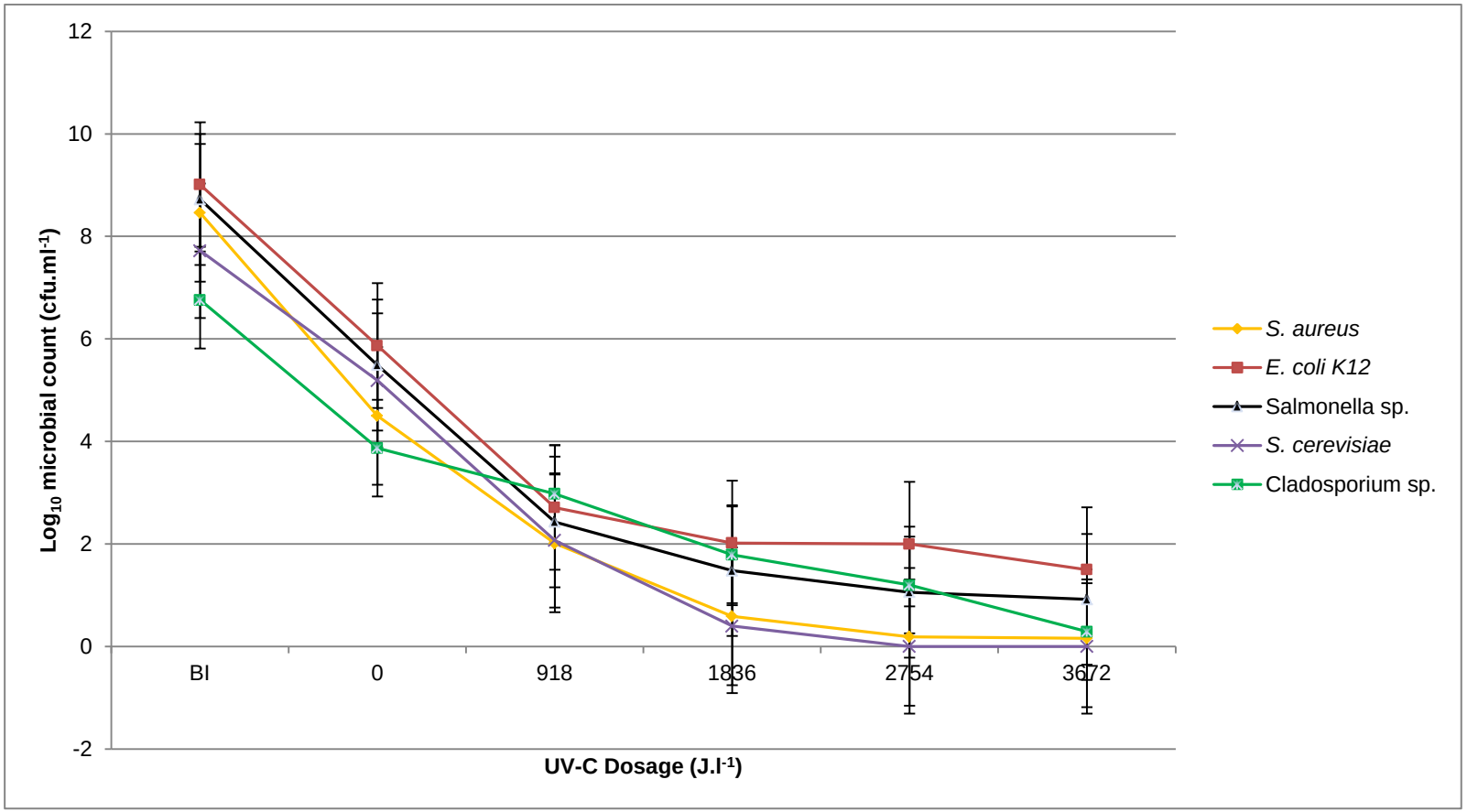

Figure 4.2: The average $\log_{10}$ microbial counts of *S. aureus*, *E. coli* K12, *Salmonella* sp., *S. cerevisiae* and *Cladosporium* sp. that were inoculated into RIT after exposure to different UV-C dosages

Table 4.4: Statistical pairwise comparison of microorganisms at different UV-C dosages

UV-C	Microorganisms	*p*-values				
		E. coli K12	*S.aureus*	*Salmonella* sp.	*S. cerevisiae*	*Cladosporium* sp.
BI*	*E. coli* K12	N/A	0.014	0.410 [a]	0.000	0.000
	S. aureus	0.014	N/A	0.728 [b]	0.001	0.000
	Salmonella sp.	0.410[a]	0.728[b]	N/A	0.000	0.000
	S. cerevisiae	0.000	0.001	0.000	N/A	0.000
	Cladosporium sp.	0.000	0.000	0.000	0.000	N/A
0**	*E. coli* K12	N/A	0.000	0.050[c]	0.001	0.000
	S. aureus	0.000	N/A	0.000	0.001	0.001
	Salmonella sp.	0.050[c]	0.000	N/A	0.180[d]	0.000
	S. cerevisiae	0.001	0.001	0.180[d]	N/A	0.000
	Cladosporium sp.	0.000	0.001	0.000	0.000	N/A
918**	*E coli* K12	N/A	0.010	0.922[e]	0.017	1.000[i]
	S. aureus	0.010	N/A	0.203[g]	1.000[i]	0.001
	Salmonella sp.	0.922[e]	0.203[g]	N/A	0.381[h]	0.044
	S. cerevisiae	0.017	1.000[f]	0.381[h]	N/A	0.001
	Cladosporium sp.	1.000[f]	0.001	0.044	0.001	N/A
1 836**	*E. coli* K12	N/A	0.001	0.357[j]	0.000	1.000[k]
	S. aureus	0.001	N/A	0.250[l]	1.000[m]	0.003
	Salmonella sp.	0.357[j]	0.250[l]	N/A	0.007	1.000[n]
	S. cerevisiae	0.000	1.000[m]	0.007	N/A	0.001
	Cladosporium sp.	1.000[k]	0.003	1.000[n]	0.001	N/A
2 754**	*E. coli* K12	N/A	0.000	0.001	0.000	0.004
	S. aureus	0.000	N/A	0.003	0.321[o]	0.001
	Salmonella sp.	0.001	0.003	N/A	0.000	1.000[p]
	S. cerevisiae	0.000	0.321[o]	0.000	N/A	0.000
	Cladosporium sp.	0.004	0.001	1.000[p]	0.000	N/A
3 672**	*E. coli* K12	N/A	0.002	0.292[q]	0.000	0.004
	S. aureus	0.002	N/A	0.077[r]	1.000[s]	1.000[t]
	Salmonella sp.	0.292[q]	0.077[r]	N/A	0.006	0.208[u]
	S. cerevisiae	0.000	1.000[s]	0.006	N/A	0.568[v]
	Cladosporium sp.	0.004	1.000[t]	0.208[u]	0.568[v]	N/A

[a-v]Average mean ± SD in the same block followed by the same letters are not significantly different (p>0.05)

* Before Inoculation

** UV Dosages ($J.l^{-1}$)

N/A Not Applicable

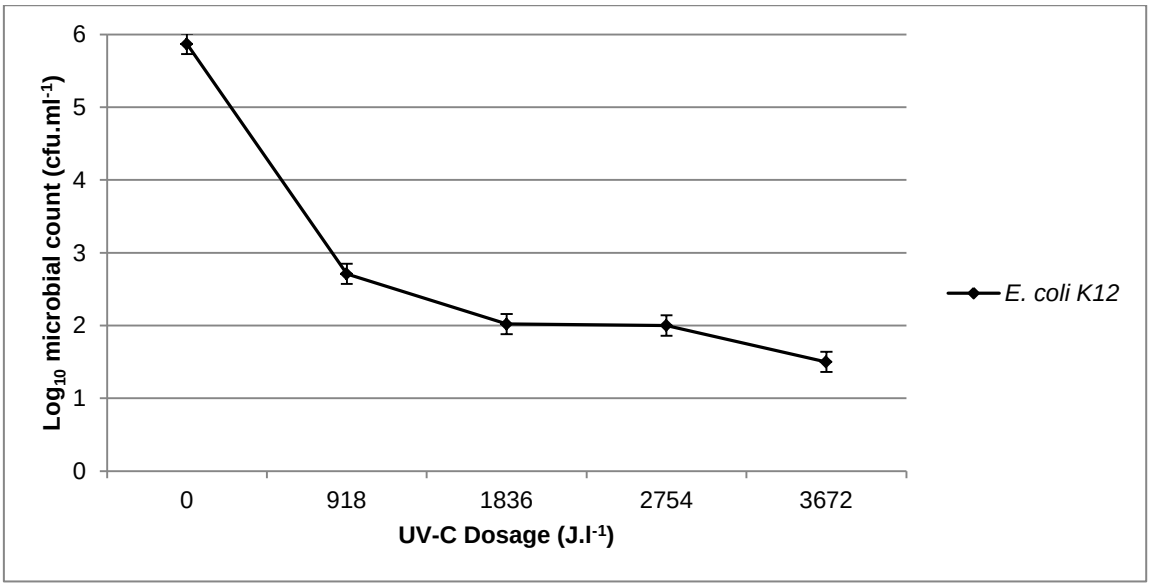

Figure 4.3: UV-C inactivation of *E. coli* K12 inoculated in RIT

Table 4.5: Effect of different UV-C dosages on *E. coli* K12

UV Dosage ($J.l^{-1}$)	Log_{10} Average mean ± SD	Log reduction (log_{10})
0	5.87 ± 0.14^{a}	NA
918	2.71 ± 0.14^{b}	3.16
1 836	2.02 ± 0.14^{bcd}	3.85
2 754	2.00 ± 0.14^{cd}	3.87
3 672	1.50 ± 0.14^{cd}	4.37

[a-d] Average mean ± SD in the same column followed by the same letters are not significantly different (p>0.05)

Escherichia coli are have been studied in UV-C investigations across many different liquid foods (Basaran *et al*., 2004; Keyser *et al*., 2008; Lu *et al*., 2010; Unluturk *et al*., 2010; Müller *et al*., 2011; Bandla *et al*., 2012; Gabriel, 2012). *Escherichia coli* are Gram-negative and rod-shaped bacteria with a size of approximately 0.5 x 1-3 micrometers (Moseley, 2012). Various strains of *E. coli*, that have been studied widely, have been found to have UV-C sensitivity varying from ranges of 2 to 12.5 $mJ.cm^{-2}$ to achieve a 1 log reduction (Koutchma, 2009). These Gram-negative bacteria are generally found to be more sensitive to UV-C as opposed to Gram-positive bacteria (Koutchma *et al*., 2009).

Unluturk *et al*. (2010) investigated the effect of UV-C on *E. coli* K12, *E. coli* O157:H7 and *Listeria innocua* in liquid egg white and found that the K12 strain of *E. coli* was more resistant as compared to the O157:H7 strain. It was found that UV-C of 26.44 $mJ.cm^{-2}$ reduced *E. coli* K12, *E. coli* O157:H7 and *L. innocua* by 0.896, 1.403 and 0.96 log_{10} respectively. The study concluded that different strains of *E. coli* exhibit different UV-C sensitivities. *Escherichia coli* K12 inoculated in grapefruit juice was treated at a UV-C dose of 19 $mJ.cm^{-2}$ and resulted in a reduction of 5 log_{10} as required and proposed by the US Food and Drug Administration to ensure microbiological safety of processed juices (Geveke & Torres, 2012). Although the effect of UV-C on the *E. coli* K12 was successful, similarly to the present study, it was less sensitive as compared to *S. cerevisiae*. Amongst other factors, the difference in the inactivation media might have had an important role to play in influencing the differing outcomes of the various studies.

Figure 4.4 shows the effect of UV-C on *Salmonella* sp. The initial log count of 5.49 was reduced by 3.06 log_{10} at the lowest UV-C dosage of 918 $J.l^{-1}$. Following UV-C dosages of 1 836 and 2 754 $J.l^{-1}$, the species was reduced by 4.01 and 4.43 log_{10}. At the highest UV-C dosage of 3 672 $J.l^{-1}$ the highest microbiological reduction of 4.57 log_{10} was obtained. Table 4.6 shows no significant difference ($p>0.05$) between UV-C treatment of 1 836 and 2 754 $J.l^{-1}$ and 3 672 $J.l^{-1}$.

UV-C inactivation studies on *Salmonella* sp. in liquid foods and beverages are extremely limited and remain unpublished. The main reason that may limit the investigations of such nature in liquid foods and beverages could be that *Salmonella* usually reside in the gastrointestinal tract and faeces of reptiles, amphibians, birds, humans and livestock (Hurd, 2004). However, most research pertaining to this is conducted in meat and fresh produce studies. Amongst the few studies available in water and liquid foods is the inactivation of *Salmonella* spp., *E. coli* and *Listeria monocytogenes* in phosphate-buffered saline (PBS) and apple juice by UV-C (Gabriel & Nakano, 2009). *Salmonella* Typhimurium showed less resistance in apple juice whilst in the PBS, *E. coli* O157:H7 and *Salmonella* enteritidis were more sensitive to the UV-C treatment. The

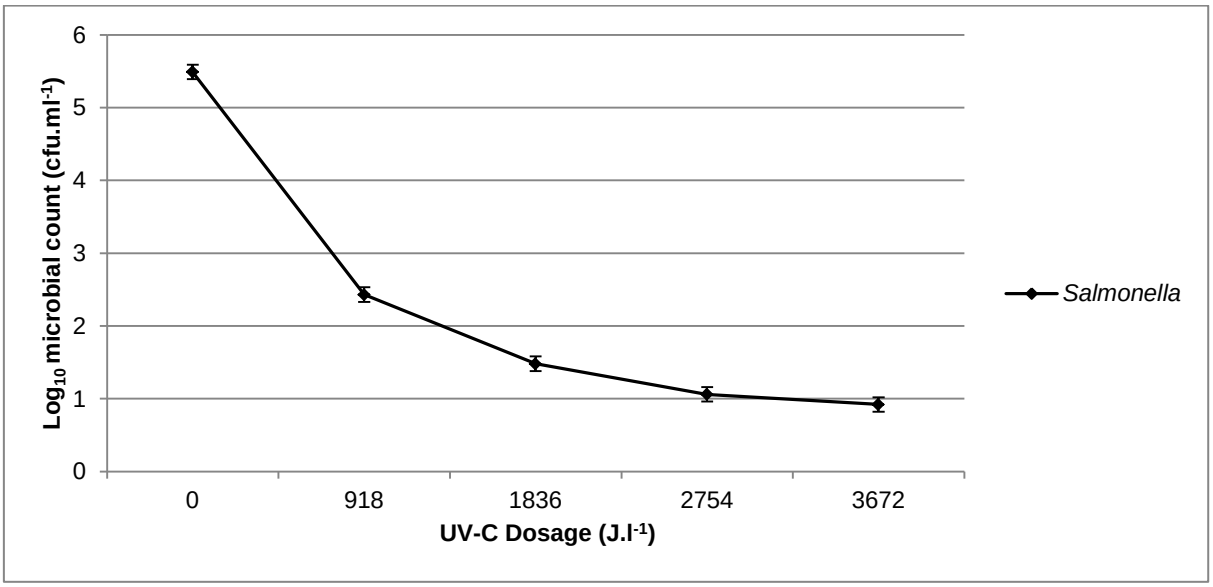

Figure 4.4: UV-C inactivation of *Salmonella* sp. inoculated in RIT

Table 4.6: Effect of UV-C on *Salmonella* sp.

UV Dosage ($J.l^{-1}$)	Log_{10} Average mean ± SD	Log reduction (log_{10})
0	5.49 ± 0.10^{a}	NA
918	2.43 ± 0.10^{b}	3.06
1 836	1.48 ± 0.10^{c}	4.01
2 754	1.06 ± 0.10^{cd}	4.43
3 672	0.92 ± 0.10^{d}	4.57

[a-d] Average mean ± SD in the same column followed by the same letters are not significantly different (p>0.05)

study was conducted using UV-C light transmitted in a biological safety unit where the inoculated mediaums were suspended in Petri dishes. Similarly, Koivunen & Heionen-Tanski (2005) investigated the use of UV-C to inactivate microorganisms that included *S.* Enteritidis, *Enterococcus faecalis* and *E. coli* in peptone water. Dosage treatments of 6 and 10 $mWs.cm^{-2}$ resulted in log reductions of 0.87 and 2.61, respectively for *S.* Enteritidis. The UV-C dose of 10 $mWs.cm^{-2}$ was however needed to reduce *E. faecalis* and *E. coli* by 1.20 and 0.55 log_{10} which clearly indicated the high sensitivity of *S.* Enteritidis to UV-C in comparison with the other microorganisms. Dizer *et al*. (1993) tested the effectiveness of UV-C against artificially seeded *Streptococcus facealis* and *S.* Enteritidis in waste-water pretreated by flocculation and filtration. A full-scale UV-C system with a flow rate of 180 $m^3.h^{-1}$ and dose 47 $mWs.cm^{-2}$ was used. The treatment of the waste- water resulted in the elimination of the *S.* Enteritidis by 4 log_{10} which showed more sensitivity than *S. faecalis*. In 1989, Lee *et al*. conducted a study on thin films (0.5, 1.0 and 2.0 mm) of chocolate inoculated with *S. eastbourne* treated with UV-C dosage of 17.1×10^5 $erg.cm^{-2}.s^{-1}$. The chocolate was treated at films of 0.1 and 0.5 mm and resulted in a complete destruction of the *S. eastbourne* after 1.5 min of exposure to UV. All the above mentioned studies indicate the degree of sensitivity of *Salmonella* to UV-C. *Salmonella* is Gram-negative and its sensitivity as seen in the mentioned studies agrees with the statement, as cited by Gailunas (2003), that Gram-negative bacteria are more sensitive to UV-C than Gram-positive bacteria (Farkas, 2001). Chang *et al*. (1985) found 0.5 cm suspension of vegetative bacteria (*E. coli*, *S. aureus*, *Shigella sonnei* and *S.* Typhi) with the exception of *S. faecalis* to have similar resistance to surface UV-C where they required about the same dose for a 3 log_{10} (99.9%) inactivation. The *S. faecalis* required about 1.4 times higher dose for 3 log_{10} units of inactivation. This indicates that different strains of microorganisms have different sensitivities to UV-C.

Figure 4.5 illustrates the resulting effect of UV-C on *S. aureus* inoculated in the RIT. The *S. aureus* had an initial log count of 4.50. After treatment with the lowest UV-C dosage of 918 $J.l^{-1}$, it resulted in a log reduction of 2.49. Upon exposure to UV-C of 1 836 $J.l^{-1}$, there was a 3.91 log_{10} reduction. A log reduction of 4.31 and a further 4.34 were attained when the dosages were increased to 2 754 and 3 672 $J.l^{-1}$ respectively. The UV-C dosage of 2 754 $J.l^{-1}$ was observed to be the optimum for the reduction of *S. aureus* since it resulted in a significant reduction of more than 4 log_{10}, although there was statistically no significant difference ($p>0.05$) between 1 836, 2 754 and 3 672 $J.l^{-1}$. Table 4.7 shows the statistical effect of UV-C on *S. aureus* inoculated in RIT.

An increase in the UV-C dosage showed a decrease in the S. *aureus* microbial load. A similar effect was seen in apple juice (Walkling-Ribeiro *et al*., 2008) where an increase in

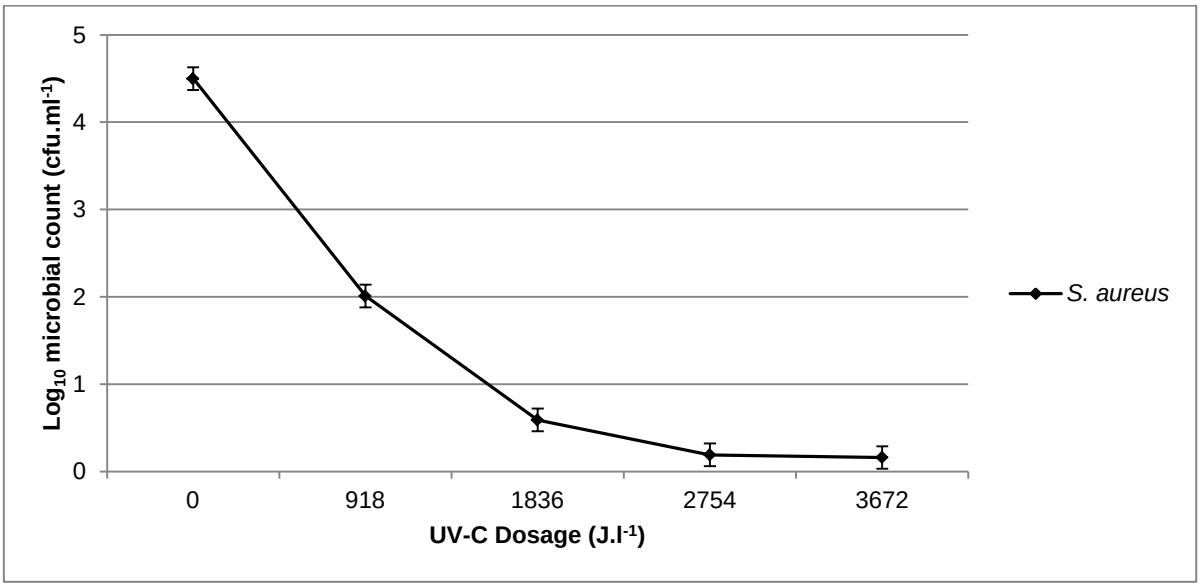

Figure 4.5: UV-C inactivation of *S. aureus* inoculated in RIT

Table 4.7: Effect of UV-C on *S. aureus*

UV Dosage ($J.l^{-1}$)	Log_{10} Average mean ± SD	Log reduction (log_{10})
0	4.50 ± 0.13^{a}	NA
918	2.01 ± 0.13^{b}	2.49
1 836	0.59 ± 0.13^{cd}	3.91
2 754	0.19 ± 0.13^{cd}	4.31
3 672	0.16 ± 0.13^{cd}	4.34

[a-d] Average mean ± SD in the same column followed by the same letters are not significantly different (p>0.05)

the UV-C treatment time showed a decrease in the microbial load of the *S. aureus*. The experiment was conducted in a laminar flow cabinet where the juice was treated in Petri dishes. Kuda *et al*. (2012) investigated the inactivation of *S.* Typhimurium and *S. aureus* in wet and dried suspensions on surface with egg residues by UV-C. The results in this case indicated no survival of cells of both microorganisms after UV-C treatment of 0.16 $mW.cm^{-2}$ for 1 min in suspensions without egg compounds. However, the suspensions with 15% albumen, 15% yolk and 30% whole egg had an effect on the absorption of the UV-C and following the treatment the log reductions were 4.7, 6.6 and 6.2 respectively for *S. aureus*. This clearly indicates that suspended solids within food have protective effects on microorganisms which results in the UV-C being less effective in their inactivation. UV-C effect on greywater was studied by Gilboa & Friedler (2008) using several microorganisms, amongst which were *S. aureus*. The *S. aureus* was found to be the most sensitive microorganism.

As already mentioned, there are a lot of factors that affect the effect of UV-C on microorganisms in liquid foods such as pH, dissolved solids, absorbance, suspended solids, temperature and wavelength (Koutchma *et al*., 2009). Different UV-C equipment were used in treating different liquids in the various studies and this makes it difficult to compare the results. The flow pattern inside the UV reactor has a great influence on the total UV-C dose applied since the position and the residence time of the microorganisms in certain regions of the irradiance can vary considerably (Koutchma, 2009). Different flow patterns of liquid in reactors can have a great influence on the success of the UV-C. Turbulent flow increases the turbulence within a UV reactor and to bring all liquid into close proximity of the UV light during treatment. Improved homogeneity of the flow when the fastest flowing particle travels 1.1-1.2 times faster than the volume averaged particle is provided by the higher flow rates that are achieved under turbulent conditions, thus theoretically, each volume of the product will be exposed to UV light due to better mixing (Koutchma, 2009). In this study, including Keyser *et al*. (2008) and Fredericks *et al*. (2011), the turbulent flow reactor was used (SurePure, Milnerton, South Africa).

Figure 4.6 demonstrates the effect of UV-C on *S. cerevisiae*. Table 4.8 indicates the statistical results thereof. An initial log count of 5.19 resulted in a log reduction of 3.12 at the lowest UV-C treatment dosage of 918 $J.l^{-1}$. A UV-C treatment of 1 836 $J.l^{-1}$ resulted in a log reduction of 4.79, where the desired log_{10} reduction was achieved. The *S. cerevisiae* was however effectively eliminated following treatment of 2 754 $J.l^{-1}$. No significant difference was noted for UV-C dosages of 1 836, 2 754 and 3 671 $J.l^{-1}$.

Yeasts have been reported to be more resistant to UV-C than bacteria (Koutchma *et al*., 2009) however, in this study the *S. cerevisiae* was the most sensitive to UV-C of all

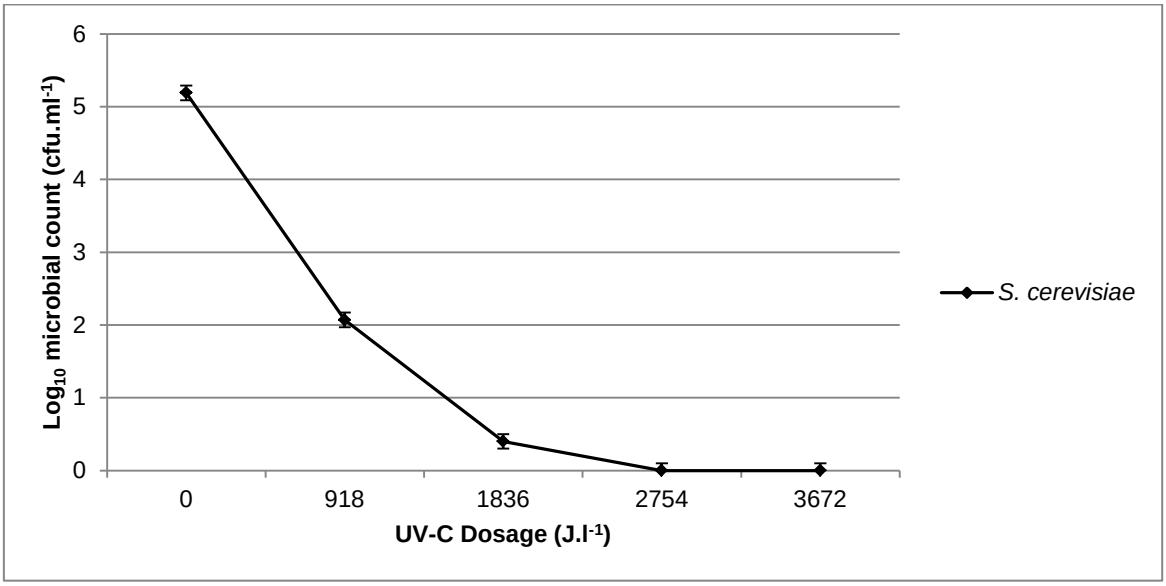

Figure 4.6: UV-C inactivation of *S. cerevisiae* inoculated in RIT

Table 4.8: Effect of different UV-C dosages on *S. cerevisiae*

UV Dosage ($J.l^{-1}$)	Log_{10} Average mean ± SD	Log reduction (log_{10})
0	5.19 ± 0.10^{a}	NA
918	2.07 ± 0.10^{b}	3.12
1 836	0.40 ± 0.10^{cd}	4.79
2 754	0.00 ± 0.10^{cd}	5.19
3 672	0.00 ± 0.10^{cd}	5.19

[a-d] Average mean ± SD in the same column followed by the same letters are not significantly different (p>0.05)

five organisms investigated as it was the only one eliminated. Yeasts have been reported to exhibit resistance due to their structural characteristics, where the yeast cells have been found to be larger in size than bacteria (Guerrero-Beltrán & Barbosa-Cánovas, 2004). Various studies have shown differing outcomes with regards to the resistance and sensitivity of *S. cerevisiae* to UV-C. The DNA has been found to have a lesser pyrimidine base. According to Müller *et al*. (2011) the arrangement of the DNA inside the nucleus which is packed around the histone proteins may have an effect on the different sensitivity of the eukaryotic yeast compared to the prokaryotic bacteria. The chemical composition and thicker cell walls are claimed to also contribute to their resistance to UV-C (Tran & Farid, 2004). Another factor that may have an effect on the yeast's sensitivity to UV-C is the genome size that has been found to be 12.5 million base pairs (mbp) (Link & Olson, 1991) whilst that of *E. coli* K12 is only 4.6 mbp (Blattner *et al*., 1997).

Saccharomyces cerevisiae ATCC 4026602 in grapefruit juice was reduced to a 6 $\log_{10}$ count following a UV-C treatment of only 14 $mJ.cm^{-2}$ (Geveke & Torres, 2012) and was found to be more sensitive than *E. coli* K12, a bacteria. However, Müller *et al*. (2011) found *S. cerevisiae* to be more resistant in comparison to other bacteria after UV-C treatment in cloudy apple juice. A treatment of 9.6 $kJ.l^{-1}$ resulted in a 4 $\log_{10}$ reduction of *S. cerevisiae* DSM 70478. Despite the fact that cloudy apple juice was used as an inactivation medium for UV-C treatment, *S. cerevisiae* was found to be the most sensitive microorganism at different flow rates. At the lowest flow rate of 2 $l.hr^{-1}$, a 3 - 4 $\log_{10}$ reduction was achieved. However at flow rates of 4 and 8 $l.hr^{-1}$, the organism was only reduced from 8 x 10^4 $cfu.ml^{-1}$ to 3 x 10^3 $cfu.ml^{-1}$ and 1 x 10^2 $cfu.ml^{-1}$, respectively (Franz *et al*., 2009). The same yeast was found to be more resistant to UV-C than other bacteria in beer (Lu *et al*., 2010). A 4 $\log_{10}$ reduction of *S. cerevisiae* could not be achieved even at the highest UV-C dose of 16.1 $mJ.cm^{-2}$. A UV-C dosage of 6.9 $mJ.cm^{-2}$ only reduced the yeast by 1 - 2 $\log_{10}$. Qui *et al*. (1998) attributed the resistance of *S. cerevisiae* to its ability to express proteins that are capable of resisting UV-C. These proteins are reported to play a role in the repairing of the DNA.

Fredericks *et al*. (2011) studied the effect of UV-C on grape juices and wines where *S. cerevisiae* in grape juice was found to be more sensitive to UV-C than other microorganisms (Fredericks *et al*., 2011). The equipment used in the latter experiment was similar to that used in the current study. The lowest UV-C dosage of 918 $J.l^{-1}$ yielded a 3.12 $\log_{10}$ reduction of *S. cerevisiae* in the current study whilst Fredericks e*t al*. (2011) reported the same dosage to have resulted in a 4.02 $\log_{10}$ reduction. This difference could be attributed to the different inoculation mediums used. Similarly, a complete elimination of the yeast by a UV-C treatment dosage of 1 377 $J.l^{-1}$ was observed (Fredericks *et al*., 2011) in grape juice although in the current study it was eliminated by

treatment with UV-C 1 836 $J.l^{-1}$.In a surface related UV-C study, Schenk *et al*. (2011) also found *S. cerevisiae* to be more sensitive to UV-C as compared to *E. coli* and *Listeria innocua* in their study. It seems that the question whether yeasts are more resistant to UV-C comparing to bacteria still needs to be researched further. However, it is imperative to note the difference in the inactivation mediums and strains used in the different studies as well as the lab-scale reactors and the pilot-scale equipments. It is clear in this study that *S. cerevisiae* can be effectively reduced after 1 836 $J.l^{-1}$ and completely eliminated at 2 754 $J.l^{-1}$. The optimum UV-C dosage for the significant reduction of *S. cerevisiae* in RIT is therefore be concluded to be 1 836 $J.l^{-1}$ due to the significant 4 $\log_{10}$ reduction noted after this treatment.

Figure 4.7 shows results for the treatment of *Cladosporium* sp. with UV-C. The minimum UV-C dosage of 918 $J.l^{-1}$ resulted in a log reduction of 0.89 from an initial $\log_{10}$ of 3.87. After treatment of 1 836 and 2 754 $J.l^{-1}$, it was reduced by 2.09 and 2.67 $\log_{10}$ respectively. The highest treatment dosage of 3 672 $J.l^{-1}$ reduced the *Cladosporium* sp. by 3.58 $\log_{10}$. Table 4.9 shows the statistical data of the Cladosporium sp following UV-C treatment. There was no significant difference ($p>0.05$) between UV-C treatments of 1 836 and 2 754 $J.l^{-1}$. Although the initial concentration was not enough to make up 4 $\log_{10}$, even the highest dosage did not eliminate the initial concentration of 3.87 $\log_{10}$.

According to Koutchma (2009) moulds have been reported to be the most resistant form of microorganisms to UV-C, followed by yeasts. In the current study, the *Cladosporium* sp. showed more resistance when compared to the yeast, *S. cerevisiae* but the yeast was contrarily the most sensitive of all other microorganisms. Initial log count for *Cladosporium* sp. was 3.87 and after the highest UV-C treatment of 3 672 $J.l^{-1}$, a log reduction of 3.58 was observed. Begum *et al*. (2009) investigated the effect of UV-C on four different fungi, *Aspergillus niger* FRR 5664, *Aspergillus flavus* FRR 5660, *Eurotium rubrum* FRR 5666 and *Penicillium corolophilum* FRR 5661 in aqueous Tween 80 and on agar plates where the spores were spread. The form of UV-C applied was surface UV where a dosage of 4 644 $J.m^{-2}$ was used. The results of this study showed that the fungal spores were more susceptible to UV-C inactivation when spread on agar plate than when suspended in liquid medium. The different inactivation media used could serve as an explanation for the resistance of the *Cladosporium* sp. in the current study. The effect of UV-C on fungi, like bacteria, varies from species to species. In another study conducted by Menetrez *et al.* (2010), different exposure times of 120, 180, 240 and 300 seconds at UV-C doses of 1.75 x 104, 2.63 x 104, 3.50 x 104 and 4.38 x 104 $\mu W.cm^{-2}$, respectively resulted in different inactivation percentages for four different species of moulds. The moulds used in this study included *Aspergillus versicolor*, *Aspergillus funigatus*, *Penicillium chrysogenum* and *Cladosporium cladosporoides*. The lowest exposure time of

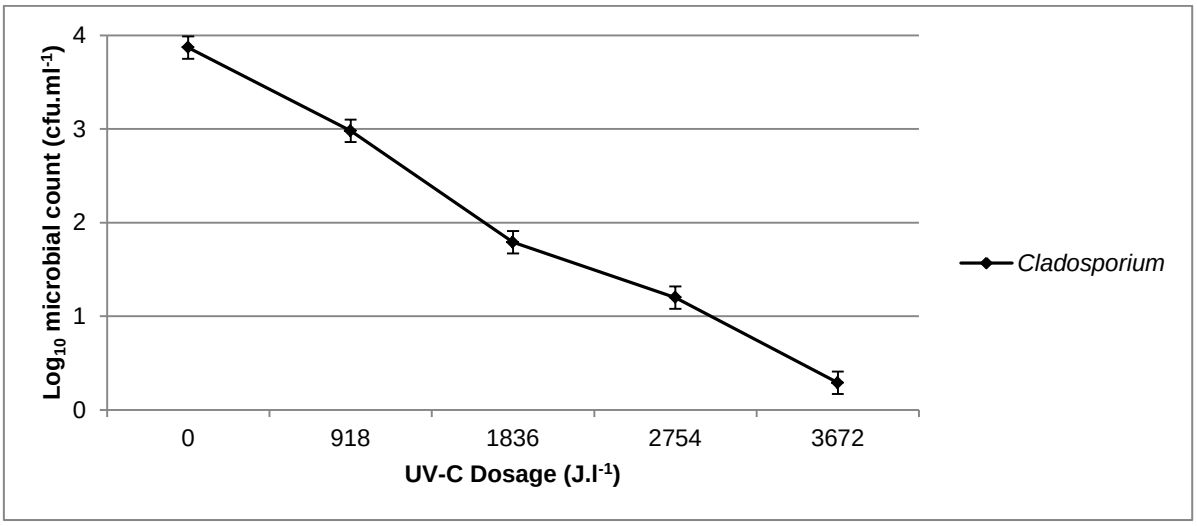

Figure 4.7: UV-C inactivation of *Cladosporium* sp. inoculated in RIT

Table 4.9: Effect of different UV-C dosages on *Cladosporium* sp.

UV Dosage ($J.l^{-1}$)	Log_{10} Average mean ± SD	Log reduction (log_{10})
0	3.87 ± 0.12^{a}	NA
918	2.98 ± 0.12^{b}	0.89
1 836	1.78 ± 0.12^{c}	2.09
2 754	1.20 ± 0.12^{c}	2.67
3 672	0.29 ± 0.12^{d}	3.58

[a-d] Average mean ± SD in the same column followed by the same letters are not significantly different (p>0.05)

120s resulted in respective inactivation of 36, 21, -2 and 8%. Amongst all four species, the *C. cladosporoides* was the most resistant to UV-C with the lowest inactivation of 23% at the highest exposure time of 300s. Some fungi have thin-walled, hyaline conidia whilst some have melanin-containing conidia, like *Cladosporium* sp. Figure 4.8 represents some of the melanin-containing *Cladosporium* sp. colonies. The melanin, which gives the olive green colour, has been found to have photoprotective properties which increase the survival and longevity of these fungal spores (Bell & Wheeler, 1986). This may be the reason for the UV-C resistant character of the *Cladosporium* sp. observed in this study. It can be concluded that the composition of the fungi together with the type of inactivation medium used had a very high contributing effect on the resistance of the *Cladosporium* sp. to UV-C. Furthermore higher UV-C dosages should be applied in order to significantly reduce or successfully eliminate the *Cladosporium* sp. As such the ideal optimum UV-C dosage for treatment of *Cladosporium* sp. in RIT is 3 754 $J.l^{-1}$.

There is currently no South African regulation on the microbiology of RIT. Microbiological specifications for RIT remain unpublished by the industry. The microorganisms tested in the current study are those associated with bulk Rooibos (Anon, 1997). Significant reduction and/or inactivation of the tested microorganisms by UV-C treatment indicate that this technology may be adopted by the RIT industry as an alternative to heat treatment. The processes involved in the production of RIT to date remain unpublished and is a property of the companies that produce it. However the ingredients involved include Rooibos tea extract, sucrose, citric acid and/or ascorbic acid. The thermal treatment and citric acid are used to inactivate the microorganisms and preserve the RIT. The application of UV-C may be beneficial in the inactivation of the spoilage microorganisms and preservation of this product and serve as a potential alternative to thermal treatment. The current shift towards the consumption of preservative-free foods may be met as the use of this technology may also reduce or eliminate the addition of citric acid in the RIT.

4.5 Conclusion

Currently there are no studies relating to the UV-C treatment of RIT as a method of microbial inactivation and preservation. The outcome of this study indicates that UV-C can inactivate and significantly reduce the microorganisms that are associated with Rooibos tea. Application of the lowest UV-C dosage of 918 $J.l^{-1}$ resulted in a >3 $\log_{10}$ reduction for S. cerevisiae, Salmonella sp. and E. coli, >2 $\log_{10}$ but for the mould which only achieved >2 $\log_{10}$ reduction following a dosage of 1 836 $J.l^{-1}$. Overall, the chosen microbiological limit of 4 $\log_{10}$ was achieved for

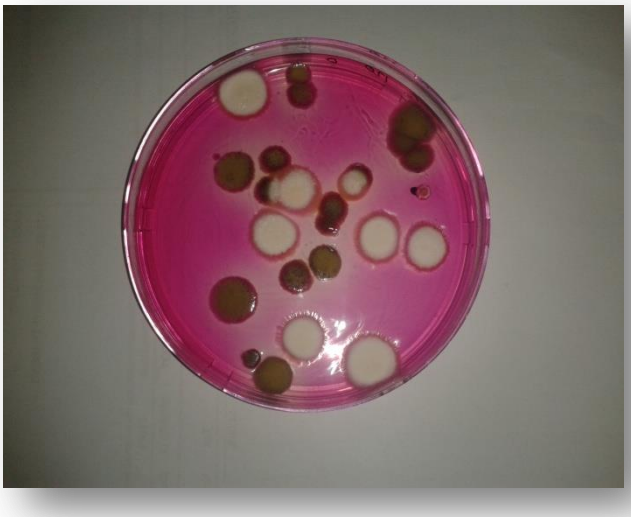

Figure 4.8: Illustration of the melanin-containing *Cladosporium spp.* denoted by the olive green moulds on Rose Bengal Chlorampenicol Agar

Salmonella sp. and *S. cerevisiae* by treatment with UV-C dosage of 1 836 $J.l^{-1}$. *S. aureus* and *E. coli* K12 were successfully reduced by treatment with UV-C dosage of 2 754 and 3 672 $J.l^{-1}$ respectively. Contrarily, the highest dosage of 3 672 $J.l^{-1}$ used could not reduce *Cladosporium* sp. significantly. Various microorganisms have different UV-C sensitivity properties. However, an increase in the UV-C dosage resulted in further reduction of the microorganisms. It is clear that higher UV-C dosages will result in better reduction of the microorganisms or even inactivation. The statistical analysis of the study indicates that various optimum UV-C dosages are applicable for the different microorganisms in RIT.

According to the results obtained, *S. cerevisiae* which is a yeast proved to be the most sensitive amongst all microorganisms. Whilst the mould, *Cladosporium* sp. was the most resistant microorganism to UV-C. The resistance of the *Cladosporium* sp. may mainly attributed to its melanin component which has been reported to have photo-protective characteristics thus protecting the DNA. On the other hand, bacteria that included *E. coli* K12, *Salmonella* sp. and *S. aureus* were significantly ($p<0.05$) reduced. The efficacy of UV-C greatly depends on a number of properties such as the physical and optical properties of the liquid. Chemical properties are capable of modifying the UV inactivation efficacy. In addition, the type of microorganisms being treated and the various strains of the same organism will yield different results. The size of the microorganism is also considered to play a role. The latter is a reflected by the results obtained in this study. Therefore the application of UV-C in RIT may need adjustment of the variables that include type of equipment, dosage, exposure time, type of lamps, wavelength and flow rate which has an effect on the turbidity. The turbulence created by turbulent flow reactors is reported to bring the entire liquid closely to the UV-C light during treatment (Koutchma, 2009). This high flow rate results in good mixing of the liquid food due to the turbulent conditions, as such exposing each volume of the liquid to the UV light (Koutchma, 2009). A modified system, SurePure Turbulator^{TM} has been recently investigated to determine its effect on the flow rate on the refreshment of fluid at the surface of UV source on milk. The results have indicated that using UV-C in combination with surface refreshment flow principle in turbulators is effective in inactivating bacteria that are associated with low UV transmittance dairy liquid foods (Alberini *et al*., 2015).

Additional studies pertaining to the effect of UV-C on RIT-associated microorganisms are essential to determine the overall effectiveness. As such determination of UV-C effectiveness on combined RIT-associated microorganisms is imperative to determine the overall optimum dosage. In addition, an investigation into various formulations of the RIT, where citric acid is added may help determine the effect of these two methods as a hurdle. In relation to the latter, the addition of ascorbic acid has been found to have enhancing properties on the effectiveness of UV-C, thus an

investigation into RIT formulation containing this compound is recommended. Lastly, pasteurisation has been found to not have any effect on the components of the RIT and perhaps a combination of the UV-C and thermal treatment may further enhance its effectiveness.

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CHAPTER FIVE
GENERAL DISCUSSION AND CONLUSIONS

Ultraviolet-C was found to be effective in the shelf life extension of RIT prepared with unfermented Rooibos extract. The chosen microbiological limit of 4 $\log_{10}$ was not reached by the two RIT formulations treated with various UV-C dosages of 918, 1 836, 2 754 and 3 672 $J.l^{-1}$. The results indicated varying effects by the different UV-C dosages, i.e. the higher the dosage, the longer the delay in microbiological growth. As such the optimum UV-C dosage was chosen to be 3 672 $J.l^{-1}$ for formulations A and B, respectively.

The UV-C treatment of RIT in order to determine its effect on shelf life of the two formulations, A and B, also indicated that the increase in shelf life was not due to the added citric acid in formulation B, which has been reported to have antimicrobial effect, proving its effectiveness. Although different, both formulations indicated a great degree of similarity in the pattern of growth of both APC and YM. Literature has attributed microbial spoilage in low acid and high sugar concentration media to YM, however, formulation A in the current study contained neither the sugar (sucrose) nor the citric acid. These findings may suggest that the spoilage of the RIT in both formulations may be due to spoilage microorganisms that are associated with Rooibos.

The pH in both formulations was not significantly affected by UV-C treatment of various dosages. The pH in formulations showed a slight gradual decrease over the duration of the shelf life. This acid production may be attributed to the growth of spoilage microorganisms, however, as already mentioned, the decrease was not found to be significant. A different observation was noted for formulation B, where the decrease was not gradual or consistent although a very slight decrease was recorded on the last day of the shelf life.

Aspalathin is the major phenolic compounds found in Rooibos together with its products, iso-orientin and orientin, has been reported to be indicative of a good quality product. There was no significant difference in the untreated and UV-C treated RIT in terms of the three phenolic compounds in both formulations. The aspalathin was however found to be slightly higher in formulation B, which may have been due to the better solubility with added citric acid. However, UV-C, as shown by other researchers, contrarily did not have an enhancing effect on the aspalathin since the untreated RIT also contained slightly increased aspalathin.

The use of UV-C treatment on the three RIT formulations resulted in a perceivable ($\Delta E^*>2$) colour change. The effect however, was not dependent on the increasing UV-C dosage in formulation B and C. Interestingly, the L* value, which is an indication of the lightness or darkness was seen to be gradually decreasing (darkness) with the increase in

UV-C dosage in all formulations. This increasing L* may be greatly attributed to the overall colour differences. The effect of the various UV-C dosages was not significantly different.

In addition, UV-C treatment indicated a significant ($p<0.05$) difference in the reduction of microorganisms that are associated with this herbal tea, which include; bacteria - *E. coli*, *S. aureus*, *Salmonella* sp., *S. cerevisiae*, a yeast and *Cladosporium* sp., a mould. Increasing UV-C dosages resulted in decreasing log counts of all the microorganisms. The microbiological reduction of the chosen of 4 $\log_{10}$ limit was achieved by the lowest UV-C dosage of 918 $J.l^{-1}$ with the exception of the mould. The highest UV-C dosage of 3 672 $J.l^{-1}$ did not effectively reduce the *Cladosporium* sp., which had an initial concentration of 3.87 $\log_{10}$. The resistance of the mould may be attributed to its melanin-containing character which literature has reported to elicit photo-protective properties on the DNA (Bell & Wheeler, 1986). On the other hand *S. cerevisiae* was completely inactivated by UV-C dosage 2 754 $J.l^{-1}$, proving to have been more sensitive to UV-C than other researchers' findings. Researchers have attributed the resistance of yeasts to UV-C to their DNA arrangement which is also protected by protein (Müller *et al*., 2011). Furthermore, the DNA has lesser pyrimidine base and a thicker cell wall (Tran & Farid, 2004). The genome size of yeasts is reported to be 12.5 mbp. The aforementioned properties of yeasts did not play a protective role on the *S. cerevisiae* in the current study. The medium of treatment has been reported to have an effect on its effectiveness, and as such, in comparison to other studies, the composition of the RIT may have contributed to the high sensitivity of the yeast. A significant difference ($p<0.05$) was recorded for all three bacteria following UV-C treatment. These results have indicated that there are various factors that may play a role during UV-C treatment as literature suggests. These may include the physical and optical properties of the liquid, the size and strain of microorganisms and the mechanical properties of the equipment used. The genome size of *E. coli* K12 has been reported to be 4.6 mbp, which may have contributed to its sensitivity towards UV-C.

The type of equipment, dosage, exposure time, type of lamps, wavelength and flow rate which has an effect on the turbulence are amongst other variables that may need to be taken into consideration during treatment of the RIT. The turbulence and good mixing that result from high flow rate (in turbulent flow reactors) have been found to bring the entire liquid closely to the UV-C light, exposing all the microorganisms.

Optimally, the investigation of UV-C effect on RIT has shown that the highest UV-C dosage of 3 672 $J.l^{-1}$ will significantly extend the shelf life of this product without compromising its phenolic components.

Recommendations

Additional studies are required in order to provide the overall functionality and benefits of UV-C of RIT. Further research is imperative on the phenolic contents and colour over the duration of the shelf life. Determining the total antioxidant activity of UV-C treated RIT will facilitate in determining whether UV-C affects the antioxidant polyphenols found in Rooibos. These studies may be further carried out using fermented Rooibos extract, which is less expensive when compared to the unfermented extract. Sensory evaluation is of utmost importance in food, the determination of this characteristic will help determine if UV-C treated RIT will be acceptable to society. It is also recommended that the effect of UV-C on a cocktail of the Rooibos-associated microorganisms be conducted in order to determine an overall optimum dosage. The latter study may also be carried out in RIT formulation that contains citric and ascorbic acids as these are sometimes constituents of commercial RIT. The dosage applied to the RIT treated does not represent the absorbed dosage, it would therefore be beneficial to conduct the same study while measuring the absorbance of RIT in order to provide more insight on the effect of UV-C on RIT. Pasteurisation has been found to not have a significant difference on the phenolic contents of RIT, therefore it may be beneficial to determine its effect in combination with UV-C as a hurdle.

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