ANTI-MICROBIAL ACTIVITY OF ROOIBOS TEA (*Aspalathus linearis*) ON FOOD SPOILAGE ORGANISMS AND POTENTIAL PATHOGENS

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously, in its entirety or in part, submitted it at any other university for a degree.

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ABSTRACT

Aspalathus linearis is an indigenous fynbos plant cultivated in the Clanwilliam area of the Western Cape, South Africa. The rooibos tea that is prepared from this plant, has become popular worldwide mainly due to the alleged health properties. Studies on the anti-microbial properties of green, black and oolong teas have shown that these teas have strong anti-microbial activity against a wide range of microbes. No studies have been done on the anti-microbial activity of rooibos tea and the aim of this study was to determine what impact rooibos tea extracts would have on the growth of different food spoilage and potential pathogenic microbes.

Water and ethyl acetate extracts of fermented and unfermented rooibos tea were used to determine the inhibitory effect on the growth of an *Escherichia coli* strain. The *E. coli* culture was grown in tea-MRS with either added fermented or unfermented rooibos tea extracts. Both the water and ethyl acetate extracts showed a strong inhibitory effect against the *E. coli* strain in that there was a decrease in the final bacterial cell density (N_{max}) (from 0.59 OD to 0.25 OD) and the maximum specific growth rate (μ_{max}) (from 1.12 h^{-1} to 0.20 h^{-1}) and an increase in the doubling time (t_d) (from 0.59 h to 1.80 h) and lag time (t_{lag}) (from 4.81 h to 6.60 h) as the concentration of the soluble solids of the tea extracts was increased from 0.5 to 5.0 $g.l^{-1}$. Furthermore, it was found that the fermented rooibos tea had a much stronger inhibitory effect (69% decrease in growth at 5.0 $g.l^{-1}$ soluble solids) compared to the unfermented rooibos tea extracts (35.1% decrease in growth at 5.0 $g.l^{-1}$ soluble solids). The resulting data indicated that rooibos tea had a very strong inhibitory effect on the growth of the *E. coli* strain. It was also found that the water extracts of rooibos tea showed a stronger inhibitory effect on the growth of the *E. coli* than the ethyl acetate extracts, indicating that the anti-microbial activity of rooibos tea is not exclusively due to the polyphenolic content – individual compounds. It was also determined that the rooibos tea water extracts showed a bacteriostatic action against the *E. coli* strain in that as soon as the tea is no longer part of the growth medium, the *E. coli* resumed a normal growth pattern. The data obtained showed that the inhibitory effect of rooibos tea water extracts (69% decrease in growth) against the growth of *E. coli* was more pronounced than that found when black tea water extracts (25.7% decrease in growth) at the same concentrations were used.

Rooibos tea water extracts (0.5 – 5.0 $g.l^{-1}$) of fermented and unfermented tea were also used to determine the inhibitory effect on other food spoilage microbes and potential pathogens. Strains of *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Streptococcus mutans, Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* were grown in the presence of fermented and unfermented rooibos tea water extracts. The effect that fermented rooibos tea had on the growth of all the microbes tested was in the following order: *Staph. aureus* (90.8% decrease in growth) > *L. monocytogenes* (89.2% decrease in growth) > *Strep. mutans* (84.1% decrease in growth) > *B. cereus* (80.3% decrease in growth) > *Sacch. cerevisiae* (77.7% decrease in growth) > *E. coli* (69.0% decrease in growth). The rooibos tea clearly had an inhibitory effect on the growth of all the microbes, with the exception of the *Z. rouxii* strain where the presence of the tea water extracts was found to enhance the growth.

The inhibitory effect of rooibos tea on the growth of these microbes was shown by changes in the growth parameters with N_{max} and μ_{max} showing decreases, while the t_d and t_{lag} increased as the concentration of the tea soluble solids was increased. As with *E. coli*, the fermented rooibos tea water extracts showed the stronger inhibitory effect on the growth of the various microbes.

The data obtained in this study suggests that rooibos tea is not effective as an anti-microbial agent against all yeast species, but will strongly retard the growth of specific Gram-positive and Gram-negative bacteria. As long as rooibos tea is present, strong anti-microbial activity will be observed at a cup of tea concentration of 2.5 $g.l^{-1}$ soluble solids. These results may be of value to support the health claims associated with rooibos tea and may in the future lead to the use of rooibos tea as a "natural" food preservative.

UITTREKSEL

Aspalathus linearis is `n inheemse fynbosplant wat gekultiveer word in die Clanwilliam area van die Wes Kaap, Suid-Afrika. Rooibostee, wat gemaak word van hierdie plante, het baie gewild geword wêreldwyd a.g.v. die gesondheidsaspekte van hierdie tee. Studies toon dat groen, swart en oolong tee sterk anti-mikrobiese aktiwiteit het teen `n wye reeks mikrobes. Aangesien daar voorheen geen studies gedoen is op die anti-mikrobiese aktiwiteit van rooibostee nie, was die doel van hierdie studie om die effek van rooibostee te bepaal op die groei van verskillende voedselbederwers en potensiële patogeniese mikrobes.

Water- en etielasetaat-ekstrakte van gefermenteerde en ongefermenteerde rooibos tee is gebruik om die inhiberende effek op die groei van *Escherichia coli* te bepaal. *Escherichia coli* is gegroei in tee-MRS met bygevoegde gefermenteerde of ongefermenteerde rooibostee-ekstrakte. Beide die water- en etielasetaat-ekstrakte van rooibostee het `n sterk inhiberende effek gewys teen *E. coli* en dit word gestaaf deur `n afname in die finale bakteriese seldigtheid (N_{max}) (vanaf 0.59 OD tot 0.25 OD) en die maksimum spesifieke groeitempo (μ_{max}) (vanaf 1.12 h^{-1} tot 0.20 h^{-1}) en `n toename in die verdubbelingstyd ($t_d$) (vanaf 0.59 h tot 1.80 h) en die sloerfase ($t_{lag}$) (vanaf 4.81 h tot 6.60 h) soos wat die konsentrasie van oplosbare vastestowwe van die tee toeneem van 0.5 tot 5.0 $g.l^{-1}$. Verder is daar gevind dat die gefermenteerde rooibostee `n baie sterker inhiberende effek het (69% afname in groei by 5.0 $g.l^{-1}$ oplosbare vastestowwe) in vergelyking met die ongefermenteerde rooibostee-ekstrakte (35.1% afname in groei by 5.0 $g.l^{-1}$ oplosbare vastestowwe). Die resultate van die data dui aan dat rooibos tee `n baie sterk inhiberende effek het op die groei van die *E. coli* spesie. Die water-ekstrakte van rooibostee het `n sterker inhibisie getoon teen die groei van *E. coli* as die etielasetaat-ekstrakte, wat aandui dat die anti-mikrobiese aktiwiteit van rooibostee nie eksklusief toegeskryf kan word aan die polifenoliese samestelling nie. Daar is ook gevind dat rooibostee water-ekstrakte `n bakteriostatiese effek het teen *E. coli*, want sodra die tee ekstrakte nie meer teenwoordig is in die groeimedium nie, hervat *E. coli* normale groei. Die data wys ook dat die inhiberende effek van rooibostee water-ekstrakte (69.0% afname in goei) teen *E. coli* baie sterker is as dié van swart tee water-ekstrakte (25.7% afname in groei) by dieselfde konsentrasies.

Rooibostee water-ekstrakte (0.5 – 5.0 $g.l^{-1}$) van gefermenteerde en ongefermenteerde rooibostee is ook gebruik om die inhiberende effek te bepaal teen ander voedselbederwers en potensiële patogene. Spesies van *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Streptococcus mutans, Saccharomyces cerevisiae* en *Zygosaccharomyces rouxii* is gegroei in die teenwoordigheid van gefermenteerde en ongefermenteerde rooibostee water-ekstrakte. Die effek wat gefermenteerde rooibostee het op die groei van die getoetste mikrobes is soos volg: *Staph. aureus* (90.8% afname in groei) > *L. monocytogenes* (89.2% afname in groei) > *Strep. mutans* (84.1% afname in groei) > *B. cereus* (80.3% afname in groei) > *Sacch. cerevisiae* (77.7% afname in groei) > *E. coli* (69.0% afname in groei). Rooibostee het `n duidelike inhiberende effek gehad teen al die organismes, behalwe teen *Z. rouxii* spesie, waar die teenwoordigheid van rooibostee die groei van die organisme bevorder het.

Die inhiberende effek van rooibostee teen die groei van hierdie mikrobes word ondersteun deur die groei parameters waar die N_{max} en μ_{max} afgeneem het terwyl die t_d en t_{lag} toegeneem het soos wat die konsentrasie van die oplosbare vastestowwe toeneem. Die gefermenteerde rooibostee water-ekstrakte het ook `n sterker inhiberende effek op die groei van die verskillende mikrobes net soos met *E. coli*.

Die data wat verkry is van hierdie studie dui aan dat rooibostee nie effektief sal wees as `n anti-mikrobiese middel teen alle gis spesies nie, maar dit sal die groei van spesifieke Gram-positiewe en Gram-negatiewe bakterië sterk vertraag. So lank as wat rooibostee teenwoordig is, sal sterk anti-mikrobiese aktiwiteit waargeneem word by `n koppie-tee konsentrasie van 2.5 $g.l^{-1}$ oplosbare vastestowwe. Hierdie resultate kan help om die gesondheidseienskappe geassosieer met rooibostee te ondersteun en help om die gebruik van rooibostee as `n "natuurlike" preserveermiddel te bevorder.

dedicated to my parents

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CONTENTS

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

CHAPTER 1

INTRODUCTION

Rooibos tea is a unique beverage and this rooibos plant is only cultivated in the Western Cape of South Africa. Processing of the *Aspalathus linearis* plant to produce rooibos tea involves a 'fermentation/oxidation' process that is triggered by cutting and bruising the plant material between rollers. The distinctive colour, aroma and flavour of rooibos tea is then released during the 'fermentation' process (Morton, 1983).

Rooibos tea, an infusion of the fermented leaves and stems, contains polyphenols, no caffeine and has a low tannin content (Rabe *et al.*, 1994). The most abundant substances in rooibos tea are the phenolics, including flavonoids and phenolic acids. The flavonoid composition of rooibos tea is unique, since it contains aspalathin, which is believed to be enzymatically oxidised to dihydro-2,3-orientin and dihydro-2,3-iso-orientin during processing (Ferreira *et al.*, 1995; Joubert & Ferreira, 1996). Another rare flavonoid is nothofagin, which together with aspalathin may be responsible for the typical natural sweet taste of rooibos tea (Rabe *et al.*, 1994; Ferreira *et al.*, 1995). Other flavonoids present in rooibos tea are iso-quercitrin, rutin, quercetin, luteolin, chrysoeriol, orientin, vitexin, iso-orientin and iso-vitexin (Rabe *et al.*, 1994; Ferreira *et al.*, 1995).

Rooibos tea has been shown to have strong antioxidant activities and it has been reported that the flavonoids present in rooibos tea may be responsible for the antioxidant activity (Joubert & Ferreira, 1996; Winterton, 1999). It was also found that unfermented rooibos tea had a stronger antioxidant activity than fermented rooibos tea (Von Gadouw, 1996, Standley *et al.*, 2001). Rooibos tea also shows strong antimutagenic activity and this is also attributed to the flavonoid composition of rooibos tea (Marnewick *et al.*, 2000; Standley *et al.*, 2001).

Many studies have been done on the anti-microbial activity of green and black teas (Toda *et al.*, 1989; Sakanaka *et al.*, 1990; Diker *et al.*, 1991; Fukai *et al.*, 1991; Diker & Hascelik, 1994; Yeo *et al.*, 1995; Yoshino *et al.*, 1996; Yam *et al.*, 1997). Catechins are reported to play a major role in the anti-microbial effect of the black and green teas, especially (-)-epicatechin gallate and (-)-

epigallocatechin gallate which showed the strongest anti-microbial activity against a wide range of microbes (Sakanaka *et al.*, 1990; Ahn *et al.*, 1991; Fukai *et al.*, 1991; Hamilton-Miller, 1995; Tezuka *et al.*, 1997). Some of the bacteria tested included the species *Bacillus cereus*, *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Clostridium perfringens* and *Pseudomonas fluorescens,* but it was found that these teas are not effective against yeasts and moulds (Toda *et al.*, 1989; Ahn *et al.*, 1991; Yeo *et al.*, 1995; Oh *et al.*, 1999). Many studies have also been done on the anti-microbial effect on cariogenic bacteria, like *Streptococcus mutans* (Sakanaka *et al.*, 1989; Yeo *et al.*, 1995; Yoshino *et al.*, 1996) and *Porphyromonas gingivalis* (Sakanaka *et al.*, 1996). It was reported that green tea strongly inhibits the growth of these cariogenic bacteria, which could be the reason why the Japanese believe that those who drink large volumes of green tea have a low incidence of tooth decay (Kubo *et al.*, 1992).

Just as it is believed that green tea is good for health, rooibos tea is also believed to possess health-giving properties and these health aspects are mainly linked to the polyphenolic compounds and the associated antioxidant activity (Niwa & Miyachi, 1986; Yoshikwa *et al.*, 1990; Von Gadow, 1996; Von Gadow *et al.*, 1997). South African consumers regard rooibos tea as beneficial to the body, improving appetite, calming digestive disorders, reducing nervous tension and promoting sound sleep (Morton, 1983). Although some studies have been done on the health aspects of rooibos tea, no research has been reported on the bacteriology of rooibos tea or the anti-microbial properties as was done with green, black and oolong teas. Selected compounds, that may be responsible for the anti-microbial activity of rooibos tea, can in the future be used as natural preservatives which, in recent years, have become more popular. The modern trend is that products carrying "*natural*" labels attract much more consumer attention than those carrying "healthy food" claims (Sloan, 2000). Therefore, these types of "natural preservatives" will probably gain popularity in the future.

The aim of this study was to determine the anti-microbial activity of water and ethyl acetate extracts of fermented and unfermented rooibos tea on the growth characteristics of related food spoilage organisms and potential pathogens.

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CHAPTER 2
LITERATURE REVIEW

A. BACKGROUND

Rooibos tea is a unique beverage that can be consumed hot or cold. It has a natural sweetish taste and a clear red-brown colour, with an orange-yellow tint. Rooibos tea is a popular beverage in South Africa and is becoming more popular world-wide (Joubert, 1994) with a 10% increase in sales from 1995 to 1997 (Anon., 1997). The tea is currently exported to many countries, including Britain, Germany, Japan, the Far East and the United States of America (Dr. E. Joubert, 2001, ARC Infruitec-Nietvoorbij, personal communication).

Rooibos tea is prepared from the leaves and stems of the rooibos tea plant (*Aspalathis linearis)*, which is a leguminous shrub, indigenous to South Africa (Morton, 1983). It is grown in the mountainous areas of the north-western Cape and is one of the major crops that are cultivated in the Clanwilliam region. The winter rainfall and the coarse sandy soil of the north-western Cape are the ideal conditions for the cultivation of *A. linearis* (Morton, 1983), which can grow up to 2 meters and has yellow flowers and slender red-brown branches (about 60 cm long), with needle-like leaves that are 2 to 6 cm long.

The rooibos tea stems and leaves are harvested mainly from mid summer to early autumn when the plants are cut with scythes or sickles and processed immediately to prevent the loss of moisture (Cheney & Scholtz, 1963). The harvested green rooibos stem and leaf bundles are then cut into 3 – 4 mm lengths and heaped on a cement drying yard in order to enzymatically and chemically "ferment". The polyphenols present in the plants are oxidized during this fermentation and this leads to the unique brick-red colour, smell and taste of rooibos tea (Joubert, 1996). The heaps are then mixed with water, approximately 10 kg of water per 35 kg of tea (Joubert, 1994), as the water is essential for efficient fermentation. This is followed by the bruising of the wet tea by running a tractor over the fermentation heaps. The bruising is done preferably in the late afternoon to allow for the fermentation of the tea during the night and the subsequent drying of the tea during the next day. Water is again added to the fermentation heaps after bruising (Cheney & Scholtz, 1963). The heaps are

turned 2 to 3 times during the night to ensure uniform oxidation of the wet tea. The recommended temperature for fermentation is about 38-42°C, and in the case of lower temperatures, the heaps are covered with jute bags (Cheney & Scholtz, 1963; Joubert, 1994). The average fermentation time of the rooibos tea is 12 to 18 h, depending on the climate, the composition of the plant material, the degree of bruising and the amount of water added to the fermentation heaps (Joubert, 1994).

As soon as the characteristic, sweet aroma develops, the fermented tea is spread open in thin layers of about 15 - 20 mm using a mechanical spreader and left to dry in the sun (Cheney & Scholtz, 1963). The spreading of the tea is done before dawn, so that on a hot day the tea is dried by noon. In the case of fog or rain, the drying can take up to 24 h or longer, resulting in a tea that lacks flavour (Cheney & Scholtz, 1963). Over-fermentation can occur when the tea stays moist, which causes the tea to smell sour. Brushing is sometimes done to increase the drying rate of the wet tea, but this can result in the formation of tea dust, which lowers the economic ratings and creates pollution (Joubert, 1994). After drying, the tea is collected (by means of a vacuum system) (Morton, 1983), sieved through 3 mm mesh sieves and bagged (Joubert, 1994). At Rooibos (Pty) Ltd., the major bulk supplier of rooibos tea, it is graded in accordance with the company's standards, steam pasteurised and again dried over an air-bed drier. Finally, the tea is weighed, packed and marketed, either in tea bags or in the loose leaf form.

In 1998, about 23 090 hectares of rooibos plantations were harvested, yielding about 7 500 tons of tea (Anon., 1998). More than 90% of this tea is processed and marketed by Rooibos (Pty) Ltd., while the rest is processed by smaller companies, including Cape Natural Tea Products, Redbush Herbal Tea Traders, Kings Products and Khoisan Products (Anon., 1998).

It is generally believed that rooibos tea possesses health giving properties and these health aspects are mainly linked to the polyphenolic compounds and the associated antioxidant activity (Niwa & Miyachi, 1986; Yoshikwa *et al.*, 1990; Von Gadow, 1996; Von Gadow *et al.*, 1997). Rooibos tea has been shown to improve the health conditions of illnesses such as insomnia, allergies, loss of appetite and nervous complaints (Morton, 1983). The tea extracts also influence dermatological diseases such as Bechet`s disease, sweet disease and photosensitive dermatitis, and also exhibit anti-viral and anti-inflammatory

properties as well as antimutagenic activities (Shindo & Kato, 1991; Standley, 1999).

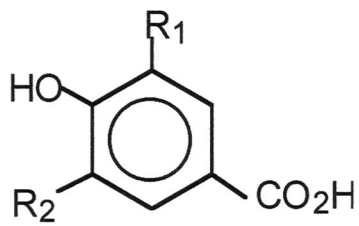
B. CHEMICAL COMPOSITION OF ROOIBOS TEA

Rooibos tea contains many different chemical substances that posses functional groups that are beneficial to our health (Morton, 1983; Rabe *et al.*, 1993; Ferreira *et al.*, 1995). The most abundant substances are the phenolics, and they can be classified into two groups, namely flavonoids and phenolic acids (Koeppen, 1970). The flavonoids found in rooibos tea can be divided into three groups, namely flavones, flavonols and the flavanones (Ferreira *et al.*, 1995). Other substances isolated from rooibos tea extracts are volatile components and minerals (Rabe *et al.*, 1994). Some of the major volatile components isolated from rooibos tea extracts include guaiacol, damascenone, geranylacetone and phenylethyl alcohol (Habu *et al.*, 1985).

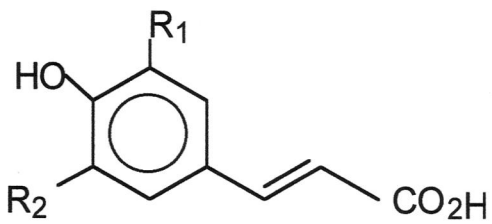
Phenolic acids that have been isolated from rooibos tea include 4-coumaric, protocatechuic, vanillic, syringic, caffeic, ferulic, cinnamic and 4-hydroxybenzoic acid (Table 1) (Rabe *et al.*, 1994; Ferreira *et al.*, 1995). All these phenolic acids present in rooibos tea show a strong anti-microbial activity (Eklund, 1985), while protocatechuic, syringic, caffeic and ferulic acid have antioxidant activities (Larson, 1988). Some of these phenolic acids also have therapeutic properties; caffeic acid has anti-ulcer and anti-mutagenic activities, while ferulic acid exhibits anti-tumor properties (Onyeneho & Hettiarachy, 1992).

Another group of phenolic substances isolated from rooibos tea is the flavonoids, which consist of a C_6-C_3-C_6 skeleton with two aromatic rings that are joined by an aliphatic three-carbon chain with hydroxyl, methoxyl or glycosyl groups attached at various positions on the carbon skeleton (Kuhnau, 1976). Flavonoids are water-soluble and occur naturally in vegetables, fruits and beverages, such as tea and wine (Kuhnau, 1976; Hollman & Arts, 2000; Tomàs-Barberàn & Clifford, 2000). The flavonoids also have important health-giving properties in that they have the potential to prevent certain forms of cancer and reduce the risk of coronary heart disease (Ruth *et al.*, 1989; Wang *et al.*, 1989).

Table 1. Phenolic acids isolated from rooibos tea (Rabe *et al.,* 1994; Ferreira *et al.*, 1995).



Phenolic carboxylic acid



Hydroxycinnamic acid

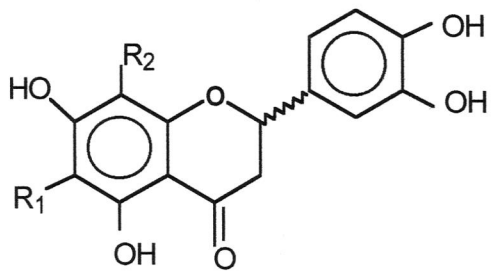
Acid	**R_1**	**R_2**
Phenolic carboxylic acid:		
4-hydroxybenzoic acid	H	H
Protocatechuic acid	OH	H
Vanillic acid	OCH_3	H
Syringic acid	OCH_3	OCH_3
Hydroxycinnamic acid:		
4-coumaric acid	H	H
Caffeic acid	OH	OH
Ferulic acid	OCH_3	H
4-hydroxy-3,5-dimethoxycinnamic acid	OCH_3	OCH_3

The flavonoid composition of rooibos tea is unique, since it contains aspalathin, a dihydrochalcone (2',3,4,4',6'-pentahydroxy-3-C-β-D-glycopyranosyldihydrochalcone) (Koeppen & Roux, 1966). Aspalathin is the main flavonoid in unprocessed tea, but during processing it is enzymatically oxidised to the flavanones, dihydro-2,3-orientin and dihydro-3,4-iso-orientin (Table 2) (Ferreira *et al.*, 1995; Joubert & Ferreira, 1996). Nothofagin, another rare β-hydroxydihydrochalcone, has also been isolated from rooibos tea, and has only previously been isolated from *Nothofagus fusca* (red beech) (Hillis & Inoue, 1976). Aspalathin and nothofagin are structurally very similar, with the only difference being the hydroxylation pattern of the B-ring (Table 3). The presence of aspalathin and nothofagin may be responsible for the natural sweet taste of rooibos tea (Rabe *et al.*, 1994; Ferreira *et al.*, 1995). Other flavonoids that are present in rooibos tea are the flavones, orientin, iso-orientin, vitexin, iso-vitexin, chrysoeriol, 8,7,4'-trihydroxy–3-methoxyflavone and luteolin (Rabe *et al.*, 1994).

The flavonols in rooibos tea include isoquercitrin, rutin and their aglycone, quercetin (Joubert & Ferreira, 1996). The most common flavonoids isolated from rooibos tea, namely the flavones and flavonols are shown in Tables 4 and 5 (Rabe *et al.*, 1994; Ferreira *et al.*, 1995). Luteolin and quercetin were among the first compounds that were isolated from rooibos tea, and it has been shown that these two flavonoids have anti-spasmodic properties (Snyckers & Salemi, 1974). Quercetin causes the prevention of the oxidation of low-density lipoproteins (LDL) and it may also have anti-antherosclerotic activity (Varma, 1986; Ferreira *et al.*, 1995). Isoquercitrin and rutin have been shown to exhibit antioxidant properties. Rutin occurs abundantly in plants and because of its pharmacodynamic properties is included in a variety of medical formulations (Herrman, 1976; Ferreira *et al.*, 1995).

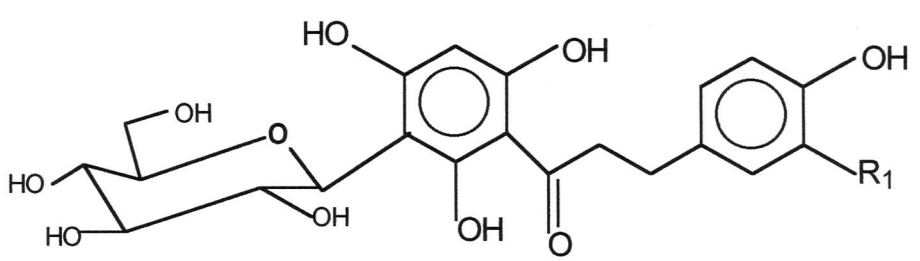
Although it is generally considered that tannins have an adverse affect on human health (Chung *et al.*, 1998), it is documented that low concentrations of tannins in the diet have a beneficial effect on human health (Petereit *et al.*, 1991). Three types of condensed-tannin metabolites have been found in rooibos tea and include (+)-catechin, procyanidin B3 and the profistinidin triflavanoid, bis-fisetinidol-

Table 2. The C-C linked flavanone glycosides found in rooibos tea (Rabe *et al.,* 1994; Ferreira *et al.*, 1995).



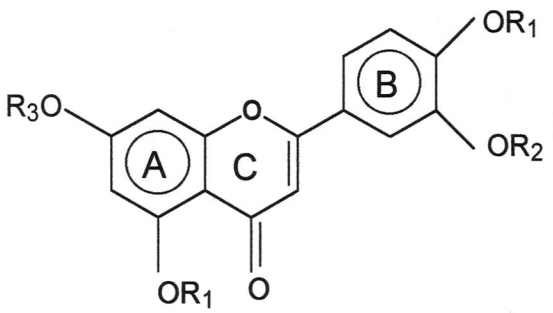
Flavanone	**R_1**	**R_2**
Dihydro-iso-orientin	C-ß-D-glucopyranosyl	H
Dihydro-orientin	H	C-ß-D-glucopyranosyl

Table 3. The C-C linked dihydrochalcone glycosides found in rooibos tea (Rabe *et al.,* 1994; Ferreira *et al.*, 1995).

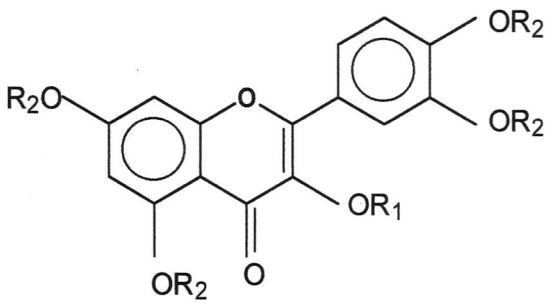


Dihydrochalcone	**R_1**
Aspalathin	OH
Nothofagin	H

Table 4. Flavonoid compounds isolated from rooibos tea (Rabe *et al.*, 1994; Ferreira *et al.*, 1995).



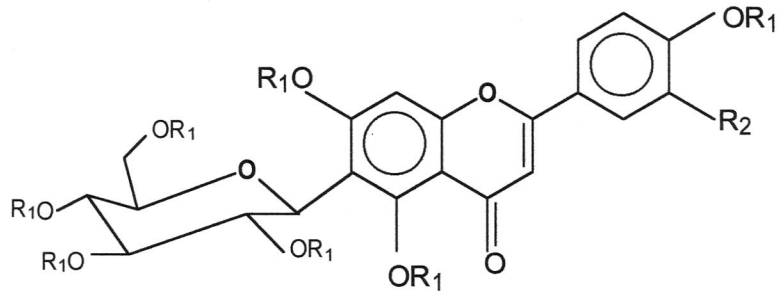
Flavone



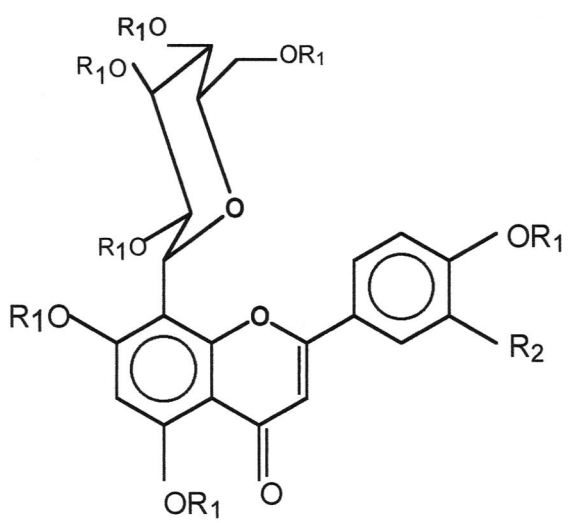
Flavonol

Flavonoid	**R_1**	**R_2**	**R_3**
Flavone:			
Luteolin	H	H	H
Chrysoeriol	H	CH_3	H
Luteolin-7-O-ß-D-alucopyranoside	H	H	β-D-glucopyranosyl
Flavonol:			
Quercetin	H	H	
Isoquercitrin	ß-D-glucopyranosyl	H	
Rutin	Rutinosyl	H	

Table 5. The C-C linked flavone glycosides found in rooibos tea (Rabe *et al.*, 1994; Ferreira *et al.*, 1995).



Flavone	**R_1**	**R_2**
Iso-orientin	H	OH
Iso-vitexin	H	H



Flavone	**R_1**	**R_2**
Orientin	H	OH
Vitexin	H	H

(4β,b:4βB,8)-catechin (Table 6). However, these compounds are found in very low concentrations, thus resulting in the rooibos tea beverage having a low tannin content (Ferreira *et al.*, 1995).

The non-phenolic metabolites from rooibos tea include the inositol, (+)-pinitol, the nucleoside, uridine, and a phenylpyruvic acid-O-β-D-glucopyranosyl derivative (Table 6) (Ferreira *et al.*, 1995). The inositols are claimed to have anti-viral properties and they play an important role in cellular communication (Petereit *et al.*, 1991). The phenylpyruvic acid plays a key role in the biosynthesis of the C_6-C_3-C_6 backbone of flavonoids and it is used for the prevention of dermatological diseases (Ferreira *et al.*, 1995).

C. ANTIOXIDANT ACTIVITY OF ROOIBOS TEA

Antioxidants are "substances capable of delaying, retarding or preventing the oxidation process" (Schuler, 1990). In recent years, interest in antioxidants has increased because an imbalance in the oxidative levels may be an important factor in causing diseases, such as atherosclerosis, arthritis, heart disease, Alzheimer's disease and cancer (Halliwell *et al.*, 1992; Kinsella *et al.*, 1993; MacCord, 1994; Frei, 1995). Natural antioxidants have received much attention for their use as inhibitors of lipid peroxidation or for the protection of the damage caused by free radicals (Yen *et al.*, 1997). Rooibos tea extracts have a protective action against H_2O_2-induced tissue (Ito *et al.*, 1991), an inhibitory affect on X-ray induced damage (Komatsu *et al.,* 1994) and scavenging effects on superoxide radicals ($O_2^{\bullet}$) (Yoshikwa *et al.*, 1990). Fermented rooibos tea contains less antioxidant activity than green and black teas, but through manipulating preparation procedures the antioxidant activity of rooibos tea can be increased (Von Gadow, 1996; Von Gadow *et al.*, 1997). During normal aerobic respiration superoxide radicals are formed which are usually removed by superoxide dismutase, an enzyme responsible for catalyzing superoxide to hydrogen peroxide (H_2O_2). The hydrogen peroxide is rapidly removed by glutathione peroxidase, since it causes damage in biological systems (Bowler *et al.*, 1992). The superoxide formed during respiration, as well as the hydrogen peroxide can

Table 6. Some of the minor compounds found in rooibos tea (Rabe *et al.*, 1994; Ferreira *et al.*, 1995).

Compound
C-C linked chromone glycoside:
Chromone
Condensed tannin-type compounds:
Catechin
Procyanidin B_3
Bis-fisetinidol-(4ß,6:4ßB,8)-catechin
Non-phenolic metabolites:
(+)-pinitol
Uridine
Phenylpyruvic acid-O-ß-D-glucopyanosyl

form hydroxyl radicals ($OH^{\bullet}$) in the presence of metal ions (e.g. iron) through the Fenton and Haber-Weiss reactions (Namiki,1990). A hydrogen atom is required for the stabilisation of the hydroxyl radical and in biological systems this is often removed from various parts of the cell, causing lipid peroxidation, protein denaturation and DNA mutation (Imlay & Linn, 1988; Halliwell & Aruoma, 1991; Halliwell *et al.*, 1995). Under specific circumstances, for example, high levels of air pollution, tobacco smoke, pesticides, certain drugs, alcohol and transition metals, more damaging free radicals are formed (Thomas, 1995). Biological systems are protected against these oxidative substances by antioxidant enzymes and antioxidant nutrients, including vitamin A, C and E (Reinton & Rogstad, 1981; Larson, 1988; Halliwell *et al.*, 1995). The radical defence mechanisms can be weakened as a result of ageing and inadequate nutrition, resulting in oxidative stress and in severe cases it can cause cell damage and death (Halliwell *et al.*, 1995).

Antioxidants can generally be classified into two major groups (Gordon, 1990). The first group is the primary, chain-breaking antioxidants which react with lipid radicals to form stable products by the rapid donation of hydrogen atoms. This group of antioxidants also have the ability to bind active oxygen species (Gordon, 1990). The second group of antioxidants includes the secondary antioxidants. These compounds slow down the rate of lipid peroxidation through mechanisms such as the chelation of metals, scavenging of oxygen, quenching of singlet oxygen and the reduction of hydroperoxides to non-radical species (Gordon, 1990).

One group of phytochemicals, the polyphenols, and specifically the flavonoids, has recently received scientific interest (Hollman & Arts, 2000; Tomàs-Barberàn & Clifford, 2000). Flavonoids have been reported to exhibit potent primary antioxidant characteristics because of the scavenging of superoxide, hydroxyl radicals and peroxyl radicals. Secondary antioxidant activity is also displayed in the quenching of singlet oxygen, as well as in the metal chelating ability (Hudson & Lewis, 1983; Hussain *et al.*, 1987). Flavonoids occur naturally in plants and leafy materials, such as teas and these are a rich source of flavonoids and phenolic acids (Von Gadow *et al.*, 1996).

During the processing of rooibos tea the percentage of polyphenols decreases because of oxidation (Joubert, 1996). Aspalathin is initially converted

to dihydro-2,3-orientin and dihydro-3,4-iso-orientin during the processing (Koeppen & Roux, 1966) and only about 7% of the original aspalathin concentration is still present after oxidation (Joubert, 1996). It has been claimed that aspalathin has the potential to scavenge active oxygen species, as well as the products that are formed from the oxidation process (Ferreira *et al.*, 1996)

Several of the other flavonoids found in rooibos tea display antioxidant activity, including quercetin, luteolin, rutin, isoquercitrin and iso-vitexin (Namiki, 1990; Winterton, 1999). The ene-diol functionality in the electron rich, aromatic β-ring system could supply the electrons required for the reduction of the active oxygen species and these ene-diol functionality is present in luteolin, quercetin, rutin, isoquercitrin, orientin, iso-orientin, 2,3-dihydro-orientin, 2,3-dihydro-iso-orientin and aspalathin (Hussain *et al.*, 1987; Ramarathnam *et al.*, 1989; Haraguchi *et al.*, 1992; Igile *et al.*, 1994; Joubert & Ferreira, 1996).

The antioxidant activity of fermented and unfermented rooibos tea was recently compared and the results indicated that unfermented tea has an inhibition of 86.6%, while fermented tea has an inhibition of 83.4%, based on the scavenging of the DPPH radical by compounds in the tea. The higher inhibition of the unfermented tea is probably due to the fact that aspalathin is an active scavenger of DPPH (Von Gadow, 1996) and the major flavonoid of unfermented rooibos tea (Ferreira *et al.*, 1995). In Table 7 the antioxidant activity of different teas are given as a measure of their DPPH radical scavenging ability.

D. ANTI-MICROBIAL ACTIVITY

Background

An anti-microbial agent is a chemical or substance, either synthetic or natural (Brock *et al.*, 1994), that retards microbial growth or even kills micro-organisms (Pelczar *et al.*, 1993; Brock *et al.*, 1994). More specifically, there are anti-bacterial, anti-viral, anti-fungal and anti-protozoan agents depending on the type of micro organism affected by the anti-microbial agent (Pelczar *et al.*, 1994) as anti-microbial agents can vary in their selective toxicity. Certain of these chemicals act

Table 7. Antioxidant activity of different teas as assessed with the DPPH radical scavenging methods (Von Gadouw *et al.*, 1997).

Type of tea	Inhibition (%)
Green	90.8
Oolong	71.2
Black	81.7
Unfermented rooibos	86.6
Semi-fermented rooibos	81.9
Fermented rooibos	83.4

in a non-selective manner and have similar effects on all types of cells. The action of anti-microbial agents can either be highly selective or even more toxic to a specific microbial species. The action spectrum of each anti-microbial agent is, therefore, different (Brock *et al.*, 1994).

Anti-microbial agents affect the growth of microbes in a variety of ways and when an anti-bacterial agent is added to an exponentially growing bacterial culture, the effect may be either bacteriostatic, bactericidal or bacteriolytic (Brock *et al.*, 1994). A bacteriostatic effect is observed when growth is inhibited, but the microbes are still viable as illustrated in Figure 1. As soon as the anti-microbial agent is removed or its activity neutralised the organism present can, under favourable conditions, re-initiate growth (Brock *et al.*, 1994). Bactericidal agents in contact kill cells, but lysis or cell rupture does not occur (Fig. 2). Bacteriolytic agents induce cell death by lysis, which is observed as a decrease in the cell numbers or even in an increased turbidity after the agent has been added (Fig. 3) (Brock *et al.*, 1994; Gustafson *et al.*, 1998).

Some anti-microbial agents damage microbial cells by altering the normal selective permeability of the cytoplasmic membrane and the cell wall, causing leakage of vital intracellular substances (Pelczar *et al.*, 1993). Protein denaturation also occurs when the permeability of the membranes are influenced, resulting in the inactivation of the proteins and enzymes involved in the normal functioning of the cell (Pelczar *et al.*, 1993).

Determination of anti-microbial activity

Anti-microbial activity is generally measured by determining the smallest amount of agent needed to inhibit the growth of a test organism and this is referred to as the minimum inhibitory concentration (MIC) (Pelczar *et al.*, 1993; Brock *et al.*, 1994). One method of determining the MIC of an anti-microbial agent is the tube dilution technique (Kim *et al.*, 1995; Sakanaka *et al.*, 1996) where a series of culture tubes are prepared, each containing medium with a different concentration of the anti-microbial agent. All the tubes are inoculated with the same microbial species and incubated at a suitable temperature. After incubation, the tubes

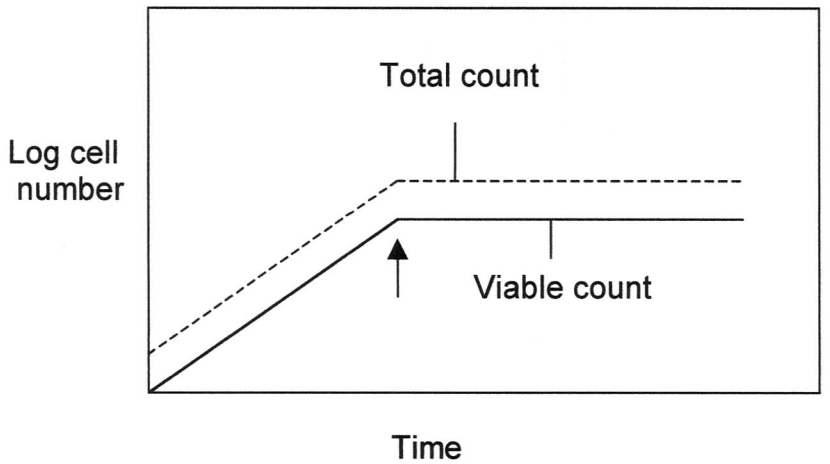


Figure 1. The bacteriostatic action of an anti-bacterial agent (Brock *et al.*, 1994).

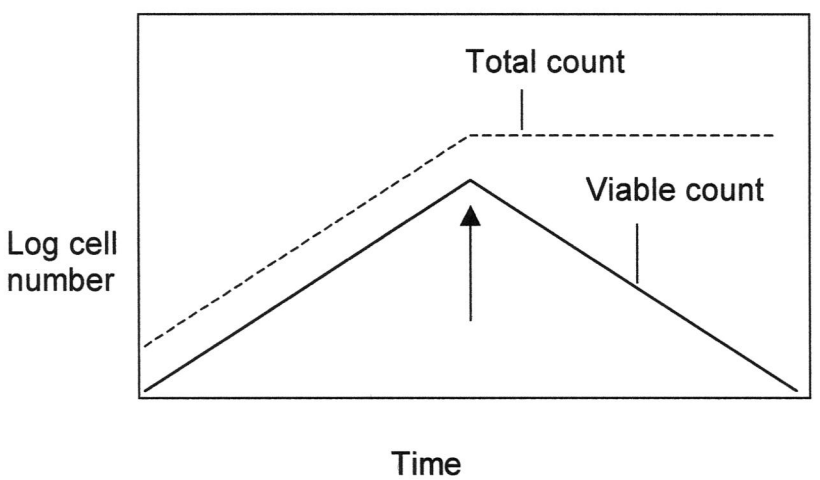


Figure 2. The bactericidal action of an anti-bacterial agent (Brock *et al.*, 1994).

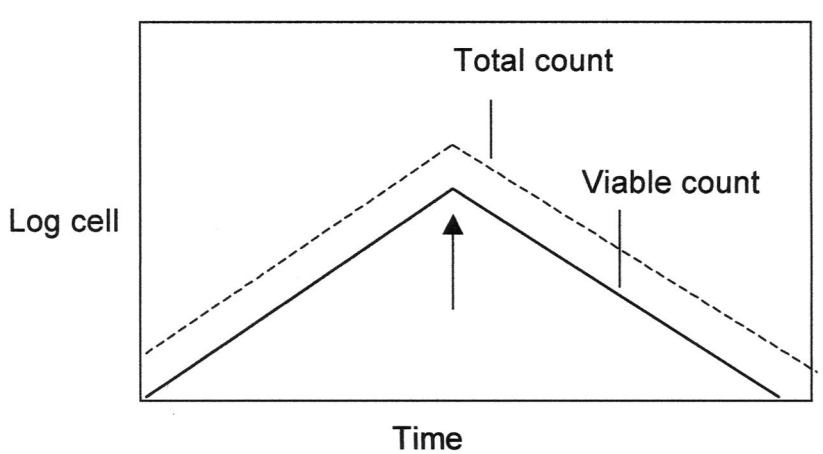


Figure 3. The bacteriolytic action of an anti-bacterial agent (Brock *et al.*, 1994).

where no growth has occurred (indicated by absence of visible turbidity) is noted and this concentration where no growth occurs, is referred to as the MIC. Another procedure for studying anti-microbial action is the agar diffusion method (De Pooter *et al.*, 1995). This is done by preparing a petri dish containing an agar medium that has been evenly inoculated with the test organism. A known amount of the anti-microbial agent is added to filter paper discs, which are then placed on the surface of the agar. The zone of inhibition is measured and the size of the diameter is proportional to the concentration of anti-microbial agent added to the disc and the MIC is calculated.

Anti-microbial compounds

Many naturally occurring compounds such as those found in edible and medicinal plants, herbs and spices have been shown to posses anti-microbial characteristics and could serve as a source of anti-microbial agents against food pathogens and spoilage organisms (Kim *et al.*, 1995; Hammer *et al.*, 1999a). The anti-microbial activity of essential oil extracts from herbs, spices and plants are also well recognised (Shapiro *et al.*, 1994; Kim *et al.*, 1995; De Pooter *et al.*, 1995; Bagki & Dïgrak, 1996; Hammer *et al.*, 1999a). Essential oil compounds are membrane active and attack the cytoplasmic membrane of the microbes, releasing the intracellular constituents (Cox *et al.*, 1998; Gustafson *et al.*, 1998; Cox *et al.*, 2000), resulting in a bactericidal or bacteriostatic action (Nychass & Tassau, 2000). Essential oils have activity against different Gram-positive and Gram-negative bacterial species, yeasts and mycelial fungi (Shapiro *et al.*, 1994; Cerutti & Alzamora, 1996; Carson *et al.*, 1998; Hammer *et al.*, 1999a; Cox *et al.,* 2000). Essential oils of almond, basil, bay, cinnamon, clove, coriander, grapefruit, onion, oregano, pepper, rosemary, sage, spearmint, tea tree and thyme have been tested for anti-microbial activity (Carson *et al.*, 1998; Hammer *et al.,* 1999a; Hammer *et al.,* 1999b; Harkenthal *et al.*, 1999; Marino *et al.*, 1999). Clove was found to have the strongest anti-fungal activity, while thyme and oregano showed strong activity against *Aspergillus niger* and *Penicillium chrysogenum* (Marino *et al.*, 1999; Nychas & Tassau, 2000).

Some known compounds that are effective anti-microbial agents are thymol from thyme and oregano, cinnamic aldehyde from cinnamon, euganol from cloves (Dorman & Deans, 2000; Nychas & Tassau, 2000) and terpinen–4–ol, α -

terpined and linalool from tea tree oil (Shapiro *et al.*, 1994; Carson & Riley, 1995; Harkenthal *et al.*, 1999). It has also been reported that essential oils have anti-bacterial activity against foodborne pathogens. Cinnamon, clove, garlic, onion, oregano and thyme were some of the essential oils that showed inhibitory activity against pathogens like *Escherichia coli, Listeria monocytogenes, Salmonella thyphimurium, Staphylococcus aureus* and industrial yeasts, like *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii* (Kim *et al.*, 1995; Cerutti & Alzamora, 1996; Hammer *et al.*, 1999a; Marino *et al.*, 1999).

Other compounds that show anti-microbial activity are proteins of low molecular weight and they are generally known as bacteriocins (Dillon, 2000). Certain strains of *Lactococcus lactis* produce a bacteriocin, nisin (Rodriguez, 1996), which is non-toxic and exhibits inhibition against a broad range of Gram-positive organisms such as *Clostridium* and *Listeria* species (Rodriguez, 1996; Dillon, 2000). This compound acts on the cytoplasmic membrane of the bacterial cell, binding to it and then causing pores in the membrane which results in the dissipation of the membrane potential and the pH gradients (Rodriguez, 1996; Nilsson *et al.,* 2000). Nisin is generally used as a preservative because of its strong anti-microbial properties and its heat stability (Dillon, 2000). In combination with other substances like carvacrol (Pol & Smid, 1999) and CO_2 (Nilsson *et al.*, 2000), nisin has a synergistic action on bacteria like *Bacillus cereus* and *Lysteria monocytogenes* (Pol & Smid, 1999; Nilsson *et al.*, 2000). Sublethal ultra high pressure and reduced temperatures, with combinations of nisin, show a better anti-microbial effect against bacteria and yeasts like *Lactobacillus plantarum, E. coli* and *S. cerevisiae* (Ter Steeg *et al.*, 1999). In kimchi, a fermented vegetable dish, nisin is used as a selective compound in the control of the Lactobacilli that are responsible for the over-ripening of kimchi (Choi & Park, 2000). Brochocin-C, a two-peptide bacteriocin that is produced by *Brochothrix campestris,* is an example of a bacteriocin that has anti-bacterial activity against a broad spectrum of Gram-positive bacteria (Gao *et al.*, 1999). Over the last few years the interest in bacteriocins have increased and many anti-microbial studies have been done on a wide range of bacteriocins against foodborne microbes (Muriana, 1996).

Organic acids are also used to inhibit certain bacteria that causes food spoilage (Bui & Cooper, 1987; Surekha & Reddy, 2000). Many of these acids are produced by starter cultures during the fermentation process and the drop in pH,

Table 8. Anti-microbial agents used in food packaging (Han, 2000).

Organic acid	Potassium sorbate, Calcium sorbate, Propionic sorbate, Acetic acid, Benzoic acid, Sodium benzoate, Sorbic acid
Fungicide/bacteriocin	Benomyl, Imazalil, Nisin (peptide)
Peptide/protein/enzyme	Lysozyme, Glucose oxidase, Alcohol oxidase
Alcohol/thiol	Ethanol, Hinokithiol.
Oxygen absorber/antioxidant	Reduced iron complex, butylated hydroxy toluene (BHT)
Gas	Carbon dioxide (CO_2), Sulphur dioxide (SO_2)
Other	UV irradiation, Silver zeolite, Grapefruit seed extract

as a result of the fermentation process, prevents the growth of Gram-positive and Gram-negative bacteria (Dillon, 2000; Garotte *et al.*, 2000). Organic acids, like benzoic acid and propionic acid can be used to inhibit certain bacteria that causes food spoilage (Eklund, 1985) and many of the organic acids are also used in anti-microbial food packaging (Han, 2000). The major anti-microbial agents used in the food industry are listed in Table 8.

E. ANTI-MICROBIAL ACTIVITY OF TEA

Tea, which is the most widely consumed beverage in the world and is just an infusion of processed leaves from the plant, *Camellia sinensis* (Stagg, 1980). Green tea differs from black tea in that with this black tea an oxidation step, also generally referred to as fermentation, is arrested (Hamilton-Miller, 1995). Although tea has little nutritional value it is very refreshing (Hamilton-Miller, 1995) and is also known to have physiological and pharmacological effects that is beneficial to our health (Stagg & Millin, 1995).

Many studies have been undertaken on the anti-bacterial effects of green, oolong and black teas (Toda *et al.*, 1989; Sakanaka *et al.*, 1990; Diker *et al.*, 1991; Fukai *et al.*, 1991; Diker & Hascelik, 1994; Yeo *et al.*, 1995; Yoshino *et al.*, 1996; Yam *et al.*, 1997). These studies have been done using a wide range of micro organisms with different types of extracts of green, black and oolong teas. It was found that these extracts showed a strong anti-microbial activity against Gram-positive and Gram-negative bacteria including members of the species of *Bacillus cereus*, *S. aureus, E. coli, Salmonella typhimurium, Clostridium perfingens* and *Pseudomonas fluorescens,* but were found not to be effective against yeasts and moulds (Toda *et al.*, 1989; Ahn *et al.*, 1991; Yeo *et al.*, 1995; Oh *et al.*, 1999). Many studies have also been done on the anti-microbial effect on cariogenic bacteria, like *Streptococcus mutans* (Sakanaka *et al.*, 1989; Yeo *et al.*, 1995; Yoshino *et al.*, 1996) and *Porphyromonas gingivalis* (Sakanaka *et al.*, 1996). It was reported that green tea strongly inhibits the growth of these cariogenic bacteria, which could be the reason why the Japanese believe that those who drink large volumes of green tea have a low incidence of tooth decay (Kubo *et al.*, 1992).

The chemical composition of tea is complex (Hamilton-Miller, 1995) and although black tea has many more components than green tea, partly due to the oxidation process, much interest has been shown in the polyphenolic composition of tea (Hamilton-Miller, 1995). The simplest compounds of the polyphenolic class are the catechins, which mainly consists of four compounds, (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG) (Hamilton-Miller, 1995). It is these polyphenolic fractions, and specifically the purified catechin fractions from green and black teas, that inhibit the growth of many bacterial species (Sakanaka *et al.*, 1989; Sakanaka *et al.*, 1990; Sakanaka *et al.*, 1996; Mabe *et al.*, 1999; Hara, 2000). Tea catechins have also been found to show inhibitory effects against phytopathogenic bacteria such as *Erwinia, Pseudomonas, Clavibacter, Xanthomonas* and *Agrobacterium* and results have shown that EGC and EGCG are more effective than EC and ECG (Fukai *et al.*, 1991). The microbial inhibition of green and black tea are, therefore, mainly as a result of the catechins EGC and EGCG at 'cup-of-tea' concentrations (Sakanaka *et al.*, 1990; Ahn *et al.*, 1991; Fukai *et al.*, 1991; Hamilton-Miller, 1995; Tezuka *et al.*, 1997). Tea catechins at an MIC value of 32 $\mu g.ml^{-1}$ are also able to inhibit the growth of *Helicobacter pylori*, which causes chronic gastritis and gastric cancer (Diker & Hascelik, 1994; Mabe *et al.*, 1999), *Campylobacter jejuni* (Diker *et al.*, 1991), which causes enteric infections and several *Clostridium* species. (Ahn *et al.*, 1991). It has also been reported that small amounts of flavonols, such as quercetin, kaempferol and myricetin present in tea, have anti-microbial activity against Gram-positive bacteria and phytopathogenic fungi and it was determined that quercetin had a MIC value of 37 $\mu g.ml^{-1}$ against *S. aureus* (Hamilton-Miller, 1995).

Tannins have been shown to inhibit the growth of many filamentous fungi (Scalbert, 1991). The MIC in this case is usually higher than 0.5 $g.l^{-1}$ for *Fomus annosus* and often reaches 10 – 20 $g.l^{-1}$ for *Merulius lacrymans* and *Penicillium* species. Yeasts appear to be more resistant and some species are inhibited at a tannic level of 25 $g.l^{-1}$, where as others require levels as high as 125 $g.l^{-1}$. The MIC for bacteria is usually lower and can vary between 0.012 and 1.0 $g.l^{-1}$ (Scalbert, 1991; Chung *et al.*, 1998). The tannins that are toxic to foodborne bacteria such as *E. coli, L. monocytogenes, Proteus vulgaris, Salmonella paratyphy, S. aureus, Streptococcus faecalis* and *Yersinia enterocolitica*, are

tannic acid and propyl gallate, while in contrast, gallic acid shows no anti-bacterial activity (Chung *et al.*, 1998). These compounds have also been shown to be active against fungi (Scalbert, 1991; Chung *et al.*, 1998). Evaluations have also been performed with several tannins and related compounds such as the A-type compounds and the 5 – deoxy compounds. The A-type compounds' A-ring is similar, but when hydroxylation structural variations occur on the A-ring, it is known as the B-types and 5-deoxy analogs (Chung *et al.*, 1998; Kolodziej *et al.*, 1999). Some of these compounds showed only moderate anti-bacterial activity against *Bacillus subtilus, S. aureus, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and *Candida albicans* with most of the MIC values between 1 000 and 2 000 $\mu g.ml^{-1}$ (Kolodziej *et al.*, 1999). It must be stressed that tea tannins are not harmful to humans and that tea does not contain tannic acid (Stagg, 1980).

Volatile flavour compounds play an important role in the taste of black and green teas (Hamilton-Miller, 1995). It was also reported that these compounds show anti-bacterial activity, but not at a 'cup-of-tea' concentration against bacteria like *B. subtilis, Staph. aureus, Strep. mutans, E. coli* and *Saccharomyces cerevisiae* (Kubo *et al.*, 1992). The total activity of a cup of green tea was reported to be enough to control *Strep. mutans*, but the volatile components alone do not appear to be potent enough (Kubo *et al.*, 1992). The flavour components of green tea in combination with each other, and indole, enhances the anti-bacterial activity of these components (Muroi & Kubo, 1993). It was reported that 6.25 $\mu g.ml^{-1}$ δ-cadinene combined with 400 $\mu g.ml^{-1}$ indole was bactericidal against *Strep. mutans* (Muroi & Kubo, 1993). In addition to its anti-bacterial activity, the green tea flavour compounds also show anti-fungal activity and this may contribute to the fact that these compounds can be used as anti-microbial agents for cosmetic and food products (Kubo *et al.*, 1992).

F. CONCLUSIONS

The growing demand for safe and nutritious food has necessitated the improvement of the quality of food. As a result of large population increases, especially in developing countries, it is also becoming more essential to prevent spoilage caused by the activity of microbes. In recent years, the demand for

natural ingredients as food preservatives have been emphasized. Natural anti-microbial agents have been isolated from essential oils, plant tannins and green tea as well as black tea where the most abundant anti-microbial substances in these products are the polyphenols. Polyphenols are naturally occuring major compounds in rooibos tea, suggesting that rooibos tea may posses anti-microbial activity and are also responsible for the antioxidant activity, as well as the anti-mutagenic properties contributing to rooibos tea as being a healthy beverage. Since the demand for natural food preservatives is increasing, rooibos tea may just help to fulfill this demand. However, no literature was found on any studies done on the anti-microbial activity of rooibos tea.

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CHAPTER 3

INHIBITORY EFFECT OF ROOIBOS TEA (*ASPALATHUS LINEARIS*) EXTRACTS ON THE GROWTH OF *ESCHERICHIA COLI*

Abstract

Rooibos tea extracts contain unique phenolic metabolites that exhibit antioxidant and anti-microbial activity. The phenolic compounds include the flavonoids, aspalathin and nothofagin as well as flavones, flavonols and flavanones. The inhibitory effect of rooibos tea water and ethyl acetate extracts on the growth of *Escherichia coli* was studied. Growth studies were performed over a period of 12 h in the absence or presence of different concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 $g.l^{-1}$ soluble solids) of fermented and unfermented rooibos tea extracts. The water and ethyl acetate extracts of rooibos tea inhibited the growth of *E. coli*. This was supported by the growth profiles, which showed decreases in the N_{max} and μ_{max} values with constant increases in the t_d and the t_{lag} as the soluble solid concentration of the tea in the growth medium increased. The water extracts of rooibos tea showed the strongest inhibitory activity, more so than the ethyl acetate extracts. It was also found that the fermented and unfermented rooibos tea water extracts had a more bacteriostatic effect on *E. coli*. In the absence of the rooibos tea extracts, the *E. coli* was able to resume its growth.

Introduction

Rooibos tea (*Aspalathus linearis*) is an indigenous shrub that grows in the mountainous areas around Clanwilliam in the Western Cape, South Africa (Morton, 1983). The world-wide consumption of rooibos tea has increased due to the alleged health properties of the tea (Morton, 1983; Niwa & Miyachi, 1986; Joubert, 1994). The beneficial effects of rooibos tea can be linked to its polyphenolic composition and associated antioxidant activity (Yoshikwa *et al.*, 1990; Fereirra *et al.*, 1995; Von Gadow *et al.*, 1997).

The flavonoid composition of rooibos tea is unique as it contains aspalathin that has only been isolated from rooibos tea (Koeppen & Roux, 1966). Another rare flavonoid that is present in rooibos tea is nothofagin, which has previously

only been isolated from red beech (*Nothofagus fusca*) (Hillis & Inoue, 1976). The flavonoid fraction of rooibos tea, apart from aspalathin and nothofagin, also includes the flavones orientin, iso-orientin, vitexin, iso-vitexin, chrysoeriol, 8,7,4' – trihydroxy-3-methoxyflavone and luteolin and the flavonols iso-quercitrin, rutin and quercetin (Rabe *et al.*, 1993; Joubert & Fereirra, 1996). During the processing of the tea the aspalathin and nothofagin content decreases and other flavonoids are formed from aspalathin, such as the flavanones, dihydro-2.3-orientin and dihydro-3,4-iso-orientin (Joubert, 1996; Marais *et al.*, 2000).

In the past, interest has been shown in the polyphenolic fraction of tea as an anti-microbial agent (Hamilton-Miller, 1995). The simplest phenolic compounds of green and black tea extracts are the catechins and these compounds inhibit the growth of many microbial species (Sakanaka *et al.*, 1989; Sakanaka *et al.*, 1990; Sakanaka *et al.*, 1996; Mabe *et al.*, 1999; Hara, 2000). The microbial inhibition by green and black tea is mainly due to the action of the catechins, (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG) at cup-of-tea concentrations (Sakanaka *et al.*, 1990; Fukai *et al.*, 1991; Ahn *et al.*, 1991; Hamilton-Miller, 1995; Tezuka *et al.*, 1997). Toda *et al.* (1989) found that tea polyphenols inhibited the growth of both Gram-positive and Gram-negative bacteria. It was also found that commercially available tea polyphenols had a strong anti-microbial effect on harmful food-borne microbes such as *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Candida utilis* (Toda *et al.*, 1989; Yeo *et al.*, 1995; Oh *et al.*, 1999; Hara, 2000), but that the tea polyphenols from green and black tea had little effect on the growth of *Escherichia coli* (Toda *et al.*, 1989; Yeo *et al.*, 1995; Hamilton-Miller, 1995; Hara, 2000).

The aim of this study was to determine the influence of rooibos tea water and ethyl acetate extracts on the growth of *E. coli*. From this data, the initial bacterial cell density (N_0), the final bacterial cell density (N_{max}), the maximum specific growth rate (μ_{max}), lag phase (t_{lag}) and doubling time (t_d) were determined.

Materials and methods

Plant material

Processed ("fermented") rooibos tea was obtained from farms in the Clanwilliam region, South Africa. Unprocessed plant material ("unfermented") was harvested during 1999 from a rooibos tea plantation in the Clanwilliam region, South Africa, and air-dried at 40°C in a drying tunnel (Continental Fan Works, Parow, South Africa). The dried, unfermented and fermented rooibos tea samples were finely grounded with a hammermill (Retch KG, Type SK1) using a 1.0 mm sieve. The tea was stored in sealed plastic containers, at room temperature in the dark. Black tea was locally purchased in the loose leaf form.

Water and ethyl acetate extracts

Water extracts of fermented and unfermented rooibos tea as well as black tea were prepared by adding 1 litre of boiling distilled water to 100 g samples of black tea and the finely grounded rooibos tea. The mixture was simmered for 30 min on a steambath (for black tea it was simmered for only 10 min), after which the tea extract was filtered through a 125 µm mesh cloth (Polymer PES D25/35, Swiss Silk Bolting Cloth Mfg. Co. Ltd., Zurich, Switzerland) to remove the grounded tea. The extracts were then filtered using Whatman no. 3mm Chr paper, the filtrate was frozen at -18°C and lyophilized using an Atlas commercial freeze-drier (Copenhagen, Denmark).

The ethyl acetate extractions were performed on the freeze-dried water extracts by dissolving 20 g samples of the lyophilized rooibos tea water extracts in 500 ml distilled water. Liquid-liquid extractions were performed in a separating funnel using 250 ml ethyl acetate (Saarchem). This mixture was gently mixed and left to separate. After separation the ethyl acetate layer was removed and the extraction procedure was repeated 10 times for exhaustive extraction. The ethyl acetate layers were pooled and evaporated at 60°C in a Büchi rotavapour, 250 ml distilled water added to the residue and the mixture frozen and lyophilized.

Soluble solid content

The soluble solid content of each of the tea extracts was determined gravimetrically. A 15 ml aliquot of each of the tea extracts was pipetted into a pre-weighed nickel moisture dish and dried on a steambath. Final drying was done in a vacuum oven at 70°C for 18 h, after which the samples were cooled and weighed.

Growth studies

The authentication of the *E. coli* (ATCC 11775 = USFSCC 58) strain obtained from the University of Stellenbosch Food Science Culture Collection (USFSCC) was done using the API 20E system (API system S.A., La Balme le Grottes, 38390 Montalieu Vercieu, France) and the morphology of the strain was microscopically investigated after Gram-staining. The *E. coli* used in the growth assays was inoculated into MRS broth (Biolab) and incubated overnight at 37°C.

A tea-MRS broth (MRS with added lyophilized tea extracts at different concentrations) was used as the growth medium in the growth studies. Both fermented and unfermented rooibos tea water and ethyl acetate extracts were used in the tea-MRS broth at different soluble solid concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 $g.l^{-1}$). The tea-MRS broth was sterilised in a small autoclave for 10 min to prevent the discolourisation of the medium (light brown to dark). The tea-MRS broth, as well as MRS broth without tea (control), was inoculated with 2% (v/v) of the *E. coli* culture which is equivalent to McFarland Standard 2 (6.0×10^8 $cfu.ml^{-1}$) from overnight cultures and these were incubated at 37°C for the duration of the growth assay. At one hour intervals, the broth was gently shaken and samples were taken to determine the optical density at 600 nm over a period of 12 h, using a spectrophotometer (Spectronic 20, Genesys, Spectronic Instruments, USA).

Serial dilutions (10^{-1} to 10^{-8}) were done of the *E. coli* grown in the fermented and unfermented tea-MRS broth, as well as the MRS broth without the tea extracts. The serial dilutions were done in sterile saline solution (0.85% (w/v) NaCl) and plated on MRS agar using the pour plate method. The plates were incubated overnight at 37°C and the number of colonies determined.

Escherichia coli was also grown in MRS and tea-MRS with 3.0 $g.l^{-1}$ soluble solids rooibos tea extracts added as described previously. After 12 h of incubation

the cells were centrifuged for 20 min at 3 000 rpm. The supernatant was removed and the cells were washed with 40 ml sterile saline solution and centrifuged. The pellet was sealed and kept at 4°C for about 10 h and then inoculated into fresh MRS broth. The *E. coli* was grown for 12 h and hourly samples were taken and the optical density at 600 nm determined using a spectrophotometer (Spectronic 20, Genesys, Spectronic Instruments, USA).

Determining the Minimum Inhibitory Concentration (MIC)

A 0.1 ml overnight culture of *E. coli* was spread uniformly on MRS agar. Paper discs were saturated with fermented and unfermented rooibos tea water extracts at different concentrations (1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 $g.l^{-1}$) and placed on the MRS agar. A control paper disc that was not saturated with tea was also placed on the plates. The plates were incubated at 35°C for 16 h and the zones around the paper discs were visually compared.

Growth parameters

The data obtained from the growth studies were analysed using the Microfit Software program (Institute of Food Research, Microfit©, Version 1.0). The initial bacterial cell density (N_0) (OD), final bacterial cell density (N_{max}) (OD), maximum specific growth rate (μ_{max}) (h^{-1}), lag phase (t_{lag}) (h) and the doubling time (t_d) (h) values of each growth study were determined.

Results and discussion

Soluble solid content of rooibos tea water extracts

The water extracts of fermented and unfermented rooibos tea contained *ca.* 0.90% and 1.90% ($m.v^{-1}$) soluble solids, respectively. The lower soluble solid concentration of the fermented rooibos tea is attributed to the oxidative changes that occur during the chemical fermentation and is in agreement with the findings reported by Joubert (1996).

Minimum inhibitory concentration (MIC)

No inhibition zones were observed around the paper discs that had been saturated with either the fermented or unfermented tea at all the concentrations

tested (1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 $g.l^{-1}$). These tests were done several times and it was thus concluded that the MIC could not be determined using this method.

Growth studies

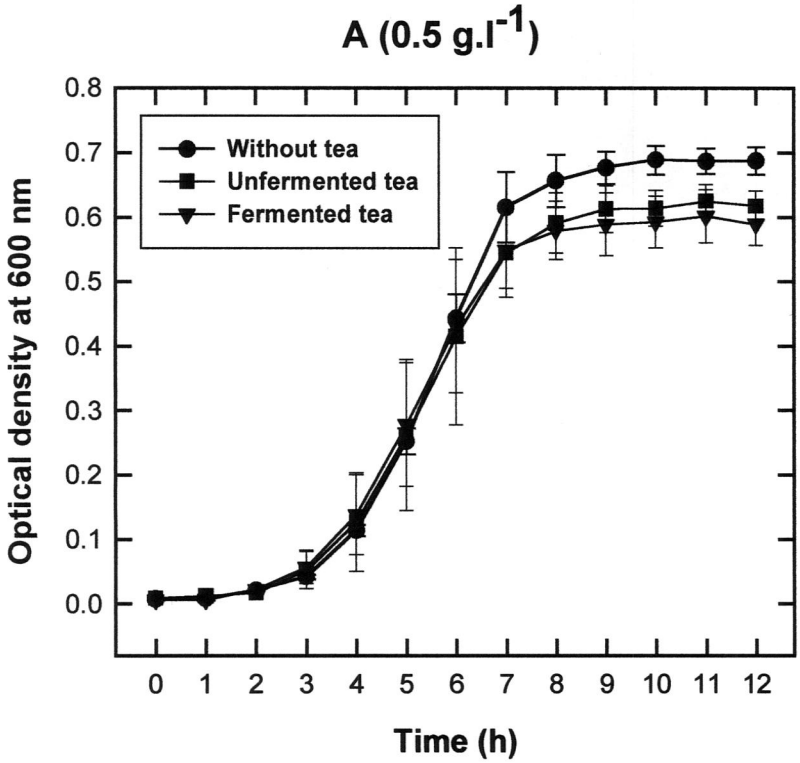
Growth studies of *E. coli* in the presence of rooibos tea water extracts

The inhibitory effect of fermented and unfermented rooibos tea water extracts was clearly observed from the growth profiles of *E. coli* at the different soluble solid concentrations (Fig.1). These growth studies show that an increase in the soluble solid concentration resulted in an increased inhibitory activity against *E. coli*. Furthermore, the fermented rooibos tea water extracts had a stronger inhibitory effect than the unfermented rooibos tea water extracts (Fig. 1A – F). The decreases in the *E. coli* growth in the fermented and unfermented tea extracts are shown in Table 1. A tea concentration (soluble solids) higher than 5.0 $g.l^{-1}$ could not be used due to the dark discolouration of the broth, making spectrophotometric measurements impossible. Concentrations lower than 0.5 $g.l^{-1}$ were also not tested due to the low level of inhibition, as well as the fact that a cup of tea contains *ca.* 2.5 $g.l^{-1}$ soluble solids (Dr. E. Joubert,1999, ARC Infruitec-Nietvoorbij, personal communication).

Bacterial growth is generally characterised by a low specific growth rate within the first few hours of growth and this then accelerates to a maximal value (μ_{max}), resulting in a lag time (Zwietering *et al.*, 1990). From growth profiles the μ_{max} can be estimated from either viable cell counts or the turbidimetric measurements (OD) (Dalgaard *et al.*, 1994). Other parameters that can be determined from growth profiles include the lag phase (t_{lag}) and the doubling time (t_d) of the specific bacterial strain used (Whiting & Cygnarowicz-Provost, 1992).

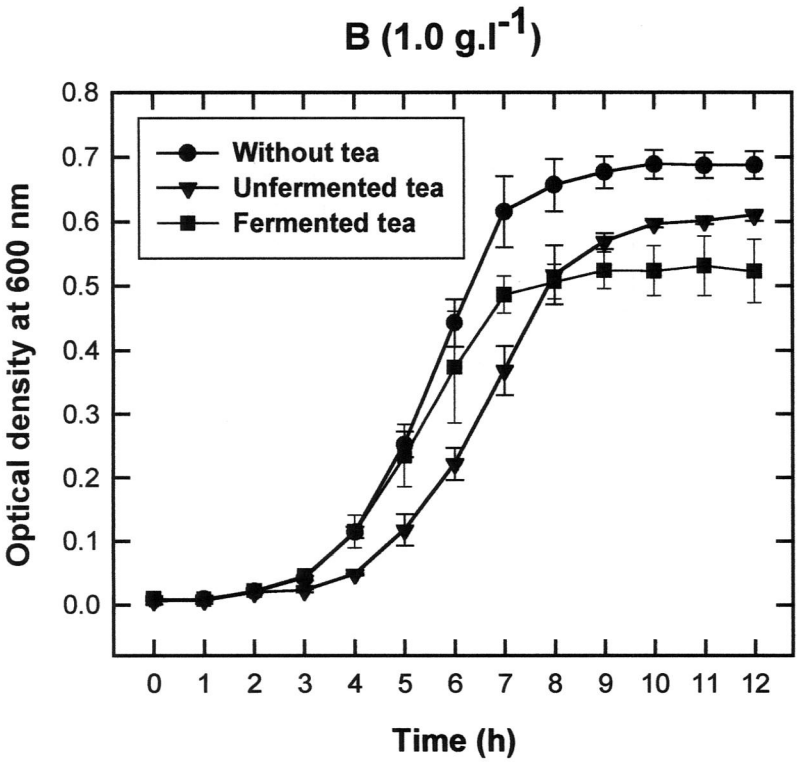
In this study it was found that, as the concentration of the rooibos tea water extracts increased, the N_{max} decreased (Fig. 2). The N_{max} for the unfermented rooibos tea decreased from 0.69 OD at 0.5 $g.l^{-1}$ soluble solids to 0.58 OD at 5.0 $g.l^{-1}$ soluble solids, while the fermented rooibos tea showed a larger decrease from 0.59 OD at 0.5 $g.l^{-1}$ soluble solids to 0.25 OD at 5.0 $g.l^{-1}$ soluble solids.

The maximum specific growth rate of the *E. coli* in the presence of the rooibos tea water extracts also decreased as the concentration of the different tea



	N_O	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.03	0.80	1.23	4.42	0.61
Uft	0.03	0.69	1.19	4.63	0.60
Ft	0.03	0.59	1.12	4.81	0.57

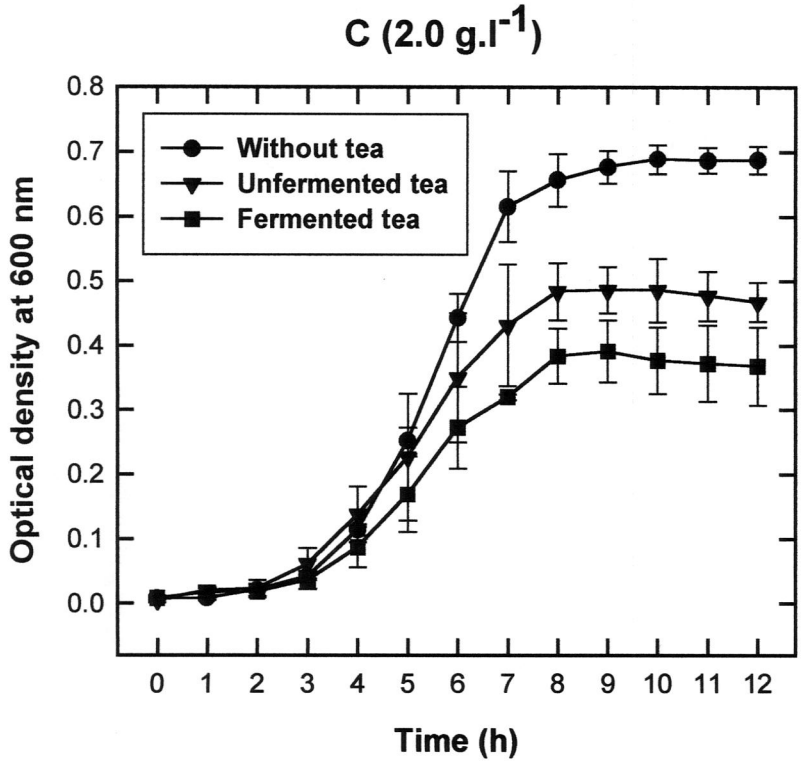
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_O	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.03	0.80	1.23	4.42	0.61
Uft	0.02	0.60	0.88	4.89	0.66
Ft	0.03	0.52	0.73	4.83	1.00

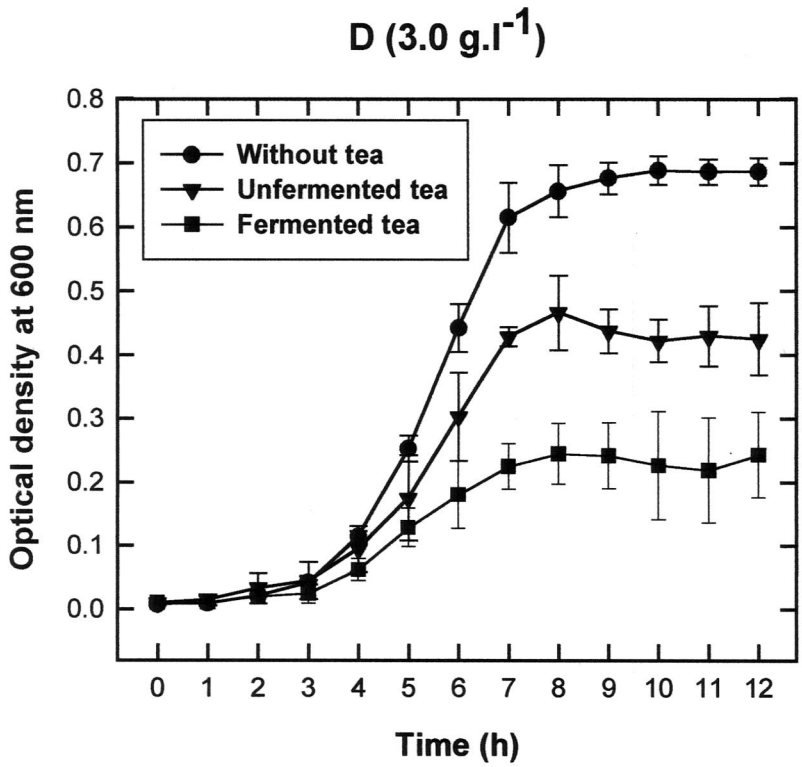
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 1. Growth profiles of *E. coli* (USFSCC 58) in the presence of MRS plus different concentrations fermented and unfermented rooibos tea water extracts. Error bars represent the standard deviation of three repeats.



	N_O	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.03	0.80	1.23	4.42	0.61
Uft	0.02	0.56	0.84	4.93	0.69
Ft	0.02	0.37	0.59	5.05	1.24

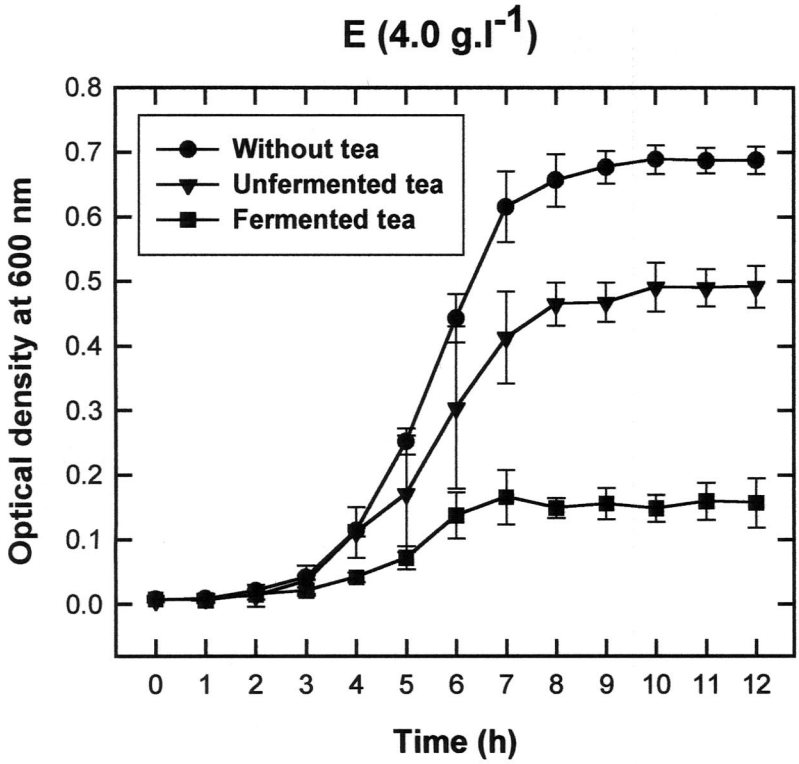
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_O	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.03	0.80	1.23	4.42	0.61
Uft	0.03	0.57	0.72	5.09	0.72
Ft	0.02	0.36	0.39	5.20	1.56

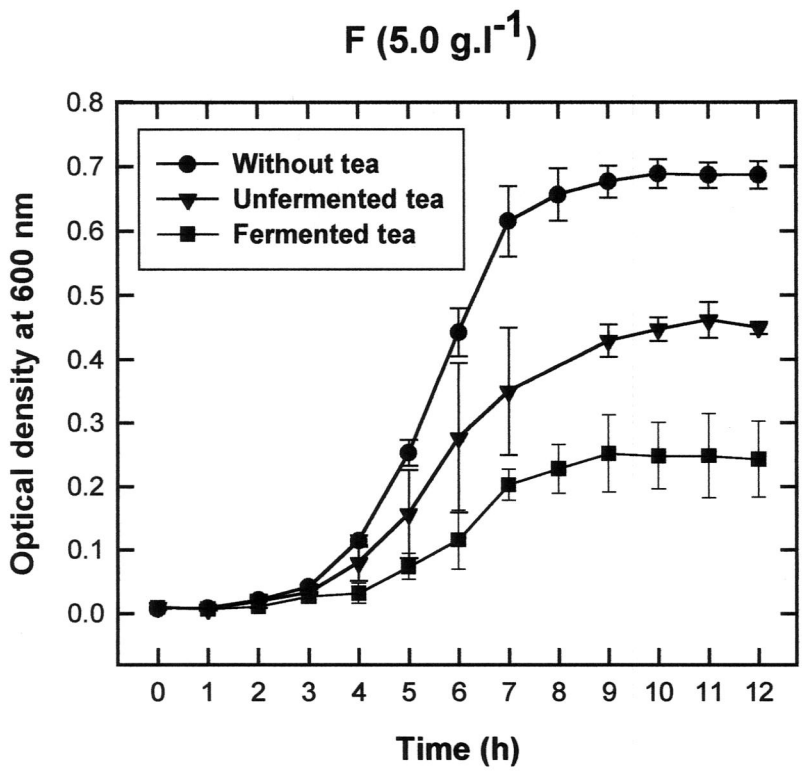
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 1. (continue)



	N_O	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.03	0.80	1.23	4.42	0.61
Uft	0.03	0.62	0.50	5.07	1.18
Ft	0.02	0.25	0.20	5.40	1.67

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_O	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.03	0.80	1.23	4.42	0.61
Uft	0.03	0.58	0.40	5.29	1.20
Ft	0.02	0.25	0.20	6.60	1.80

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 1. (continue)

Table 1. The decrease (%) in *E. coli* growth in the presence of the water extracts of the fermented and unfermented rooibos teas at different soluble solid concentrations compared to the growth in the MRS in the absence of the rooibos tea extracts after 12 h.

Soluble solid Concentration ($g.l^{-1}$)	**Unfermented tea***	**Fermented tea***
0.5	10.2	14.3
1.0	12.8	22.4
2.0	31.6	46.4
3.0	36.0	52.7
4.0	33.0	68.6
5.0	35.1	69.0

* Average value of triplicate values

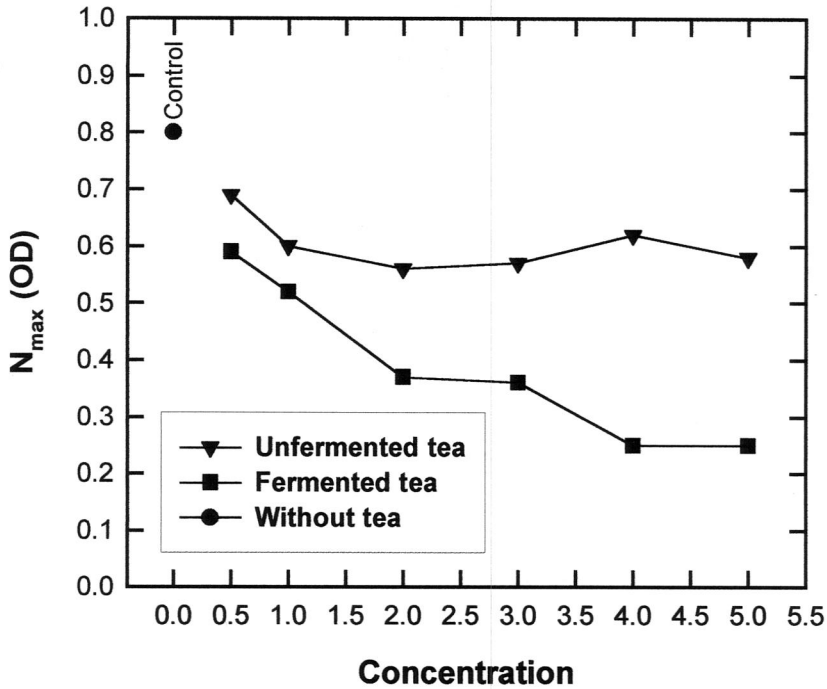


Figure 2. The decrease in the final bacterial cell density (N_{max}) of *E. coli* (USFSCC 58) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

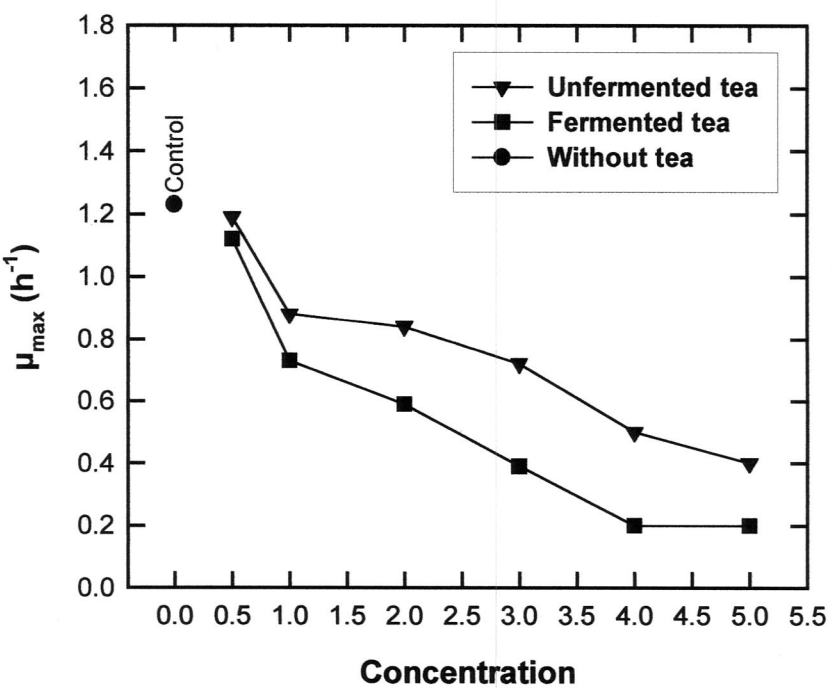


Figure 3. The decrease in the maximum growth rate (μ_{max}) of *E. coli* (USFSCC 58) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

extracts was increased. The μ_{max} of *E. coli* grown in unfermented rooibos tea water extracts decreased from 1.19 h^{-1} at a soluble solid tea concentration of 0.5 $g.l^{-1}$ to 0.40 h^{-1} at a tea concentration of 5.0 $g.l^{-1}$. The μ_{max} of the fermented rooibos tea water extracts decreased steadily from 1.12 h^{-1} for 0.5 $g.l^{-1}$ soluble solids to 0.20 h^{-1} for 5.0 $g.l^{-1}$ soluble solids (Fig. 3). The data from the growth profiles showed that the μ_{max} for the *E. coli* grown in fermented rooibos tea extracts was in all cases much slower than for the *E. coli* grown in the presence of unfermented tea extracts.

The t_d of the *E. coli* also increased for both unfermented and fermented rooibos tea water extracts from 0.60 h (0.5 $g.l^{-1}$) to 1.20 h (5.0 $g.l^{-1}$) and from 0.59 h (0.5 $g.l^{-1}$) to 1.80 h (5.0 $g.l^{-1}$), respectively (Fig. 4).

The t_{lag} values of the *E. coli* strain were also found to increase in the presence of fermented and unfermented rooibos tea water extracts. The t_{lag} for the *E. coli* grown in unfermented rooibos tea water extracts increased slightly from 4.63 h (0.5 $g.l^{-1}$ soluble solids) to 5.29 h (5.0 $g.l^{-1}$ soluble solids), while the t_{lag} of the *E. coli* grown in fermented rooibos tea water extracts increased from 4.81 h (0.5 $g.l^{-1}$ soluble solids) to 6.60 h (5.0 $g.l^{-1}$ soluble solids) (Fig. 5).

Growth studies of *E. coli* in the presence of rooibos tea ethyl acetate extracts

The results of the growth of *E. coli* in MRS plus fermented and unfermented rooibos tea ethyl acetate extracts, are shown in Fig. 6. The inhibitory activity of the unfermented rooibos tea ethyl acetate extracts was found to remain fairly constant irrespective of the concentration used, while the inhibitory activity of the fermented rooibos tea ethyl acetate extracts increased slightly with each increase in the concentration of the tea. The N_{max} of the *E. coli* grown in MRS plus unfermented rooibos tea ethyl acetate extracts remained fairly constant at the different concentrations (0.60 OD for 0.5 $g.l^{-1}$ and 0.61 OD for 5.0 $g.l^{-1}$ soluble solids), while the N_{max} for the *E. coli* grown in MRS plus fermented rooibos tea ethyl acetate extracts was found to decrease from 0.56 OD for 0.5 $g.l^{-1}$ to 0.40 OD for 5.0 $g.l^{-1}$ (Fig. 7).

The μ_{max} decreased, after a slight increase, for both the unfermented and fermented rooibos tea ethyl acetate extracts from 1.24 h^{-1} (0.5 $g.l^{-1}$) to 0.64 h^{-1} (4.0 $g.l^{-1}$) and 1.36 h^{-1} (0.5 $g.l^{-1}$) to 0.69 h^{-1} (5.0 $g.l^{-1}$), respectively (Fig. 8).

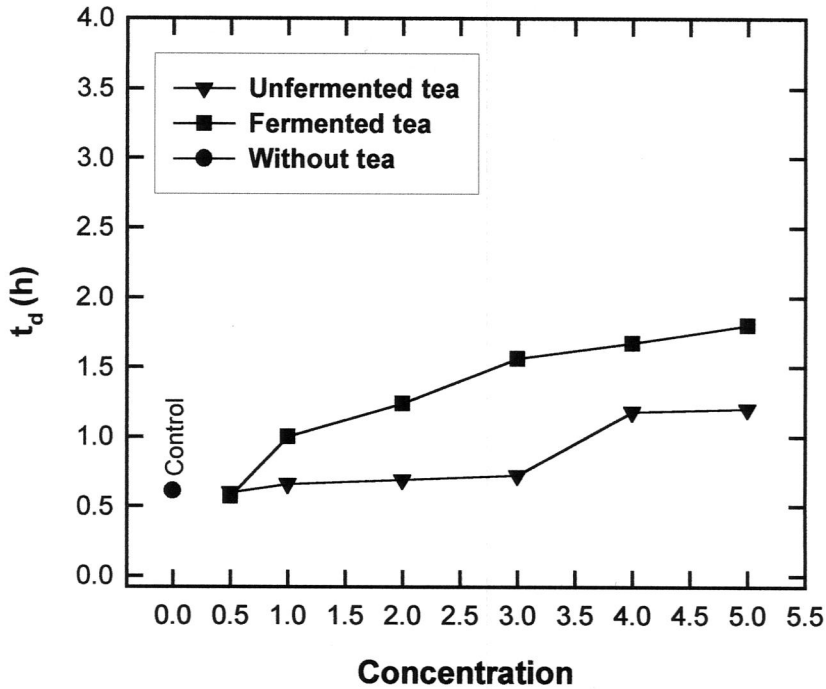


Figure 4. The increase in the doubling time (t_d) of *E. coli* (USFSCC 58) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

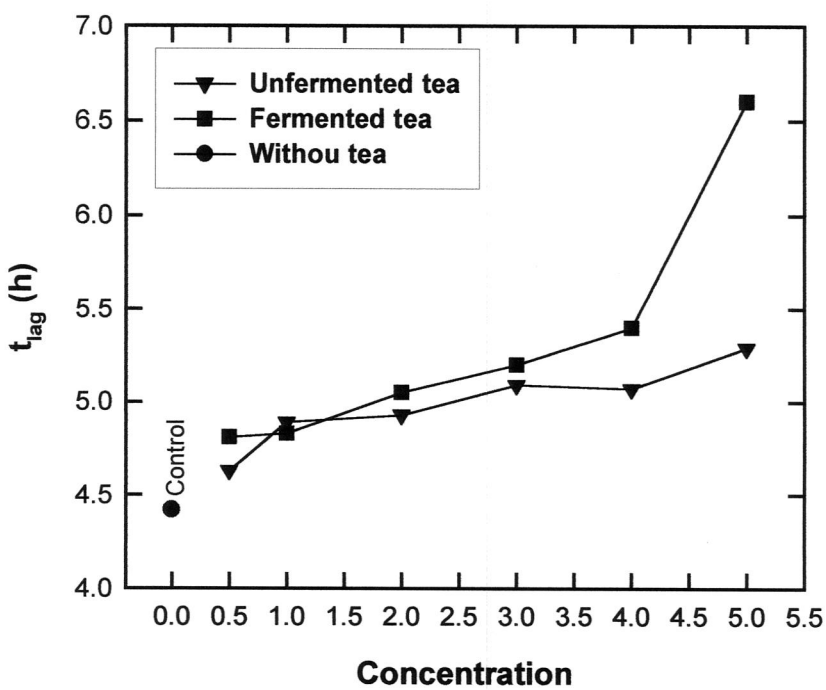
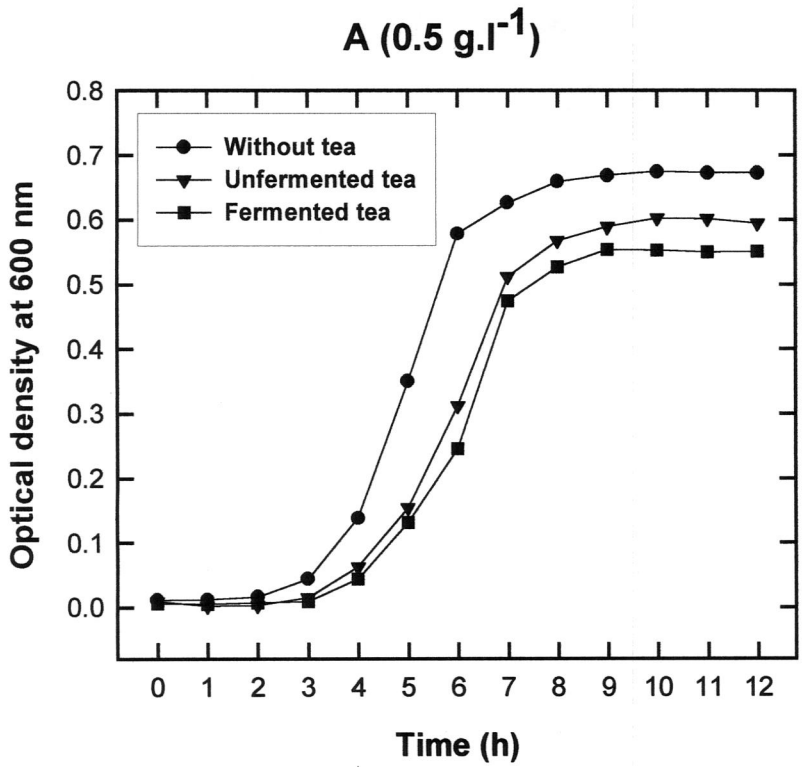
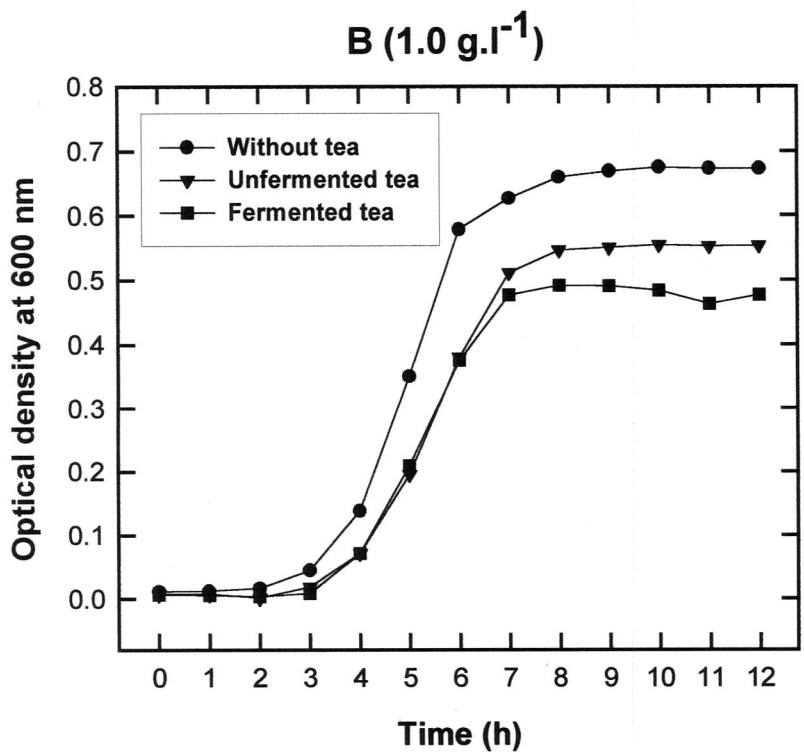


Figure 5. The increase in the lag time (t_{lag}) of *E. coli* (USFSCC 58) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.



	N_O	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.02	0.64	1.46	4.50	0.63
Uft	0.02	0.60	1.24	5.23	0.62
Ft	0.02	0.56	1.36	5.30	0.58

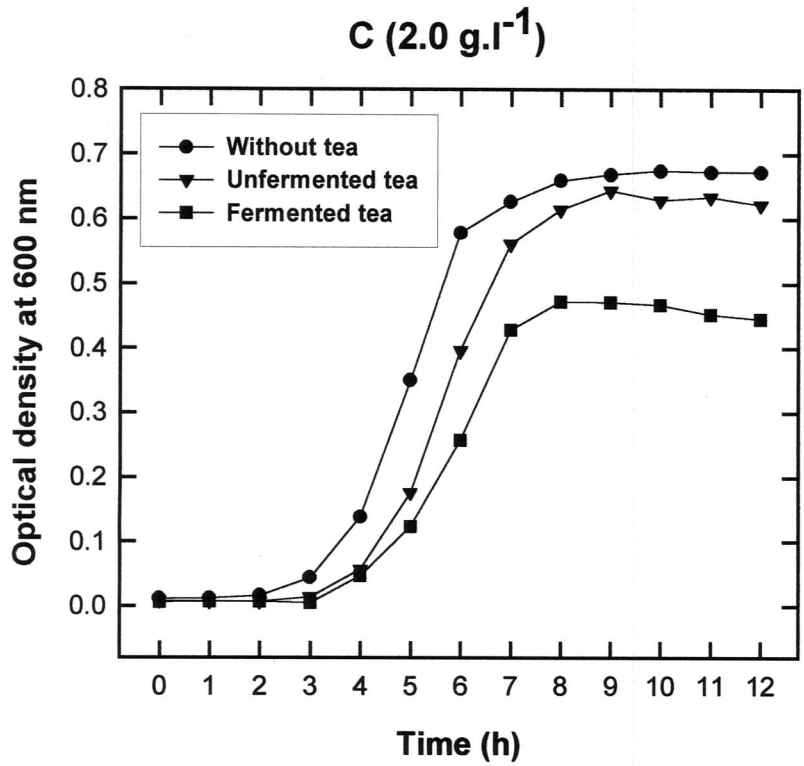
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_O	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.02	0.64	1.46	4.50	0.63
Uft	0.02	0.54	1.31	4.99	0.48
Ft	0.02	0.48	1.42	5.00	0.49

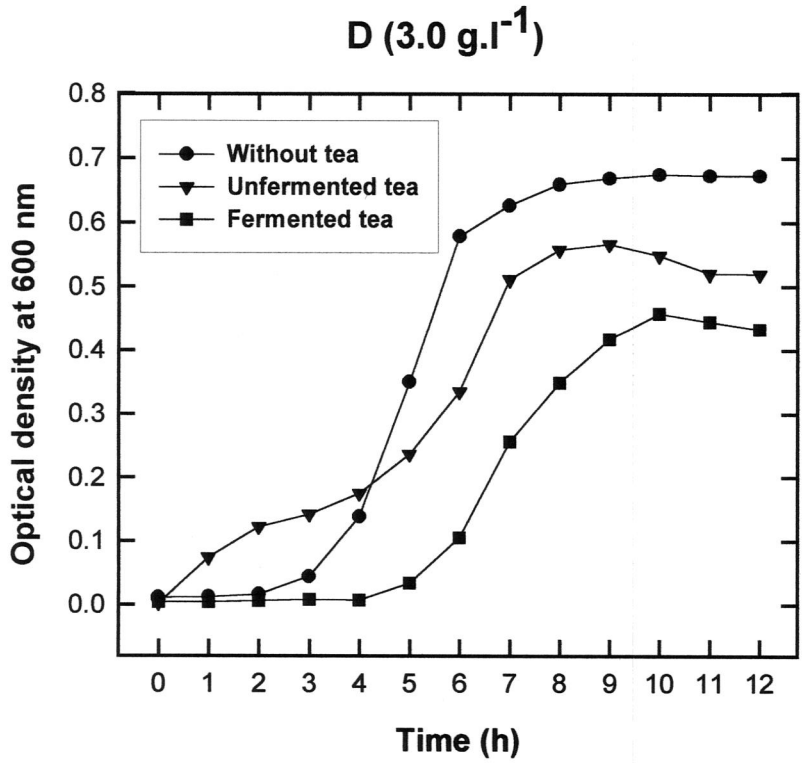
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 6. Growth profiles of *Escherichia coli* (USFSCC 58) grown in MRS plus ethyl acetate extractions of fermented and unfermented rooibos tea at different concentrations.



	N_O	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.02	0.64	1.46	4.50	0.63
Uft	0.02	0.62	1.34	5.17	0.45
Ft	0.02	0.46	1.13	5.39	0.53

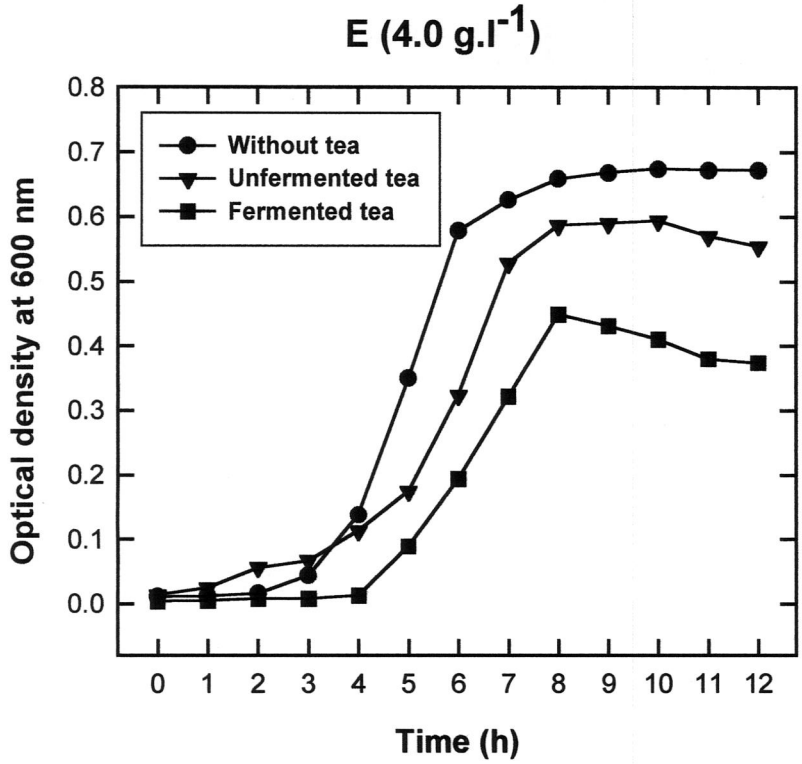
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_O	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.02	0.64	1.46	4.50	0.63
Uft	0.02	0.56	-	-	-
Ft	0.02	0.45	0.60	5.70	1.41

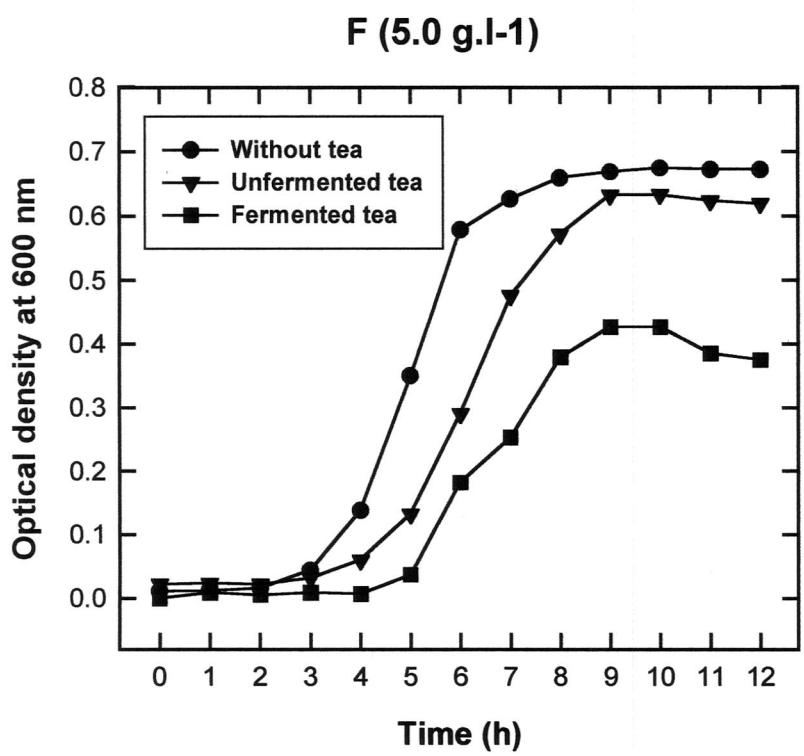
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 6. (continue)



	N_O	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.02	0.64	1.46	4.50	0.63
Uft	0.02	0.60	0.70	5.30	1.34
Ft	0.02	0.41	0.52	5.84	1.45

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_O	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.02	0.64	1.46	4.50	0.63
Uft	0.02	0.61	0.64	5.35	1.31
Ft	0.02	0.40	0.96	6.15	1.49

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 6. (continue)

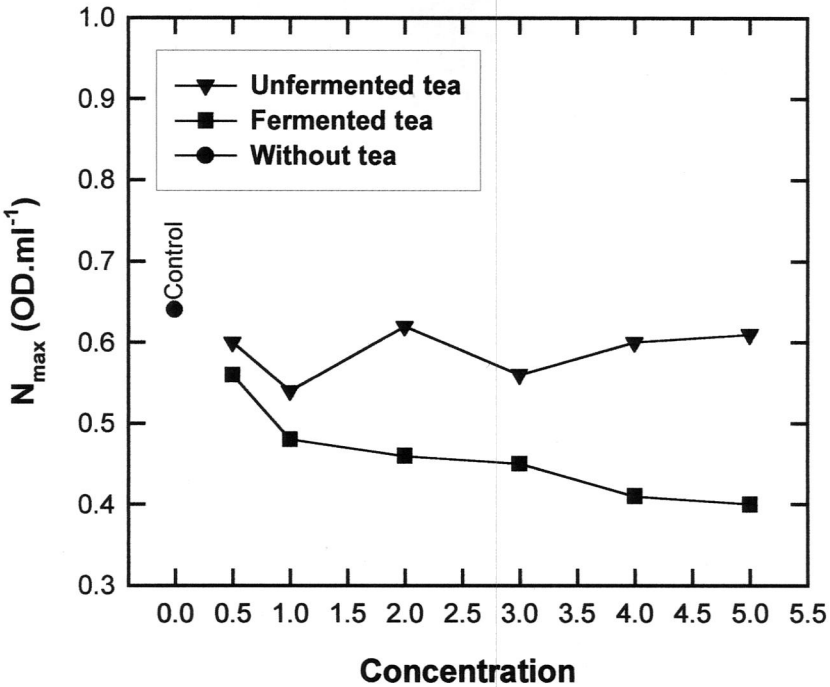


Figure 7. The changes in the final bacterial cell density (N_{max}) of *E. coli* (USFSCC 58) grown in MRS plus fermented and unfermented rooibos tea ethyl acetate extracts at different concentrations.

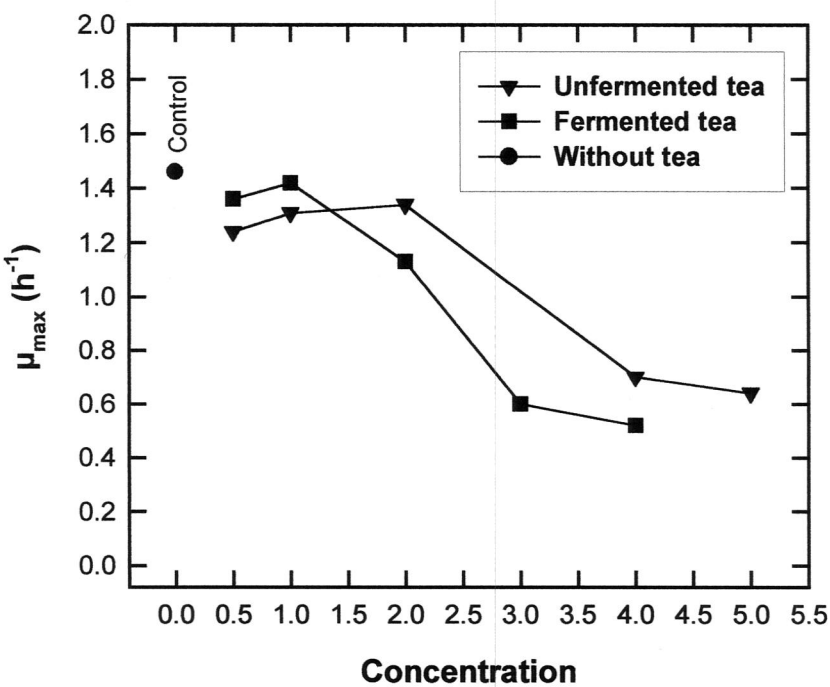


Figure 8. Changes in the maximum growth rate (μ_{max}) of *E. coli* (USFSCC 58) grown in MRS plus fermented and unfermented rooibos tea ethyl acetate extracts at different concentrations.

The t_d of the *E. coli* grown in the presence of unfermented tea was found to firstly shorten from 0.62 h and then increase to the final value of 1.31 h. The t_d of the *E. coli* grown in the presence of fermented tea showed a similar profile with an initial shorting followed by sharp lengthening to 1.49 h (Fig. 9). In contrast, the t_{lag} increased slightly for the *E. coli* grown in unfermented rooibos tea ethyl acetate extracts from 5.23 h at 0.5 $g.l^{-1}$ to 5.35 h at 5.0 $g.l^{-1}$ and for the *E. coli* grown in fermented rooibos tea ethyl acetate extracts from 5.30 h at 0.5 $g.l^{-1}$ to 6.15 h at 5.0 $g.l^{-1}$ (Fig. 10).

The results of the growth studies of *E. coli* in unfermented tea were not as would be expected since the inhibition activity of the unfermented rooibos tea ethyl acetate extracts does not decrease steadily as the concentration of the tea extracts increases. Furthermore, even though identical ethyl acetate extracts were used for all the growth studies, the growth of *E. coli* in the unfermented rooibos tea ethyl acetate extracts was not consistent (Table 2).

According to Winterton (1999), ethyl acetate extracts of both fermented and unfermented rooibos tea have a higher total polyphenol content than that of rooibos tea water extracts of both the fermented and unfermented rooibos tea. This suggests that the inhibitory effect that rooibos tea extracts have on *E. coli* growth can not only be due to the action of the polyphenols that are present in higher concentrations in the ethyl acetate extracts. The greater inhibition of the water extracts suggests that other compounds probably play an important role in the inhibitory effect of rooibos tea. For the water extracts of the unfermented tea, the growth of *E. coli* decreased with 35.1% at 5.0 $g.l^{-1}$ soluble solids but for the ethyl acetate extracts the growth inhibition remained fairly constant for all the tea concentrations tested. The presence of the water extracts of the fermented rooibos tea in the growth medium led to a decrease in the growth of the *E. coli* of 69.0% at 5.0 $g.l^{-1}$ soluble solids while the rooibos tea ethyl acetate extracts decreased the growth by 42.6% the same concentration.

Growth study of *E. coli* in the presence of black tea extracts (*Camellis sinensis*)

Escherichia coli was also grown in MRS plus black tea water extracts to determine the inhibitory effect of black tea on the growth of *E. coli* (Fig. 11). This was done to compare the growth in the presence of MRS plus black tea with the growth of *E. coli* in MRS plus rooibos tea water extracts. The results indicated that

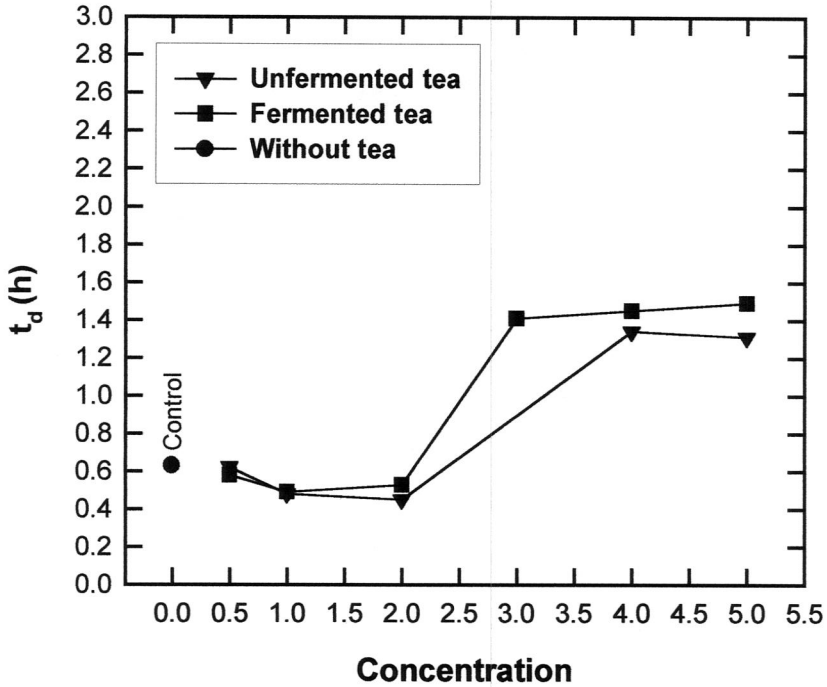


Figure 9. The changes in the doubling time (t_d) of *E. coli* (USFSCC 58) grown in MRS plus fermented and unfermented rooibos tea ethyl acetate extracts at different concentrations.

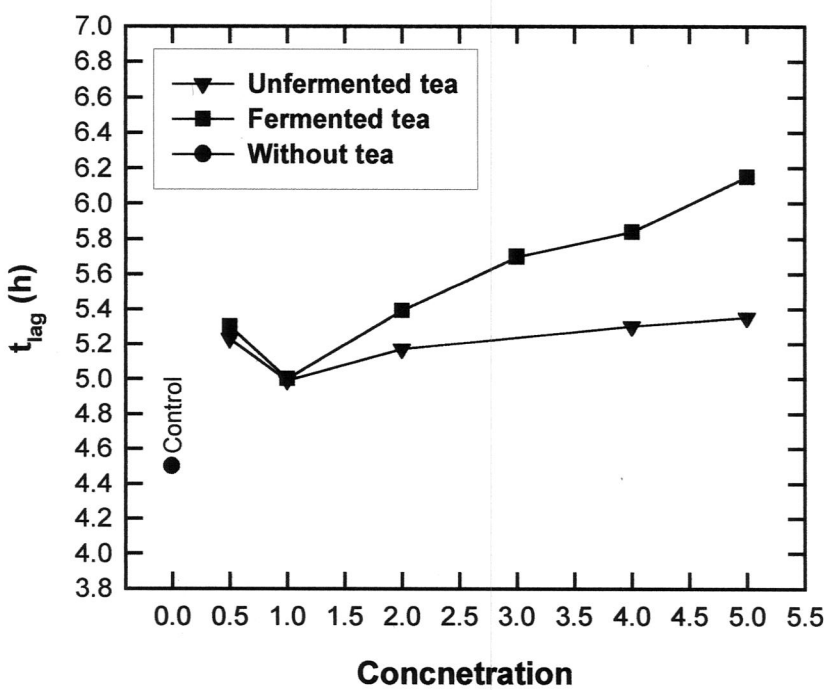


Figure 10. The changes in the lag time (t_{lag}) of *E. coli* (USFSCC 58) grown in MRS plus fermented and unfermented rooibos tea ethyl acetate extracts at different concentrations.

Table 2. The decrease (%) of *E. coli* growth in the presence of ethyl acetate extracts of fermented and unfermented rooibos teas at different soluble solid concentrations when compared to the growth in MRS in the absence of the rooibos tea ethyl acetate extracts after 12 h.

Soluble solid Concentration ($g.l^{-1}$)	**Unfermented tea***	**Fermented tea***
0.5	9.0	15.8
1.0	15.3	27.1
2.0	4.8	31.9
3.0	17.5	33.7
4.0	15.2	42.7
5.0	5.2	42.6

* Average values of triplicate values

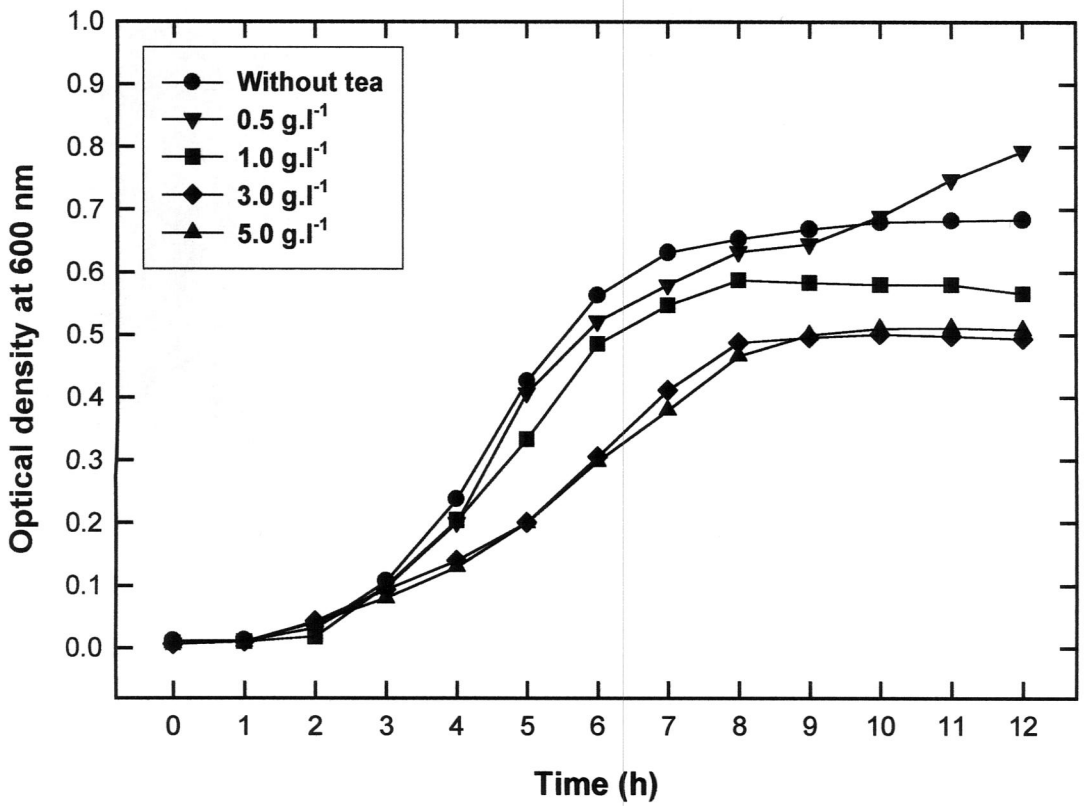


Figure 11. Growth profiles of *E. coli* (USFSCC 58) in MRS plus black tea water extracts at different soluble solid concentrations.

the inhibitory activity of the black tea water extracts was not as pronounced as the effect that rooibos tea had on the growth of *E. coli*. The weaker effect that black tea has on *E. coli* in comparison to inhibition of other microbes, has also been reported in the literature by Toda *et al.* (1989), Hamilton-Miller (1995), Yeo *et al.* (1995) and Hara (2000).

Determining the bacteriostatic action of rooibos tea on the growth *E. coli*

The data from this study (Fig. 1 – 10) clearly show that rooibos tea has an inhibitory action on the growth of *E. coli*, but the question does arise whether this action is bacteriostatic or bactericidal? To answer this question, the growth of the *E. coli* strain was studied in MRS and tea-MRS (with fermented and unfermented rooibos tea water extracts) for 12 h, after which the cells were separated from the growth media by centrifugation, washed twice, and placed in MRS without tea extracts (Fig. 12). The resulting growth profiles clearly show that the inhibitory effect of the tea is bacteriostatic.

Conclusions

The results of this study show that both the rooibos tea water and ethyl acetate extracts have an inhibitory effect on the growth of *E. coli* strain 58. As the concentration of the tea extracts was increased, the inhibitory effect also increased, with the fermented rooibos tea extracts having a stronger effect than the unfermented rooibos tea extracts. In contrast, the ethyl acetate extracts resulted in a lower inhibition compared to the water extracts, suggesting that the inhibitory activity of rooibos tea could not only be ascribed to the polyphenol fraction present in the rooibos tea.

In this study it was also found that, for both the fermented and unfermented rooibos tea, the highest concentration (5.0 $g.l^{-1}$) showed the strongest activity against the growth of this *E. coli* strain as was indicated by an increase in the t_d and the decrease in the μ_{max}. Furthermore, the inhibitory activity of rooibos tea was found to be bacteriostatic and thus the extracts did not cause the death of the *E. coli* cells.

The soluble solid content of rooibos tea at a cup of tea concentration is *ca.* 2.5 $g.l^{-1}$ (Dr. E. Joubert, 1999, ARC Infruitec-Nietvoorbij, personal communication)

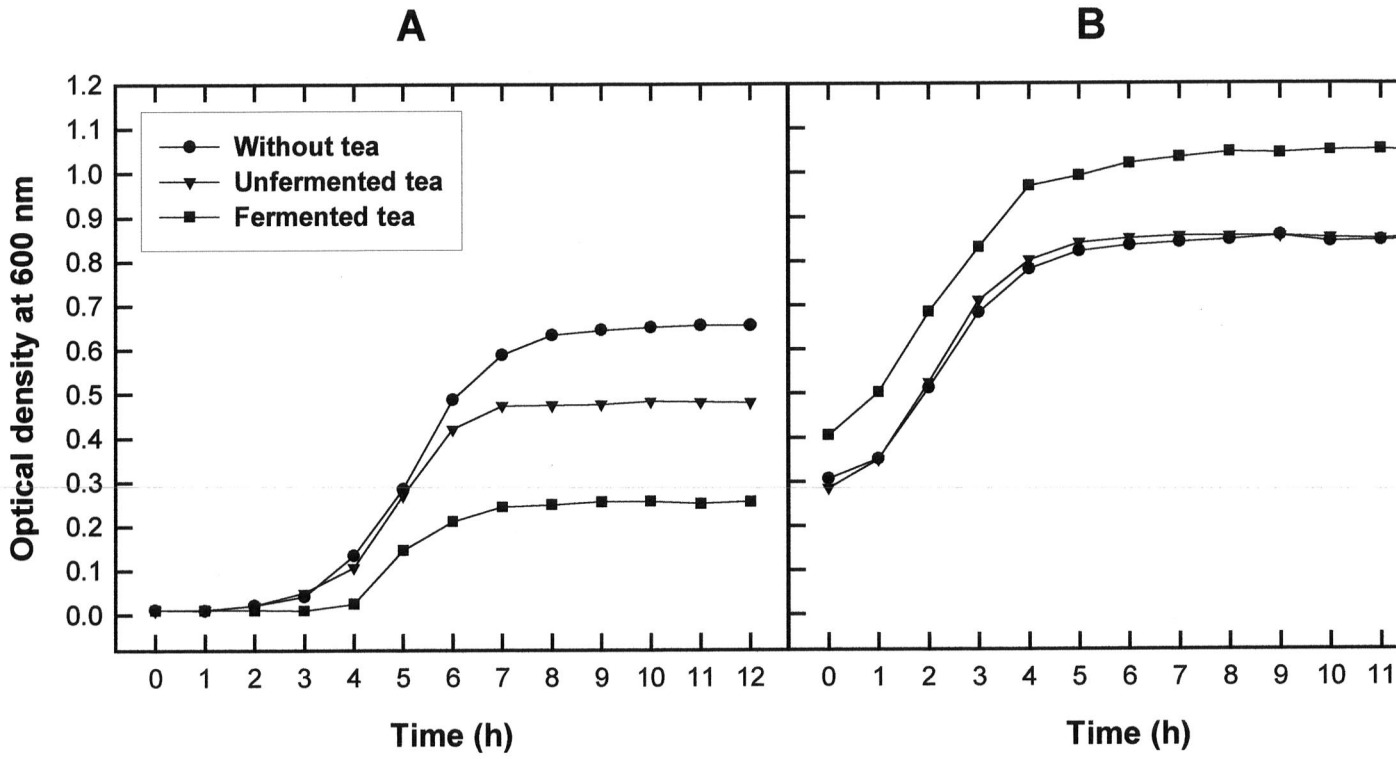


Figure 12. Growth profiles of *E. coli* (USFSCC 58) in fermented and unfermented rooibos tea water extracts at a concentration of 3.0 $g.l^{-1}$ (**A**). Growth after the *E. coli* cells were removed by centrifugation from the tea-MRS media after 12 h of growth and then re-inoculated into only MRS medium for 12 h (**B**).

and from this data it is clear that a cup of rooibos tea would show an inhibitory effect on the growth of *E. coli*. Based on the data obtained in this study, the inhibitory effect of rooibos tea against other potential spoilage and pathogenic microbes that are of importance to the food industry has to be tested to asses the importance of rooibos tea as a natural preservative.

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CHAPTER 4

INHIBITORY EFFECT OF ROOIBOS TEA (*ASPALATHUS LINEARIS*) WATER EXTRACTS ON POTENTIAL FOOD SPOILAGE AND PATHOGENIC MICROBES

Abstract

Rooibos tea (*Aspalathus linearis*) is unique to South Africa and the tea beverage is rich in volatile components and minerals, is caffeine free and has a low tannin content. The aim of this study was to determine the inhibitory effect of Rooibos tea water extracts on different microbes, including *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Streptococcus mutans, Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii*. Different concentrations soluble solids (0.5, 1.0, 2.0, 3.0 4.0, 5.0 $g.l^{-1}$) of both fermented and unfermented rooibos tea water extracts were used and the growth studies were performed over different time periods for each individual strain. Both types of the rooibos tea water extracts inhibited the growth of *Staph. aureus, B. cereus, L. monocytogenes, Strep. mutans* and *Sacch. cerevisiae*. The growth of Z. *rouxii* in contrast, was not inhibited and the rooibos tea extracts were found to even enhance the growth of this specific strain. The inhibition of the other strains was confirmed by the growth profiles that showed a decrease in N_{max} and μ_{max} values and an increase in the t_d and t_{lag}. It was also found that the fermented rooibos tea water extracts exhibited a stronger inhibitory effect than the unfermented rooibos tea water extracts. It was thus concluded that rooibos tea has a strong inhibitory effect against food spoilage and pathogenic microbes evaluated in this study and it was found that the growth of *Staph. aureus* was inhibited the strongest, but was not effective against all the yeasts species tested.

Introduction

Aspalathus linearis is unique to South Africa and grows in the mountainous areas around Clanwilliam in the Western Cape (Morton, 1983). This winter rainfall area with its coarse sandy soil is ideal for the cultivation of the tea bush (Morton, 1983).

The rooibos tea plant has red-brown branches and needle-like leaves which are used to produce rooibos tea (Morton, 1983). The plant material is cut into smaller pieces, bruised and enzymatically and chemically "fermented", resulting in a product that is naturally sweet with a brick-red colour (Morton, 1983; Joubert, 1996).

Plant phenolics, such as the dietary flavonoids found in black and green teas are of growing consumer interest due to their proposed functional properties in promoting health (Rauha *et al.*, 2000; Wang *et al.*, 2000). One of the classes of these flavonoids is the flavanols, which in part consists of catechins, the main component of black and green teas (Hamilton-Miller, 1995; Balentine *et al.*, 1997). It is mainly these catechins that are responsible for the anti-microbial effect of black and green teas, and it was found that these compounds inhibit a range of microbes, including *Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium* and *Clostridium perfringens* (Toda *et al.*, 1989; Ahn *et al.*, 1991; Yeo *et al.*, 1995; Oh *et al.*, 1999; Sakanaka *et al.*, 2000). It has also been shown that the polyphenols in green tea can prevent tooth decay by inhibiting the activity of the cariogenic streptococci, *Streptococcus mutans* and *Strep. sobrinus* (Sakanaka *et al.*, 1989; Yeo *et al.*, 1995; Yoshino *et al.*, 1996; Wang *et al.*, 2000) and *Porphyromonas gingivalis* (Sakanaka *et al.*, 1996).

Although a lot of research has been done on the anti-microbial effect of green and black teas, very little has been done on the anti-microbial effect of rooibos tea. However, it was found in the previous study (Chapter 3 of this thesis) that rooibos tea showed a stronger inhibitory effect on the growth of *Escherichia coli* than black tea and it was, therefore, decided to evaluate the anti-microbial effect of rooibos tea on other potential food spoilage and pathogenic microbes. The aim of this study was to determine the inhibitory effect of rooibos tea water extracts on the growth of different bacteria and yeasts, including *Staph. aureus, B. cereus, Listeria monocytogenes, Strep. mutans, Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii.*

Materials and methods

Rooibos tea water extracts

The plant material used for the preparation of the water extracts was the same material as used in Chapter 3 of this thesis. One litre of boiling distilled water was added to 100 g of the finely grounded fermented and unfermented rooibos tea and placed on a steambath for 30 min. The extract was filtered through a 125 μm mesh cloth (Polymer PES D25/35, Swiss Silk Bolting Cloth Mfg. Co. Ltd., Zurich, Switzerland) and then filtered through Whattman no. 3mm Chr paper, frozen at -18°C and then lyophilised using an Atlas commercial freeze-drier (Copenhagen, Denmark).

Growth studies

The microbial cultures used for the growth studies were obtained from the University of Stellenbosch Food Science Culture Collection (USFSCC) and included *Staph. aureus* (ATCC 12600 = USFSCC 29), *B. cereus* (DSM 31 = USFSCC 39), *L. monocytogenes* (ATCC 15313 = USFSCC 1273), *Strep. mutans* (AA 17 = USFSCC 1277), *Sacch. cerevisiae* (USFSCC 1035) and *Z. rouxii* (NRRLY-998 = USFSCC 1310). All the microbes used in the growth studies were inoculated into MRS broth (Biolab) and incubated overnight at 37°C, except for the *Z. rouxii* strain which was cultivated in DYE-medium which consisted of 20 $g.l^{-1}$ Dextrose (Biolab) and 5 $g.l^{-1}$ Yeast extract (Biolab) and incubated for 3 days at 30°C.

A tea-MRS broth (containing either fermented or unfermented rooibos tea water extracts) was used as the growth medium in the growth studies at different rooibos tea soluble solid concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 $g.l^{-1}$). In the case of the *Z. rouxii* strain, the growth studies were done in DYE-medium with either added fermented or unfermented rooibos tea water extracts. The tea-MRS and DYE-tea and MRS and DYE media (without any tea and used as controls), were inoculated with 2 % (v/v) of each microbe used (equivalent to McFarland Standard 2 = microbial concentrations of 6.0×10^8 $cfu.ml^{-1}$) from the overnight cultures (3 day culture for *Z. rouxii*) and incubated at 37°C and at 30°C, respectively, for the duration of the growth assay. Samples were taken at one hour intervals and the optical density was determined at 600 nm using a spectrophotometer (Spectronic 20 Genesys, Spectronic Instruments, USA). The

determination of the cell concentration was done over a specific time period for each individual microbe studied (*Staph. aureus* – 18 h; *B. cereus* – 12 h; *L. monocytogenes* – 14 h; *Strep. mutans* and *Sacch. cerevisiae* – 16 h; *Z. rouxii* – 56 h).

Determining growth profiles

The data obtained from the growth studies were analysed using the Microfit Software program (Institute of Food Research, Microfit©, Version 1.0). The initial cell concentration (N_0) (OD), final cell concentration (N_{max}) (OD), maximum specific growth rate (μ_{max}) (h^{-1}), lag time (t_{lag}) (h) and doubling time (t_d) (h) values for each study, were determined.

Results and discussion

This study resulted in the generation of a large volume of data and to simplify the discussion of the results, the data illustrated in Fig. 1 – 30 have been included as an Appendix at the end of this chapter. A summary of the growth parameters at concentrations of 0.5 and 5.0 $g.l^{-1}$ is given in Table 1.

Staphylococcus aureus

The inhibitory effect of rooibos tea water extracts on the growth of *Staph. aureus* is clear from the growth studies (Fig. 1 – Appendix A), with the fermented rooibos tea water extracts showing a stronger inhibitory effect. At soluble solid concentrations of 4.0 and 5.0 $g.l^{-1}$ fermented rooibos tea the growth of *Staph. aureus* was strongly inhibited. In Table 1, the growth parameters of *Staph. aureus* are given at soluble solid concentrations of 0.5 and 5.0 $g.l^{-1}$. These results show that with an increase in the rooibos tea soluble solid concentration, the N_{max} (Fig. 2 – Appendix A) and the μ_{max} (Fig. 3 – Appendix A) decreased, while the t_d (Fig. 4 – Appendix A) and t_{lag} (Fig. 5 – Appendix A) showed an increase.

In Table 2, the decrease found after growth (%) of *Staph. aureus* in the presence of fermented and unfermented rooibos tea water extracts is given. A 50.1% decrease occurred when *Staph. aureus* was grown in the unfermented tea-MRS, while, in the presence of the fermented rooibos tea, the growth decreased remarkably by 90.8%.

Table 1. The growth parameters of the selected microbes evaluated with the control (C) (media without any tea) and in the presence of 0.5 and 5.0 $g.l^{-1}$ fermented and unfermented rooibos tea water extracts.

	Staph. aureus			*B. cereus*			*L. monocytogenes*			*Strep. mutans*			*Sacch. cerevisiae*			*Z. rouxii*		
Soluble solid concentration ($g.l^{-1}$)	C	0.5	5.0	C	0.5	5.0	C	0.5	5.0	C	0.5	5.0	C	0.5	5.0	C	0.5	5.0
N_{max}																		
Unfermented	1.25	1.23	0.69	0.87	0.88	0.61	0.90	0.82	0.48	1.92	1.81	1.39	1.47	1.34	1.10	0.83	0.89	0.66
Fermented	1.25	1.20	0.18	0.87	0.85	0.23	0.90	0.79	0.09	1.92	1.80	0.36	1.47	1.25	0.44	0.83	0.99	0.96
μ_{max}																		
Unfermented	0.35	0.38	0.32	0.64	0.55	0.43	0.43	0.43	0.41	1.38	1.28	0.73	0.57	0.66	0.44	0.14	0.16	0.17
Fermented	0.35	0.35	0.19	0.64	0.47	0.32	0.43	0.41	0.21	1.38	0.96	0.23	0.57	0.51	0.24	0.14	0.16	0.18
t_d																		
Unfermented	1.98	1.84	2.41	1.21	1.40	1.67	1.63	1.60	1.74	0.63	0.64	1.03	1.22	1.04	1.59	4.71	4.28	3.95
Fermented	1.98	1.97	4.13	1.21	1.51	2.30	1.63	1.63	2.56	0.63	0.76	1.95	1.22	1.36	2.20	4.71	4.50	3.95
t_{lag}																		
Unfermented	6.44	7.57	8.51	5.41	5.90	7.30	7.14	7.66	10.03	5.07	5.30	6.28	7.74	8.20	8.66	34.75	34.60	35.60
Fermented	6.44	6.95	14.59	5.41	5.98	7.85	7.14	7.45	12.10	5.07	5.60	7.55	7.74	8.03	10.89	34.75	34.98	35.44

Table 2. The decrease (%) in *Staphylococcus aureus* growth in the presence of the water extracts of fermented and unfermented rooibos tea at different soluble solid concentrations after 12 h. The growth in MRS without added tea extracts was used as base control.

Soluble solid Concentration ($g.l^{-1}$)	**Decrease in growth in unfermented tea* (%)**	**Decrease in growth in fermented tea* (%)**
0.5	5.7	8.2
1.0	14.1	25.5
2.0	21.0	56.7
3.0	35.3	74.0
4.0	37.9	90.7
5.0	50.1	90.8

* Average of triplicate values

Bacillus cereus

A summary of the growth parameters at concentrations 0.5 and 5.0 $g.l^{-1}$ is given in Table 1. The growth studies done on *B. cereus* clearly show that rooibos tea has an inhibitory effect on its growth (Table 1). The results of these growth studies (Fig. 6 – Appendix A) showed that an increase in the soluble solid concentration of the rooibos tea water extracts had an increased inhibitory effect on the growth of *B. cereus*. Furthermore, with an increase in rooibos tea soluble solids, the N_{max} (Fig. 7 – Appendix A) and μ_{max} (Fig. 8 – Appendix A) decreased, while an increase was observed in the t_d (Fig. 9 – Appendix A) and t_{lag} (Fig. 10 – Appendix A). As was found with *Staph. aureus*, the fermented rooibos tea water extracts showed a stronger inhibitory effect than the unfermented rooibos tea water extracts. These results are supported by the decrease in growth (%) of *B. cereus* in the presence of fermented and unfermented rooibos tea, as given in Table 3. For the unfermented rooibos tea the growth of *B. cereus* decreased by 47.2%, while for the fermented rooibos tea the growth decreased by 80.3%.

Listeria monocytogenes

A summary of the growth parameters for the *Listeria* strain at concentrations 0.5 and 5.0 $g.l^{-1}$, is given in Table 1. The growth of *L. monocytogenes* was also inhibited by fermented and unfermented rooibos tea water extracts (Fig. 11 – Appendix A), and, as with *Staph. aureus* and *B. cereus*, it can clearly be seen that the fermented rooibos tea water extracts have a stronger effect on the growth of *L. monocytogenes* than that of the unfermented rooibos tea water extracts (Table 1). The growth parameters of *L. monocytogenes* showed that the inhibitory effect of rooibos tea increases as the concentration of the tea increase (Table 1). With an increase in soluble solid concentration, the N_{max} (Fig. 12 – Appendix A) and μ_{max} (Fig. 13 – Appendix A) decreased, while the t_d (Fig. 14 – Appendix A) and t_{lag} (Fig. 15 – Appendix A) increased. In the presence of unfermented rooibos tea the growth of *L. monocytogenes* decreased by 51.6%, while in the presence of the fermented rooibos tea the growth decreased by 89.2% (Table 4).

Table 3. The decrease (%) in *Bacillus cereus* growth in the presence of the water extracts of fermented and unfermented rooibos tea at different soluble solid concentrations after 12 h. The growth in MRS without added tea extracts was used as base control.

Soluble solid concentration ($g.l^{-1}$)	Decrease in growth in unfermented tea* (%)	Decrease in growth in fermented tea* (%)
0.5	9.4	14.4
1.0	21.2	26.4
2.0	35.3	44.8
3.0	32.2	66.3
4.0	37.1	79.1
5.0	47.5	80.3

* Average of triplicate values

Table 4. The decrease (%) in *Listeria monocytogenes* growth in the presence of the water extracts of fermented and unfermented rooibos tea at different soluble solid concentrations after 12 h. The growth in MRS without added tea extracts was used as base control.

Soluble solid concentration ($g.l^{-1}$)	Decrease in growth in unfermented tea* (%)	Decrease in growth in fermented tea* (%)
0.5	9.3	12.4
1.0	21.3	30.4
2.0	37.2	55.0
3.0	44.0	74.4
4.0	44.4	87.6
5.0	51.6	89.2

* Average of triplicate values

Streptococcus mutans

A summary of the growth parameters of the *Strep. mutans* strain obtained at concentrations 0.5 and 5.0 $g.l^{-1}$, is given in Table 1. *Streptococcus mutans* is a cariogenic streptococci and is known to be responsible for dental carries (Wang *et al.*, 2000), but green tea has been shown to inhibit the growth of *Strep. mutans* (Sakanaka *et al.*, 1989; Yeo *et al.*, 1993; Yoshino *et al.*, 1996; Wang *et al.*, 2000). The results of the growth studies of *Strep. mutans* in the presence of rooibos tea water extracts showed that rooibos tea also has an inhibitory effect against this species (Fig. 16 – Appendix A). The growth parameters of *Strep. mutans* showed that the growth of this strain was inhibited by an increase in the rooibos tea extracts (Table 1). The N_{max} (Fig. 17 – Appendix A) and μ_{max} (Fig. 18 – Appendix A) also clearly showed a decrease and the t_d (Fig. 19 – Appendix A) and t_{lag} (Fig. 20 – Appendix A) showed an increase as the soluble solid concentration of the tea was increased. The percentage decrease in growth of the *Strep. mutans* strain is shown in Table 5. In the presence of unfermented rooibos tea, the growth decreased by 30.1%, while in the presence of the fermented rooibos tea, the growth decreased by 84.1%.

Saccharomyces cerevisiae

The anti-microbial effect of rooibos tea was also tested against yeasts that may pose a food spoilage problem and a summary of the growth parameters of the *Sacch. cerevisiae* strain at concentrations 0.5 and 5.0 $g.l^{-1}$, is given in Table 1. The results of the growth studies of *Sacch. cerevisiae* are shown in Figure 21 (Appendix A). These results show that rooibos tea water extracts had an inhibitory effect on *Sacch. cerevisiae* and that the fermented rooibos tea had a stronger effect than the unfermented rooibos tea, which was similar to the results for the other bacteria included in this study. With an increase in the soluble solid concentration of the rooibos tea water extracts, a decrease was observed in the N_{max} (Fig. 22 – Appendix A) and μ_{max} (Fig. 23 – Appendix A), while an increase occurred in the t_d (Fig. 24 – Appendix A) and t_{lag} (Fig. 25 – Appendix A) (Table 1). The decrease in growth of *Sacch. cerevisiae* in the presence of fermented and unfermented rooibos tea water extracts is shown in Table 6. A percentage decrease in growth of 38.1% was observed for the unfermented rooibos tea, while for the fermented rooibos tea there was a decrease of 77.7%.

Table 5. The decrease (%) in *Streptococcus mutans* growth in the presence of the water extracts of fermented and unfermented rooibos tea at different soluble solid concentrations after 12 h. The growth in MRS without added tea extracts was used as base control.

Soluble solid concentration ($g.l^{-1}$)	Decrease in growth in unfermented tea* (%)	Decrease in growth in fermented tea* (%)
0.5	5.1	9.3
1.0	13.1	20.4
2.0	22.7	47.5
3.0	24.3	58.7
4.0	30.6	76.9
5.0	30.1	84.1

* Average of triplicate values

Table 6. The decrease (%) in *Saccharomyces cerevisiae* growth in the presence of the water extracts of fermented and unfermented rooibos tea at different soluble solid concentrations after 12 h. The growth in MRS without added tea extracts was used as base control.

Soluble solid concentration ($g.l^{-1}$)	**Decrease in growth in unfermented tea* (%)**	**Decrease in growth in fermented tea* (%)**
0.5	4.5	17.0
1.0	11.5	34.8
2.0	21.4	53.1
3.0	24.8	68.4
4.0	26.7	80.0
5.0	38.1	77.7

* Average of triplicate values

Zygosaccharomyces rouxii

Zygosaccharomyces rouxii is also a yeast that is known to cause spoilage problems in especially high sugar content food products and it was thus decided to evaluate the impact of rooibos tea on the growth of this organism. A summary of the growth parameters at tea concentrations of 0.5 and 5.0 $g.l^{-1}$ is given in Table 1. The rooibos tea water extracts were found not to have an inhibitory effect on the growth of *Z. rouxii* and the data showed that this strain even grows better in the presence of rooibos tea (Fig. 26 – Appendix A). The results also showed the *Z. rouxii* stain grew better in the fermented rooibos tea extracts than in the unfermented rooibos tea water extracts. It was also found that at a soluble solid concentration of 3.0 $g.l^{-1}$ for the unfermented rooibos tea, the growth of *Z. rouxii* suddenly decreased after 44 h and at 4.0 $g.l^{-1}$ and 5.0 $g.l^{-1}$ the growth of *Z. rouxii* decreased after 48 h. The growth parameters for the *Z. rouxii* strain at these concentrations was determined before the sudden decrease in growth. From the data summarised in Table 1 it is clear that the fermented rooibos tea enhanced the growth of this yeast strain and with an increase in the soluble solid concentration of the tea the N_{max} (Fig. 27 – Appendix A) showed a slight decrease, while the μ_{max} (Fig. 28 – Appendix A) showed a slight increase. The t_d (Fig. 29 – Appendix A) decreased, while the t_{lag} (Fig. 30 – Appendix A) showed a slight increase. This indicates that rooibos tea, specifically the fermented rooibos tea, enhanced the growth of *Z. rouxii*, while the unfermented rooibos tea showed only a slight effect on the growth of this yeast.

Conclusions

The results of this study showed that rooibos tea water extracts do have an inhibitory effect on the growth of *Staph. aureus, B. cereus, L. monocytogenes, Strep. mutans* and *Sacch. cerevisiae*, but a growth stimulatory effect on the *Z. rouxii* strain. It was found that as the concentration of the tea extracts was increased, the inhibitory effect subsequently increased, with the fermented rooibos tea extracts having a stronger negative impact than the unfermented rooibos tea extracts. In contrast, the growth of the *Z. rouxii* strain was enhanced by the presence of the fermented rooibos tea water extracts.

It is clear that rooibos tea does not inhibit the growth of the different species tested to the same extent. For the unfermented rooibos tea water extracts the

order of inhibition was as follows: *L. monocytogenes* > *Staph. aureus* > *B. cereus* > *Sacch. cerevisiae* > *Strep. mutans*. For the fermented rooibos tea, the order of inhibition was as follows: *Staph. aureus* > *L. monocytogenes* > *Strep. mutans* > *B. cereus* > *Sacch. cerevisiae*.

In this study it was also found that, for both the fermented and unfermented rooibos tea, the highest concentration (5.0 $g.l^{-1}$) showed the strongest activity against the growth of the different microbes. This was indicated by an increase in the t_d and t_{lag}, and with a decrease in N_{max} and μ_{max} values for all the tested strains used the growth assays with the exception of the *Z. rouxii* strain.

It is generally accepted that a cup of rooibos tea contains about 2.5 $g.l^{-1}$ soluble solids (Dr. E. Joubert, 1999, ARC Infruitec,Nietvoorbij, personal communication) and based on the data obtained in this study, it can be concluded that at this concentration rooibos tea will have an inhibitory effect on the growth of *Staph, aureus, B. cereus, L. monocytogenes, Strep. mutans* and *Sacch. cerevisiae*. However, it will be necessary in future studies to identify the compound or compounds responsible for the anti-microbial effect of rooibos tea and to test the inhibitory effect of these compounds on the different potential spoilage and pathogenic organisms before any further conclusions or recommendations can be made on the direct use of rooibos tea extracts in the food industry.

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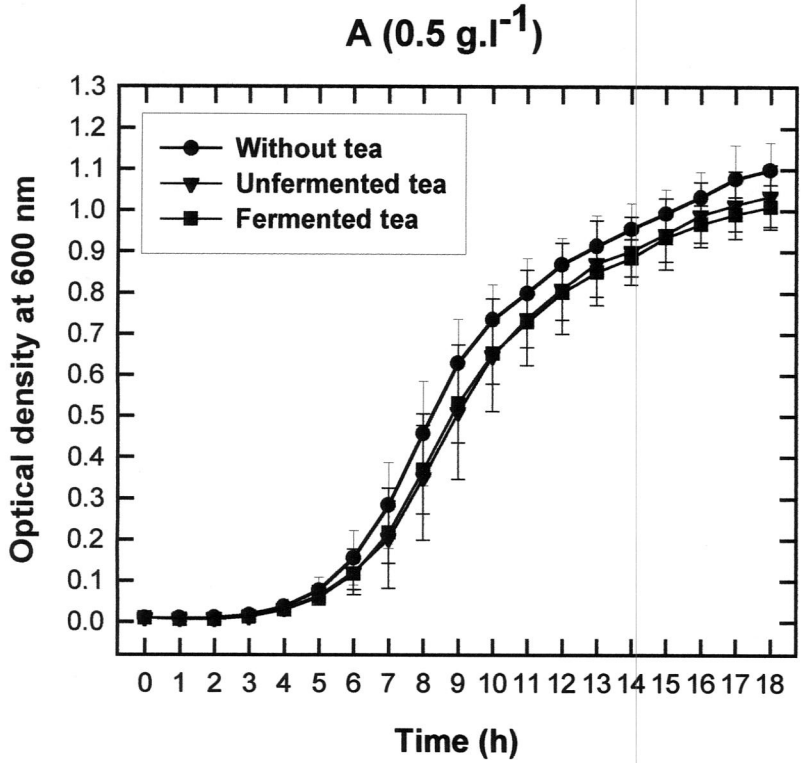
APPENDIX

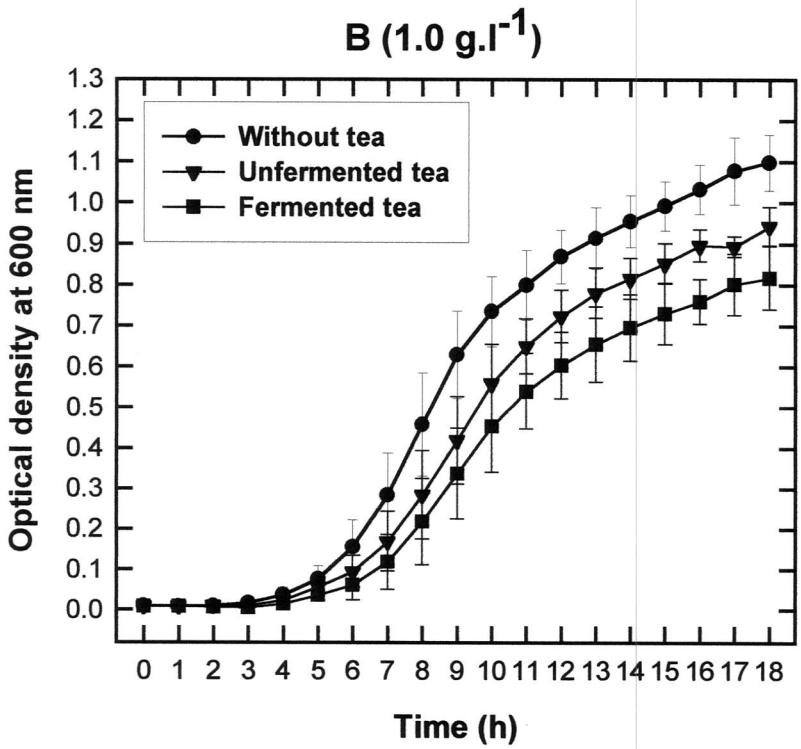
TO CHAPTER 4

To simplify the discussion of the results, the data illustrated in Fig. 1 – 30 have been included in this Appendix.



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.07	1.25	0.35	6.44	1.98
Uft	0.06	1.23	0.38	7.57	1.84
Ft	0.05	1.20	0.35	6.95	1.97

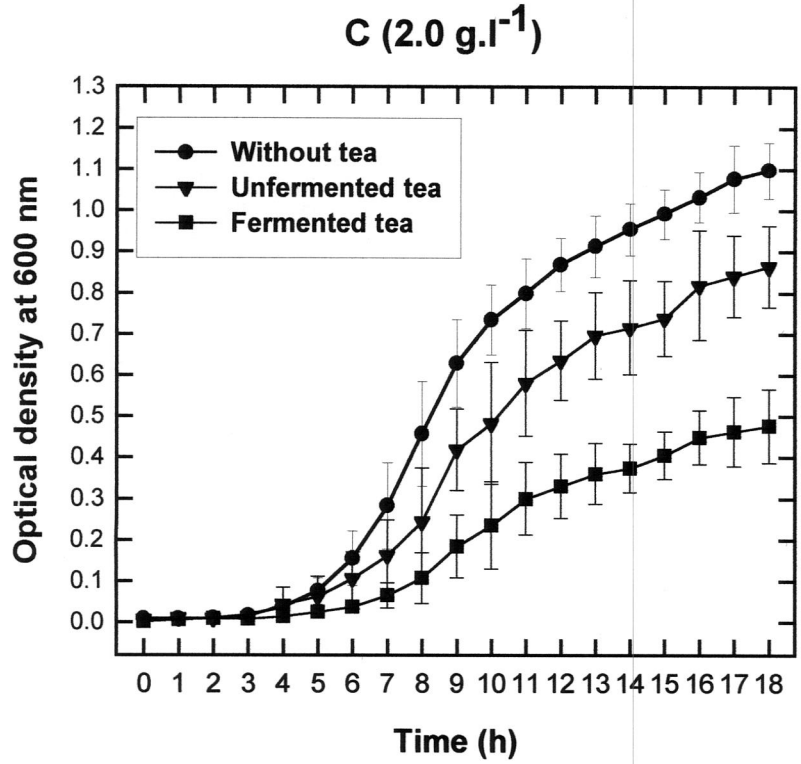
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.07	1.25	0.35	6.44	1.98
Uft	0.03	1.13	0.35	7.11	1.91
Ft	0.03	0.97	0.36	8.36	2.03

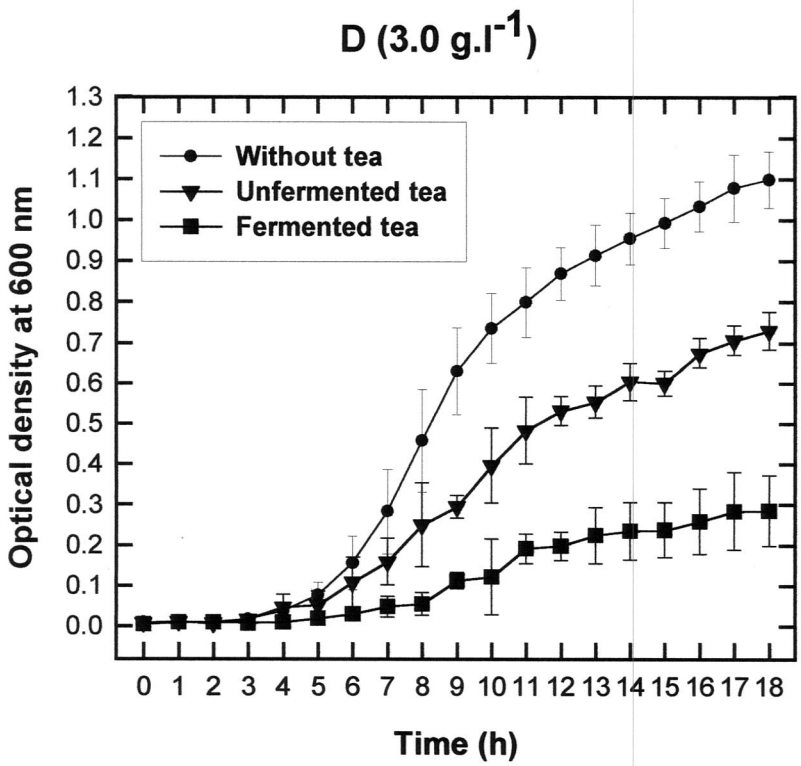
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 1. Growth of *Staphylococcus aureus* (USFSCC 29) in the presence of MRS plus different concentrations of fermented and unfermented rooibos tea water extracts. The error bars represent the standard deviation of three repeats.



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.07	1.25	0.35	6.44	1.98
Uft	0.03	1.03	0.35	7.66	1.80
Ft	0.02	0.64	0.32	10.36	2.23

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

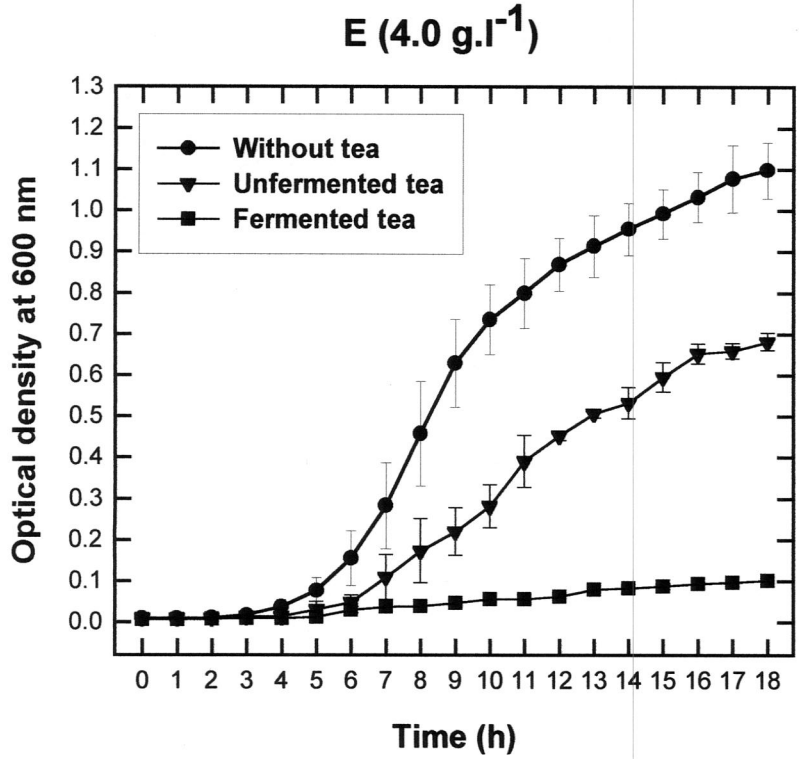


	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.07	1.25	0.35	6.44	1.98
Uft	0.02	0.83	0.33	7.79	1.84
Ft	0.01	0.34	0.27	10.60	2.42

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

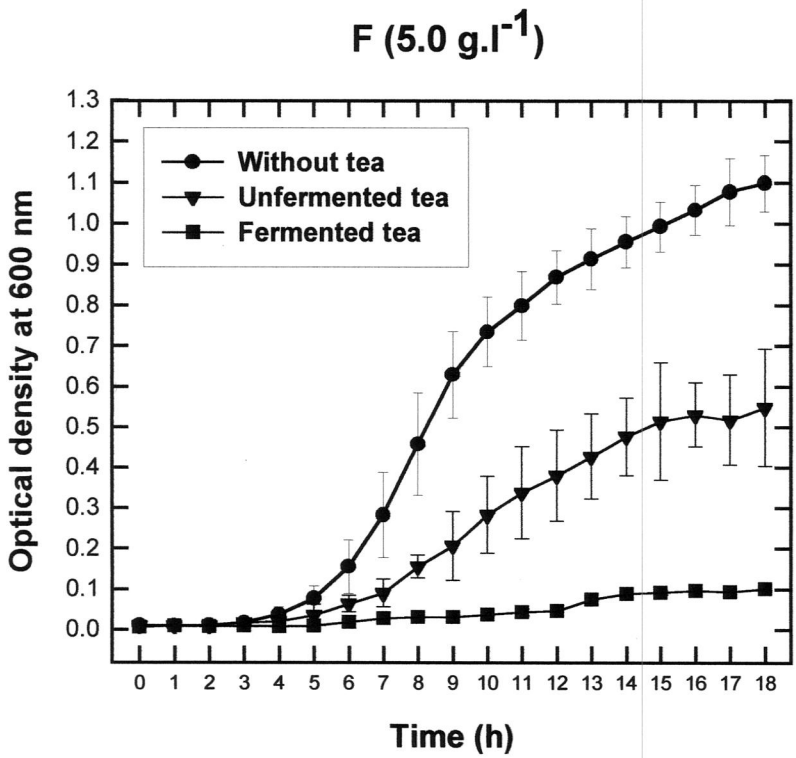
Figure 1. (Continue)

groei55b



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.07	1.25	0.35	6.44	1.98
Uft	0.02	0.79	0.33	8.35	2.04
Ft	0.00	0.18	0.25	12.58	4.12

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.07	1.25	0.35	6.44	1.98
Uft	0.01	0.69	0.32	8.51	2.41
Ft	0.00	0.18	0.19	14.59	4.13

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 1. (Continue)

maxstaph

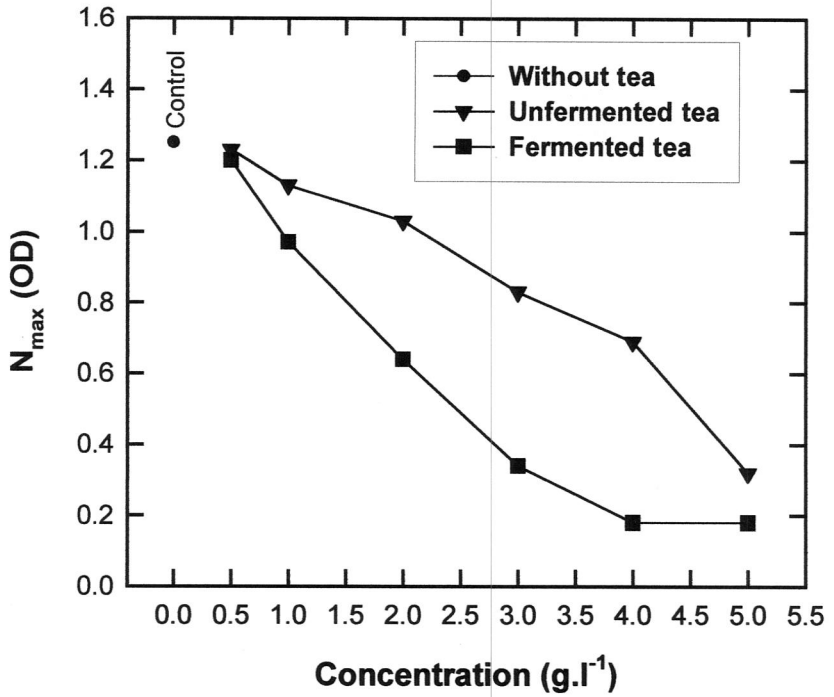


Figure 2. The decrease in the final bacterial cell density (N_{max}) of *Staph. aureus* (USFSCC 29) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

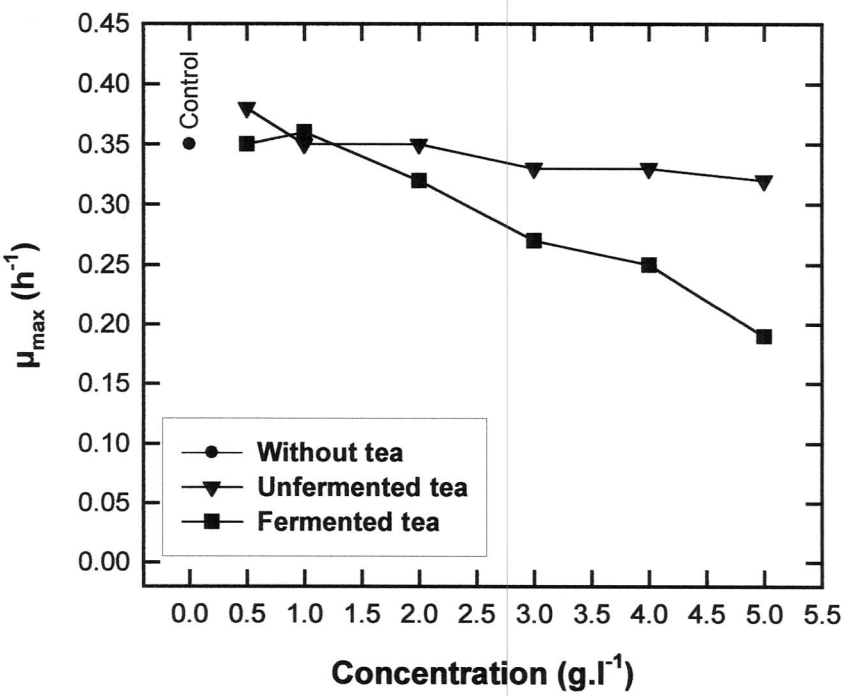


Figure 3. The decrease in maximum growth rate (μ_{max}) of *Staph. aureus* (USFSCC 29) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

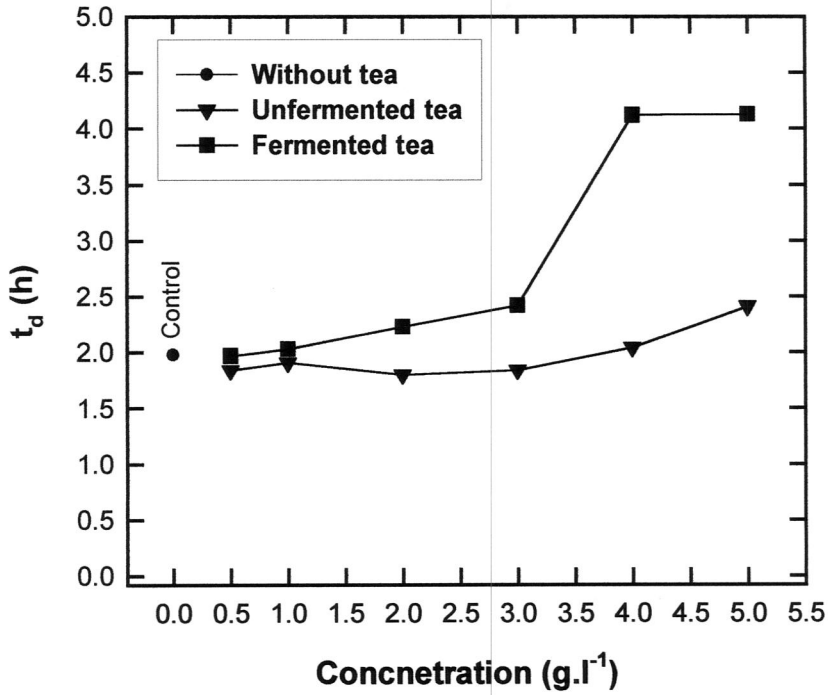


Figure 4. The increase in doubling time (t_d) of *Staph. Aureus* (USFSCC 29) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

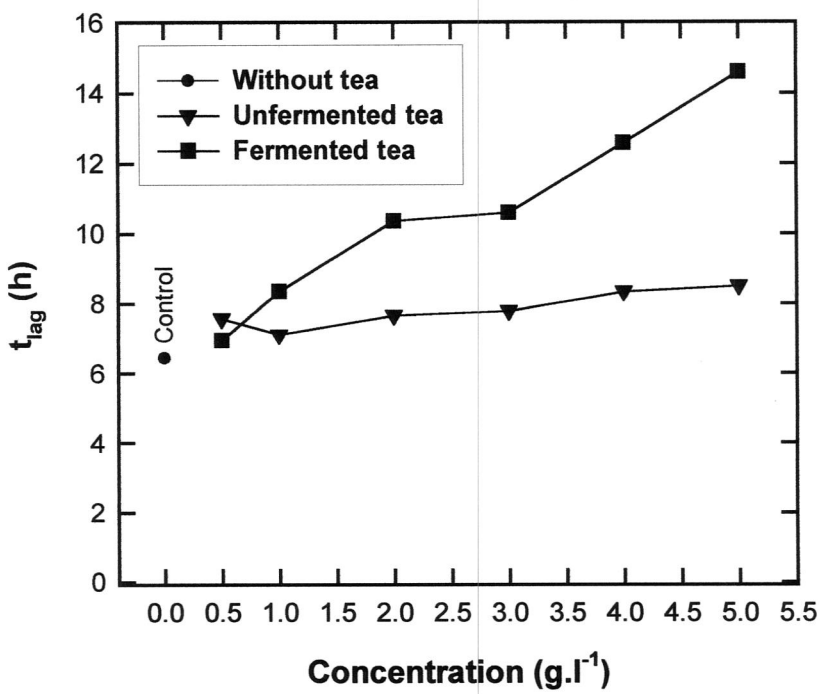
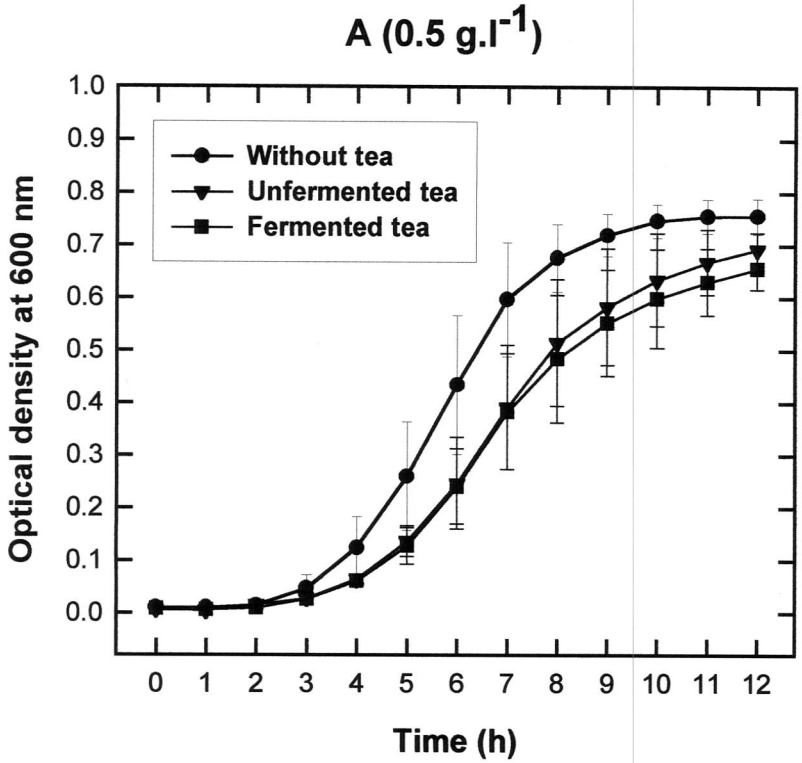
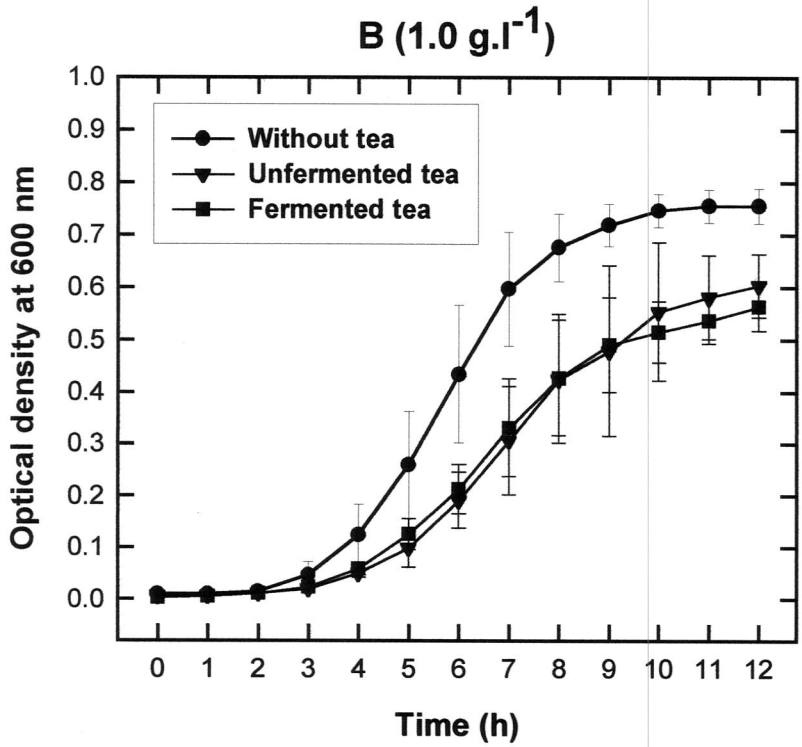


Figure 5. The increase in lag time (t_{lag}) of *Staph. aureus* (USFSCC 29) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.04	0.87	0.64	5.41	1.21
Uft	0.03	0.88	0.55	5.90	1.40
Ft	0.03	0.85	0.47	5.98	1.51

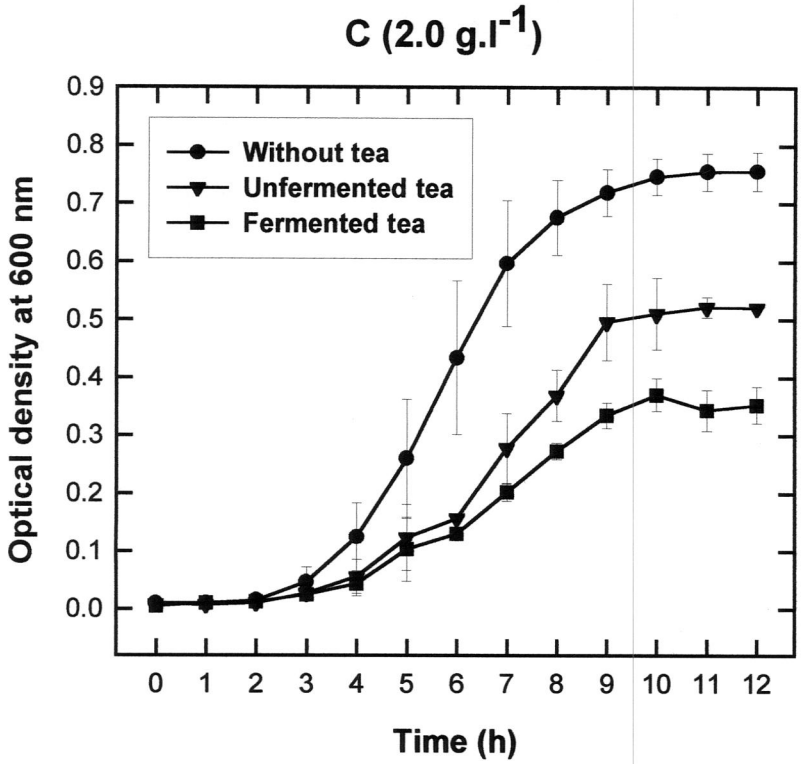
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.04	0.87	0.64	5.41	1.21
Uft	0.03	0.79	0.49	6.38	1.44
Ft	0.04	0.77	0.43	6.13	1.54

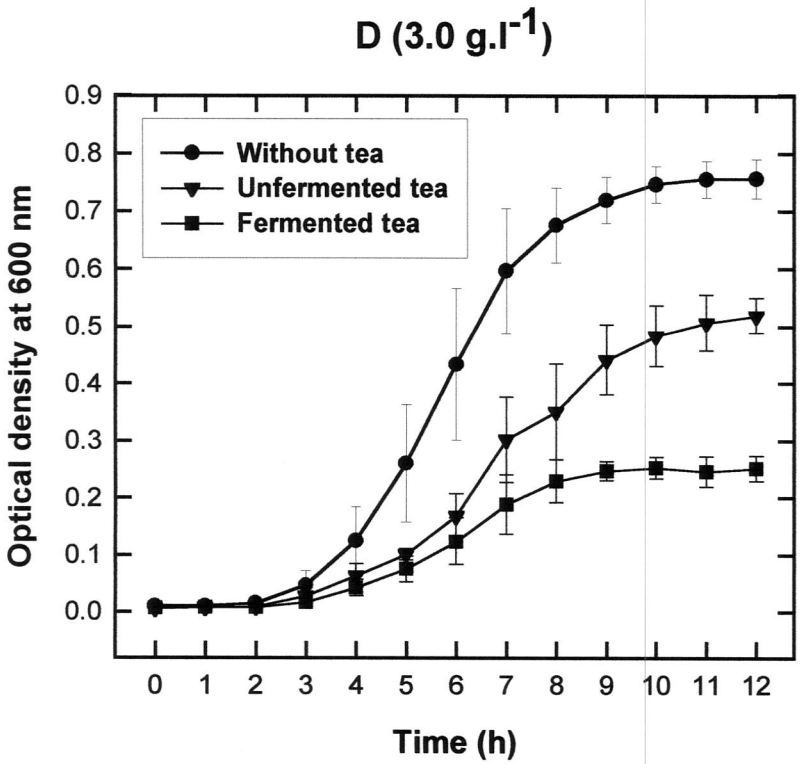
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 6. Growth of *Bacillus cereus* (USFSCC 39) in the presence of MRS plus different concentrations fermented and unfermented rooibos tea water extracts. The error bars represent the standard deviation of three repeats.



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.04	0.87	0.64	5.41	1.21
Uft	0.03	0.63	0.48	6.44	1.45
Ft	0.03	0.50	0.40	6.12	1.54

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

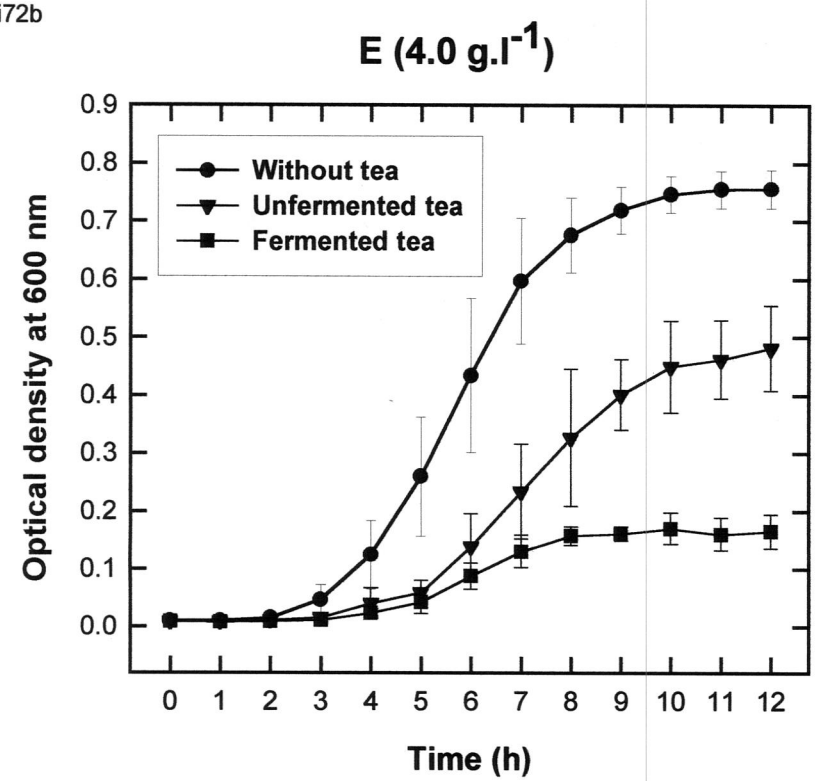


	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.04	0.87	0.64	5.41	1.21
Uft	0.03	0.61	0.43	6.46	1.51
Ft	0.03	0.32	0.38	6.36	1.89

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

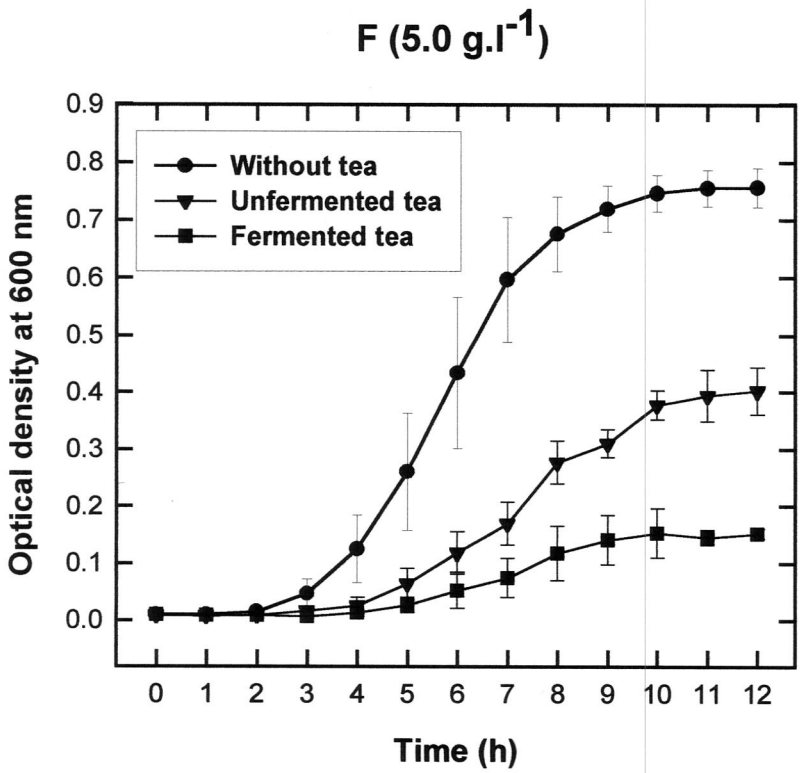
Figure 6. (continue)

groei72b



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.04	0.87	0.64	5.41	1.21
Uft	0.03	0.61	0.42	6.80	1.62
Ft	0.03	0.24	0.32	6.91	2.18

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.04	0.87	0.64	5.41	1.21
Uft	0.03	0.61	0.43	7.30	1.67
Ft	0.03	0.23	0.32	7.85	2.30

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 6. (continue)

maxbac

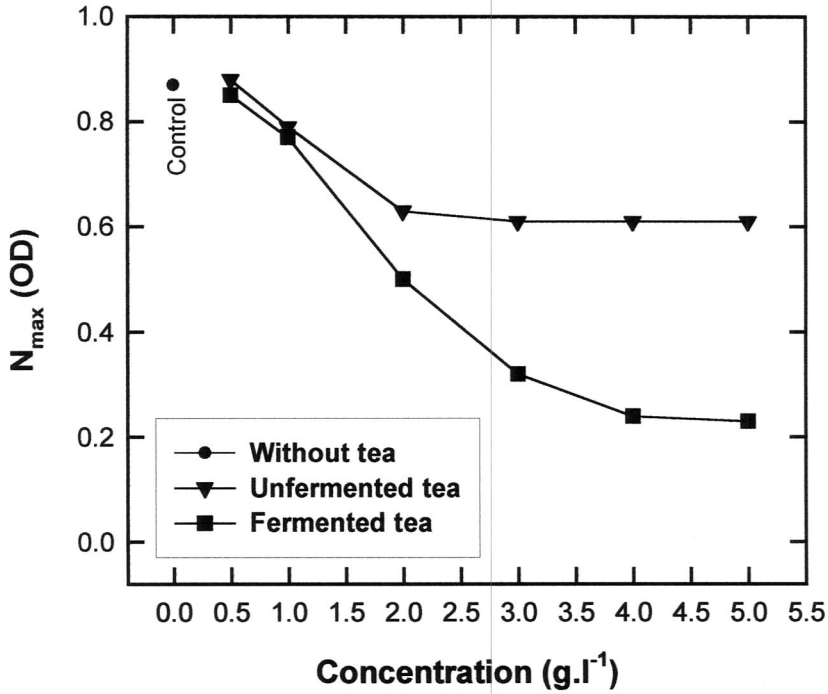


Figure 7. The decrease in final bacterial cell density (N_{max}) of *B. cereus* (USFSCC 39) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

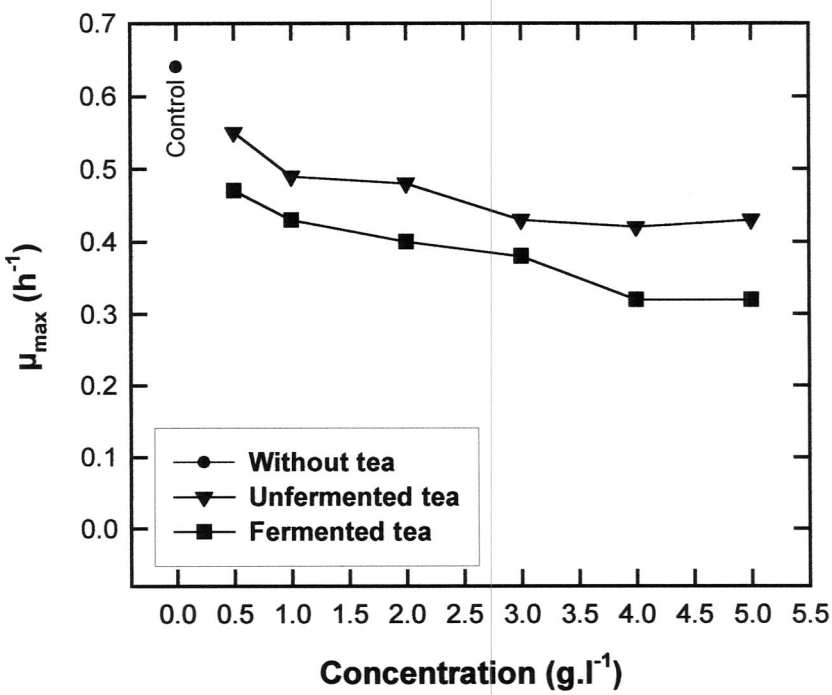


Figure 8. The decrease in maximum growth rate (μ_{max}) of *B. cereus* (USFSCC 39) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

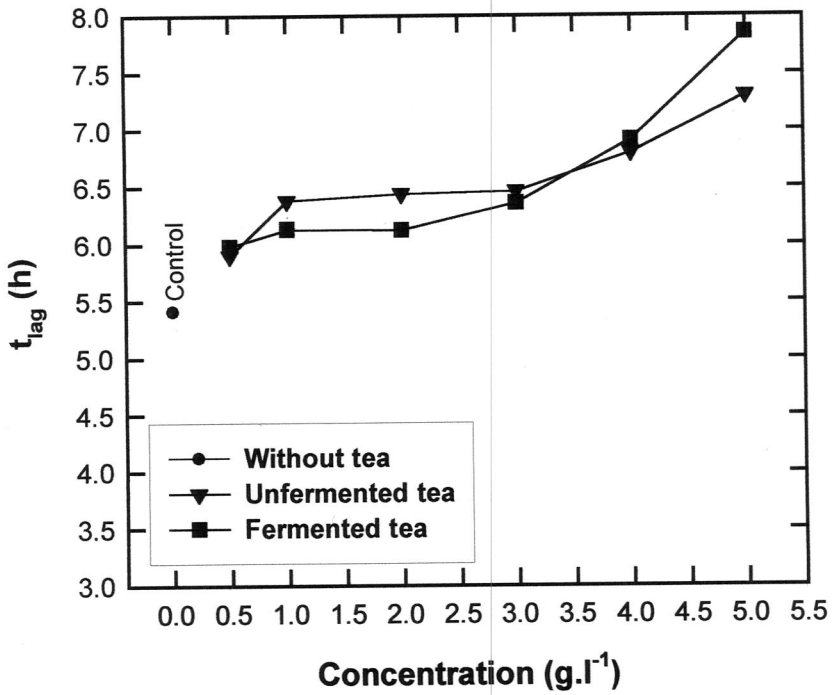


Figure 9. The increase in lag time (t_{lag}) of *B. cereus* (USFSCC 39) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

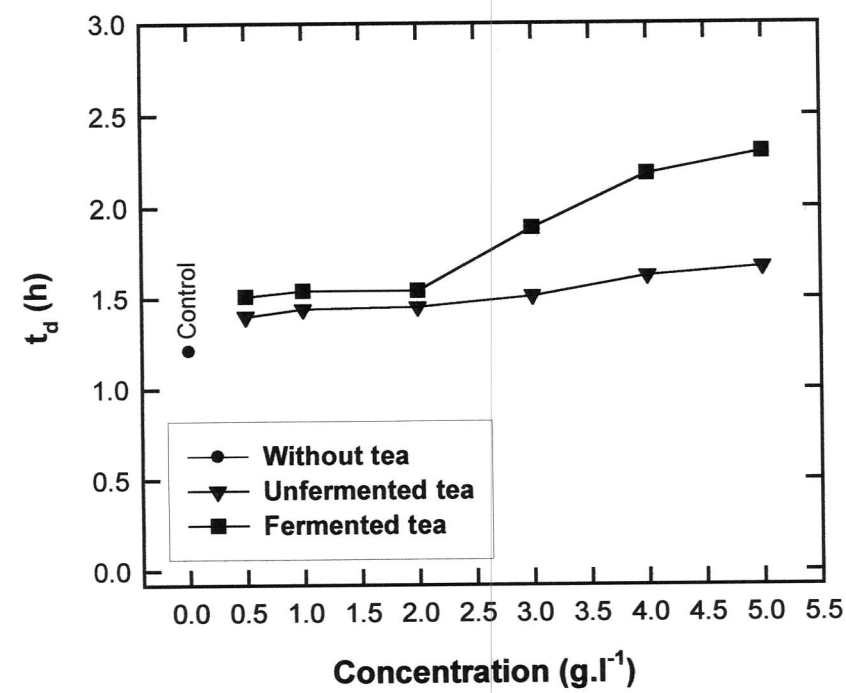


Figure 10. The increase in doubling time (t_d) of *B. cereus* (USFSCC 39) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

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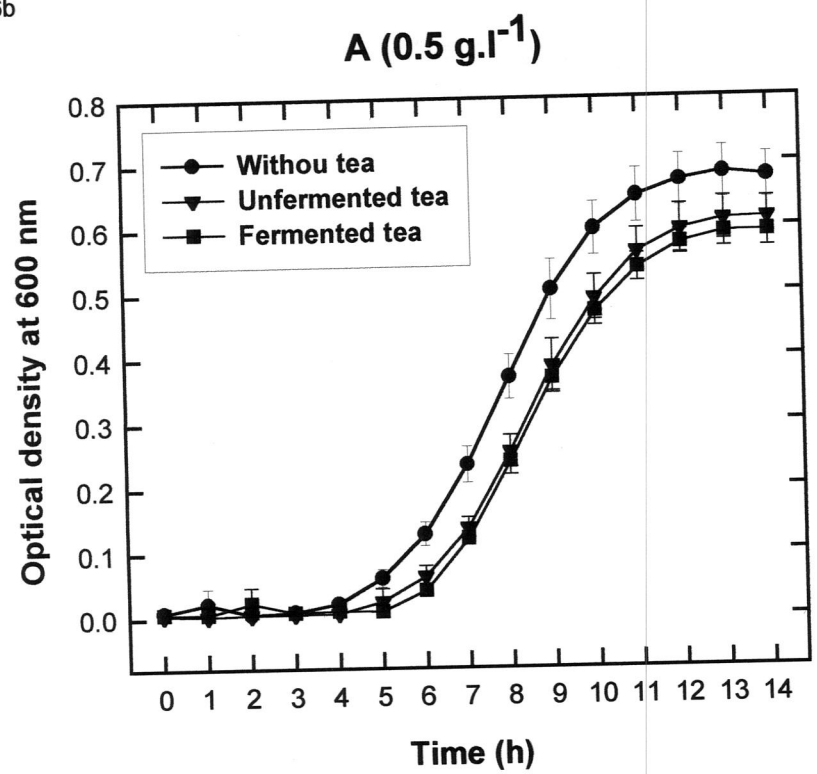
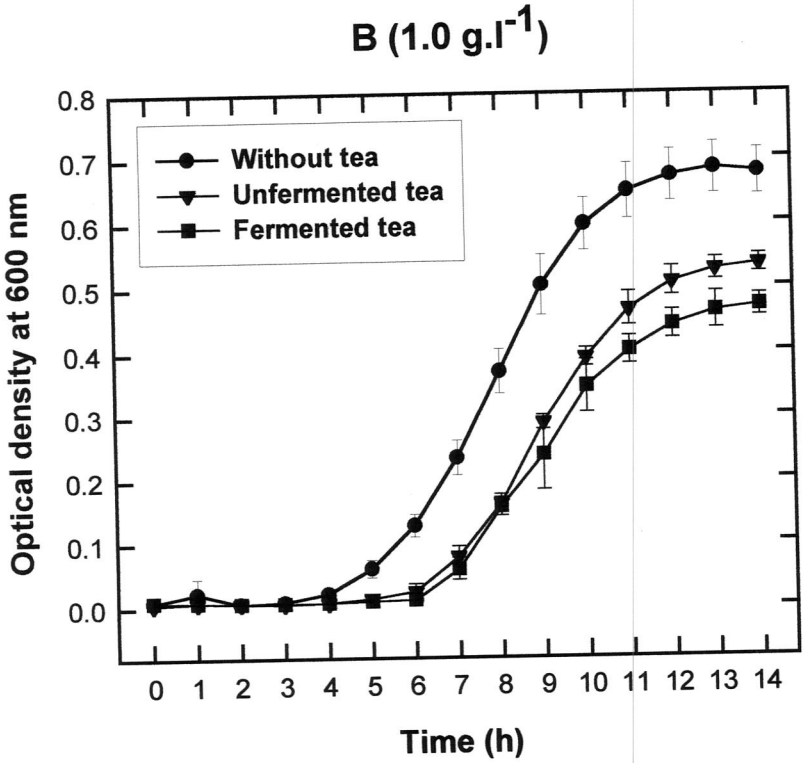


	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.03	0.90	0.43	7.14	1.63
Uft	0.01	0.82	0.43	7.66	1.60
Ft	0.00	0.79	0.41	7.45	1.63

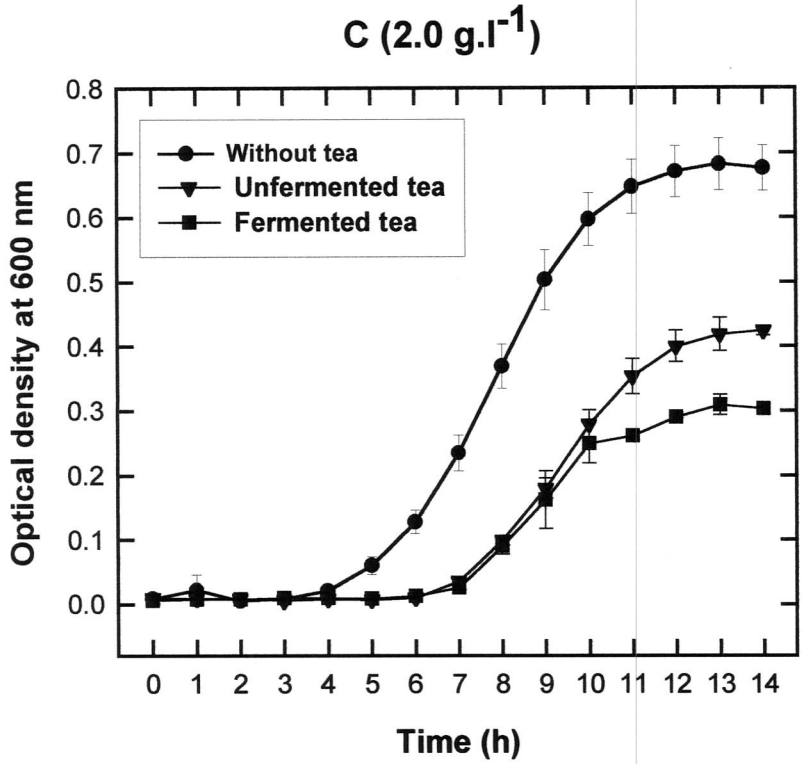
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.03	0.90	0.43	7.14	1.63
Uft	0.01	0.71	0.47	8.79	1.44
Ft	0.01	0.63	0.41	8.96	1.46

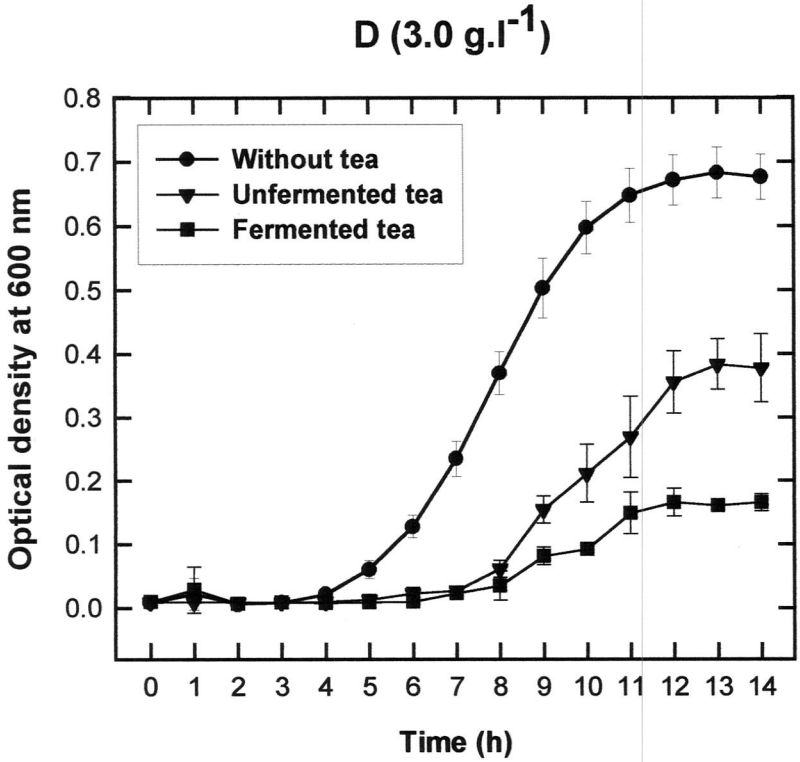
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented te

Figure 11. Growth of *Listeria monocytogenes* (USFSCC 1273) in the presence of MRS plus different concentrations fermented and unfermented rooibos tea water extracts. The error bars represent the standard deviation of three repeats.



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.03	0.90	0.43	7.14	1.63
Uft	0.00	0.61	0.44	9.41	1.50
Ft	0.00	0.39	0.35	9.65	1.50

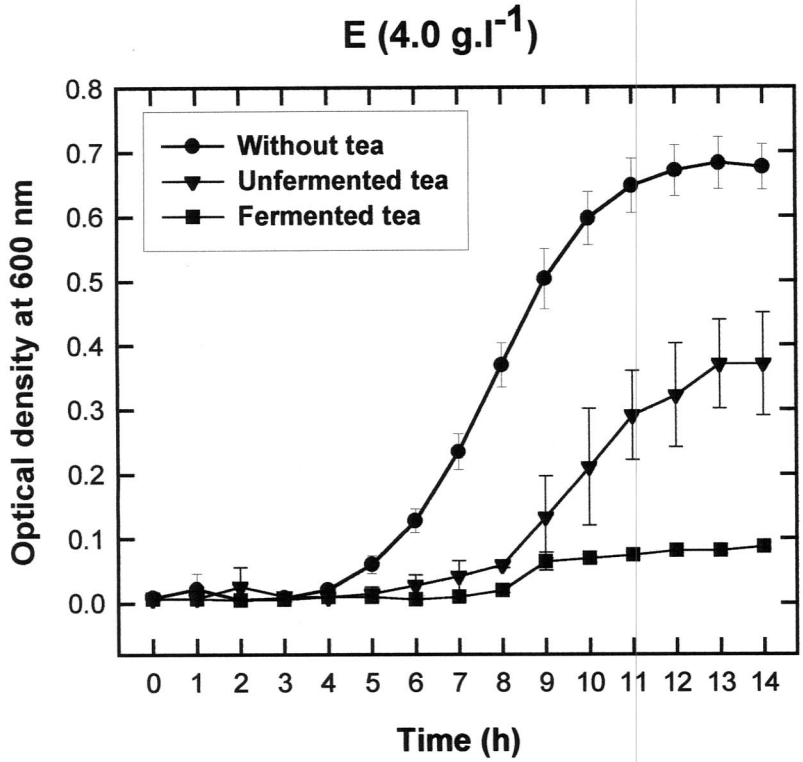
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.03	0.90	0.43	7.14	1.63
Uft	0.01	0.56	0.45	10.00	1.49
Ft	0.00	0.30	0.34	11.13	2.05

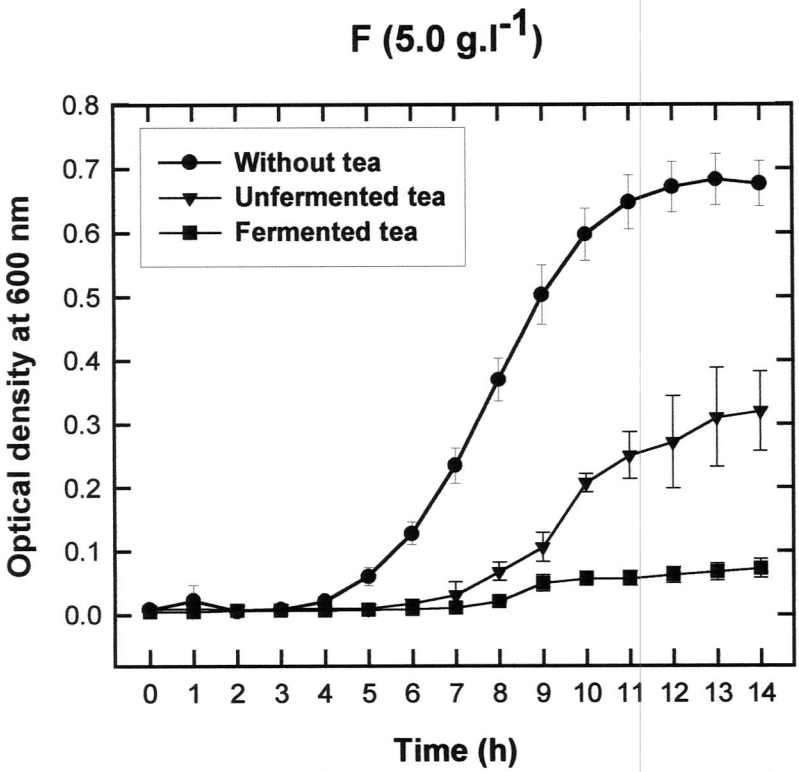
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 11. (continue)



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.03	0.90	0.43	7.14	1.63
Uft	0.00	0.54	0.43	10.01	1.54
Ft	0.00	0.14	0.28	11.98	2.55

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.03	0.90	0.43	7.14	1.63
Uft	0.00	0.48	0.41	10.03	1.74
Ft	0.00	0.09	0.21	12.10	2.56

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 11. (continue)

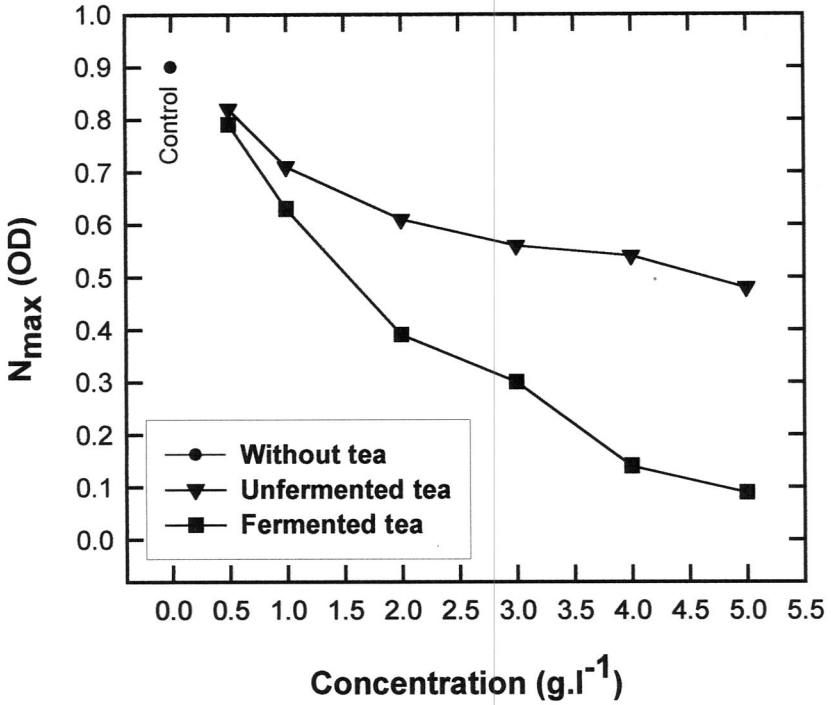


Figure 12. The decrease in final bacterial cell density (N_{max}) of *L. monocytogenes* (USFSCC 1273) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations

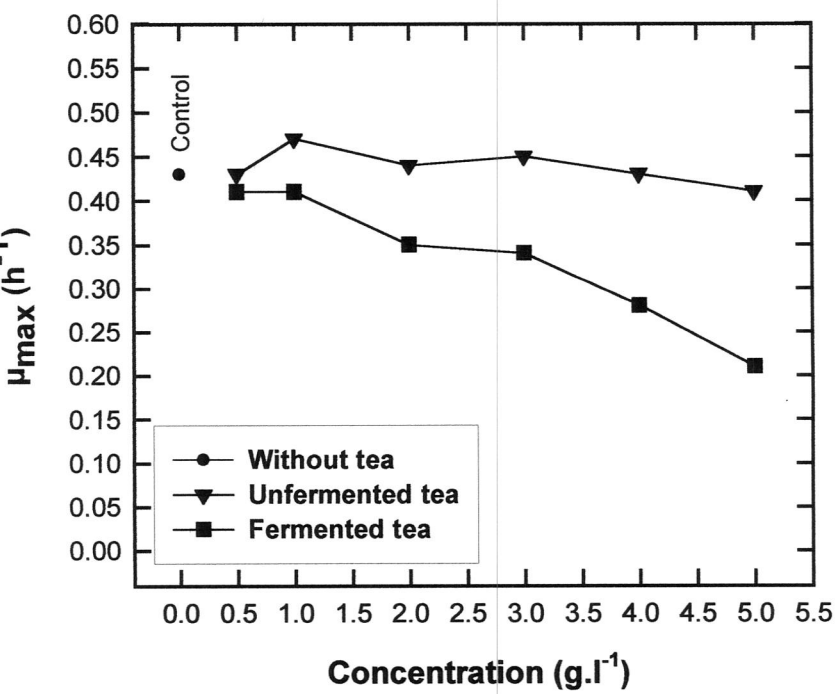


Figure 13. The decrease in maximum growth rate (μ_{max}) of *L. monocytogenes* (USFSCC 1273) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

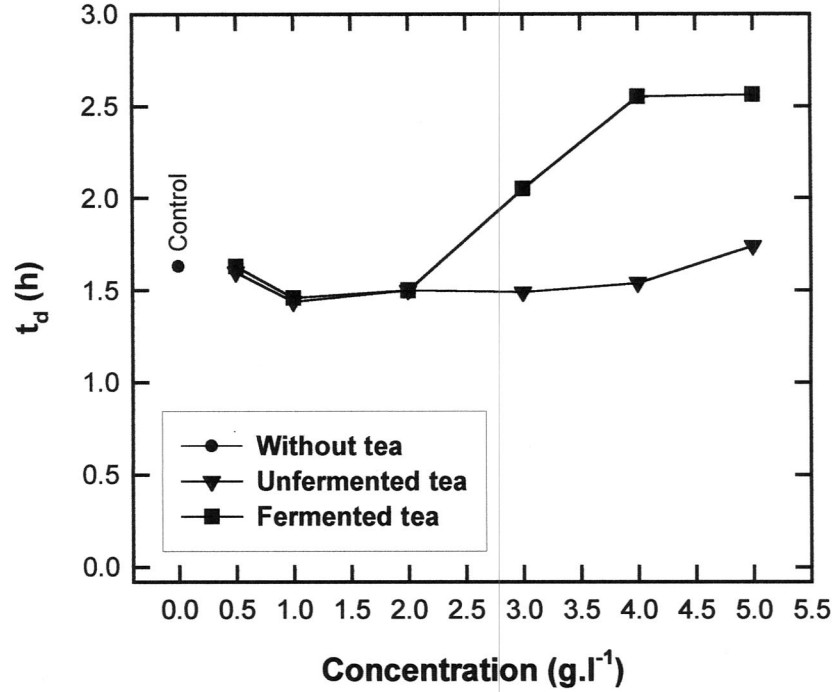


Figure 14. The increase in doubling time (t_d) of *L. monocytogenes* (USFSCC 1273) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

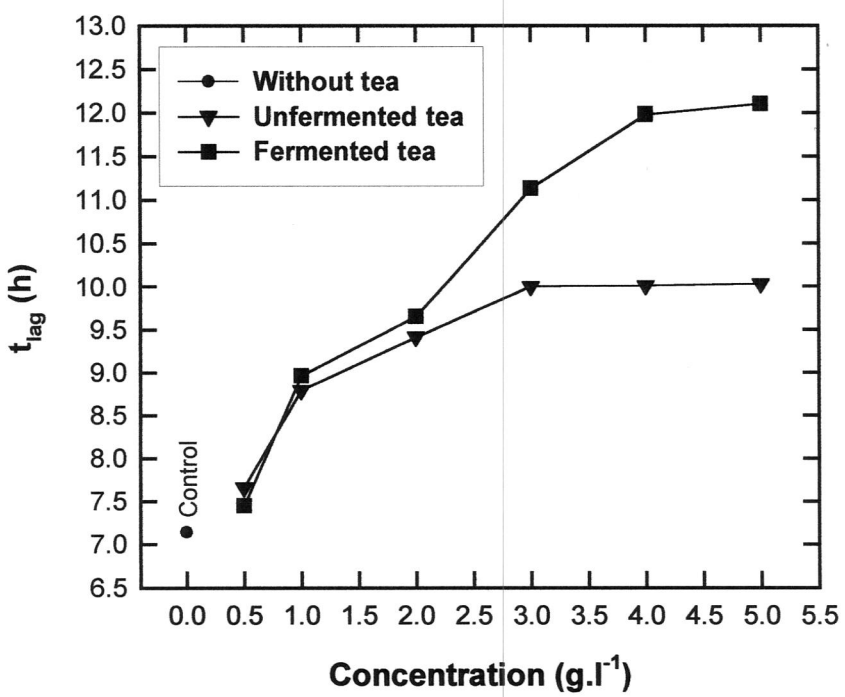
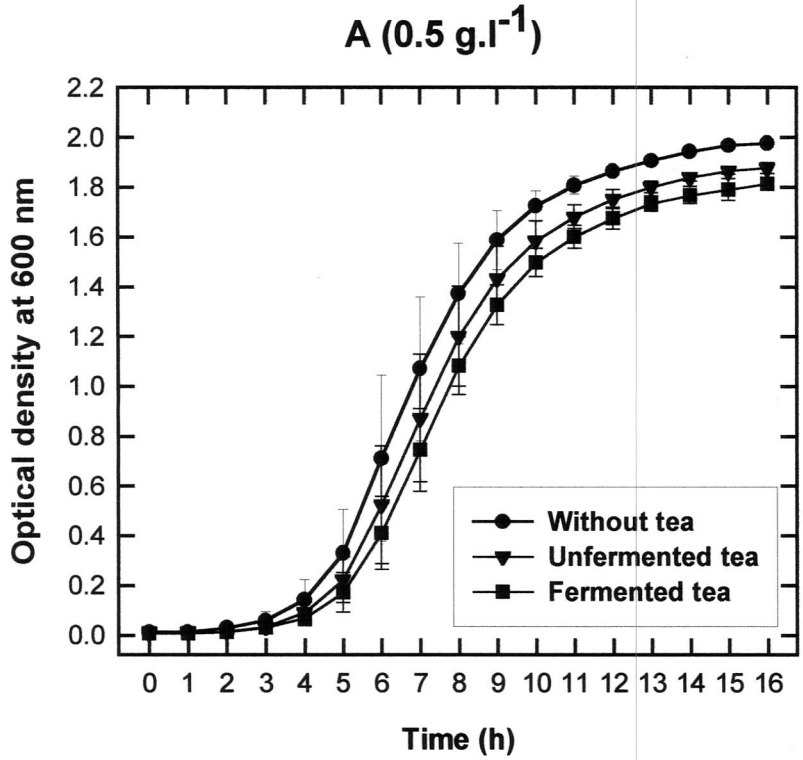
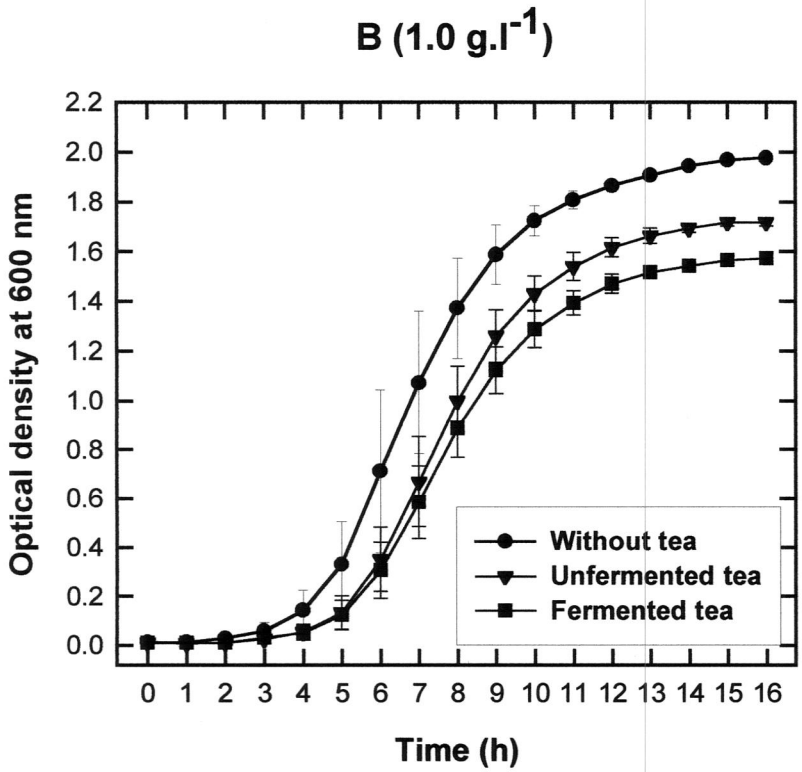


Figure 15. The increase in lag time (t_{lag}) of *L. monocytogenes* (USFSCC 1273) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.10	1.92	1.38	5.07	0.63
Uft	0.05	1.81	1.28	5.30	0.64
Ft	0.05	1.80	0.96	5.60	0.76

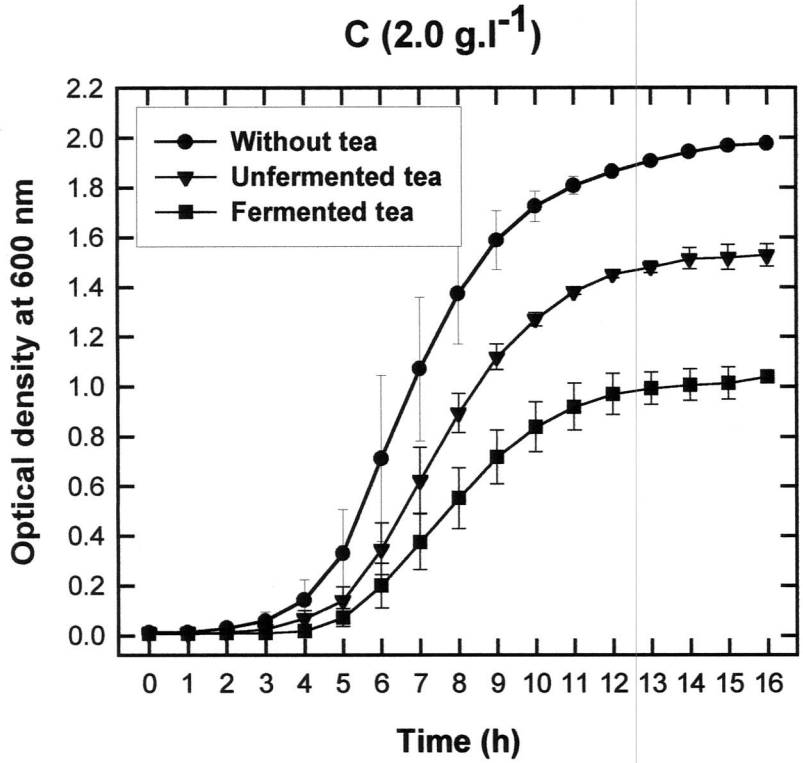
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.10	1.92	1.38	5.07	0.63
Uft	0.06	1.71	0.97	5.71	0.72
Ft	0.07	1.58	0.90	6.23	0.78

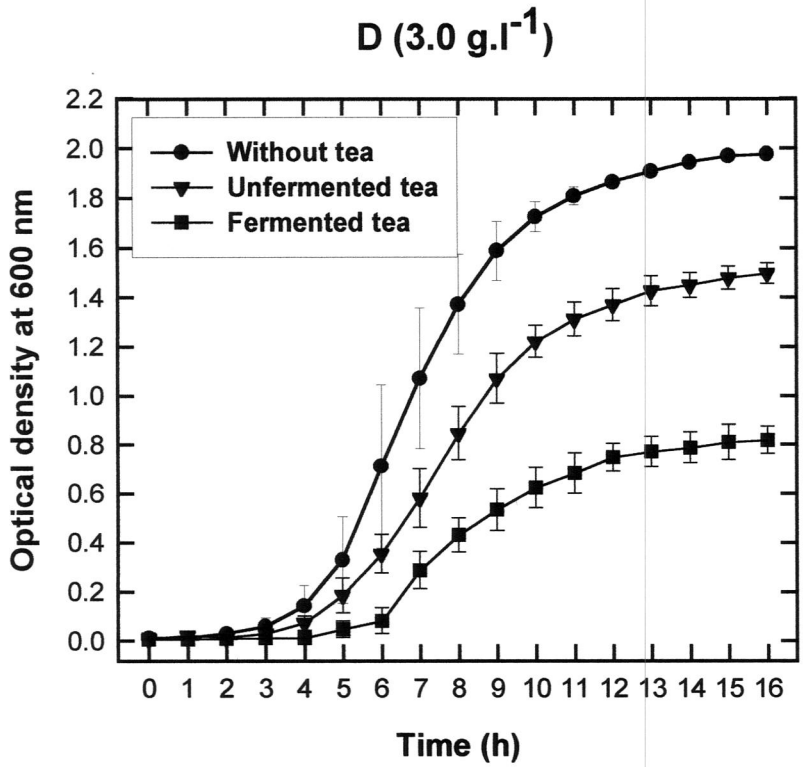
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 16. The growth of *Streptococcus mutans* (USFSCC 1277) in the presence of MRS plus different concentrations fermented and unfermented rooibos tea water extracts. The error bars represent the standard deviation of three repeats.



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.10	1.92	1.38	5.07	0.63
Uft	0.09	1.62	0.77	5.65	0.86
Ft	0.05	1.23	0.45	6.34	1.57

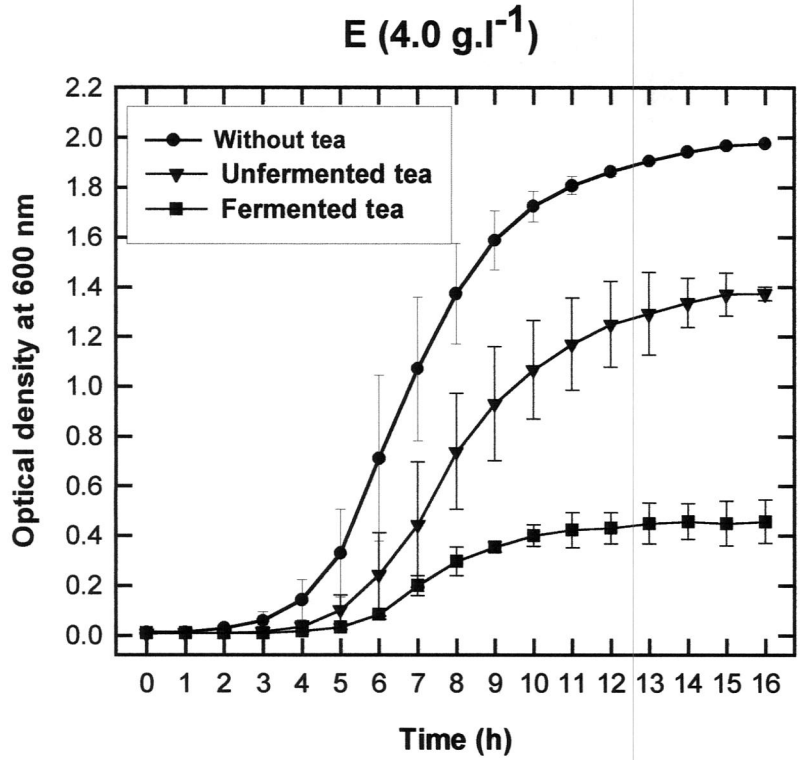
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.10	1.92	1.38	5.07	0.63
Uft	0.08	1.48	0.78	5.92	0.89
Ft	0.02	1.03	0.35	6.58	1.96

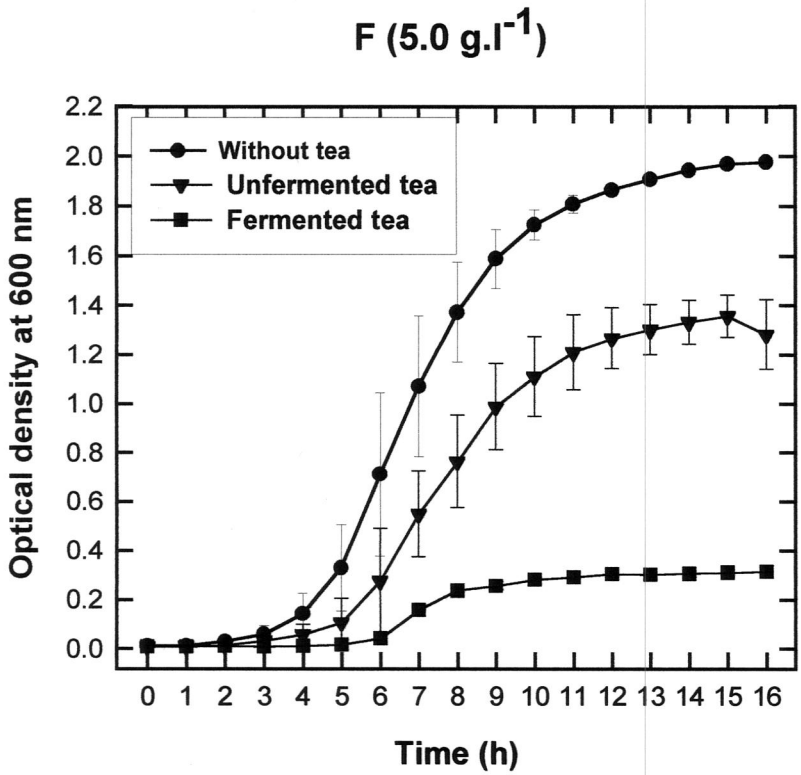
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 16. (continue)



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.10	1.92	1.38	5.07	0.63
Uft	0.04	1.41	0.75	6.11	0.98
Ft	0.02	0.60	0.27	7.07	2.00

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.10	1.92	1.38	5.07	0.63
Uft	0.05	1.39	0.73	6.28	1.03
Ft	0.02	0.36	0.23	7.55	1.95

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 16. (continue)

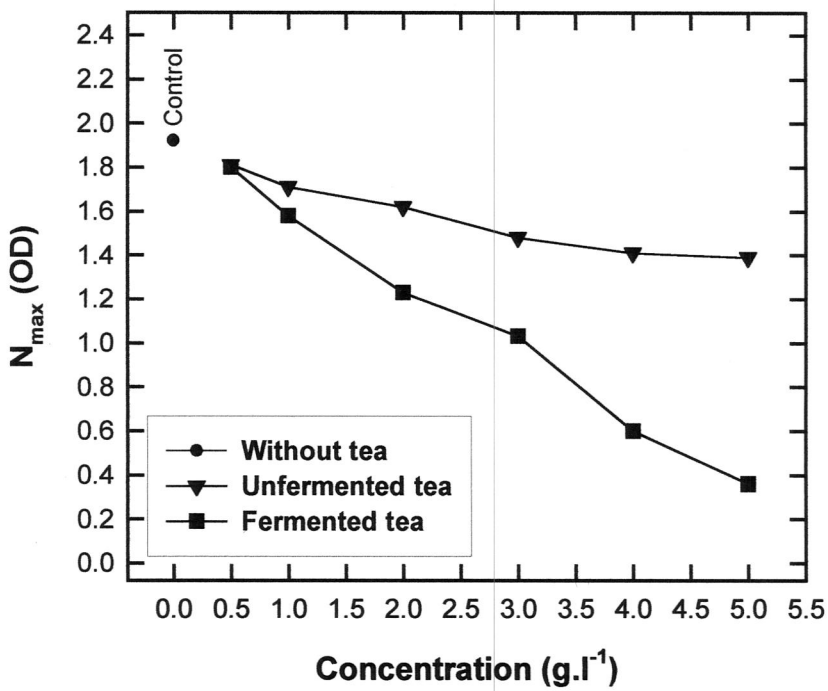


Figure 17. The decrease in final bacterial cell density (N_{max}) of *Strep. mutans* (USFSCC 1277) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

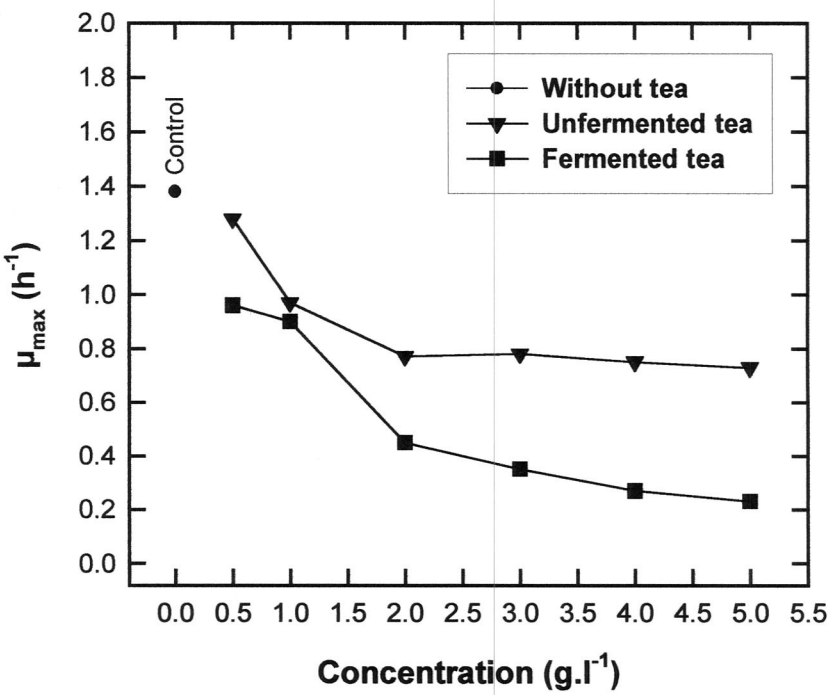


Figure 18. The decrease in maximum growth rate (μ_{max}) of *Strep. mutans* (USFSCC 1277) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

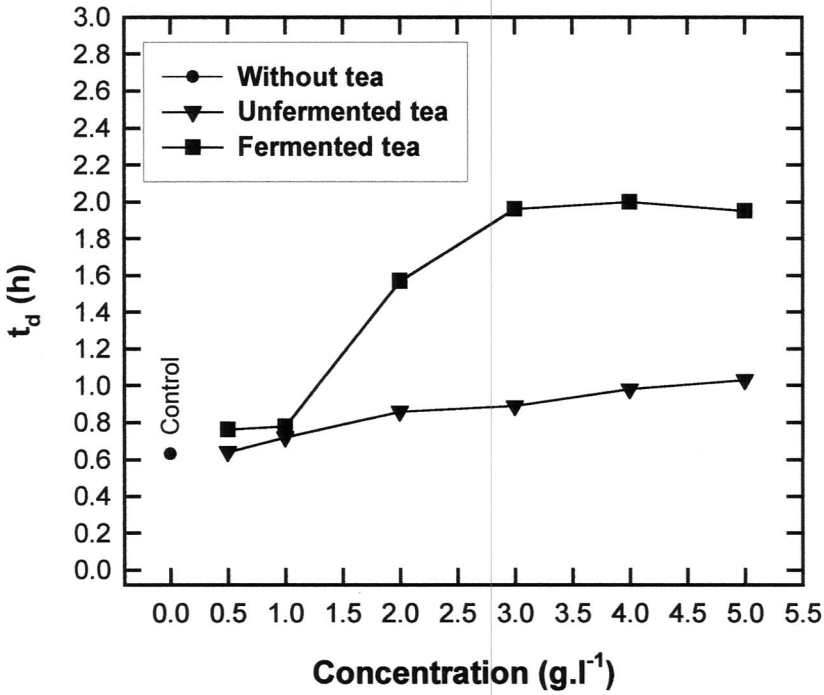


Figure 19. The increase in doubling time (t_d) of *Strep. mutans* (USFSCC 1277) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

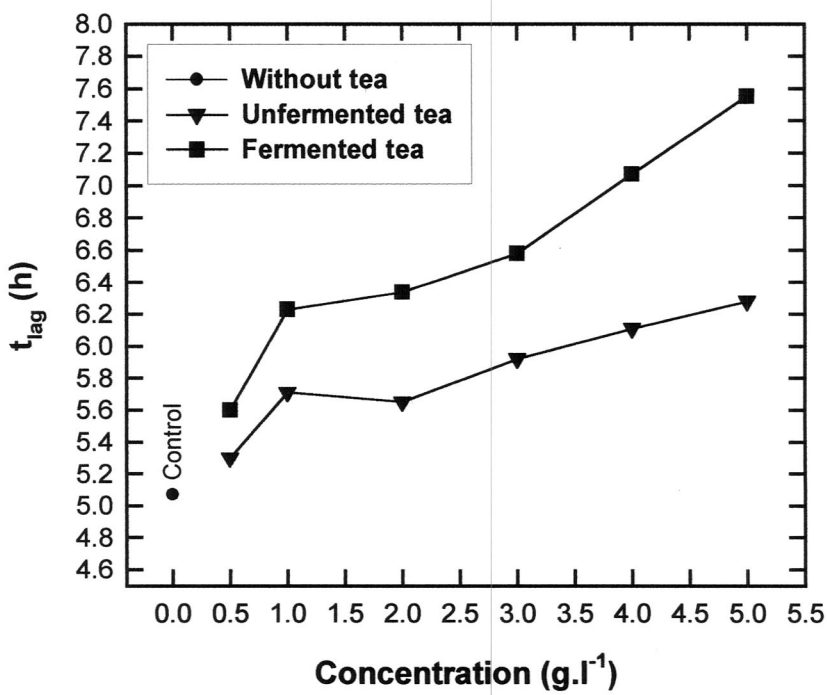
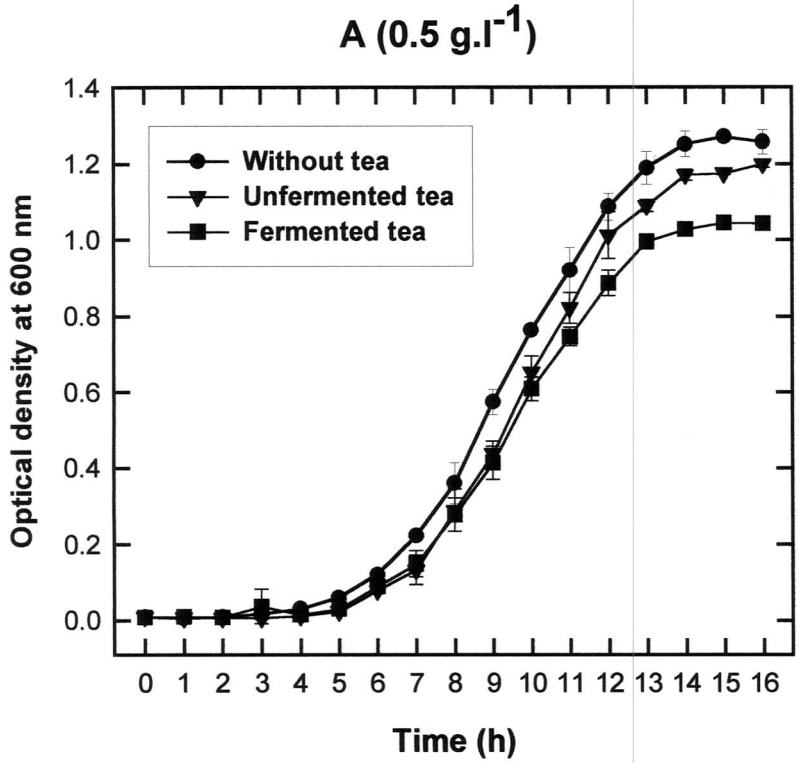
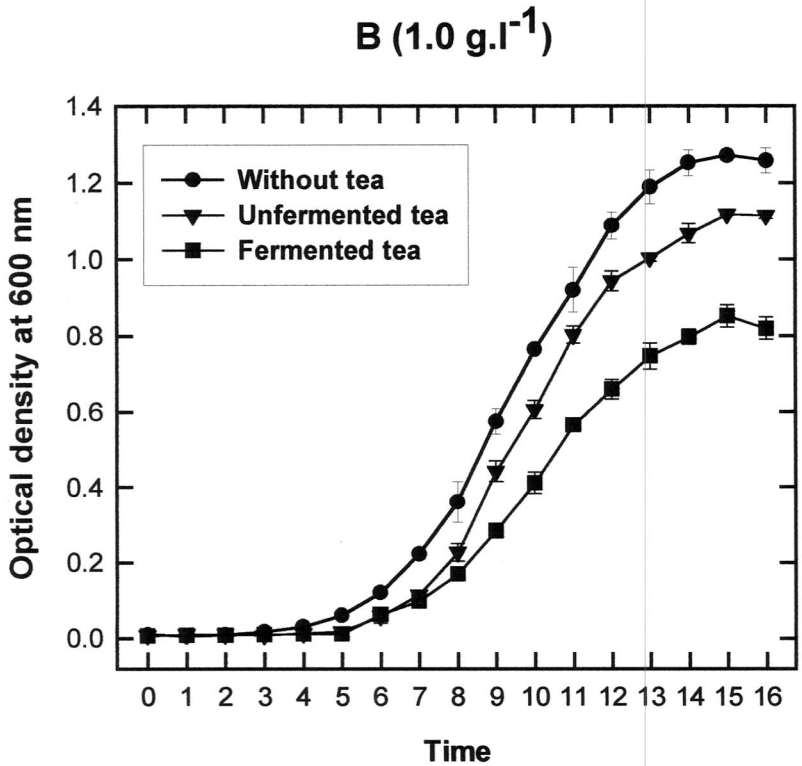


Figure 20. The increase in lag time (t_{lag}) of *Strep. mutans* (USFSCC 1277) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.06	1.47	0.57	7.74	1.22
Uft	0.03	1.34	0.66	8.20	1.04
Ft	0.04	1.25	0.51	8.03	1.36

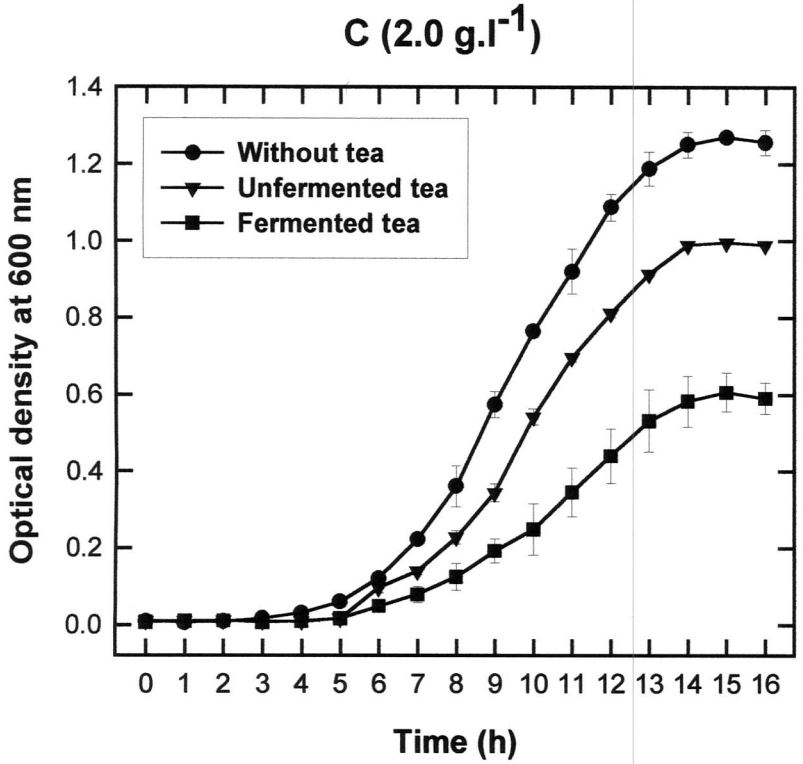
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.06	1.47	0.57	7.74	1.22
Uft	0.03	1.32	0.55	8.21	1.26
Ft	0.01	1.03	0.46	8.56	1.51

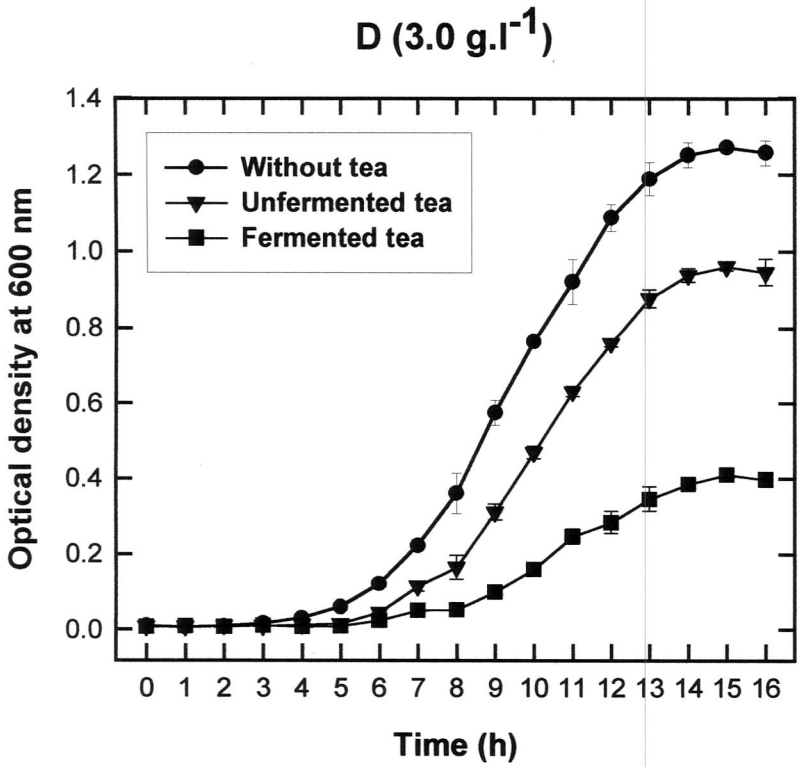
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 21. The growth of *Saccharomyces cerevisiae* (USFSCC 1035) in the presence of MRS plus different concentrations of fermented and unfermented rooibos tea water extracts. The error bars represent the standard deviation of three repeats.



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.06	1.47	0.57	7.74	1.22
Uft	0.03	1.20	0.51	8.36	1.34
Ft	0.01	0.79	0.39	9.31	1.75

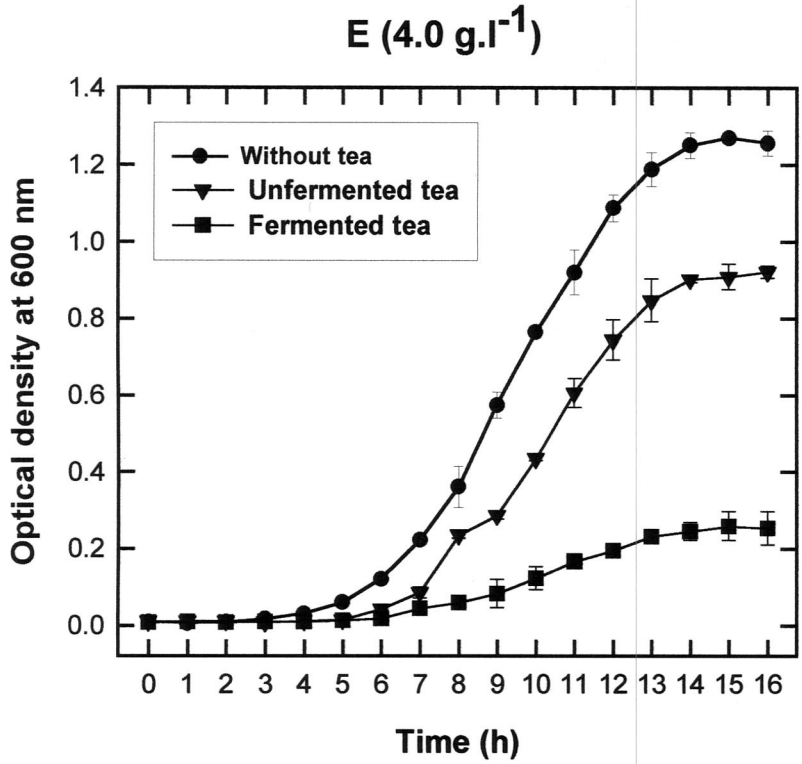
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.06	1.47	0.57	7.74	1.22
Uft	0.04	1.17	0.54	8.57	1.34
Ft	0.00	0.59	0.36	10.40	1.94

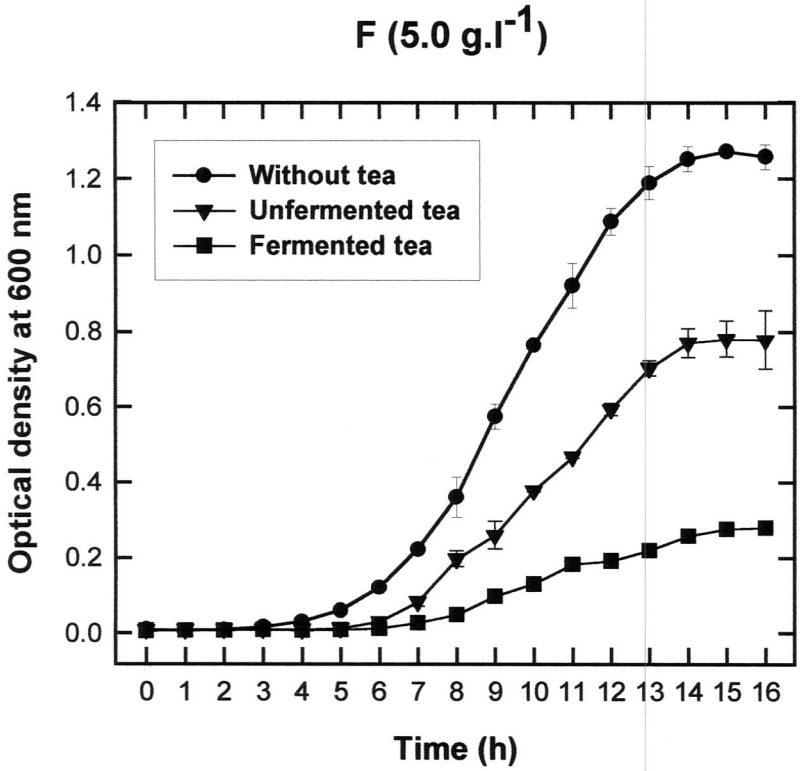
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 21. (continue)



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.06	1.47	0.57	7.74	1.22
Uft	0.01	1.11	0.50	8.60	1.44
Ft	0.00	0.36	0.28	10.66	1.93

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.06	1.47	0.57	7.74	1.22
Uft	0.00	1.10	0.44	8.66	1.59
Ft	0.00	0.44	0.24	10.89	2.20

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 21. (continue)

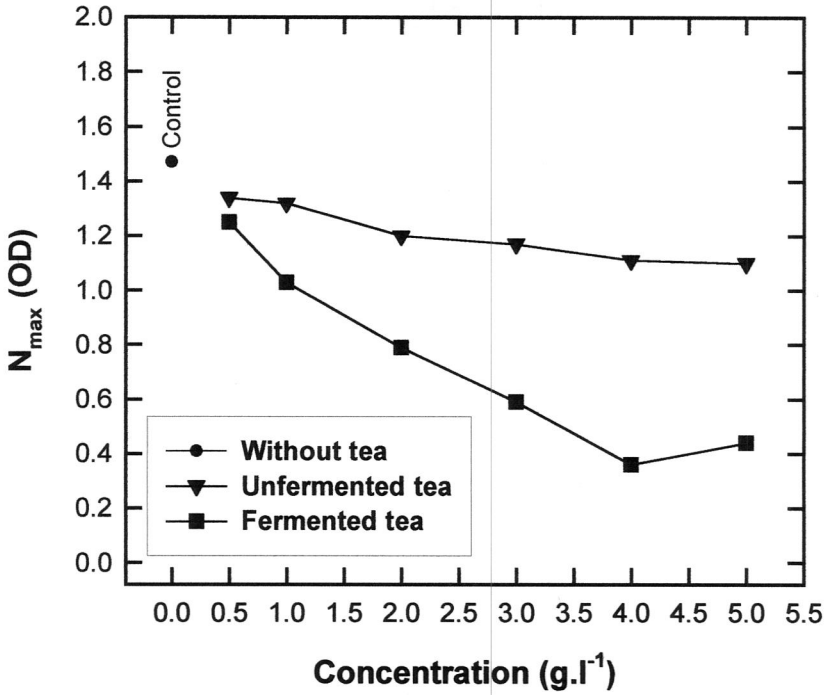


Figure 22. The decrease in final bacterial cell density (N_{max}) of *Sacch. cerevisiae* (USFSCC 1035) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

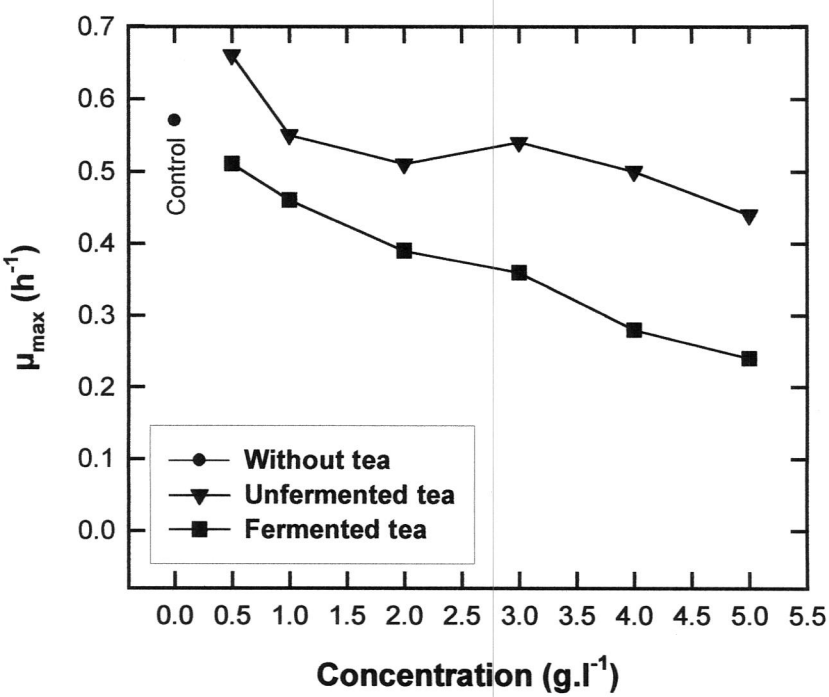


Figure 23. The decrease in maximum growth rate (μ_{max}) of *Sacch. cerevisiae* (USFSCC 1035) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

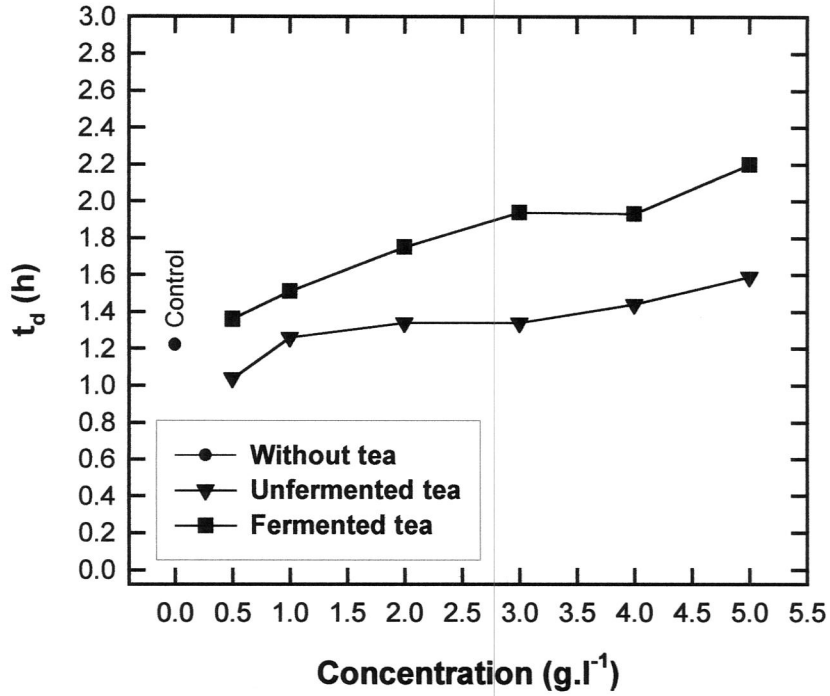


Figure 24. The increase in doubling time (t_d) of *Sacch. cerevisiae* (USFSCC 1035) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

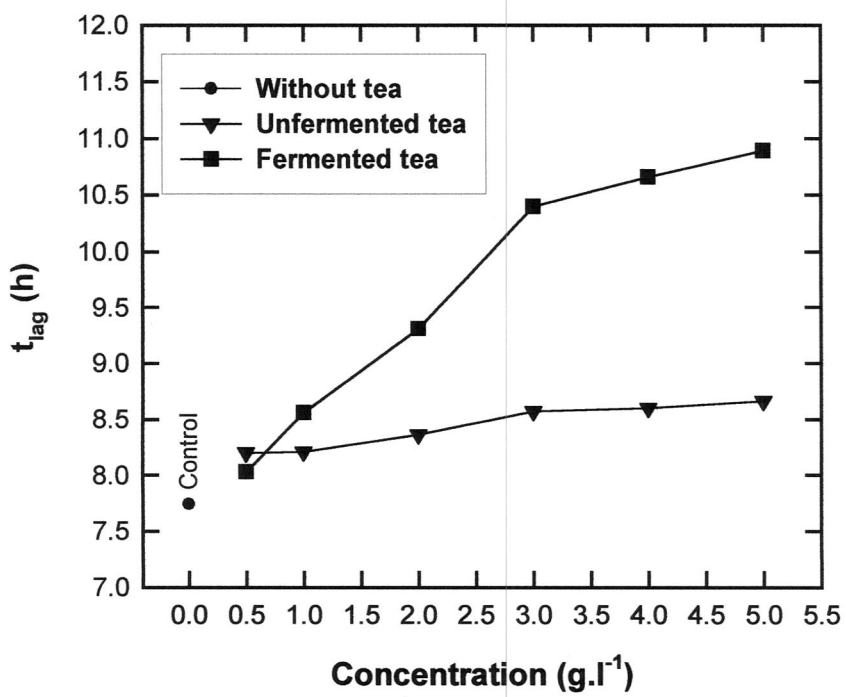
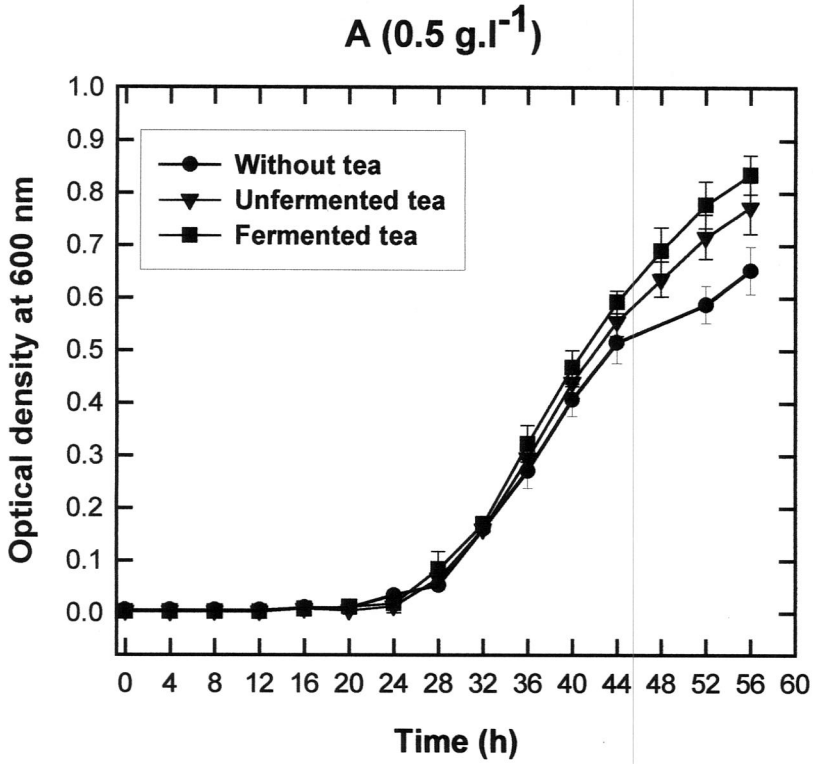
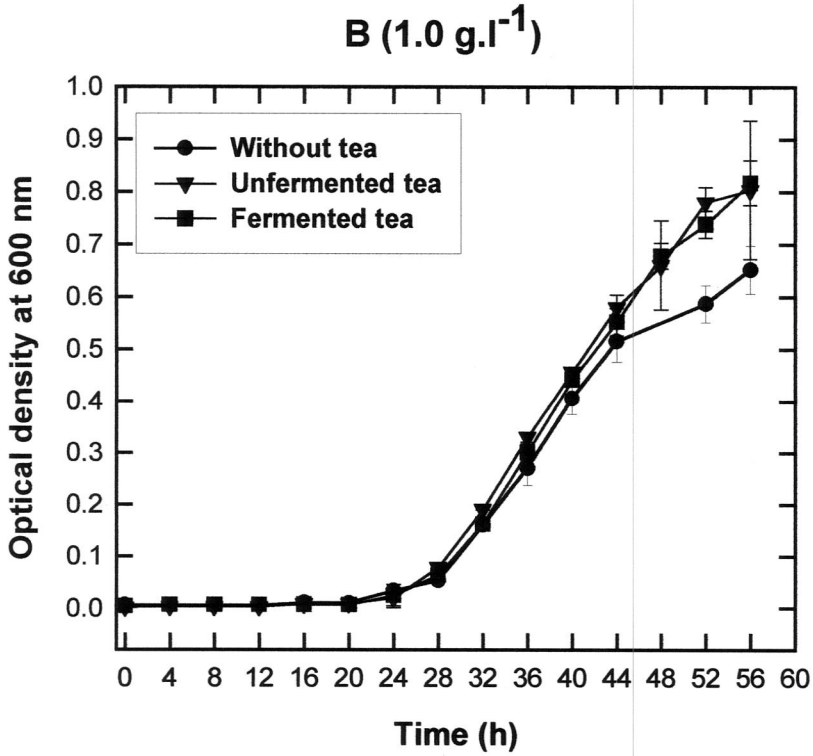


Figure 25. The increase in lag time (t_{lag}) of *Sacch. cerevisiae* (USFSCC 1035) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.02	0.83	0.14	34.75	4.71
Uft	0.01	0.89	0.16	34.60	4.28
Ft	0.01	0.99	0.16	34.98	4.50

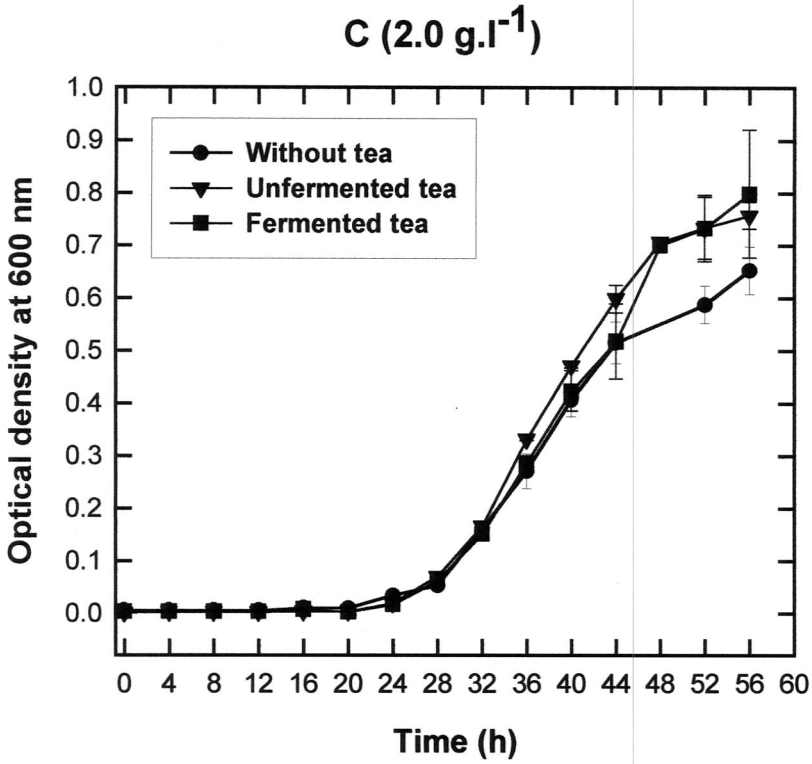
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.02	0.83	0.14	34.75	4.71
Uft	0.01	0.90	0.17	34.70	4.21
Ft	0.01	0.97	0.16	34.92	4.56

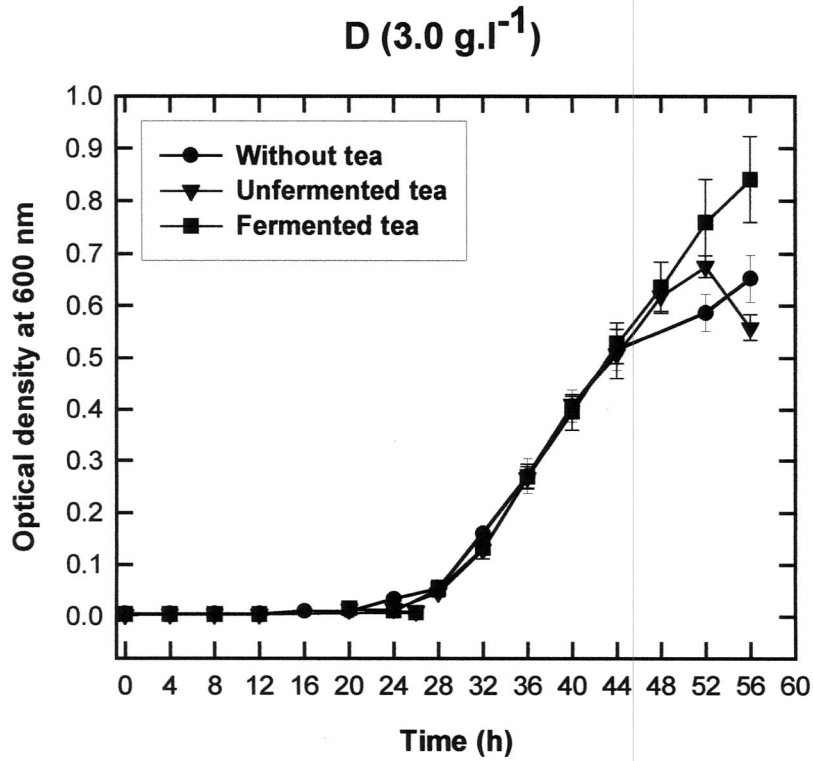
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 26. The growth of *Zygosaccharomyces rouxii* (USFSCC 1310) in the presence of MRS plus different concentrations fermented and unfermented rooibos tea water extracts. The error bars represent the mean of two repeats.



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.02	0.83	0.14	34.75	4.71
Uft	0.01	0.89	0.17	34.81	4.10
Ft	0.01	0.96	0.16	34.81	4.47

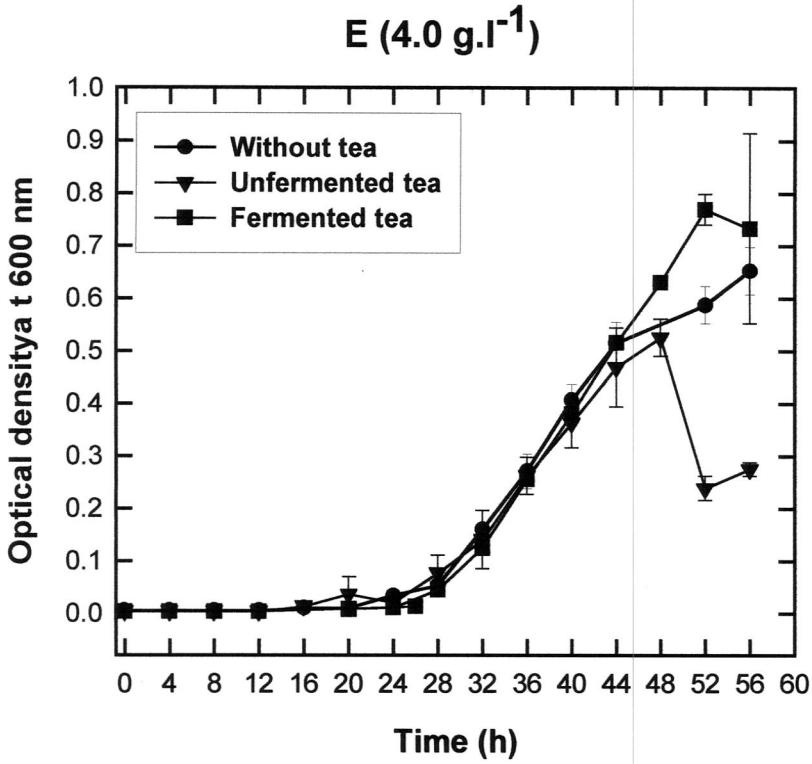
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.02	0.83	0.14	34.75	4.71
Uft	0.01	0.84	0.17	35.45	4.04
Ft	0.01	0.87	0.18	35.08	3.77

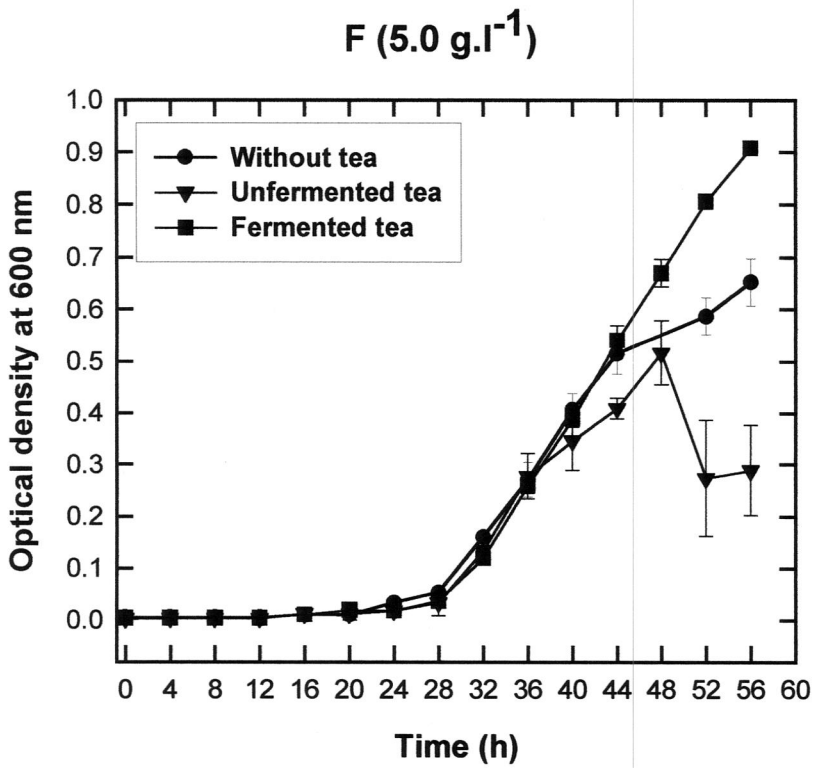
Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 26. (continue)



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.02	0.83	0.14	34.75	4.71
Uft	0.01	0.68	0.17	35.58	3.97
Ft	0.01	0.83	0.18	35.48	3.80

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea



	N_0	N_{max}	μ_{max}	t_{lag}	t_d
Wt	0.02	0.83	0.14	34.75	4.71
Uft	0.01	0.66	0.17	35.60	3.95
Ft	0.01	0.96	0.18	35.44	3.95

Wt - Without tea, Uft - Unfermented tea, Ft - Fermented tea

Figure 26. (continue)

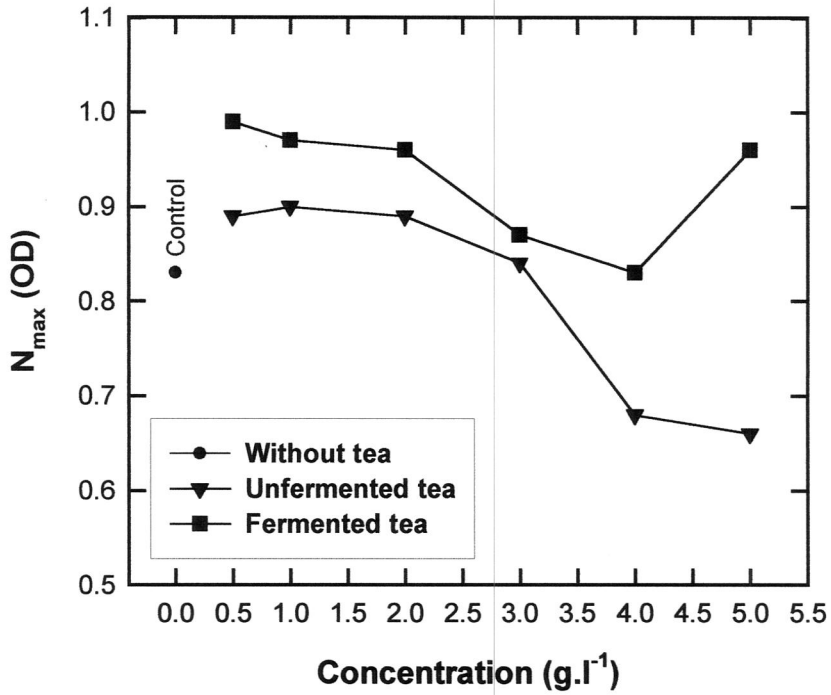


Figure 27. The decrease in final bacterial cell density (N_{max}) of *Z. rouxii* (USFSCC 1310) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

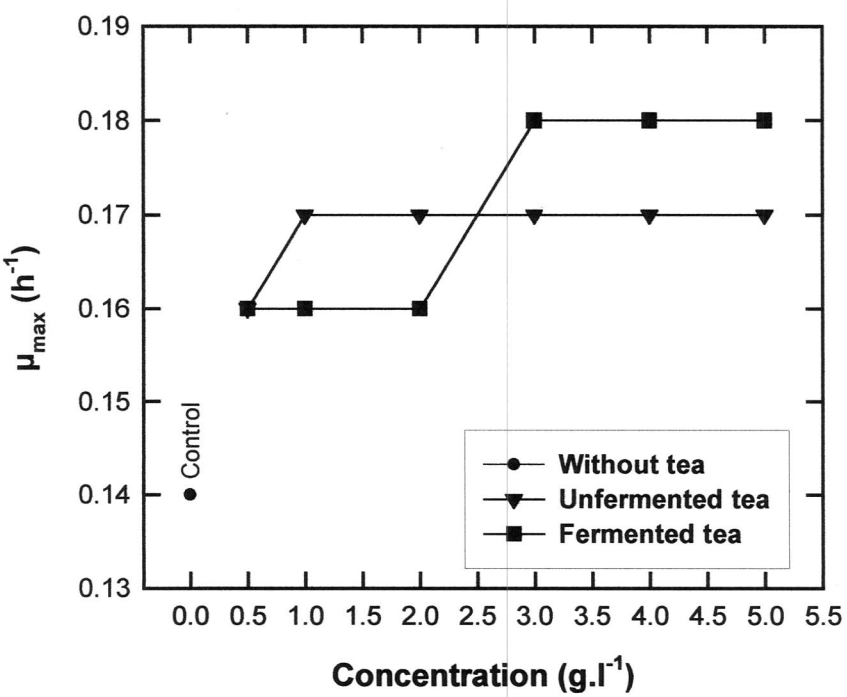


Figure 28. The maximum growth rate (μ_{max}) of *Z. rouxii* (USFSCC 1310) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

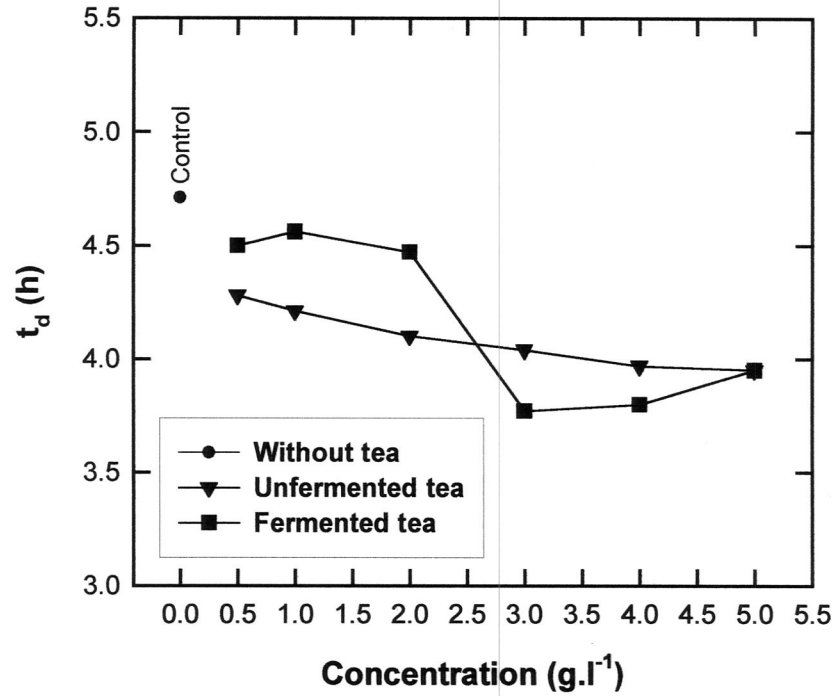


Figure 29. The doubling time (t_d) of *Z. rouxii* (USFSCC 1310) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

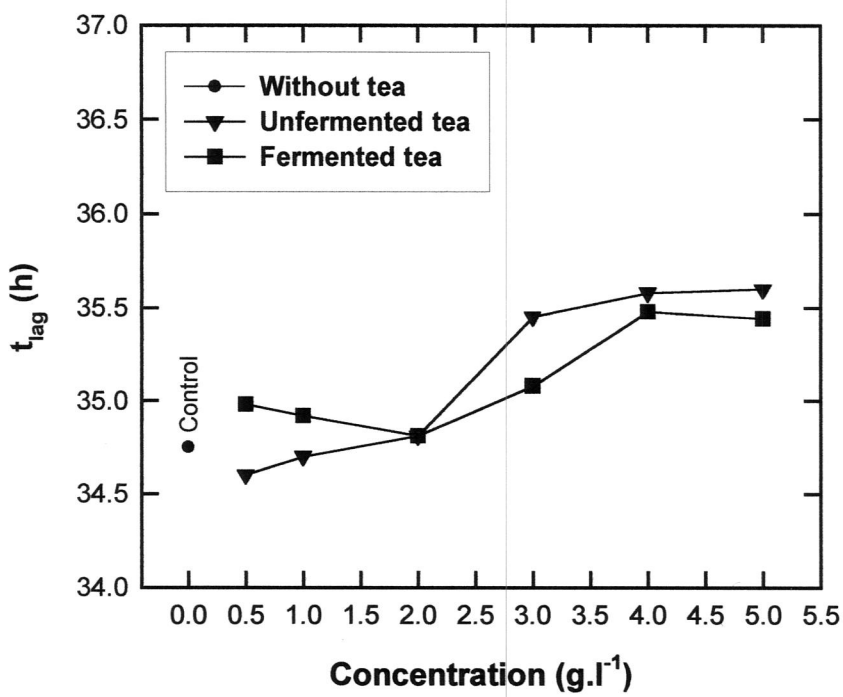


Figure 30. The increase in lag time (t_{lag}) of *Z. rouxii* (USFSCC 1310) grown in MRS plus fermented and unfermented rooibos tea water extracts at different concentrations.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Rooibos tea, a beverage unique to South Africa, is prepared from the leaves and stems of *Aspalathus linearis* and has a natural sweetish taste and a clear red-brown colour with an orange-yellow tint. This tea has become increasingly popular over the past decade due to its alleged health properties and it has been shown to improve the symptoms of insomnia, allergies and nervous complaints (Morton, 1983; Joubert & Ferreira, 1996). These health aspects have mainly been ascribed to the polyphenolic compounds and the associated antioxidant activity of rooibos tea (Niwa & Miyachi, 1986; Von Gadow *et al.*, 1997).

Similarly, green and black teas have also been reported to possess various positive biological and pharmacological effects (Yen & Chen, 1995) and, furthermore, are known to possess strong anti-microbial activities (Toda *et al.*, 1989; Sakanaka *et al.*, 1990). As far as can be assessed, there are no reports in the literature on the anti-microbial activity of rooibos tea, although there are several on the antioxidant, antimutagenic and anticarcinogenic activities (Von Gadow, 1996). The aim of this study was thus to determine the anti-microbial activity of rooibos tea against *Escherichia coli* and other food spoilage and potential pathogenic microbes.

Rooibos tea water and ethyl acetate extracts were used to determine the effect of rooibos tea on the growth of *E. coli*. The data obtained with the water extracts showed a stronger anti-microbial activity than the ethyl acetate extracts, suggesting that the anti-microbial activity of rooibos tea is not exclusively due to the flavonoids. The fermented rooibos tea water extracts (69% decrease in growth at a soluble solid concentration of 5.0 $g.l^{-1}$) showed a stronger inhibitory effect on *E. coli* growth than the unfermented rooibos tea water extracts (35.1% decrease in growth at a soluble solid concentration of 5.0 $g.l^{-1}$). A decrease in the N_{max} and the μ_{max} and an increase in the t_{lag} and the t_d values were a clear indication of the inhibitory impact. It was furthermore found that the effect of the rooibos tea on *E. coli* growth was strongly bacteriostatic, because when the rooibos tea water extracts were removed, *E. coli* resumed a normal growth profile. Data obtained in this study showed that the inhibitory effect of rooibos tea on the growth of the *E.*

coli strain was much stronger than that found with black tea on the same *E. coli* strain. This suggests that rooibos tea may have a stronger anti-microbial effect than black tea. Although rooibos tea inhibits the growth of *E. coli* more than black tea, it does not kill the *E. coli* strain because of the bacteriostatic action of the tea.

The anti-microbial activity of rooibos tea water extracts on the growth of other food spoilage and potential pathogens was also determined. The data showed the strongest anti-microbial rooibos activity against the *Staphylococcus aureus* strain, resulting in a 90.8% decrease in growth. Anti-microbial activity was also observed against strains of *Bacillus cereus, Listeria monocytogenes* and *Saccharomyces cerevisiae*. As with *E. coli*, the fermented rooibos tea showed a stronger inhibitory effect on the various microbes than the unfermented rooibos tea water extracts, which led to a decrease in the N_{max} and the μ_{max} and an increase in the t_{lag} and t_d values for all the tested microbes. In contrast, it was found that the rooibos tea water extracts enhanced the growth of the *Zygosaccharomyces rouxii* strain. The data obtained in this study suggests that rooibos tea will not be effective as an anti-microbial agent against all the yeasts, but will strongly retard the growth of Gram-positive and Gram-negative bacteria. As long as rooibos tea is present, strong anti-microbial activity will be observed at a cup of tea concentration (2.5 $g.l^{-1}$ soluble solids).

A cariogenic bacterium (*Streptococcus mutans*) was also tested to determine the effect of rooibos tea on a bacterium that may be responsible for tooth decay. In the literature it has been shown that green tea strongly inhibits the growth of cariogenic bacteria such as *Streptococcus mutans* and *Porphyromonas gingivalis*. This could be the reason why the Japanese believe that those who drink large volumes of green tea have a low incidence of tooth decay (Kubo *et al.*, 1992). In this study, it was found that rooibos tea did inhibit the growth of *Strep. mutans* and that the fermented rooibos tea decreased the growth by 84.1%. At a cup of tea concentration (2.5 $g.l^{-1}$ soluble solids), rooibos tea showed strong inhibitory activity, but it is possible that due to the bacteriostatic effect, the microbes could resume growth if the tea is no longer present.

Concluding Remarks

From the data obtained in this study it is clear that rooibos tea has a strong anti-microbial activity against a variety of food spoilage and potential pathogenic

microbes. Results from this study also showed that water extracts of fermented rooibos tea, in all the cases, showed a stronger anti-microbial activity than found with unfermented rooibos tea. This suggests that the qualitative differences in polyphenol composition of the two rooibos tea extracts are important. It is thus necessary that further research on rooibos tea be done to determine which compound or compounds present in rooibos tea are responsible for the anti-microbial activity. It is also important to determine the MIC of the compounds to determine the toxicity of rooibos tea against the microbes tested. This will be of value in determining the different concentrations needed to be bactericidal against different microbes and whether rooibos tea could be effective as a food preservative.

The results of this study clearly provide a valuable basis for the evaluation of rooibos tea as a potential "natural" anti-microbial agent for use in the food industry. The potential of rooibos tea to inhibit the growth of food spoilage microbes and potential pathogens may make rooibos tea a very useful tool as a "natural" preservative, as the current consumer trend is that of "natural" preservatives. "Natural" preservatives will certainly be in demand in the near future and if compounds isolated from rooibos tea show a strong and stable anti-microbial activity, it should be possible to use rooibos tea extracts as a "natural" food preservative.

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