Effects of maturity and processing on quality properties of the watermelon (*Citrullus lanatus*) fruit juice

By

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Declaration

I, Maoto Makaepea Mossa (11575457), hereby declare that I have developed and completed enclosed intellectual work of masters' dissertation titled "*Effects of maturity and processing on quality properties of the watermelon fruit juice*" by myself with guidance and inputs from my supervisors. Furthermore, I certify that this research dissertation has not been previously submitted to achieve an academic grading or is being published elsewhere. Moreover all the reference material contained therein have been duly acknowledged.

Date: Signature:

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Dedication

This study is dedicated to my parents, Mr Maboragane J. and Mrs Malesele C. Maoto for their continuous support and their sacrifices during my studies. None of this would have been possible without their faith in me and for their pure, unconditional and everlasting love and support. Lastly, I dedicate it to my children and little brother M. Maoto, for their future academic inspiration.

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Abbreviations

AA	Ascorbic acid
CVD	Cardiovascular diseases
FRSA	Free radicals scavenging activity
DNA	Deoxyribonucleic acid
HPLC	High pressure liquid chromatography
HPP	High pressure processing
HDL	High-density lipoprotein
LDL	Low density lipoprotein
NO	Nitric oxide
ROS	Reactive oxygen species
RSM	Response surface methodology
RDA	Recommended dietary allowance
TP	Thermal processing
TC	Total cholesterol
TPC	Total polyphenolic content
µm	Microns

General Abstract

Watermelon (*Citrullus lanatus*) juice is rich in phytochemicals that possess antioxidant properties which are known to have a positive contribution to human health. Colour, pH, total soluble solids, lycopene, β-carotene, ascorbic acid, total polyphenolic content and antioxidants activity are quality properties that characterise watermelon juice. However, these quality properties undergo some chemical changes throughout the stages of maturity of the fruit as well as during thermal processing. The aim of this study was to investigate the effects of maturity and filtration on the physicochemical properties (colour, pH, total soluble solids) and the phytochemicals (lycopene, β-carotene, ascorbic acid, total polyphenolic content and antioxidants activity) of fresh watermelon juice. It further investigated thermosonication effects on these quality properties using response surface methodology (RSM). RSM considering temperature (25 – 52°C), time (2 – 10 min) and amplitude level (24.1 – 60 µm) at a constant frequency of 20 kHz were the independent variables while colour variables (L*, a* and b*, C* and h*), pH, total soluble solids, lycopene, β-carotene, ascorbic acid, total polyphenolic content and antioxidants activity were the dependent variables. Results showed that colour, pH, total soluble solids, lycopene, β-carotene, ascorbic acid, total polyphenolic content and antioxidants activity of watermelon fruit juice are maturity dependent. All the physicochemical and phytochemical properties increased with maturity. The combination of the CIELAB parameters (L*, a*, b*) was clearly explained by the Chroma (C*) and hue (hº). The (C*) and hº values of the watermelon fruit juice were significantly ($p < 0.05$) affected by the stages of maturity. The red colour was observed to be more saturated in the fully - ripe sample as indicated by the highest Chroma (7.35) values. The half - ripe sample which was observed to be pink - red had a C* of 5.88 while the lowest (4.32) value was observed from the unripe sample. The highest hº value was obtained from unripe sample (83.89), while lower value was observed from the fully - ripe (76.03) sample because of the saturation of the red colour in the fully - ripe sample. The total soluble solids also increased with stages of maturity 6.66 to 6.60 at half - ripe while at fully - ripe redness changed from 9.3 to 8.99 and TSS 8.37 to 8.02. Filtration has shown a significant ($p < 0.05$) on the C* and hº values. There was a higher retention of all dependent variables at lower thermosonication treatment (25°C; 24.4 µm), especially the lycopene which increased by 127%. Model predictions for the colour properties and phytochemicals were closely correlated to the experimental results obtained. Prediction models were found to be significant ($p < 0.05$) with low standard errors and high coefficients of determination (R^2).

Keywords: Watermelon, phytochemical, antioxidants, maturity, filtration, thermosonication, response surface methodology

CHAPTER 1: INTRODUCTION

1.1 Background information

Functional foods and nutraceuticals are gaining immense importance in the prevention of various ailments and chronic diseases. As a result, incorporation of fruits and vegetable as well as their products in a diet is recommended due to the presence of phytochemicals (Naz *et al*., 2014). In this context, watermelon juice (*Citrullus lanutus*) contains health promoting phytochemicals such as lycopene, β-carotene, ascorbic acid and total polyphenols that are known to possess antioxidant, anti-inflammatory, antimicrobial, antiulcer and laxative properties (Zhao *et al*., 2013; Bianchi *et al*., 2018; Hong *et al*., 2018). Watermelon fruit juice is a common beverage preferred by consumers to quench the summer thirst (Olaniyan and Adigun, 2017). It is used as refreshing, aphrodisiac, diuretic and a digestive stimulant (Dieng *et al*., 2017).

Antioxidants are compounds, which are beneficial in preventing life threatening ailments (Abid *et al*., 2014; Guo *et al*., 2015). Watermelon is a unique source of lycopene in the form of *cis*-isomeric lycopene (Choudhary *et al*., 2015). Lycopene is the most efficient oxygen radical scavenger among all the phytochemicals (Goula and Adamopoulos. 2005). Owing to these properties, the consumption of watermelon juice is associated with reduction of oxidative stress (Hong *et al*., 2015) which leads to prevention of non-communicable diseases such as atherosclerosis, stroke, diabetes, obesity, cancer insurgence, erectile dysfunctions, cardiovascular disorders and muscular diseases (Johary *et al*., 2012; Elumalai *et al*., 2013; Choudhary *et al*., 2015).

In addition, watermelon is a rich source of citrulline that is converted into the amino acid arginine, which is important in vasodilation which is the dilation of blood vessels resulting in decreasing the blood pressure (Olaniyan and Adigun, 2017; Oberoi and Sogi, 2017a). Although watermelon juice is a rich source of phytochemicals, physiological changes in gene expression occurs during maturity, these physiological changes affects the sensory properties as well as the phytochemical properties of the watermelon fruit juice (Guo *et al*., 2015). In addition, changes in watermelon quality properties are intensified during thermal processing (Oberoi and Sogi, 2017b; Wang *et al*., 2018). Continuously increasing consumer demands for processed beverages with fresh like attributes has encouraged food industries to adopt innovative food processing technologies to preserve the essential nutrients of these beverages (Rawson *et al*., 2011a).

Therefore, research interest has accelerated into developing non-thermal processing approaches that can retain quality and nutritional properties of the processed product (Lv *et al*., 2015). Among these methods, ultrasounds, which are also referred to as sonication, have the advantages of enhancing product quality (Abid *et al*., 2013). They use sound waves above the threshold of human hearing (Aguilar *et al*., 2017). The combination of ultrasounds with thermal treatment is called thermosonication (Abid *et al*., 2013; Ijah *et al*., 2015). The effectiveness of thermosonication is attributed to cell disruption caused by acoustic cavitation which is determined by the frequency, power intensity and treatment time (Anaya-Esparza *et al*., 2017). Application of thermosonication is more efficient in increasing the shelf-life of beverages, which is very important for economic consideration (Abid *et al*., 2014). It has been observed to retain the sensory properties of mango and strawberry juice (Santhirasegaram *et al*., 2013; Bhat and Goh, 2017). Aadil *et al*. (2017) as well as Khandpur and Gogate (2016) reported that thermosonication improved the phytochemical properties of grapefruit and orange juice. In the present study, the effects of maturity and filtration on quality properties were investigated. Furthermore, the effects of thermosonication using a response surface methodology (RSM) were determined on colour, pH, total soluble solids (TSS), lycopene, β-carotene, total polyphenolic content and antioxidants activity of fully - ripe watermelon fruit juice.

1.2 Problem statement

Watermelon juice undergoes changes such as accumulation of total soluble solids and pigments as well as chemical changes in phytochemical quantity during maturity (Guo *et al*., 2015). Fresh watermelon fruit juice is highly perishable; therefore, it has to be processed to increase the shelf-life and expand utilisation (Santos *et al*., 2015). However, watermelon fruit juice is thermo-sensitive in nature (Oberoi and Sogi, 2017b); its chemical changes are intensified during processing methods such as filtration and thermal treatment (Lv *et al*., 2015). More importantly, there is less work done on the effects of maturity and processing on the quality properties of the watermelon fruit juice.

1.3 Aim

To investigate the effects of maturity (unripe, half - ripe and fully - ripe) and filtration on the physicochemical (colour, pH and total soluble solids) and the phytochemical (lycopene, β-carotene, ascorbic acid, total polyphenols and antioxidants activity) properties of the watermelon fruit juice. To further investigate the effects of thermosonication on the same physicochemical and phytochemical properties of fully - ripe watermelon fruit juice using response surface methodology.

1.4 Specific objectives

1.4.1 To determine the effects of maturity at unripe, half - ripe and fully - ripe stages on the physicochemical (colour, pH and total soluble solids) and the phytochemical (lycopene, β-carotene, ascorbic acid, total polyphenols and antioxidants activity) properties of the watermelon fruit juice.

1.4.2 To determine the effects of filtration on the physicochemical (colour, pH and total soluble solids) and the phytochemical (lycopene, β-carotene, ascorbic acid, total polyphenols and antioxidants activity) properties of the watermelon fruit juice at the unripe, half - ripe and fully - ripe stages of maturity.

1.4.3 To determine the effects of thermosonication on the physicochemical (colour, pH and total soluble solids) and phytochemical (lycopene, β-carotene, ascorbic acid, total polyphenols and antioxidants activity) properties of the watermelon fruit juice at fully - ripe stage using response surface methodology.

1.5 Hypotheses

1.5.1 Stages of maturity and filtration may have significant effects physicochemical (colour, pH and total soluble solids) and phytochemical (lycopene, β-carotene, ascorbic acid, total polyphenols and antioxidants activity) properties of the watermelon fruit juice. Lv *et a*l. (2015) provided estimation of changes in phytochemical profiles during fruit maturity.

1.5.2 Using response surface methodology for optimisation of thermosonication variables may predict the optimum processing variables, which may retain the physicochemical and phytochemical properties of the watermelon juice (Oberoi and Sogi, 2017b).

1.6 Significance of the study

Consumer's interest on healthy and nutritious food has influenced the food industries to produce functional foods which are rich in vitamins, minerals and phytochemicals to help maintain good health. Watermelon fruit juice is an example of such foods (Oberoi and Sogi, 2017b). This study identifies how maturity and processing affects the important physicochemical and phytochemical properties of the watermelon fruit juice. The data presented in this study could be a source of information to consumers in order to expand their knowledge on quality properties of the watermelon fruit juice with respect to change during stages of maturity, it could also to be useful to food industries in terms of expanding the thermosonication processing method in a large processing scale in order to produce safe and quality watermelon fruit juice.

CHAPTER 2: LITERATURE REVIEW

2.1 Fruits consumption: health and nutritional benefits

There has been 60% increase in global fruits and vegetables production in the last two decades compared to the preceding decade (Parajuli *et al*., 2019). The production increase is attributed to the rising demands by consumers for fresh produce and minimally processed foods (Oberoi and Sogi, 2015a), which is associated with health benefits (Choudhary *et al*., 2015; Kyriacou *et al*., 2018). Epidemiological studies have indicated that; an increase in the consumption of fruits provides health-promoting benefits due to their antioxidant and anti-ulcer properties (Gardner *et al*., 2000; Pacier and Martirosyan, 2015).

The presence of natural antioxidants in fruits is of nutritional importance in the prevention of chronic diseases such as cardiovascular disease (CVD), diabetes and various cancers (Bramley, 2000; Tlili *et al*., 2011). Phytochemicals present in fruits, such as organic acids, ascorbic acid (AA) and vitamin E have biological activity against oxidation and free radical formation (Morales *et al*., 2012). The human body is unable to synthesise phytochemicals; therefore, it is important to consume foods rich in natural phytochemicals. Watermelon is a locally grown fruit, consumers prefer it for its nutritional content, high satiety index, thirst-quenching and refreshing capability (Choudhary *et al*., 2015).

2.2 Background information of watermelon fruit

2.2.1 Origin and classification

Native to tropical Africa, watermelon is a vine-like fruit; it is a member of *Cucurbitaceae* family (Dane *et al*., 2006). Researchers relate watermelon to fruits that grow on vines on the ground such as squash, pumpkin and cantaloupe (Mandel *et al*., 2005). In South Africa, watermelon is planted in hot regions of the country, i.e. Mpumalanga, Limpopo, North West and some parts of the Eastern Cape Province (DAFF, 2013). It is known as *legapu* (Sepedi), *bvani* (Tshivenda*), kalavatla* (Xi Tsonga) *and likhebe* (Isi Swati). External features include deep green or yellow colour smooth rind with light-green coloured vertical stripes all over its outer surface (Figure 1). Watermelon juice is extracted from the flesh of a watermelon through squeezing of a juice by hand or extracting equipment (Perkins-Veazie *et al*., 2003).

Figure 1: Ripe watermelon fruit; Source: Naz *et al*. (2014)

Watermelon juice contains different phytochemicals that have antioxidants properties such as lycopene, β-carotene, ascorbic acid, phenols and amino acids (Perkins-Veazie *et al*., 2007a). Watermelon juice is consumed raw, freshly squeezed from flesh to quench thirst, directly from flesh as a snack, as a fruit salad or as a ready processed product (Figure 2).

a) b) c)

Figure 2: Watermelon food products: a = Juice, b = Iced tea and c = Jam
Source:https://www.google.co.za/search?q=watermelon+food+products&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiYhaf7u4DcAhUCLMAKHY8QDwoQ_AUICigB&biw=1536&bih=723#imgrc=LmzfJkxVmJAkjM (2018)

Choudhary *et al*. (2012) reported that; although the most common and well-known watermelon is deep red colour; there are many varieties that feature orange, yellow or white flesh (Table 1).

Table 1: Different watermelon cultivars

Cultivar type	Illustration	Characteristics	References
Golden honey		Takes 90 days to mature, orange flesh, 8.9 - 9.2 °Brix and ≥ 99% β-carotene and only traces of lycopene	Poole and Grimball (1945)
Colorado preservative		Takes 90 days to mature, white flesh with bright red seeds, 8.9 °Brix and 0.03 total carotenoid and no lycopene content	Robisons *et al*. (1976); Mohr and Sandhu (1975)
Dessert King		Takes 85 days to mature, yellow flesh, 8 - 8.2 °Brix and 82.87% - 89.99% total carotenoid and 12.16% lycopene	Liu and Loy (1972)
Sugar baby		Takes 68 - 86 days to mature, red flesh, ≥ 10.2 °Brix and ≥ 84% lycopene, 2% - 11% β-carotene	Mohr and Sandhu (1975)
Crimson sweet		Takes 68 - 86 days to mature, red flesh, ≥ 10.2 °Brix and ≥ 84% lycopene, 2% - 11% β-carotene	Mohr and Sandhu (1975)

2.2.2 Nutritional composition of watermelon juice

Watermelon fruit juice contains major health-enhancing nutrients (Table 2). It is one of the rich sources of major chemical compounds of foods (Mehra *et al*., 2015; Bianchi *et al*., 2018). It has recently received attention for its low-fat content (Choo and Sin 2012); therefore, it is considered a constituent of a healthy diet that is low in cholesterol, saturated fat and sodium as compared to other fruits such as avocados (Tong *et al*., 2010; Rawson *et al*., 2011a; Araújo *et al*., 2018). As a fruit, it has low energy density therefore, it is recommended for weight management (Jumde *et al*., 2015; Hong *et al*., 2018). Adedeji and Oluwalana (2013) indicated that watermelon is a good source of minerals and vitamins. It contains 11 minerals, 9 vitamins and fibre, which are essential for human health (Guo *et al*., 2015). This includes vitamins such as thiamine, riboflavin, niacin and folate (Adedeji and Oluwalana, 2013) and minerals such as potassium, magnesium, calcium, phosphorus and iron (Sivudu *et al*., 2014; Ijah *et al*., 2015).

Table 2: Nutritive composition of fresh watermelon juice, per 100 g

Nutritional content	Nutrient value per 100 g	Percentage of RDA (%)	References
Energy	30 – 46.2 Kcal	1.5	Shahzad *et al*. (2014)
Carbohydrates	7.6 – 11.6 g	6	Naz *et al*. (2014)
Protein	0.6 – 0.9 g	1	Naz *et al*. (2014)
Total fat	0 - 0.15 g	0.5	Choudhary *et al*. (2015)
Cholesterol	0. 00 mg	0	USDA National nutrient data base, (2015)
Dietary fiber	0.4 - 0.61 g	1	Shahzad *et al*. (2014)
Vitamin A	569 – 864.88 IU	19	USDA National nutrient data base, (2015)
Vitamin C	8.1 – 12.31 mg	13.5	USDA National nutrient data base, (2015)
Sodium	1 – 1.5 mg	0	USDA National nutrient data base, (2015)
Lycopene	6888.64 mg	23	Shahzad *et al*. (2014)

Owing to these properties, watermelon consumption can be useful in maintaining acid-base balance in the body, playing a major role in normal physiology, maintaining appetite and normal digestion (Choudhary *et al*., 2015). In addition, Adedeji and Oluwalana (2013)

reported that minerals such as calcium and potassium play an important role in cell regulation, maintenance of the cell structure and cell differentiation process. Bailey *et al*. (2016) reported that, supplementation with watermelon fruit juice improves aspects of vascular health in individual with hypertension. Consumption of watermelon fruit juice is also associated with supporting normal vision, skin health, managing cholesterol, supporting normal appetite and nervous system function and may be involved in normal muscle contraction due to presence of ascorbic acid and β-carotene (Adedeji and Oluwalana, 2013). Olaniyan and Adigun (2017) reported that watermelon produces nitric oxide, which is associated with relaxing the blood vessels and further plays a vital role in increased sexual satisfaction in men and women as well as alleviation of erectile dysfunction.

2.2.3 Watermelon utilisation

The lycopene-rich nature and health benefits of the watermelon fruit juice makes it an excellent choice for preparing additional functional foods to increase utilisation. Watermelon juice concentrate (WJC) is used as an additive in different food formulation such as yoghurt, quick bread and muffins; it has a positive effect on the sensory qualities of these products (Perkins-Veazie *et al*., 2007b; Jumde *et al*., 2015). The fresh-cut industry also introduced the fresh cut watermelon cubes on the shelves for the convenience of the consumer (Perkins-Veazie and Collins, 2004). The pulp (residue left after juice extraction) has a potential to be utilised as a value-added product in the manufacturing industries. It can be used to produce jams, sweets, powders, and additive paste for desserts.

2.3 Free radicals and formation of diseases in a human body

Oxidation reactions leads to the formation of free radicals in the human body (Figure 3). Free radicals are unstable molecules that affect normal functioning of the DNA and cell membrane (Gardner *et al*., 2000). They are generated in a form of reactive oxygen species (ROS) in a healthy human body (Devasagayam *et al*., 2004), which cause the oxidation of various cellular constituents like DNA, protein, lipids, resulting in alterations that produces a range of cellular damage, which can ultimately lead to cell death (Ruiz *et al*., 2019). Free radicals play a significant role in the development of chronic diseases like cancer, heart attacks and diabetes (Zhu *et al*., 2019), which constitutes a significant public health problem (Chen *et al*., 2017). Approximately 30% of all deaths worldwide results from chronic diseases (Castro-López *et al*., 2017).

Initiation RH→R• + H• (1)

Propagation R• + O2 → ROO• (2)

ROO• + RH→ R• + ROOH

Termination R R (3)

R ROO

ROO ROO (Non-radical products)

Figure 3: Free radical formation in the body. Source: Lipinski (2001) and Tadmor *et al*. (2005)

2.4 Benefits of consuming natural antioxidants

A way of inhibiting the adverse effects of free radicals is through improvement of antioxidant status in the body (Niki *et al*., 2005). Antioxidants are molecules capable of preventing oxidation (Kagan *et al*., 2002; Ko *et al*., 2005), by trapping ROS and preventing their formation (Niki *et al*., 2005). In addition, antioxidants are capable of neutralising free radicals and their actions in order to repair the damaged cell membrane and prevent damage (Devasagayam *et al*., 2004; Zhu *et al*., 2019). Natural antioxidants are presumed to be safe and are more desirable than their synthetic counterparts (Bianchi *et al*., 2018) therefore consumption of watermelon juice as a natural source of antioxidants is beneficial to human health (Figure 4).

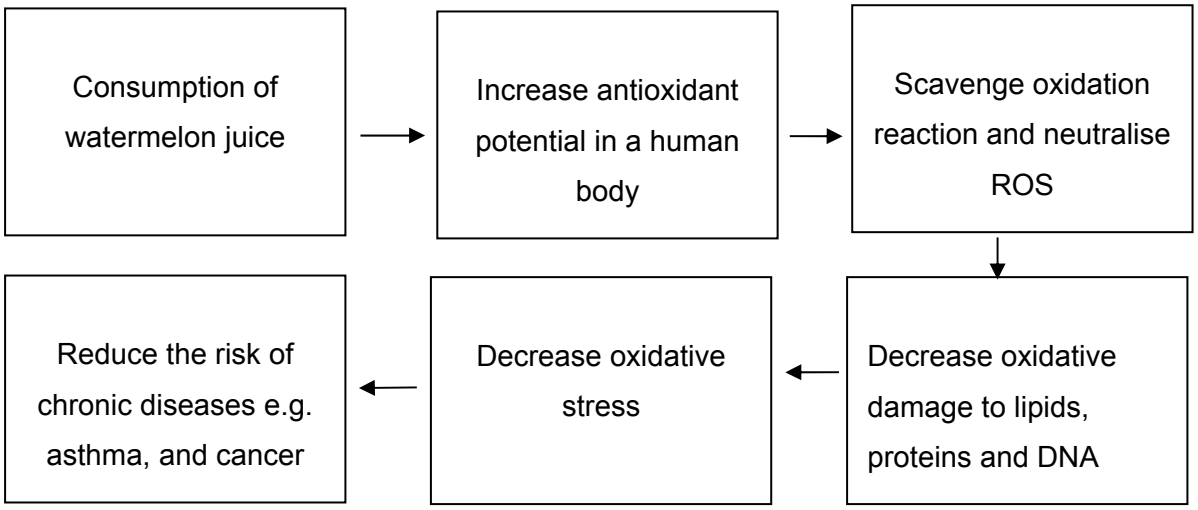


Figure 4: Effectiveness of antioxidants in reducing the risk of chronic diseases. Source: Herber and Lu (2003)

The consumption of watermelon juice can contribute significantly to the prevention of diseases (Table 3) due to the antioxidants: presence of lycopene, β-carotene and polyphenols.

1 **Table 3:** Effectiveness of antioxidants found in watermelon juice on diseases

Parameter	Disease	Outcomes	Reference
Citrulline	Obese postmenopausal women	Improved cardiac autonomic function in sedentary obese postmenopausal women, increase plasma arginine	Sai'd (2014); Wong *et al*. (2016)
Lycopene	Hypertension	Improved plasma agitation	Choudhary *et al*. (2015)
Ascorbic acid	Stroke	Stroke reduction	Pacier and Martirosyan (2015)
Lycopene	Breast cancer patients	Serum lycopene associated with decreased risk	Kun *et al*. (2006); Dia *et al*. (2016)
Lycopene	Osteoporosis	can counteract the damaging effects of oxidative stress which causes osteoporosis	Choudhary *et al*. (2015)
Ascorbic acid	Liver disease	Decrease of 58.2% serum alanine aminotransferase and 49.4% of high-sensitivity C-reactive protein and also minimise damage and slow disease progression	Elumalai *et al*. (2013)
Ascorbic acid	Human metabolism	Least risk of inadequacy or adverse health effects.	Pacier and Martirosyan (2015)
β-carotene	Cardiovascular mortality	Reduced the hazard ration in cardiovascular diseases and Coronary heart diseases	Choudhary *et al*. (2015)
Lycopene	High cholesterol in macrophage cell line	Lowered cholesterol synthesis	Kulczynski *et al*. (2017)
Citrulline	Low libido	Improve erectile functions	Soteriou *et al*. (2014)
Ascorbic acid	Flue and scurvy	Prevents and treats variety of ailments, scurvy and a simple cold	Choudhary *et al*. (2015)
Vitamin A	Visual system	Enhances optimal eye functioning	Xiao *et al*. (2019)

2.4.1 Lycopene

Lycopene ($C_{40}H_{56}$) is a phytochemical with the highest degree of unsaturation among all phytochemicals (Heber and Lu, 2003; Kehili *et al*., 2017); it is a straight chain hydrocarbon with a total of 13 double bonds, 11 of which are conjugated (Figure 5). Choudhary *et al*. (2009) described lycopene as a bright red pigment belonging to the carotenoid family. It is available in *trans* configuration from natural sources while in a human plasma, lycopene is an isomeric mixture containing 50% of the total lycopene as *cis* isomers (Goula and Adamopoulos, 2005; Elumalai *et al*., 2013). It has received great research attention due to its enormous biological activities associated with the stimulation of cell-to-cell communication (Poojary and Passamonti, 2015).

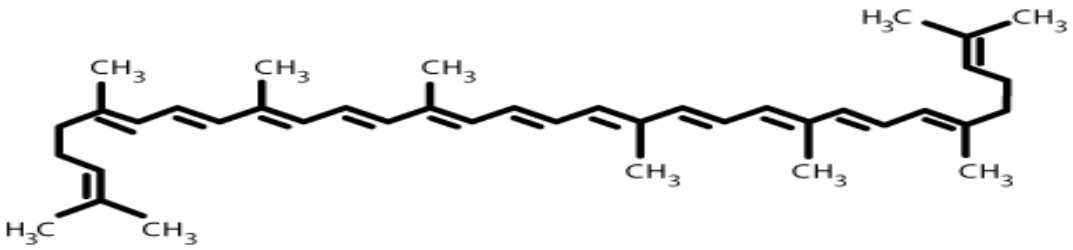


Figure 5: Chemical structure of lycopene. Source: Choudhary *et al*. (2009*)* and Soteriou *et al.* (2014)

Although it is reported to be beneficial due to its therapeutic effect, the mechanisms of this action are not yet clear (Prakash and Kumar, 2014). Studies suggested that lycopene has the highest antioxidant activity of all the phytochemicals (Wang, 2012; Soteriou *et al*., 2014), thus making it a compound of interest amongst medical and nutrition researchers. In the human body, lycopene is found in the blood plasma, adipose, adrenal tissues, testes, human serum and prostate tissue, it has also been detected in the ocular tissues (Bohm, 2012; Brat *et al*., 2006; Choudhary *et al*., 2015), as well as the liver, lungs, breast, cervix, skin, ciliary body and retinal pigment epithelium (Halliwell and Gutteridge, 2007; Oberoi and Sogi, 2015a). Lycopene is more prominent in watermelon and tomato but also available in lower quantities in some other fruits and vegetables (Table 4). It is used as a colouring ingredient in many food products (Giovannucci, 2002; Naz *et al*., 2014; Jamal *et al*, 2016). Edwards *et al*. (2003) reported that, fresh watermelon fruit juice is a great natural source of highly bioavailable lycopene for human nutrition. Bioavailability from fresh watermelon fruit juice is in fact higher than that of heat-processed tomatoes (Rao and Rao, 2007). Moreover, watermelon contains 60% more lycopene (per 100 g) than raw ripe tomatoes (Fish *et al*., 2001). Lycopene is insoluble in water and poorly soluble in organic solvents, which limits its

removal from raw plant material (Ho *et al*., 2015). Due to this limitation, pure lycopene is often expensive (Ascenso *et al*., 2013). Lycopene has been shown to protect important biomolecules such as lipids against oxidative damage and oxidative stress (Rao, 2002; Giovannucci, 2002), which results in prevention of cell proliferation (Weisburger, 2002; Stahl *et al*., 2006).

Table 4: Dietary sources of lycopene

Source	µg/100 g wet weight	References
Raw tomato	8.8 - 42	Rao and Rao (2007); Fish *et al*. (2001)
Tomato juice	23 - 72	Rao and Rao (2007)
Watermelon	86 - 100	Rawson *et al*. (2011)a
Pink grapefruit	3.6 - 34	Rao and Rao (2007)
Pink guava	54 - 60	Rao and Rao (2007)
Rosehip puree	7.8-8.99	Bohm *et al*. (2003)
Apricot	<0.1	Elumalai *et al*. (2013)
Papaya	20-53	Elumalai *et al*. (2013)
Passion fruit	8.87-8.91	Mourvaki *et al*. (2005)
Jackfruits	1.44-1.56	Suwanaruang (2016)
Grapes	3.18-3.20	Suwanaruang (2016)
Orange	1.01-1.02	Suwanaruang (2016)
Banana	3.1-3.35 -	Suwanaruang (2016)
Red Bell pepper	5.0-5.4	Suwanaruang (2016
Red cabbage	<0.1	Charoensiri *et al*. (2009)
Dragon fruit	3.4- 3.9	Charoensiri *et a*l. (2009)
Mango	3.7 - 4.1	Charoensiri *et al*. (2009)
Rose apple	17.1 - 18.0	Charoensiri *et al*. (2009)

Evidence in literature shows that lycopene has tumor suppressing activity, it decreases cellular proliferation induced by insulin-like growth factors that has an effect on mitogens in various cancer cell lines and further inhibits abnormal cellular growth in the body (Johary *et al*., 2012; Giovannucci *et al*., 2002). In addition, regulation of gap-junction communication in embryo fibroblast cells gives lycopene its anti-carcinogenic effects (Elumalai *et al*., 2013). As a result, intake of lycopene containing-products is associated with a reduced incidence of cervical, breast, bladder and prostate cancer (Bianchi *et al*., 2018). Despite watermelon being the major source of lycopene, tomatoes and tomato products provides more than 85%

of our dietary lycopene in the food market (Bramley, 2000; Edwards *et al.*, 2003). This opens a greater market scope for watermelon-based products.

2.4.2 β-carotene

β-carotene belongs to the carotenoid family and is found in plant-based foods such as carrots, sweet potatoes, spinach, watermelon and mangos (Chen *et al*., 2017). It consists of 40 carbon atoms, contains 11 conjugated and 2 unconjugated double bonds (Figure 6). β-carotene is a precursor of vitamin A (Livny *et al*., 2003) and is an insoluble vitamin, which includes a group of unsaturated nutritional organic compounds (Rao, 2006; Kulczynski *et al*., 2017; Xiao *et al*., 2019) such as retinoic, retinal and retinol (Feng *et al.,* 2006; Saeeduddin *et al*., 2015).

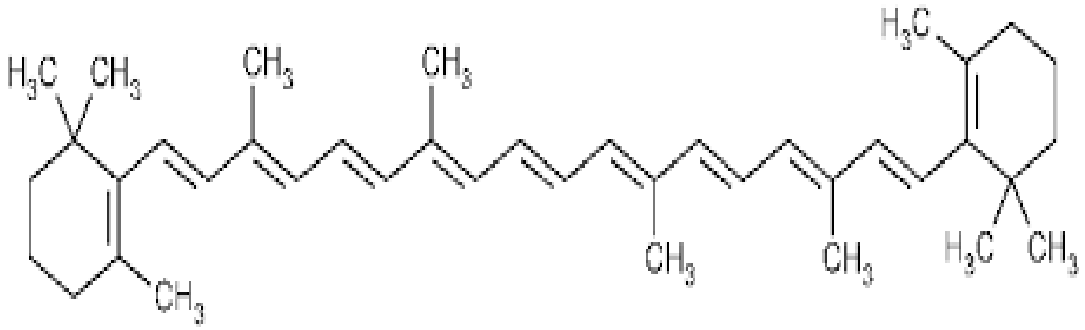


Figure 6: Chemical structure of β-carotene. Source: Livny *et al*. (2003)

Incorporation of β-carotene in the diet is important for maintaining human health and wellbeing because it is enzymatically converted to retinol (vitamin A) in the human intestine by the β-carotene 15,15'- monoxygenase thus constituting the most common and safe source of vitamin A (Chen *et al*., 2017). Retinol and its derivatives are used in the body to absorb light in the eyes (Livny *et al*., 2003), therefore β-carotene is an essential nutrient needed to help in the normal functioning of the visual system (Xiao *et al*., 2019). In addition to its pro-vitamin A function, β-carotene exhibits antioxidant properties (Johary *et al*., 2012; Jayathunge *et al*., 2017). As an antioxidant, β-Carotene is a potent quencher of singlet oxygen, it protects the body against oxidation, low density lipoprotein (LDLP) and lowers the incidence of metabolic syndrome in middle-aged adults (Hozawa *et al*., 2007; Bianchi *et al*., 2018).

In addition, β-carotene is important in the maintenance of the immune system; reduces the risk of type 2 diabetes and plays a role in the formation and maintenance of the heart, kidney

and other organs (Johary *et al*., 2012; Chen *et al*., 2017; Kulczynski *et al*., 2017). Moreover, β-carotene functions as growth factor for epithelial cells; it is important for reproduction and modulates gene function (Sahin *et al*., 2010). The first symptom of lack of β-carotene in diet is night blindness (Choudhary *et al*., 2009). Even though consumption of β-carotene is associated with health promoting properties, its utilisation as a functional ingredient is limited because of its poor water-dispersibility, chemical stability and bioavailability (Chen *et al*., 2017).

2.4.3 Ascorbic acid

Ascorbic Acid (AA) is an essential nutrient present in most fruits and vegetables such as citrus, spinach and carrots (Lee and Kader, 2000; Odriozola-Serrano *et al*., 2009; Pacier and Martirosyan, 2015). It is frequently added to a variety of food products for nutrient enhancement. It is one of the water-soluble vitamins that plays a role in the biosynthesis of pro-collagen, immune response, growth and malformations (Ruiz *et al*. 2019), and it has a strong antioxidant effects (Saeeduddin *et al*., 2015; Oberoi and Sogi, 2017a). It is mostly refered to as vitamin C. Odriozola-Serrano *et al*. (2007); reported that the term vitamin C is used as the general description for all organic compounds exhibiting the biological activity of AA (Figure 7).

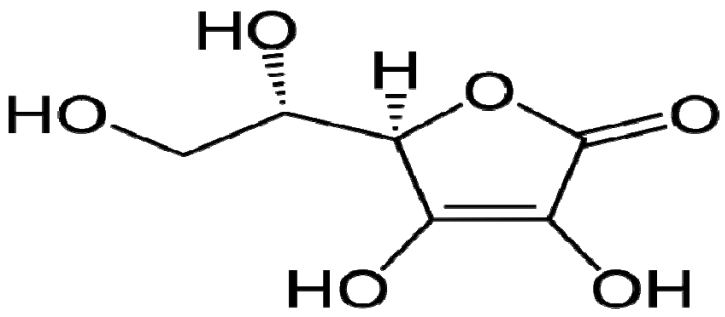


Figure 7: Chemical structure of ascorbic acid, Source: Odriozola-Serrano *et al*., 2007

As an antioxidant, AA is effective in scavenging superoxide radical anion, hydrogen peroxide, the hydroxyl radical, reactive nitrogen oxide (NO) and singlet oxygen (Halliwell and Gutteridge, 2007). It has a potential role in modifying low density lipoprotein (LDL) oxidation and the NO synthetic pathway (Odriozola-Serrano *et al*., 2009; Pacier and Martirosyan, 2015). In addition, AA plays a significant role in stress resistance (Davey *et al*., 2000) and it is believed to be of major importance for protection against diseases and degenerative processes caused by oxidative stress (Choudhary *et al*., 2015). Ascorbic acid contributes substantially towards the prevention of many diseases including prenatal health problems, eye disease and even skin wrinkling (Sahin *et al*., 2010; Pacier and Martirosyan, 2015). AA

is an antioxidant that has long been reported to help prevention of a variety of ailments, from a simple cold to chronic cancer (Sánchez-Moreno *et al*., 2003; Sahin *et al*., 2010), prevent free radical-induced damage (Johary *et al*., 2012), quenching oxidants which can lead to the development of cataracts (Morales *et al*., 2012).

2.5 Polyphenols and antioxidant activity

Dane *et al*. (2006) described polyphenols as the most abundant micronutrients in our diet; they are secondary metabolites that cover several classes of natural products (Figure 8) biogenetically derived from the shikimate-phenylpropanoids-flavonoids pathways (Edwards *et al*., 2003). The structure of polyphenols consists of an aromatic benzene ring with one or more hydroxyl substituents (Fawole *et al*., 2013a; Zhu *et al*., 2019). These compounds which are widely distributed in plants; are needed by plants for pigmentation, growth, reproduction, resistance to pathogens and for many other functions (Feng *et al*., 2006). They are also important determinants of the sensory and nutritional quality of fruits (Nowicka *et al*., 2019).

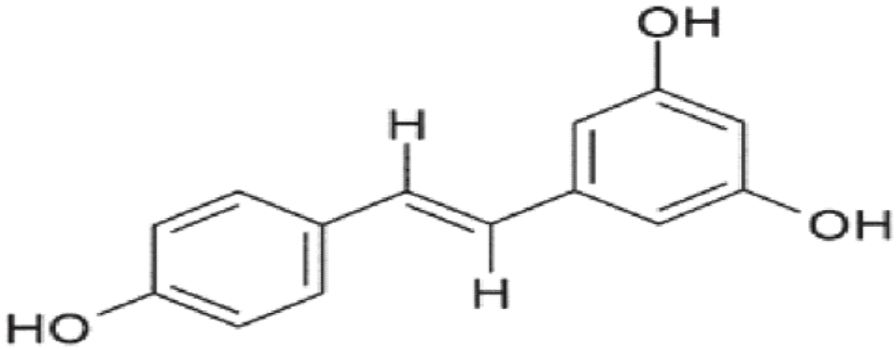


Figure 8: Chemical structure of polyphenol, Source: Dane *et al.* (2006)

Watermelon fruit juice is a natural source of polyphenols which includes luteolin, lariciresinol, medioresinol and pinoresinol (Rao and Rao, 2007). Polyphenols show evidence of antioxidant properties (Saura-Calixto *et al*., 2007; Singh *et al*., 2017), which inhibit the development of disease conditions by free radical activities. It functions in the maintenance of immune system to prevent cellular damage or cell death (Fish *et al*., 2001).

Antioxidant activity is an important quality property for the nutritional quality of foods. It has redox properties (Gil *et al*., 2006), which allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Choudhary *et al*. (2015) reported that, antioxidants scavenge free-radicals by preventing formation and breaking chain propagation by binding to the metal ions (Abushita *et al*., 2000; Fawole *et al*., 2013b), reducing hydrogen peroxide and quenching superoxide and singlet oxygen (Dane *et al*., 2006). Both polyphenols and

antioxidants activity have been proven to be effective for their role in preventing degenerative diseases such as cancer and CVD. Edwards *et al*. (2003) and Choudhary *et al*. (2015) described that among other functions polyphenols and antioxidant releases and delays growth hormones such as auxin and prevent deterrence of sensory properties and prevent microbial infections.

2.6 Development of watermelon and stages of maturity (Sugar baby cultivar)

Watermelon fruit maturity usually starts out as a densely woolly mass with yellowish-brown hairs, which disappear as the plant ages (Dane *et al*., 2006). Flesh and juice colour changes with fruit development. It is white at the initial stage and changes continuously to a red colour until fruit is fully matured (Figure 9).

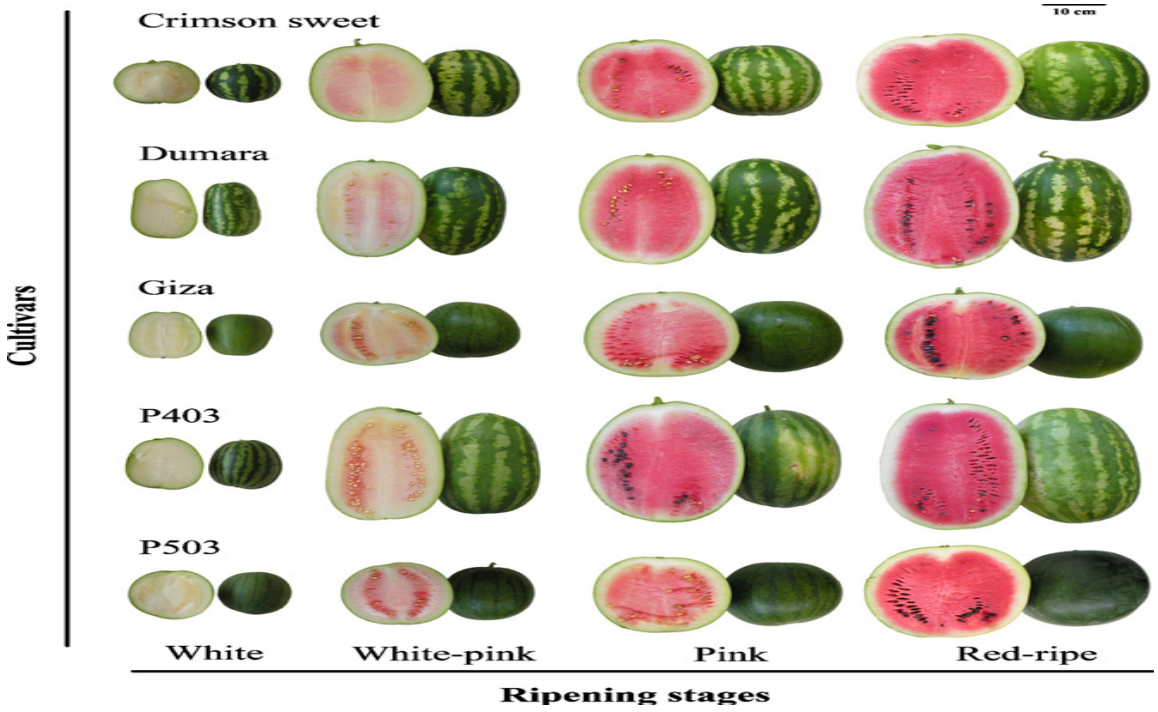


Figure 9: Watermelon colours at maturity stages, Source: Tlili *et al.* (2011)

Leaves are stemmed, alternate, large, pinnately lobed, stiff and rough when old (Dane *et al*., 2006; Davis *et al*., 2013). Firmness reflects changes in tissue texture during growth, maturation and storage (Taniwaki *et al*., 2009a). Watermelons should be harvested when completely mature to achieve the best taste and texture. Characteristics that are often affected by maturity are fruit appearance (e.g. size, shape and colour), firmness, texture, flavour (e.g. sugar, acids and aroma volatiles) phenolic compounds and phytochemicals (Yoo *et al*., 2012). It is known that, the amount of each phytochemical in fruits is strongly influenced by genotype differences and external factors such as agro-technical processes,

environmental conditions, ripening degree at harvest and post -harvest manipulation (Dumas *et al*., 2003; Bianchi *et al*., 2018).

Maturity of the watermelon fruit is traditionally judged by human beings according to some typical characteristics, such as withering of tendril, changes in belly colour and a thumping test (Tlili *et al*. 2011; Davis *et al*., 2013). Indicators of fruit maturity includes a yellow spot on fruit surface which is in contact with soil or plastic mulch; and a dull surface on top of the fruit (Abushita *et al*., 2000). Drying of the stem tendril nearest attachment point of watermelon and green colour tone of the rind are also indicators of maturity (Wehner, 2008). As with other fruits, maturity is influenced by temperature, UV-radiation, CO_2 concentrations, nutritional and environmental impacts (Parajuli *et al*., 2019). Chromoplast development plays a crucial role in controlling phytochemical content in watermelon flesh. Quality in watermelon is determined during maturity through highly coordinated processes involving differential expression of specific genes during growth and differentiation of various fruit tissues, which in turn affects the sweetness, flavour, aroma, colour, texture and firmness (Guo *et al*., 2015; Bianchi *et al*., 2018).

2.7 Watermelon juice processing and its effects on antioxidants

Due to the perishable nature of fruits and vegetables, they are processed to prolong the shelf- life and give customer variety of products (Lee and Coates, 2003; Kim *et al*., 2008 Jayathunge *et al*., 2015). As a result, processing of fruits is a growing trend in the food production industry. However, studies have indicated that processing of watermelon fruit juice leads to loss of some nutrients, sensory properties and phytochemicals (Table 5). Therefore, food scientists and academics have always been in search for novel processing methods that have minimal effects on the food nutrients, sensory properties and antioxidants (Santos *et al*., 2014; Abid *et al*., 2014; Zhang *et al*., 2015; Wang *et al*., 2018). This is triggered by consumer demand for convenient, safe, delicious and nutritious food that is not detrimental to human health.

Among the many processing methods, thermosonication has been reported to be the most vital processing method that retains and improves the quality of food (Rawson *et al*., 2011b; Abid *et al*. 2013). Thermosonication is a combination of ultrasound and thermal temperature which uses sound energy which is generated from an electric current frequency (frequency ≥ 20 kHz) that exceeds the hearing limit of the human ear (Aguilar *et al*., 2017). The generated sound propagates through food materials (especially in liquid media) causing a complex phenomenon known as “cavitation” (Anaya-Esparza *et al*., 2017). Thermosonication consists

of a Sonicator probe and an external circulator water bath taking into consideration acoustic energy density (frequency, amplitude) temperature and time of the treatment (Aadil *et al*., 2017). This method can be used to enhance nutritional and phytochemical properties of foods with minimum change in colour, flavour and other quality attributes (Abid *et al*., 2013). It has been shown to improve the sensory properties of orange, cantaloupe and mango juice (Tiwari *et al*., 2009a; Fonteles *et al*., 2012). In addition, thermosonication has been shown to retain the important phytochemicals in tomato juice (Abid *et al*., 2014).

Table 5: Effects of different processing methods on phytochemicals of watermelon juice

Processing method	Effect on antioxidants	References
High-intensity pulsed electric field treatment	Decreases AA, high retention of lycopene and antioxidants capacity in watermelon juice	Aguiló-Aguayo *et al*. (2007); Oms-Oliu *et al.* (2009)
Drying	Decreases lycopene and AA, degrade antioxidants capacity and total polyphenols	Choudhary *et al*. (2015)
Thermosonication	Increase in lycopene, phenolic content is not much affected and ascorbic acid is retained, free radicals scavenging activity mostly not affected.	Rawson *et al*. (2011a)
Pasteurisation and sterilisation	Decreases AA, lycopene and polyphenols	Perez-Conesa *et al*. (2009); Zhang *et al*. (2015)
Ultra high temperature	Maintain the colour of the juice, decreases the pH,	Wang *et al*. (2018)
High pressure carbon dioxide	Inactivate polyphenol oxidase, decrease aroma	Zhang *et al*. (2015); Liu *et al*. (2012)
Pulse electric filed	Maintains the total antioxidants, maintains the colour	Aguilo-Aguayo *et al*. (2010)
Ultraviolet-c	Improves the colour of the juice, decreases β-carotene and aroma	Zhang *et al*. (2011); Feng *et al*. (2013)
High hydrostatic pressure	Changes the secondary structure of proteins, improves colour, flavour and nutritional content	Liu *et al*. (2012)
High pressure processing	Decrease colour and volatile compound	Aadil *et al*. (2017)

Response surface methodology is a method applied to determine the possible optimum processing dependent and independent variable (Myers and Montgomery, 2002), using a mathematical model to analyse problems (Onipe *et al*., 2018). Extensive research has proven RSM to be an effective tool in the prediction of processing variables for cantaloupe, melon and ginger candy juice (Fonteles *et al*., 2012; Kumar *et al*., 2018).

2.8 Conclusion

Watermelon fruit juice can be regarded as a constituent of healthy diet due to the potential health benefits especially lycopene content. The phytochemicals in watermelon fruit juice varies with stages of maturity of the fruit. Processing methods have a potential of decreasing quality properties of the watermelon fruit juice. As a result, novel processing technologies such as thermosonication are applied to preserve the watermelon fruit juice. These studies identified and quantified the variation of watermelon quality properties at different stages of maturity. It further investigated the effects of filtration on fresh watermelon fruit juice. It has also determined the effects of different processing variables on fully-ripe watermelon fruit juice. The information obtained is useful to both food processors and health conscious consumers. It can also be used in the pharmaceutical industries.

CHAPTER 3: EFFECTS OF MATURITY ON QUALITY PROPERTIES OF FRESH WATERMELON FRUIT JUICE

Abstract

Colour, acidity and total soluble solids are important physicochemical properties to be considered when evaluating the quality of the watermelon fruit juice. In addition, lycopene, β-carotene, ascorbic acid, total polyphenols and antioxidants activity are major phytochemicals with antioxidants properties that defines the quality of the watermelon. These quality properties undergo chemical changes throughout different stages of maturity. The objective of this study was to investigate changes in physicochemical and phytochemical properties of watermelon fruit juice during selected stages of maturity. A physicochemical characterisation followed by measurement of phytochemical contents was performed on the sugar baby cultivar of watermelon. All quality properties were significant ($p < 0.05$) throughout maturity especially the red colour and lycopene content. The red colour was more dominant in the fully - ripe sample as indicated by the Chroma (C*) and the hue (h°) values. The highest h° value was recorded in unripe (83.89), followed by the fully - ripe (76.03). The fully - ripe watermelon fruit juice had the highest red colour intensity as indicated by the highest (7.35) C* value. The same trend was observed for °Brix, the lowest (2.26) value was recorded from the at unripe sample while the fully – ripe sample showed a higher (8.41) value. At the unripe stage, the lycopene content was 0.04 mg/100 g but increased to 3.9 mg/100 g (half - ripe) and 6.19 mg/100 g at fully-ripe stages. No β-carotene was detected at the unripe stage; however, it increased linearly to 0.12 and 0.23 mg/100 g at half-ripe and fully - ripe stage respectively. The content of ascorbic acid was very low at the unripe stage (1.3 mg/100 g), it increased linearly until it reached 3.47 mg/100 g at fully-ripe stage. Total polyphenolic content and antioxidant activity also showed an increasing trend with maturity. It appears that maturity does not adversely affect the quality properties of the watermelon fruit juice. These results confirm the important roles played by genetic background and stages of maturity in determining antioxidant potential of watermelon fruits. They also give valuable insights into the synthesis and accumulation of phytochemicals in watermelon and furthermore move us closer to identifying the harvesting stages of maturity with the highest total soluble solids, colour and antioxidant potential.

Key words: Quality properties, watermelon, physicochemical, phytochemicals, antioxidants

3.1 Introduction

Watermelon is a delicious fruit reported to be a rich source of lycopene (Oberoi and Sogi, 2015b). It is desired for its delicate colour and flavour to extinguish the summer thirst (Bianchi *et al*., 2018). Phytochemicals possess antioxidants and anti-inflammatory properties that results in fundamental contributions to human health (Perkins-Veazie *et al*., 2003; Kyriacou *et al*., 2018). Higher intake of foods rich in natural phytochemicals such as lycopene, ascorbic acid and β-carotene have been associated with reduced incidence of CVD, various cancers, diabetes and hypertension (Choudhary *et al*., 2009; Oberoi and Sogi, 2015a). In addition to their roles as antioxidants, phytochemicals act as photosynthetic accessory pigments and colourants in plants (Nagal *et al*., 2012). As with other fruits of most plant species, watermelon maturity indices include biochemical and physiological changes such as pigment accumulation, flavour changes, fruit softening, increase in aromatic volatiles as well as conversion of starch to sugars (Karakurt and Huber 2004; Nagal *et al*., 2012; Lv *et al*., 2015).

Furthermore, the quantity of phytochemicals undergoes chemical changes throughout maturity (Abushita *et al*., 2000; Dumas *et al*., 2003). All these changes are caused by developmental and physiological changes in gene expression profiles during maturity (Guo *et al*., 2015). As a result, monitoring these quality properties during maturity is becoming very important since the state of maturity during harvest, storage and market distribution determines the quality of the product that meets the customer's satisfaction (Ahmad-Syazwan, 2012). The aim of this study was to investigate the changes in major phytochemicals of commercial watermelon cultivar grown in an open-field and harvested at three different stages of maturity. It was hypothesised that stages of maturity affect the physicochemical and phytochemical properties of watermelon fruit juice. Results obtained will be useful for both consumers and food processing companies.

3.2 Materials and Methods

3.2.1 Sample collection

Unbruised watermelons (Sugar baby *cv*) were harvested from Valley farms a division of Skywalker Trading and Projects. Three (3) watermelon per row were manually and randomly picked at different stages of maturity (unripe, half - ripe and fully ripe stage). The stages of maturity were determined according to the number of days of planting (60 days after planting recorded as unripe, 73 days after planting is recorded as half - ripe and 90

days after planting recorded as fully - ripe sample) (Tlili *et al*., 2011). External stages of maturity indices were applied at the field, these included the colour of the ground spot tendril vitality and sound of fruit by thumping (Tlili *et al*., 2011; Perkins-Veazie *et al*., 2003). Two watermelons per row were cut to assure validity of the methodology just prior to harvest (Fish *et al*., 2001). The watermelons were transported to University of South Africa (Florida campus), Food Science laboratory for processing. Upon arrival, the watermelon fruits were thoroughly washed under running tap water to remove soil residues and kept overnight at about room temperature (21 to 25˚C). The juice of the watermelon was extracted the following day. The watermelons were cut into slices; the rind and the seeds were separated from the flesh. The flesh was put in the table juice extractor (Russell Hobbs Juice Sensation Model no: RHJM01. 220-240 V - 700 W, UK) to extract the juice. Twelve (12) samples (50 ml) of fresh juice per stage of maturity were taken (Figure 10) and put at the temperature of -20ºC. Physicochemical and phytochemical analysis were done the following day. All reagents were supplied by ACE chemicals and Merck chemical suppliers.

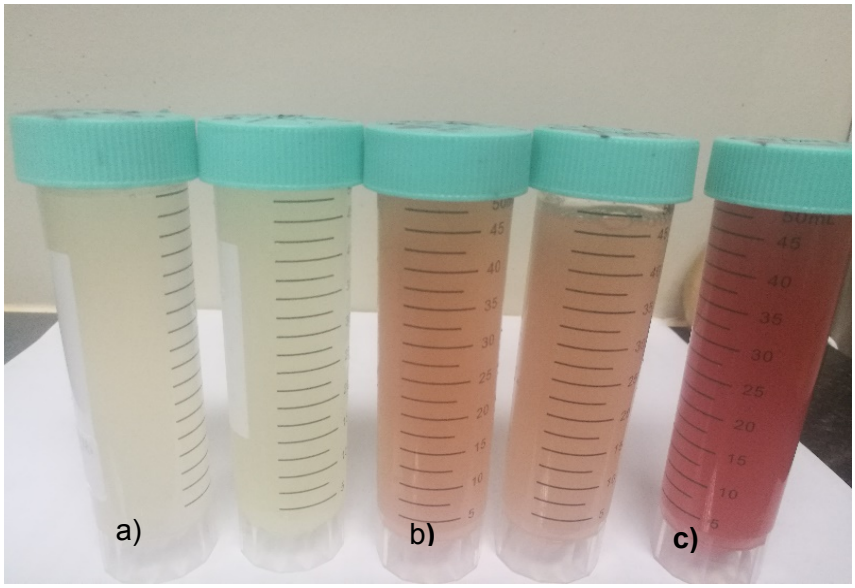


Figure 10: Fresh watermelon fruit juice at different stages of maturity: a) = unripe sample, b) = half ripe sample c) = fully ripe sample

3.2.2 Methods

Physicochemical analysis

Colour measurement of watermelon fruit juice

The colour of the watermelon fruit juice was analysed by Lovibond colorimeter (LC/00 Spectro-colorimeter & SV 100kit, UK), according to the method by Guo *et al*. (2013) and Feng *et al*. (2013). The colour of three juice samples were measured for each stage of

maturity. Juice samples were placed in cuvettes using pipette liquid droppers. The results were expressed using CIELAB parameters (L*, a*, b*); L* (100 = white; 0 = black) is an indication of lightness; a* measures chromaticity, with positive values indicating redness and negative values indicating greenness; and b* measures chromaticity, with positive values indicating yellowness and negative values indicating blueness. The colour of the samples was compared using the Chroma (C*) and the hue angle (h°) as indicted by equation equations 1 and 2 respectively, as described by (Bianchi *et al*., 2018)

$$C^* = \left(\sqrt{a^*} + b^*\right)^2 \quad \textbf{(Eq. 1)}$$

$$h^{\circ} = tan - 1\ \left(\frac{b^*}{a^*}\right)^2 \quad \textbf{(Eq. 2)}$$

Determination of total soluble solids (TSS) and pH of watermelon fruit juice

The total soluble solids content was determined by measuring °Brix (Santos *et al*., 2015) using a digital refractometer (Smart-1, ATAGO, CO, LTD, Thailand) according to method by Guo *et al*. (2013). Approximately 10 ml of watermelon fruit juice was placed on the prism of the refractometer and measured. Three replicated TSS readings were taken for each sample. The mean was used, and results were reported as °Brix (equivalent to TSS).

The pH of the sample was measured using a pH meter (LCD Display Bench-top, Sper Scientific, China) according to a method validated by Quek *et al*. (2007). The pH meter was pre-calibrated using buffers with pH 4.7 and 10 respectively. The probe was immersed in 250 ml of watermelon juice and the pH was measured. Analysis was performed in triplicate for each sample. LCD Display Bench-top

Determination of lycopene and β-carotene (vitamin A) on watermelon fruit juice

Preparation of standards

Methanol/Tetrahydrofuran (THF) (50:50 v/v) and hexane/acetone/ethanol (50:25:25 v/v/v) were prepared as extraction solvents. The stocks were prepared by methods stipulated by Scott *et al*. (1996). Thus, individual stock standard solutions were freshly prepared by adding a suitable volume of hexane to the vial containing the β-carotene standard and lycopene standard, respectively. The sample was vortexed to complete dissolution; then the solutions was transferred to different volumetric flask and the concentration was determined spectrophotometrically using a UV-VIS spectrophotometry (ThermoFinnigan/ FinniganMAT, San Jose, CA, Bremen, Germany). The absorbance of upper hexane layer was measured in a 1 cm-path length quartz cuvette at 503 nm using a spectrophotometer. An aliquot of each individual stock standard solution was evaporated to dryness using

nitrogen flow under vacuum. The sample was checked for purity using HPLC (Model RD-20A, Japan) with a detector at absorbance 503 nm (Hart and Scott, 1995 and Scott *et al*., 1996).

Extraction of lycopene and β-carotene from the watermelon fruit juice

The extraction was done using a method by Olives-Barba *et al*. (2006). Approximately 3 g of watermelon juice was placed in a vessel, protected from light by covering the vessel with aluminium foil. The watermelon juice was mixed with 100 ml of extraction solvent (methanol/Tetrahydrofuran (THF) (50:50 v/v) and hexane/acetone/ethanol (50:25:25 v/v/v) separately. The mixture was stirred for 30 min using a vortex mixer. The sample was then centrifuged (Model 5702, RH, Japan) at 1890 rpm for 15 minutes at 4˚C to separate the supernatant. The methanol/THF (50:50 v/v) extract was diluted to a concentration of THF lower than 10% to avoid peak broadening; for the hexane/acetone/ethanol extract 15 ml of distilled water was added; the upper layer was placed in a round-bottom flask and an aliquot of 10 ml of the extract was evaporated to dryness under nitrogen flow under vacuum. Different mixtures of THF/ acetonitrile (ACN)/methanol were used for the dissolution of the dry extract. The residues were dissolved to a final volume of 10 ml. The final solution was filtered through 0.45 µm membrane filters and 100 µL was injected for analysis using HPLC (Model RD-20A, Japan) with a detector at absorbance 450 nm for β-carotene and 475 nm for lycopene. The mobile phase was a mixture of methanol and acetone (90/10 v/v) at a flow rate of 0.9 ml/ min. The column temperature was 30˚C (Olives Barba *et al*., 2006). The sample was assayed by mobile phase of methanol/ACN +TEA 9 µM, the mobile phase was filtered through a 0.45 µm membrane and degassed ultrasonically prior to use.

Determination of ascorbic acid (AA) on watermelon fruit juice

AA was determined by HPLC (Model RD-20A, Japan) using the method of Soliva-Fortuny *et al*. (2004). A 25 ml of watermelon fruit juice was homogenised with 10 ml of extraction solution (10% metaphosphoric acid + 0.5% 2, 3 dimercapto-1-propanol, BAL, a thiol-reducing reagent). The homogenised mixture was centrifuged (Model 5702, RH, Japan) at 1890 rpm for 15 min at 4˚C. The supernatant was vacuum-filtered through Whatman No. 1 paper and diluted to 50 ml with distilled water. Then, samples were passed through a 0.45 µm membrane filter. An aliquot of 20 µl was injected into the HPLC system using an NH_2 Spherisorb S5 column (250 4.6 mm, 5 µm). The eluent was acetonitrile: 5 mm potassium dihydrogen phosphate buffer adjusted to pH 3.5 (40:60). The flow was isocratic at a rate of 1 ml/ min at room temperature. Detection was performed with a 486-absorbance detector at 254 nm.

Determination of TPC and antioxidants activity on watermelon fruit juice

Determination of total TPC on watermelon juice

TPC was determined according to the method of Gutfinger (1981). 1.0 ml of watermelon juice was mixed with 1.0 ml of Na_2CO_3 solution (2 g/100 ml H_2O) and kept at room temperature for 3 min. After the addition of 0.2 ml Folin-Ciocalteu reagent (2-fold diluted with H_2O), the reaction was kept for 30 min in the dark room, followed by centrifugation (Model 5702, RH, Japan) at 13.400 rpm for 5 min. The absorbance of supernatant was measured at 750 nm by using a UV-VIS spectrophotometer.

Determination of the free radical scavenging activity on watermelon juice

The 1.1-diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity was determined by the method described by Elez-Martínez and Martín-Belloso. (2007) modified by Jamal *et al*. (2016). Watermelon juice (50 ml) was centrifuged (Model 5702, RH, Japan) at 6000 rpm for 15 min at 4°C, allowed to stand for 3 min before separating the supernatant. The supernatant (approximately 0.01 ml) was mixed with 3.9 ml methanolic DPPH solution (0.025 g/ L) and 0.090 ml of distilled water. The mixture was vortexed for 30 s. The mixture was then allowed to stand for 30 min at room temperature in a dark room. Samples were measured with a UV-VIS spectrophotometer (ThermoFinnigan/ FinniganMAT, San Jose, CA, Bremen, Germany) at 515 nm against a blank of methanol without DPPH. Antioxidant capacity was calculated as follows:

$$\%\ DPPH = \frac{A\ (Sample)}{A\ (Control)} x100 \qquad \textbf{(Eq. 3)}$$

Where: A sample is the absorbance of the reaction and A control is the absorbance of the control reaction.

Statistical analysis

Data was analysed through one-way analysis of variance (ANOVA) using SPSS 24 software (IBM, Chicago, USA). When a significant ($p < 0.05$) difference was detected, comparisons were performed using Duncan's multiple range test. The processed data was presented as mean ± standard deviation of three replicates (Wang *et al*., 2018).

3.3 Results and Discussion

3.3.1 Effects of maturity on physicochemical properties of watermelon fruit juice

Consumers consider colour as an important indicator of the eating quality of fruit juices (Choudhary *et al*., 2015). Therefore, the red colour determines the approval of the watermelon fruit juice by the consumer (Oberoi and Sogi, 2015a). The colour of watermelon juice, the lightness (L^*), redness (a^*) and the yellowness (b^*) were measured throughout the selected stages of maturity (Table 6). The combination of the CIELAB parameters (L*, a*, b*) was clearly explained by the Chroma (C*) and the hue (h^o) values. A significant ($p < 0.05$) difference was observed from all the watermelon fruit juice samples collected at different stages of maturity (Table 6).

Table 6: Effects of maturity on colour properties of the watermelon fruit juice

	Colour				
Sample	L*	a*	b*	C*	h°
Unripe	50.70 ± 0.10^{c}	1.6 ± 0.21^{a}	7.47 ± 0.06^{a}	4.32 ± 0.02^{a}	83.89 ± 0.01$^{e\,c}$
Half - ripe	34.33 ± 1.07^{b}	6.67 ± 0.15^{b}	10.93 ± 0.49^{b}	5.88 ± 0.12^{b}	72.79 ± 0.01^{a}
Fully - ripe	22.27 ± 0.06^{a}	8.95 ± 0.55^{c}	17.97 ± 0.25^{c}	7.35 ± 0.21^{c}	76.03 ± 0.01^{b}

Values are mean ± SD (n = 3), means with different superscripts in the same column are significantly different at $p < 0.05$ using Duncan multiple range

The C* value which indicated the degree of colour saturation has shown to increase throughout the maturity stages, it was lower (4.32) in the unripe sample and increased to 5.88 in the half - ripe until it reached saturation in the fully - ripe (7.35) sample. The highest (7.35) C* value indicated that the red colour was more intense in the fully - ripe sample. The unripe juice sample was observed to be cream white, this was indicated by the higher h^o value observed from this sample, this was mainly due to lower traces of redness (1.6) recorded from this sample. The unripe juice colour shifted away from cream white towards the red and yellow chromaticity. This was indicated by the lower value of h^o (76.03) recorded from the fully - ripe watermelon juice sample as compared to the higher (83.89) h^o value recorded from the unripe watermelon juice sample. This was mainly due to the red colour that was intense (8.95) in the fully - ripe sample.

Watermelon fruit juice accumulated 74% of the red colour from the half - ripe to full - ripe stage making it a dominant colour than lightness and yellowness. Tlili *et al* 2011 reported that the colour changes in watermelon fruit juice samples were due to the presence of

colour pigments (lycopene extracts). These results are in accordance with those reported by Soteriou *et al*. (2014) and Bianchi *et al*. (2018), whereby it was concluded that the red colour in watermelon fruit juice is maturity dependent; it is more dominant at the fully - ripe stage. Moreover, Perkins-Veazie *et al*. (2006) observed that the red colour was present in watermelons from 12 - 16 days post-anthesis and gradually increased as maturity progressed. The changes in colour may be attributed to genes and activity of enzymes such as peroxidase and lipoxgygenase present in the watermelon (Aguilo-Aguayo *et al*., 2010). Guo *et al*. (2015) reported that carotenoid biosynthetic pathway is activated by phytoene synthase (PSY) and phytoene desaturase (PDS), these pathways are up-regulated during red-fleshed watermelon fruit development. The increase in red colour at fully - ripe maturity stage is correlated to the increase in lycopene synthesis at this stage (Guo *et al*., 2013), as the red colour is derived from the accumulation of this phytochemical in the chromoplasts (Soteriou *et al*., 2015). Furthermore, the increase in red colour of the watermelon fruit juice is in conjunction with chlorophyll degradation and anthocyanin accumulation during maturity as reported by Butkhup and Samappito (2011) on colour changes of Maoluang fruit. In addition, Kubola and Siriamornpun (2011) reported that colour change is affected by presence of phytochemicals at different stages of maturity of a Gac fruit.

Watermelon is considered a fruit of insipid acidity (Soteriou *et al*., 2014). A significant ($p < 0.05$) difference was observed throughout all maturity stages (Table 7). The pH of the watermelon fruit juice was low at unripe stage (4.3) and increased as the maturity progressed reaching 5.0 at fully - ripe stage. The 0.7 increase in pH units was observed from the unripe sample to fully - ripe sample. In accordance, Perkins-Veazie *et al*. (2007b) reported the lower pH (4.3) in an unripe watermelon fruit juice. In addition, Soteriou *et al*. (2014) observed a low pH (4.7) in an unripe and 5.0 in a fully - ripe watermelon fruit juice. These results are in accordance with those reported by Tlili *et al*. (2011) who reported that watermelon has high pH (low acid), ranging from 4.5 to 5.5 at fully – ripe stage.

Interestingly, Soteriou *et al*. (2014) reported that pH of watermelon remains stable between half - ripe and fully ripe stage. These differences in pH corroborate the suggestion by many researchers who have stated, pH of watermelon varies according to cultivar type, climatic conditions and geographic location (Lv *et al*., 2015; Santos *et al*., 2015; Bianchi *et al*., 2018). The changes in acidity may be influenced by the activity of enzymes (Aguilo-Aguayo *et al*., 2010). The change in pH can also be the results of ethylene during maturity stages, Wang *et al*. (2018) reported ethylene production affects the stability of the watermelon during maturity.

Sweetness is the most critical quality trait of watermelon juice, it determines consumer's acceptability (Tlili *et al*., 2011). The Total soluble solids (TSS) were measured as Brix as described by Santos *et al*. (2015). In this study, TSS were detectable in lower quantities (2.66) in the unripe juice. It increased linearly as the watermelon maturity progressed, thus from 6.88 to 8.41 at half - ripe and fully - ripe stage (Table 7). About 81% increase in TSS was observed at fully ripe stage.

Table 7: Effects of maturity on the pH and TSS of the watermelon fruit juice

Sample	pH	°Brix
Unripe	4.74 ± 0.04[a]	2.66 ± 0.02[a]
Half - ripe	4.94 ± 0.03[b]	6.88 ± 0.002[b]
Fully - ripe	5.50 ± 0.05[c]	8.41 ± 0.12[c]

Values are mean ± SD (n = 3), means with different superscripts in the same column are significantly different at $p < 0.05$ using Duncan multiple range

The TSS were higher in the fully - ripe sample. As a result, the sweetness of fully - ripe watermelon is concomitant with high levels of sugars. These results are in accordance with those reported by Soteriou *et al*. (2014) who reported that sugar content in most open-field watermelon cultivars peaks around 35 - 40 days from fruit setting and drops with over-maturity. Maynard (2001) also observed that TSS was affected by maturity and not by grafting when comparing the grafted and self-rooted watermelons. In addition, Nunes (2008) reported that unripe watermelon has lower total soluble solids further indicating that this property increases with maturity (3.3 to 4% in immature fruit to about 11 - 13% in mature watermelon). The variation of sugar content was dependent upon maturity; this may be because sucrose is accumulated after the 20th day of pollination and then drastically increase with ripening (Yoo *et al*., 2012). The sugar content has shown to have a correlation with the red colour of the juice. As the juice colour became redder, the TSS increased. This is in accordance with the study done by Wang, (2012) who reported that there is a correlation between TSS and colour of watermelon during maturity. Increase in soluble solids during maturity is common in many cultivars of the *cucurbit* genotypes and it is linked to their ability to accumulate sucrose at the matured stage (Perkins-Veazie *et al*., 2006; Zhang *et al*., 2015). Fructose and sucrose are key components influencing the sweetness of the juice, watermelon with higher content of sucrose than fructose is sweeter than that with lower content (Kano, 2004). Fully ripe sample had the highest amount of

TSS; this is mainly due to accumulation of 40% sucrose in the matured watermelon (Bianchi *et al*., 2018). During watermelon maturity, sucrose is the main form of carbohydrate accumulating at the expense of reducing sugars (Soteriou *et al*., 2014).

Guo *et al*. (2015) reported that the sweetness of the watermelon fruit juice at the fully - ripe stage is influenced by specific activity of sugar metabolising enzymes such as sucrose phosphate synthase (SPS), sucrose synthase (SS) and acid inveratse (AI). In addition, Kyriacou *et al*. (2018) reported that, sweetness of the watermelon fruit juice is influenced by mono and di-saccharides; it also relies partly on other solutes such as amino acids, organic acids, soluble pectin, phenolic compounds and minerals. Furthermore, Guo *et al*. (2015) reported that genes such as α-galactosidase, invertase, sugar transporter genes, UDP-galactose/glucose and pyrophosphorylase plays a potentially critical role in the determination of fruit sugar of cultivated and wild watermelon. The high TSS content of the fully - ripe watermelon fruit juice defines the good eating quality of watermelon fruit juice at this stage.

3.3.2 Effects of maturity on phytochemicals of watermelon fruit juice

Lycopene is a phytochemical, which gives watermelon their desirable red colour (Oberoi and Sogi 2017a). Lycopene was present at all stages of maturity however; it varied in quantity (Table 8), with the unripe sample having the lowest (0.04 mg/100 g). The highest value was recorded in the fully - ripe sample (6.19 mg/100 g). These results emphasise the point mentioned by Tadmor *et al*. (2005) who reported that young watermelon fruit mesocarp is usually white and contains only trace amounts of lycopene. The considerable increase of synthesis and accumulation of lycopene was detected in the transitional phase when the watermelon was harvested at the half - ripe stage (Table 8). This may be correlated to the progressive activation of the molecular mechanisms involved in carotenogenesis regulation during the transition between the unripe and half - ripe stages, followed by a feedback inhibition of the pathway by end-products towards the end of fruit maturity (Tlili *et al*., 2011). These mechanisms may include regulation at the transcriptional and post-transcriptional level, metabolite flux into the carotenoid pathway and carotenoid sequestration, as has been found in tomatoes (Bramley, 2000). Watermelon fruit juice accumulated 59% of lycopene from half - ripe stage to fully - ripe stage. Contrarily, the amount found in the watermelon fruit juice is lower than those reported by Soteriou *et al*. (2014), who reported 59.4 µg/ g of lycopene in watermelon at day 50 post-anthesis.

Table 8: Effects of maturity on phytochemicals of watermelon juice at different maturity stages

	mg/100 g				
Sample	Lycopene	β-carotene	AA	TPC mgGAE/100 g	Antioxidants activity % DPPH
Unripe	0.04 ± 0[a]	0 ± 0[a]	1.3 ± 0.1[a]	4.87 ± 0.25[a]	4.90 ± 0.02[a]
Half-ripe	3.9 ± 0.01[b]	0.12 ± 0[b]	2.27 ± 0.1[b]	9.52 ± 0.01[b]	8.28 ± 0.10[b]
Fully ripe	6.19 ± 0.01[c]	0.23 ± 0.01[c]	3.47 ± 0.1[c]	25.95 ± 0.03[c]	23.90 ± 0.02[c]

Values are mean ± SD (n = 3), means with different superscripts in the same column are significantly different at $p < 0.05$ using Duncan multiple range (AA = Ascorbic acid, TPC = Total Polyphenolic Content)

These results are in accordance with those reported by Oms-Oliu *et al*. (2009) who reported the value of 6.20 mg/100 g. The results variation may be influenced by difference in climatic conditions and the soil (Choudhary *et al*., 2009). Wehner (2017) reported that lycopene is accumulated at 10 - 12 days after pollination. This study has revealed that lycopene increases as the watermelon matured, it agrees with those reported by Jain *et al*. (2003) and Singh *et al*. (2015) who reported the increase in lycopene content during maturity of guava and teasel gourd. In addition, Ilahy *et al*. (2011) observed lycopene increase in tomato from green - orange to orange - red and orange - red to red - ripe in the Rio Grande cultivar.

Lycopene was the highest phytochemical present in the watermelon fruit juice, mainly because it accounts for 70 - 90% of total phytochemicals in the watermelon (Perkins-Veazie *et al*., 2006). There was a visible correlation between the juice colour and the lycopene content. As the red colour increased, lycopene content increased. Similar trend was observed by Bianchi *et al*. (2018). This underpins the reports, which suggested that lycopene correlates and is synchronous with changes in juice colour (Soteriou *et al*., 2015). Lycopene is a phytochemical, which gives watermelon its desirable red pigment, the very low traces of lycopene at the unripe stage, explains the reason watermelon is white at that stage. Research has expounded that tomato juice and watermelon fruit juice have higher lycopene content (Perkins-Veazie *et al*., 2006; Soteriou *et al*., 2015). These results revealed that the lycopene content in a fully - ripe sample is 6.19 mg/100 g (fw), which is higher than 30.68 µg/100 g of tomato juice reported by Jayathunge *et al*. (2017). It therefore agrees with reports, which indicated that watermelon fruit juice is a source of dietary lycopene (Choudhary *et al*., 2015).

Lycopene has shown to be maturity-dependent in nature. The increase in lycopene throughout maturity stages could be due to involvement of many genes that are present in

the fruit from unripe stage of maturity and continue to be active up to the fully - ripe stage (Ilahy *et al*., 2011). These data are consistent with a previous observation that gene expression of upstream enzyme for phytoene synthase-1 in the lycopene metabolic pathway was up-regulated during fruit maturation, whereas those of a downstream enzyme lycopene *β*-cyclase was down-regulated during maturity of watermelon fruit (Guo *et al*., 2015; Singh *et al*., 2015; Liu *et al*., 2018). In addition, these changes occur due to the decrease of chlorophyll with a concomitant rise in carotenoids as reported by Singh *et al*. (2015).

This pigment is capable of neutralising free radicals in human body with its antioxidant properties and plays a vital role in chronic diseases such as cancer, osteoporosis, diabetes and cardiovascular diseases (Aghajanzadeh *et al*., 2017; Hong *et al*., 2018). Owing to these properties, dietary lycopene consumption (4 mg/100 g or more/day) may be beneficial to human health by reducing the incidence of prostate and oral cancers and in the prevention of oxidative damage to the cells (Perkins-Veazie *et al*., 2003; Bianchi *et al*., 2018).

Watermelon juice has a considerable amount of β-carotene (Oberoi and Sogi, 2017b), the current study showed that the quantity of this antioxidant varies according to stages of maturity. There was no β-carotene observed in the unripe sample (Table 8). This observation is in accordance with the results reported by Tlili *et al.* (2011) who also did not detect any β-carotene at the unripe stage of maturity. Bianchi *et al*. (2018) reported that, the presence of β-carotene depends on the presence of lycopene in watermelon. Therefore, the absence of β-carotene at the unripe stage may be the results of only low traces of lycopene at this stage. Tlili *et al*. (2011) and Guo *et al*. (2011) reported that, lack of β-carotene during the unripe stage of maturity shows that the enzyme involved in cycling lycopene to β-carotene (lycopene-β-cyclase) is not-expressed or is absent or present in very low concentrations at the very early stage of watermelon fruit maturity. Similar β-carotene pattern was observed during fruit maturity of the β-mutant orange tomatoes (Tadmor *et al*., 2005).

β-carotene content was very low (0.12 mg/100 g) in the half - ripe watermelon juice sample. However, the quantity of β-carotene increased by 55% during the transition. Thus, from the half - ripe to fully - ripe stage reaching the highest amount of 0.22 mg/100 g (Table 8). This is because the enzyme lycopene β-cyclase is active during transition from half - ripe to fully - ripe stage (Tlili *et al*., 2011). In contrast, the amounts of β-carotene measured in fully - ripe sample were lower than those reported by Perkins-Veazie *et al*. (2006), who reported

the amount of 0.9 – 10.2 mg/ kg (fw) of the fully - ripe watermelon when investigating β-carotene content of 50 watermelons cultivars grown in Oklahoma, United Sates of America (USA). This is mainly due to agro technical and environmental conditions as well as cultivar type (Dumas *et al*., 2003; Davis *et al*., 2003).

Interestingly, β-carotene content in watermelon fruit juice increased with maturity, as opposed to fruits such as Gac fruit, which showed a sharp decrease in β-carotene content as the fruit matures (Singh *et al*., 2015). Guo *et al*. (2015) reported that, during maturity, β-carotene is influenced by the genes that regulate ACC oxidase, ethylene receptor and ethylene responsive factor that are involved with ethylene biosynthesis and therefore showed highly maturity-associated expression patterns. This indicated that ethylene might be playing a role in fruit maturity of cultivated watermelon (Guo *et al*., 2015). In addition, the changes in β-carotene content may be the result of carotenogenesis in chromoplast, Nunes, (2008) reported that, carotenogenesis contributes to the fruit carotenoid content of tomato. This data suggests that watermelon fruit juice is good source of β-carotene. Therefore, consumption of watermelon fruit juice at fully - ripe stage may contribute positively to the visual system of human being; it can improve the body's immune and reproductive system (Xiao *et al*., 2019). The presence of β-carotene at fully ripe stage indicates the potential use of watermelon for functional food items.

Watermelon is a good source of ascorbic acid (AA) (Tlili *et al*., 2011), contrarily to β-carotene, AA content was detected from the unripe stage of maturity. AA was significantly different at $p < 0.05$ at all stages. It was found to increase with maturity ranging from 1.33 to 3.47 mg/100 g (Table 8). However, the current results do not corroborate those reported by other researchers who found the highest amount of AA at the ripe stage (576.2 mg/ kg) and lower values (38.2 – 69.8 mg/ kg) (Perkins-Veazie *et al*., 2003; 2006) The variations in the value of AA content may be due to difference in environmental conditions and probably cultural practices (Dumas *et al*., 2003). These results agree with other studies which reported that AA increases with maturity in fruits such as apricot, peach, papaya and jujube (Tlili *et al*., 2011). Interestingly, Lee and Kader, (2000) reported that, citrus and mangos have high AA when they are immature, and it declines as the fruit reach peak ripeness. In fruits such as muskmelon, AA content is correlated to refractive index and with sucrose content of the juice, hence it increases during fruit maturity (Tlili *et al*., 2011).

The watermelon carotenogenic genes also influences AA variation (Karakurt and Huber, 2004; Singh *et al*., 2015). Zhao *et al*. (2013) analysed the genes at three fruit maturity stages (10, 20, and 30 days post-anthesis). Their results showed that gene expression

increases during the early stages of fruit maturity and subsequently decreased until the fruit is fully - ripe. USDA (2003) reported that, the daily recommended intake of dietary AA is between 45 and 75 mg/100 g. Based on the mean value of AA content for this study (about 3.30 mg/100 g), a cup of fresh watermelons juice should provide approximately 8 mg of AA, which is considered a good source since each serving size meets at least 10% of RDA. Therefore, consumption of watermelon fruit juice at the fully - ripe stage can contribute positively to human health because AA inhibits oxidation, which can ultimately lead to cell death (Ruiz *et al*., 2019).

Phenolic compounds are secondary metabolites in plants; they are known to be important for imparting health benefits and for developing the colour and flavour of fruit juices and wine (Feng *et al*., 2006; Choudhary *et al*., 2015). Vinson *et al*. (2010) reported that watermelon contributes about 80% and 50% of the daily phenol intake of diet. The total phenolic content of unripe sample, half - ripe sample and fully - ripe sample are 4.87, 9.54 and 25.95 mg GAE/100 g (fw) respectively. Maturity has a significant effect ($p < 0.05$) on the total phenolic content of watermelon juice. Thus, total phenolic content increased with maturity of the watermelon. The highest value of TPC in the fully ripe sample (Table 8) underpins the reports, which indicated that quantity of TPC is affected by degree of maturity (Soteriou *et al*., 2014; Bianchi *et al*., 2018).

Contrary results have been reported by Brat *et al*. (2006) who found a moderate amount (11.6 mgGAE/100 g fw) of phenolics in watermelon fruits sampled from French National markets while Perkins-Veazie *et al*. (2003) observed higher values ranging between 87 and 91 mg GAE/100 g (fw) in red-fleshed watermelon grown in Oklahoma. These differing results are mainly due to environmental and soil conditions (Tlili *et al*., 2011). Watermelon TPC increases with maturity unlike with fruits such as mangos, citrus and teasel gourd, which exhibit a linear pattern of decrease in polyphenols during maturity (Singh *et al*., 2015). The recommended daily intake of polyphenols is estimated between 2590 and 3016 mg per day (Saura-Calixto *et al*., 2007), therefore, the consumption of a cup of fully - ripe watermelon fruit juice will contribute positively to the RDA (± 10%). These compounds are also excellent antioxidants therefore they will contribute positively to the prevention of diseases such as heart attacks, cardiovascular diseases and some cancers (Dia *et al*., 2016; Nowicka *et al*., 2019).

The antioxidant activity of watermelon fruit juice was measured as free radical-scavenging capacity on DPPH radical. Higher antioxidant activity 23.90% DPPH was observed in the fully - ripe watermelon fruit juice while the unripe and half - ripe watermelon juice presented

the lowest value 4.90 and 8.28% DPPH respectively. The significant difference of ($p < 0.05$) was observed throughout all the stages of maturity (Table 8). Antioxidant activity increased by 35% between the unripe and half - ripe sample. Antioxidants activity of watermelon fruit increased with increase in maturity unlike in teasel gourd fruit that has shown decrease in antioxidant activity during the stages of maturity (Singh *et al*., 2015). Fawole *et al*. (2013a) also reported 42% decrease in the antioxidant activity of pomegranate at the fully - ripe stage of maturity. The increase in antioxidant activity from unripe stage to fully - ripe can be ascribed either to the increase in total flavonoids and tannins or to the presence of other reducing agents such as ascorbic acid which may also reduce the oxidised state of antioxidant compounds (Nagal *et al*., 2012).

The efficiency of the antioxidant activity in fruit juice is related to presence of a high concentration of total polyphenol content. Pinto *et al*. (2016) reported that phytochemicals such as phenolic compounds and AA in watermelon are the major components responsible for DPPH free radical scavenging activity. Singh *et al*. (2015) reported that antioxidant activity has a strong correlation with phenolics ($r = 0.976$; $p < 0.05$), tannin ($r = 0.984$; $p < 0.05$), β-carotene ($r = 0.951$; $p < 0.05$), carotenoids ($r = 0.923$; $p < 0.05$) and chlorophyll ($r = 0.951$; $p < 0.05$). The presence of ethylene in watermelon fruit may also contribute to the increase in antioxidant activity at the fully - ripe stage (Fawole *et al*., 2013b; Guo *et al*., 2015). In addition, the changes in antioxidants activity may be due to the inter-conversion of secondary metabolites during the maturity process (Singh *et al*., 2015; Cömert and Gökmen, 2017).

3.4 Conclusion

During fruit maturity, a linear pattern of increase was observed for phytochemicals and physicochemical properties, especially the red colour and lycopene. The degree of saturation as indicated by the C* and the h^o has shown that the red colour of the watermelon fruit is accumulated throughout the stages of maturity. The presence of lycopene, β-carotene, AA, TPC and antioxidant activity makes watermelon fruit juice a good source of antioxidants. Therefore, consumption of watermelon fruit juice at half - ripe and fully - ripe stage could constitute a predominant source of natural antioxidants in the South African diet, it could also be adopted in pharmacological industries to limit the usage of supplements and synthetic antioxidants. Moreover, these data can be useful to food manufactures and pharmaceuticals working to extend the shelf-life of watermelon juice through manufacturing of various products.

CHAPTER 4: EFFECTS OF FILTRATION ON QUALITY PROPERTIES OF FRESH WATERMELON FRUIT JUICE

Abstract

Filtration is used to obtain a clarified and smooth juice. However, filtration has a potential of changing the quality properties of the juice. The aim of this study was to determine the effects of filtration on physicochemical properties (colour, pH, and total soluble solids) and phytochemicals (lycopene, β-carotene, ascorbic acid, total polyphonic content and antioxidants activity) of fresh watermelon at different stages of maturity. The filtered and unfiltered watermelon juice were analysed for physicochemical and phytochemical properties. There was no significant ($p< 0.05$) difference observed from the colour of the unripe watermelon fruit juice. A significant ($p < 0.05$) difference was observed from the half - ripe and fully –ripe filtered watermelon fruit juice. The Chroma (C*) and the h^o values indicated that there was a decrease in colour of the watermelon fruit juice after filtration. The filtered watermelon juice of the fully - ripe sample has shown a decrease in C* value as compared to the control sample. There was a increase in pH after filtration of the fully – ripe sample of the watermelon fruit juice. Filtration has also shown to slightly decrease the ºBrix of the watermelon fruit juice. There was no significant ($p < 0.05$) difference in β-carotene, ascorbic acid and total polyphonic content between filtered and control samples at the unripe and half - ripe stages. Lycopene decreased with 0.04 mg/ 100 g, β-carotene decreased with 0.01 mg/100 g), ascorbic acid decreased with 0.41 mg/ 100, total polyphenolic content decreased with 0.79 mg GAE/ 100 g while antioxidants activity decreased with 0.88% at fully - ripe stage. Filtration retained a high percentage of quality properties of watermelon juice.

Keywords: Extracted, clarified, filtration, maturity, physicochemical, phytochemical

4.1 Introduction

Watermelon juice is mostly consumed throughout the world and it is appreciated for its sweetness to tame the summer thirst (Oberoi and Sogi, 2017a). It has recently stimulated great attention due to the presence of phytochemicals, which contribute immensely to human health, suggesting protective roles in reducing the risk of certain types of cancers, cardiovascular diseases and age-related degenerative pathologies (Soteriou *et al*., 2015; Guo *et al*., 2015; Singh *et al*., 2015; Hong *et al*., 2018). Watermelon juice is extracted from the flesh through extraction equipments (Tlili *et al*., 2011). It is filtered to obtain a clarified juice. Filtration is an important processing step, which removes solid particles to clarify the

juice (Bhattacharjee *et al*., 2017a). These finely dispersed substances must be eliminated in the production of clear juice to avoid blurring and subsequent deposition (Cassano *et al*., 2007a). Filtration results in enhanced sensory properties (taste, odour and colour) and smooth juice (Oberoi and Sogi, 2015). However, many studies have shown that, filtration decreases the colour, total soluble solids, lycopene and total polyphenols of the juice (Laorko *et al*., 2010; Bhattacharjee *et al*., 2017b). The aim of this study was to evaluate the effects of filtration on the physicochemical and antioxidant properties of the watermelon juice at different stages of maturity.

4.2 Materials and methods

4.2.1 Sample preparation

Fresh watermelon juice was filtered through a funnel into 250 ml glass bottles using a two-layer cheese cloth. The remaining paste-like filter cake fraction was rinsed with deionised water and filtered again into the glass bottle (Guo *et al*., 2013). The filtrates (juice) were transferred into pre-labelled 50 ml polyethylene bottles. Physicochemical and antioxidants analysis were carried out on both unfiltered and filtered samples (Tlili *et al*., 2011).

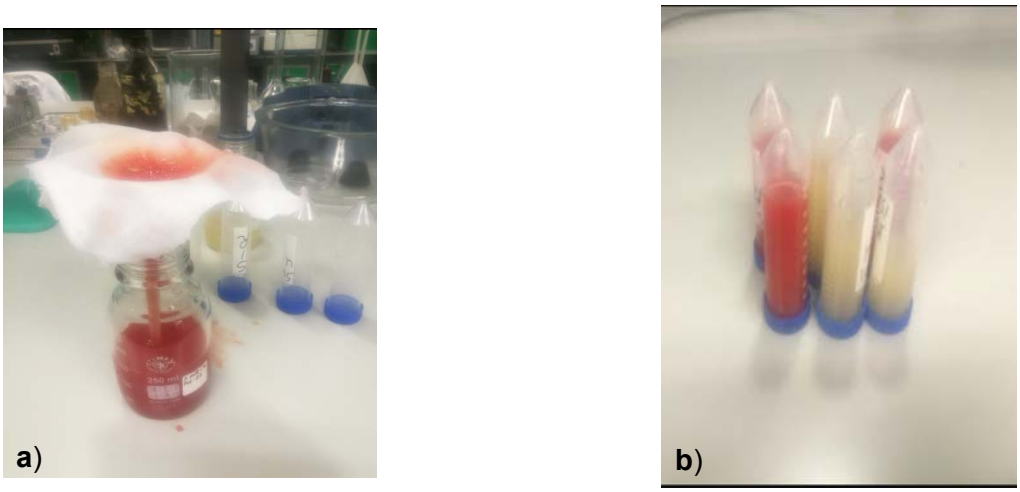


Figure 11: Fresh watermelon fruit juice preparations; a) filtration process; b) filtered watermelon juice

4.2.2 Methods

Analysis of watermelon juice quality were done using methods described in Chapter 3, Section 3.2.2

4.3 Results and Discussion

4.3.1 Effects of filtration on physicochemical of watermelon fruit juice

Filtration is an essential step in the fruit juice processing industries as it removes the unwanted solid particles from the juice (Laorko *et al*., 2011). The colour of the watermelon fruit juice was measured as L* a* and b*. The degree of saturation was explained by the Chroma (C*) and the hue (h°) values (Table 9). There was no significant ($p < 0.05$) difference observed from the watermelon fruit juices of the unripe samples. The significant difference ($p < 0.05$) were observed from the half - ripe and the fully - ripe samples (Table 9). The lower C*values after filtration of the half - ripe (5.6) and fully - ripe (7.22) samples indicates that filtration decreased the red colour of the watermelon fruit juice. Similarly, the h° has shown a decrease after filtration. The fully - ripe control sample had a higher h° (76.03) value than the filtered (75.25) sample. These means that the white solid particle which caused the juice to be cream white were removed from the juice leaving the juice to be darker because of the intensity and dominance of the red colour. The improvement in colour may be associated with the removal of solid particles from the filtrate (Laorko *et al*., 2010).

Table 9: Effects of filtration on colour of fresh watermelon fruit juice at different stages of maturity

Sample	Colour properties				
	L*	a*	b*	C*	h°
Control unripe	50.73 ± 0.12^{e}	1.67 ± 0.06^{a}	7.5 ± 0.01^{a}	4.32 ± 0.02^{a}	83.89 ± 0.01^{e}
Filtered unripe	50.73 ± 0.06^{e}	1.63 ± 0.06^{a}	7.4 ± 0.01^{a}	4.24 ± 0.02^{a}	83.67 ± 0.01^{e}
Control half - ripe	35.47 ± 0.49^{c}	6.67 ± 0.01^{c}	10.63 ± 0.25^{c}	5.88 ± 0.12^{b}	72.79 ± 0.01^{a}
Filtered half - ripe	37.49 ± 0.29^{d}	6.13 ± 0.01^{b}	10.07 ± 0.00^{b}	5.69 ± 0.13^{c}	73.09 ± 0.02^{b}
Control fully – ripe	22.27 ± 0.06^{a}	9.3 ± 0.01^{e}	17.7 ± 0.26^{e}	7.35 ± 0.21^{d}	76.03 ± 0.01^{d}
Filtered fully ripe	25.27 ± 0.29^{b}	8.99 ± 0.03^{d}	17.06 ± 0.04^{d}	7.22 ± 0.18^{e}	75.25 ± 0.03^{c}

Values are mean ± SD (n =3), different superscripts in the same column are significantly different at $p < 0.05$ using Duncan's multiple range

The results showed that about 90% of the redness (as reflected by high positive a* values) at the fully - ripe stage of watermelon remained after filtration making the red colour the most dominant. These results corroborate those reported by Laorko *et al*. (2011), where a slight decrease in colour of pineapple juice was observed after filtration. Contrarily, Rai *et al*.

(2006) reported the increase in colour of Mosambi juice after filtration. Vaillant *et al*. (2001) reported that the decrease of colour after filtration is due to loss of pectinious materials, which remains in the filter cake during filtration. In addition, the decrease may be attributed to exposure to light and oxygen during filtration process (Bhattacharjee *et al*., 2017a). Other researchers have linked the decrease in colour with the removal of suspended colloidal particles present in juice (Rai *et al*., 2006).

For the pH of the watermelon juice, no significant ($p < 0.05$) difference was observed for unripe and fully - ripe watermelon juice samples after filtration. This result corroborates those reported by Perkins-Veazie *et al*. (2007a); who observed that the pH of watermelon juice remains unchanged after filtration. However, a significant ($p < 0.05$) difference was observed in the half - ripe sample (Table 10). The pH of the unripe samples was observed to have decreased with 0.03 pH units. Contrarily, an increase (0.01) in pH was observed from the fully - ripe sample. At the fully - ripe stage the pH of filtered juice was 5.56 while the unfiltered juice had a pH of 5.55. In agreement, Laorko *et al*. (2010), reported a significant difference in pH between fresh and filtrated pineapple juice. In addition, Bhattacharjee *et al*. (2017a) reported that the effects of filtration on fruit juices depends on the chemical structure of the fruit juice. The effects of filtration at each maturity stage varies, this could be attributed to factors such as membrane pore size, solute content and size distribution, membrane material and operating conditions (Bai and Leow, 2002; Vaillant *et al*., 2001).

Table 10: Effects of filtration on pH and TSS of fresh watermelon juice at different stages of maturity

Sample	pH	ºBrix
Control unripe	4.77 ± 0.01^{a}	2.64 ± 0.05^{a}
Filtered unripe	4.77 ± 0.01^{a}	2.66 ± 0.01^{a}
Control half ripe	4.96 ± 0.01^{c}	6.66 ± 0.02^{c}
Filtered half – ripe	4.93 ± 0.02^{b}	6.63 ± 0.02^{b}
Control fully – ripe	5.55 ± 0.01^{d}	8.37 ± 0.03^{d}
Filtered fully ripe	5.56 ± 0.01^{d}	8.02 ± 0.03^{e}

Values are mean ± SD (n = 3), means with different superscripts in the same column are significantly different at $p < 0.05$ using Duncan multiple range

At the unripe and half - ripe stages, the TSS showed no significant ($p < 0.05$) change (Table 10). The significant difference ($p < 0.05$) in TSS was only observed from the fully - ripe samples of the watermelon fruit juice. At the fully ripe stage, fresh watermelon juice had a TSS of about 8.37 while 8.02 was observed after filtration. This means the TSS decrease 0.35% during filtration, 96% of the TSS remained in the filtered juice. Bhattacharjee *et al*.

(2017b) and Rai *et al*. (2006) have reported similar results, observing a slight reduction of TSS (3%) in the clarified watermelon and Mosabi (*Citrus sinensis*) juice. The loss of total soluble solids may be attributed to removal of suspended solid and soluble pectin in the juice (Laorko *et al*., 2010). Cassano *et al*. (2007b) reported that there is a high quantity of suspended solids, which is rejected by the membrane along with the pulp which ultimately interferes with the refractive index measurements during filtration. Moreover, the decrease in TSS may be attributed to pectinious materials that remains in the filter cake during filtration (Vaillant *et al*., 2008).

4.3.2 Effects of filtration on antioxidants of watermelon fruit juice

There was no significant ($p < 0.05$) difference in all phytochemical observed from the unripe sample. Similar pattern was observed for β-carotene, ascorbic acid and total polyphenolic content at half - ripe stages (Table 11). Contrarily, a significant decrease ($p < 0.05$) in lycopene was observed from half - ripe. The control sample decreased with 0.06 mg/ 100 g after filtration at this stage. A significant ($p < 0.05$) decrease in all phytochemicals was observed from the fully - ripe samples (Table 11). Lycopene decreased with 0.04 mg/ 100 g at fully - ripe stage. Lycopene content was not severely lost after filtration, this was shown by 99% of the lycopene content that was observed in the filtered juice of the fully - ripe sample (Table 11). These results agree with those reported by Bhattacharjee *et al*. (2017a) who observed 10% decrease in lycopene of the fully - ripe watermelon juice after filtration. A decrease in lycopene might be correlated to the decrease in colour during filtration as lycopene is the phytochemical that gives the red pigment to the watermelon fruit (Guo *et al*., 2015). In addition, the decrease in lycopene resulted as a part of senescence, where extended breakdown of chromoplast membranes occur in fully - ripe watermelons, releasing lycopene from its matrix and effecting a lower lycopene precipitation (Guo *et al*., 2013. Feng *et al*., 2013).

There was no significant ($p < 0.05$) difference observed for the β-carotene content of unripe and half - ripe watermelon fruit juice between the control and filtered samples. A significant ($p < 0.05$) was observed for the fully - ripe samples. This means that AA decreased with 0.01 mg/100 g after filtration. The average decrease in β-carotene was about 5% after filtration (Table 11). The decrease may be attributed to chemical residues being retained in the filter cake. A similar pattern was observed for AA. The ascorbic acid decreased with 0.41 mg/ 100 after filtration. This means that there was a 12% decrease in AA after filtration of the fully-ripe sample. This is in accordance with Bhattacharjee *et al*. (2017b) who reported 12% decrease in total AA after filtration of ripe watermelon fruit juice. The loss of AA may be

because total soluble solids were retained in the filter cake (Bhattacharjee *et al*., 2017b). In addition, this reduction can be correlated to the oxidation of this component caused by continual recycling of the juice through the filtration process (Cassano *et al*., 2007a; Vaillant *et al*., 2008).

Table 11: Effect of filtration on phytochemicals of watermelon juice at different maturity stages.

	mg/100 g				
Sample	Lycopene	β-carotene	AA	TPC mg GAE/ 100 g	Antioxidants activity, % DPHH
Control unripe	0.04 ± 0^{a}	0 ± 0^{a}	1.3 ± 0.1^{a}	4.87 ± 0.25^{a}	4.9 ± 0.02^{a}
Filtered unripe	0.04 ± 0^{a}	0 ± 0^{a}	1.23 ± 0.12^{a}	4.81 ± 0.23^{a}	4.89 ± 0.02^{a}
Control half - ripe	3.91 ± 0.01^{c}	0.12 ± 0^{b}	2.27 ± 0.06^{b}	9.52 ± 0.09^{b}	8.28 ± 0.1^{c}
Filtered half - ripe	3.85 ± 0.01^{b}	0.12 ± 0^{b}	2.2 ± 00^{b}	9.35 ± 0.01^{b}	8.03 ± 0.01^{b}
Control fully - ripe	6.19 ± 0.01^{e}	0.23 ± 0.01^{d}	3.47 ± 0.06^{d}	25.95 ± 0.03^{d}	23.9 ± 0.02^{d}
Filtered fully - ripe	6.15 ± 0.01^{d}	0.22 ± 0.1^{c}	3.06 ± 0.03^{c}	25.16 ± 0.02^{c}	23.02 ± 0.02^{e}

Values are mean ± SD (n = 3), means with different superscripts in the same column are significantly different at $p < 0.05$ using Duncan multiple range (AA =Ascorbic Acid, TPC = Total polyphenolic content)

Like the changes that were observed for the β-carotene and AA content, there was no significant ($p< 0.05$) difference observed for TPC at unripe and half - ripe stages between control and filtered samples. Similarly, Laorko *et al*. (2010) observed no significant difference of total phenolic content and antioxidant activity of filtered pineapple juice during maturity. However, the significant difference ($p < 0.05$) was observed from the fully - ripe sample. The TPC decreased with 0.79 mg GAE/ 100 g after filtration of the fully - ripe sample. The decrease in TPC could be attributed to the pore size of the filtration membrane of the watermelon fruit juice at each stage of maturity (Laorko *et al*., 2010). Decrease in TPC could be because polyphenols are associated with the other components such as AA (Bhattacharjee *et al*., 2017b) and then the membrane with smaller pore size could retain the bound phenolic compounds (Laorko *et al*., 2010).

There was no significant ($p < 0.05$) difference in antioxidants activity at the unripe stage while a significant difference ($p < 0.05$) was observed at half - ripe and fully - ripe stage (Table 11). Due to the different amounts of initial antioxidants activity of the unripe, half - ripe or fully - ripe watermelon juice, there was a greater retention for fully - ripe sample than for

half - ripe sample (88% compared to 25%). Filtration allows the passage of saccharides, acids, polyphenols and aroma compounds while insoluble solids, colloidal particles and microorganisms are retained (Bhattacharjee *et al*., 2017b). The decrease in antioxidants activity is correlated to the decrease in TPC and the availability of these compounds depends on other compounds such as AA and TPC (Pinto *et al*., 2016).

4.4 Conclusion

Filtration was quite successful in removing suspended colloidal particles from freshly produced watermelon juice; a remarkable improved and clarified watermelon juice was observed. Filtration had shown no significant difference in all physicochemical and phytochemical properties the unripe stage. The C* and h^o values have shown that filtration slightly decreases the red colour intensity of the watermelon fruit juice. The red colour decreased with 10% after filtration of the fully - ripe sample. The TSS showed a similar decreasing pattern as the colour while the pH was increased with 0.01 unit at the fully - ripe stage. The quantity of the phytochemicals varied with phytochemicals during maturity stages. The β-carotene, AA and TPC showed no difference at the half - ripe stage while the lycopene and the antioxidant activity showed a significant decrease at this stage. A significant ($p < 0.05$) difference for all the phytochemicals was observed from the fully - ripe sample. Lycopene decreased with 0.04 mg/ 100 g, β-carotene decreased with 0.01 mg/100 g), ascorbic acid decreased with 0.41 mg/ 100, total polyphenolic content decreased with 0.79 mg GAE/ 100 g while antioxidants activity decreased with 0.88% at fully - ripe stage. Filtration did not significantly affect lycopene (99%), β-carotene (92%), AA (88%) TCP (97%) in the final fully - ripe watermelon juice. These means that non-thermal filtration is a good option to clarify the watermelon fruit juice while retaining the physicochemical and phytochemical properties.

CHAPTER 5: EFFECTS OF THERMOSONICATION ON QUALITY PROPERTIES OF RIPE WATERMELON JUICE BY RESPONSE SURFACE METHODOLOGY

Abstract

Thermosonication is an emerging less energy-intensive, more cost-efficient and non-thermal feasible processing method reported to minimise loss of the quality, nutritional and sensory properties of fruit juices. Watermelon juice is highly perishable in nature due to its high pH (5.2 – 6.7) and high-water amount (92%). In this study, thermosonication was applied to preserve natural phytochemicals in watermelon juice using response surface methodology (RSM). The effect of three independent variables such as temperature (25 – 52˚C), processing time (2 – 10 min) and amplitude level (24. – 60 µm) at a constant frequency of 20 kHz was investigated on lycopene, β-carotene, ascorbic acid and total polyphenols of watermelon juice. Phytochemicals (lycopene, β-carotene, ascorbic acid and total polyphenols) were used as response variables. A quadratic model was applied to correlate independent variables for maximum retention of phytochemical content at the optimum process conditions using central composite design (CCD). A higher retention of all response variables at lower treatment conditions (25˚C; 24 µm) with special reference to increase in lycopene (127%) was observed. Lycopene, β-carotene, ascorbic acid and total polyphenols decreased significantly as temperature increased from 25 reaching 52˚C. Coefficient of determination (R^2) values were used to determine fitness of models. Optimum conditions generated were 125˚C for 2 min at an amplitude of 24 µm. The resulting predicted responses at these conditions were 7.4 mg/100 g for lycopene, 0.15 mg/100 g for β-carotene, 2.86 mg/100 g for vitamin C and 21.32 mg/100 mg/ GAE for TPC and desirability of 0.81. RSM was a useful tool to predict processing variables to preserve phytochemicals of a watermelon juice. Model equations and response surface graphs for all response variables measured were generated and they can serve as standards for any of the response measured because the experiment showed that all models were fit.

Key words: Thermosonication, watermelon juice, response surface methodology, phytochemicals

5.1 Introduction

Watermelon juice is rich in phytochemicals, which impart positive effects on human health (Liu *et al*., 2018; Bianchi *et al*, 2018). As a result, it has become one of the most popular drinks consumed to quench the summer thirst (Jumde *et al*., 2015). Like other fruits,

watermelon juice is highly perishable in nature due to its high pH (5.2 – 6.7) and high-water activity (Zhang *et al*., 2011; Santos *et al*., 2015; Lemos *et al*., 2017). Therefore, there is a need to preserve watermelon for extended shelf life and to allow its commercialisation as an ingredient in various food products. Watermelon is thermo-sensitive in nature therefore; thermal processing may affect the quality properties in several ways including loss of physicochemical, antioxidants and formation of novel compounds by Maillard or other reactions (Aguilar *et al*., 2017).

As a result, there is a need for suitable and efficient preservation methodologies that are aimed at extending the shelf life of food while retaining their natural components. An example of such technologies is thermosonication which is a combination of ultrasounds and thermal treatment (Abid *et al*., 2013). It is a novel processing technology which uses sound energy generated from an electric current that exceeds the hearing limit of the human ear (frequency ≥ 20 kHz) It breaks down molecules in food through a complex phenomenon called cavitation during sonication (Aguilar *et al*., 2017). Thermosonication has already been applied to variety of commercialised food products and have proved to be effective in achieving the required hygienic standards with minimal effects on phytochemicals in pineapple, grape and cranberry juices (Rattanathanalerk et al., 2005; Bermudes-Arguirre *et al*., 2012; Khandpur and Gogate, 2016; Aguilar *et al*., 2017; Bhat and Goh, 2017). It extends the shelf life and gives fresh-like products with less quality defects compared to thermal processing techniques (Rawson *et al*., 2011a). In addition, thermosonication is less energy-intensive and more cost-efficient than conventional thermal technology (Abdullah and Chin, 2014). RSM is an effective method used to optimise processing conditions using mathematical and statistical techniques useful for analysing problems (Onipe *et al*., 2018). It can indicate the influence of several independent variables and their interactions on the dependent variables under investigation during technological operation (Myers and Montgomery, 2002). RSM has been applied in the optimisation of processing conditions of fruit products like cantaloupe, melon and ginger candy juice (Sánchez-Moreno *et al*., 2003; Fonteles *et al*., 2012; Kumar *et al*., 2018). Therefore, the purpose of the present work was to determine the optimum thermosonication operating conditions for the preservation of watermelon fruit juice based on RSM and to evaluate the effects thermosonication processing on quality properties of the watermelon fruit juice.

5.2 Materials and Methods

5.2.1 Plant material

Fully ripe watermelon juice was extracted using a method described in Chapter 3 section 3.1. Thermosonication was done using the variables obtained from processing values (Table 12) through a combination of an ultrasound bath (waterbath, ST 30, Turkey) and a Sonicator probe (QSonica, Model Q700, USA). The treated juice was packaged in 50 ml polyethylene test tubes (Figure 12) and kept at -20ºC for analysis the following day.

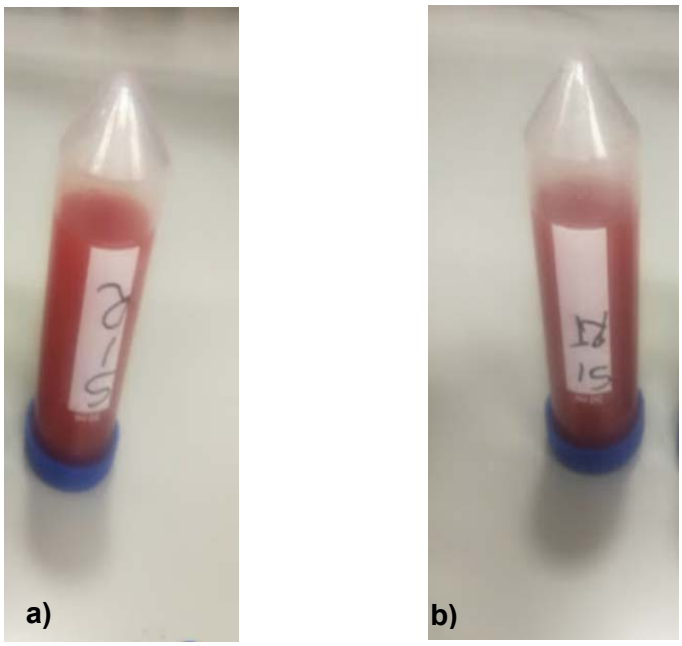


Figure 12: Watermelon fruit juice a) Fresh ripe watermelon Juice (S1R); b) treated watermelon juice (S1RT)

5.2.2 Experimental design

In this study, a central composite design (CCD) software was used to design the experiment (Aghajanzadeh *et al*., 2017). The effect of four independent (temperature (˚C), processing time (min) and amplitude (µm) frequency (kHz)) variables were analysed to examine the response pattern and to determine their optimum processing variables (Table 12). These independent variables and their optimised ranges consisted of four independent variables. To test the level of the independent variables, sixteen runs of the different combinations of the variables were generated. The measured response variables were colour variables (L*, a*, b*), pH, total soluble solids (˚Brix), lycopene, β-carotene, ascorbic acids and total polyphenols (TPC). Numerical optimisation of the thermosonication process was aimed at

maximising the levels of colour and lycopene. The 3D response surface graphs of variable-response relationship were plotted using the software.

Table 12: Optimising of thermosonication variables

	Independent variables		
Run	Temperature (˚C)	Time (min)	Amplitude (µm)
1	25	10	24.4
2	45	2	24.4
3	35	6	42.7
4	25	10	61
5	45	10	61
6	25	2	61
7	35	6	73.5
8	45	10	24.4
9	35	6	42.7
10	25	2	24.4
11	35	6	11.9
12	35	6	42.7
13	45	2	61
14	52	6	42.7
15	35	13	42.7
16	18	6	42.7

5.2.3 Methods

Analytical methods for quality properties of watermelon fruit juice were as described in Chapter 3 section 3.2.2.

5.2.4 Statistical analysis

The design of the experiment and data treatment were performed using Design Expert statistical software version 6 (Sta-ease Inc., Minneapolis MN, USA). P-value obtained from the analysis of variance (ANOVA) was used to determine significance of each independent variable. Coefficient of determination (R^2) was used to determine the adequacy of each model. Responses were predicted through quadratic polynomial regression models. Three-dimensional surface graphs were plotted for the predicted value of depended variables. Design expert software was used to find the mathematical model for any response and to present the relationship between dependent and independent variables. (Oberoi and Sogi, 2017b).

5.3 Results and Discussion

5.3.1 Effects of thermosonication on physicochemical properties of fully - ripe watermelon fruit juice

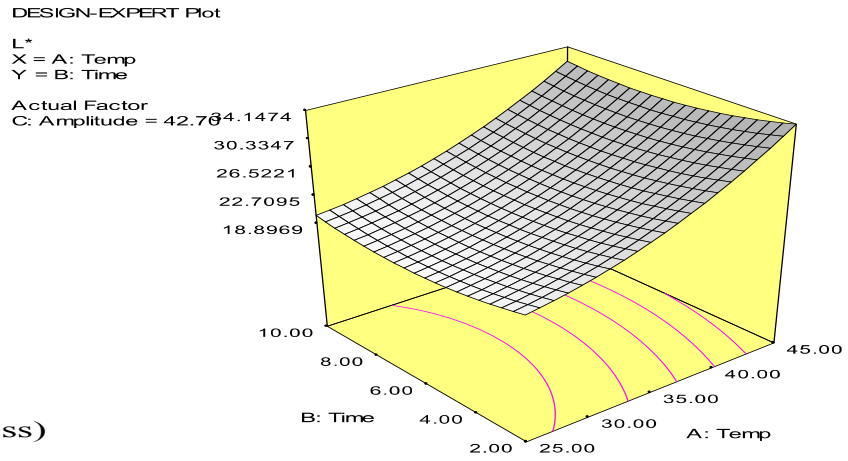
Changes in colour quality may adversely affect consumer acceptability as well as lead to a loss of marketability of watermelon juice (Santos *et al*., 2015). From the ANOVA results, the model was significant to predict the treatment variables on colour of the juice (Table 13). At the lowest temperatures of 18 and 25˚C, the red colour increased from 8.95 to 10.20 and 8.95 to 11.11 (Appendix A). The red colour was more saturated at these processing variables. The highest temperature (52˚C) and amplitude (42.7 µm) significantly ($p < 0.05$) decreased the redness of the juice from 8.9 to 3.35 (Appendix A), the decrease in red colour is correlated to the decrease in cream white colour of the watermelon fruit juice at these processing variables. This means that the watermelon fruit juice was becoming darker, this was indicated by the decrease in L* value at the higher processing variable. The coefficient (R^2) was 0.98, 0.91 and 0.93 for lightness, redness and yellowness respectively. The F and P-value of both colour attributes showed that the model was significant indicating that model is adequate to predict colour retention (Table 13). A first-order regression model fitted colour retention with accuracy (Figure 13: a, b, c). The effects are represented by response surface plots (Figure 13: a, b, c).

Temperature also showed to be an important variable affecting the redness of the juice. This is because watermelon is thermo-sensitive in nature (Oberoi and Sogi, 2017b). However, thermosonication did not cause a total loss of all the redness of the juice. About 40% of redness remained in the juice after treatment at highest temperature (52˚C) and amplitude (42.7 µm). In agreement with this result, Pinto *et al*. (2016) observed that high treatment conditions (70˚C) affected the cream white colour of the watermelon fruit juice, it increased the cream white colour of the apple juice owing to the partial precipitation of suspended particles. Isomerisation of phytochemicals, formation of brown pigments (Maillard-derived compounds), changes in physical state of lycopene are reported as possible causes of decrease in red colour of the watermelon juice at high treatment (Shi *et al*. 2000). These results are consistent with those reported by Fonteles *et al*. (2012) and Santhirasegaram *et al*. (2013) who evaluated effect of thermosonication on cantaloupe and mango juice respectively. Their studies reported the significant variations in colour resulting in a lighter colour.

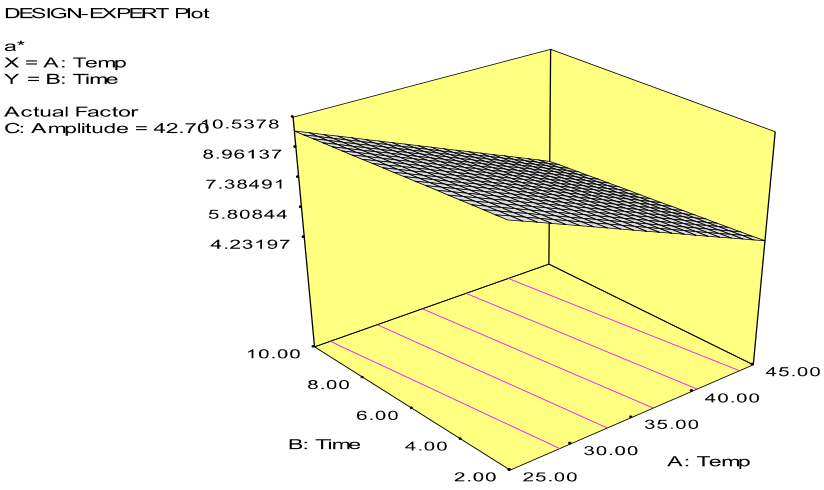
Table 13: ANOVA for response surface linear and quadratic model for colour variables of fully – ripe watermelon fruit juice

	L*			a*			b*		
Sources	F-value	P - value	Significance	F - value	P - value	Significance	F - value	P - value	Significance
Model	36.31	0.0001	significant	10.31	0.0051	significant	39.76	< 0.0001	Significant
A	264.63	< 0.0001	significant	70.72	0.0002		117.32	< 0.0001	
B	2.29	0.1809		0.11	0.7532		1.43	0.2555	
C	2.98	0.1349		1.06	0.3419		0.54	0.4774	
A^2	24.06	0.0027		12.04	0.0133				
B^2	8.30	0.0280		0.093	0.7704				
C^2	10.65	0.0172		2.34	0.1767				
AB	0.30	0.6054		0.59	0.4732				
AC	0.22	0.6573		1.230E-004	0.9915				
BC	0.047	0.8361		0.24	0.6429				
Lack of Fit	3.28	0.2470	not significant	45.24	0.0217	significant			

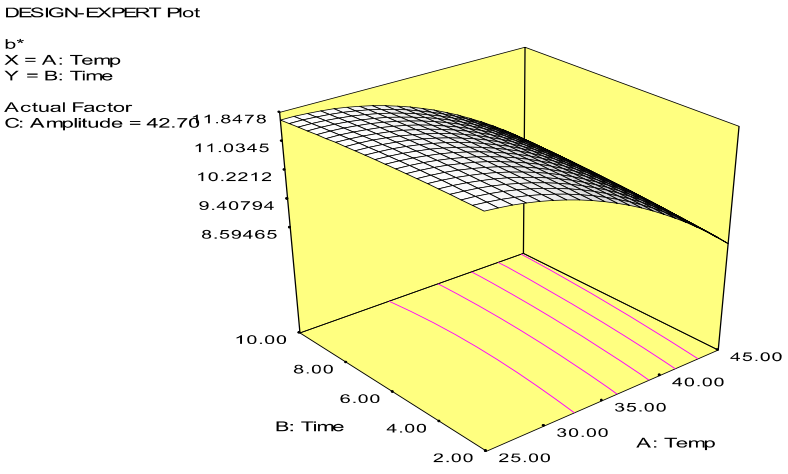
A = temperature; B = time; C = amplitude; L = lightness; a* = redness; b = yellowness



L * (Lightness)



A* redness



B* yellowness

Figure 13: Response surface plots for colour of ripe watermelon juice

Aghajanzadeh *et al*. (2017) reported that the watermelon juice became darker ($p < 0.05$) as the temperature increased during thermosonication. Rattanathanalerk *et al*. (2005) observed that pineapple juice treated at 65 - 95°C was darker than the untreated juice. A decrease in lightness of juice colour is correlated to the effects of the treatment conditions on the cloud stability of the juice (Aghajanzadeh *et al*., 2017). In addition, Rawson *et al*. (2011a) reported a maximum increase in lightness for higher treatment time in thermosonically treated watermelon juice. In agreement with this results, Martínez-Flores *et al*. (2015) attributed the increase in lightness during higher treatment to mechanical disruption of the cell wall of the juice and release of coloured compounds that occurs during sonication.

The increase in lightness makes the juice more transparent due to the destruction of coloured compounds formed during sonication (Cheng *et al*., 2017; Santhirasegaram *et al*., 2013). The sugar baby cultivar derives its red colour from phytochemicals such as lycopene, as a result, lycopene concentration is highly correlated with a^* value. As a result, accelerated lycopene isomerisation caused by high temperature and sonication may result in the decrease of red colour (Fonteles *et al*., 2012). Phytochemicals are unstable and susceptible to degradation leading to a brownish colour during storage (Santos *et al*., 2015; Pinto *et al*., 2016).

Khandpur and Gogate (2016) reported that, decrease in values of the red colour are generally attributed to the generation of brown coloured melanoidins, thus higher treatment variables increase the browning of the juice. Non-enzymatic and enzymatic browning may also influence colour of the juice (Aguilar-Rosas *et al*., 2007; Anaya-Esparza *et al*., 2017). This difference may be due to the texture and microstructure varieties of watermelon juice (Oey *et al*., 2008). Other researchers have suggested that changes in colour during thermosonication is due to accumulation of precipitated unstable particles in the juice (Santhirasegaram *et al*., 2013; Abid *et al*., 2014). The changes in colour may adversely affect the sensory properties of the juice. However, these changes could not be perceived by the naked eye and was not within the scope of the present study, as a result further research would be necessary.

The pH is a measurement of acidity of the treated juice (Perkins- Veazie *et al*., 2006). The pH of fresh watermelon juice was 5.51 indicating a neutral medium. After thermosonication, the pH ranged from 5.56 to 6.63 depending on treatment variables (Appendix A). F-value and P-value of the model was 8.23 and 0.0002 (Table 14), respectively, which suggested the model was significant. The increase of processing temperature and amplitude caused significant increase in pH of the juice as shown in response surface plot (Figure 14, a). Prolonged treatment variables also resulted on increased the pH of the watermelon fruit juice. Overall, significant differences ($p < 0.05$) were recorded in sonicated samples compared to control.

These differences can be attributed to the changes in the chemical or biochemical substances present in the juice (Khandpur and Gogate, 2016). Contrarily, Nafar *et al.* (2013), Shaheer *et al*. (2014), Bhat and Goh (2017), reported no-significant ($p < 0.05$) changes in pH values of thermosonicated red grape and strawberry juice respectively. This study agrees with Aghajanzadeh *et al*. (2017) whom stated that the appearance of watermelon juice reflecting the safety and quality is affected by the chemical reactions, which take place during juice processing.

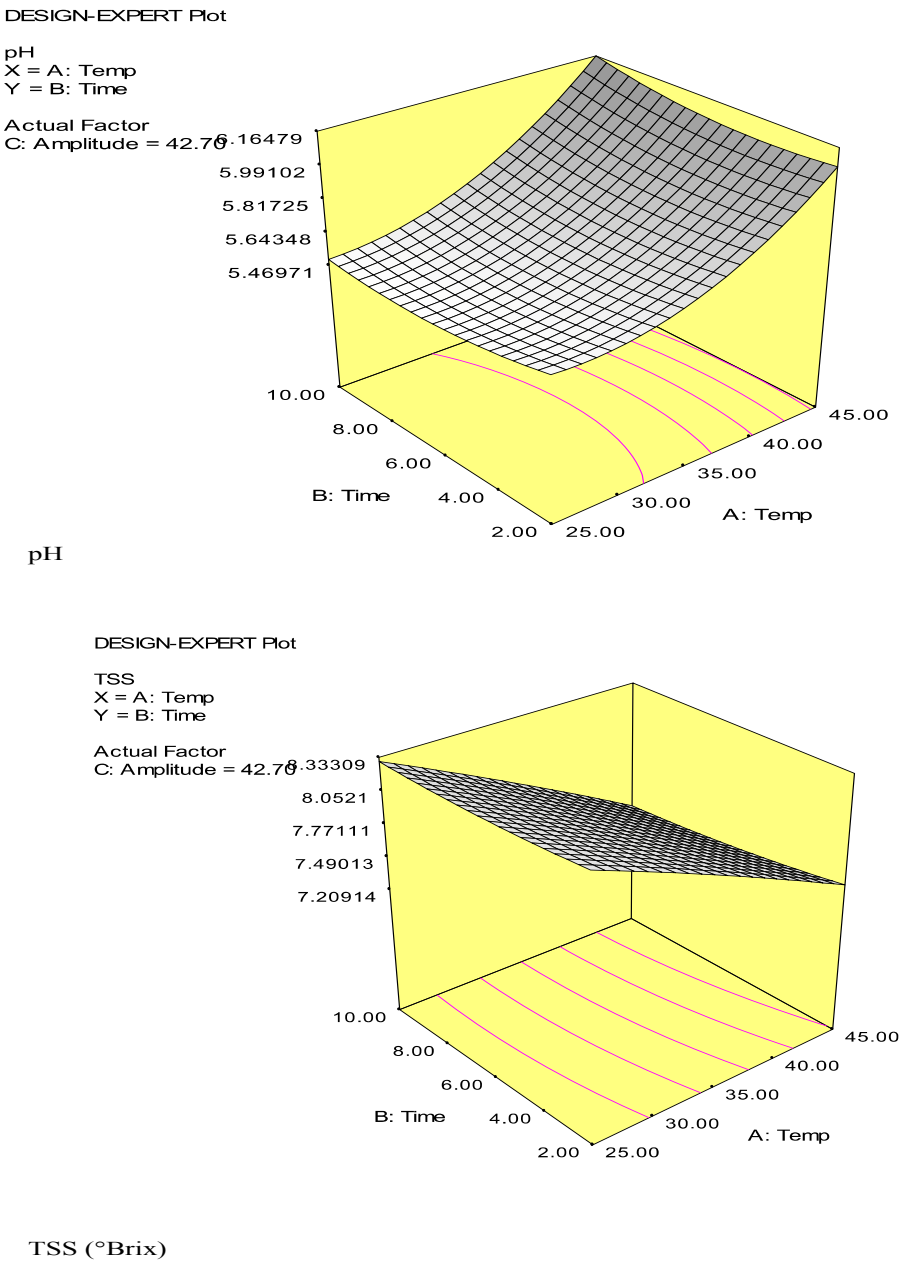


Figure 14: Response surface plots for pH and TSS

Changes in pH may be attributable to reactions accelerated by cavitation, which controls various physical, chemical, or biological reactions as oxidation of some compounds by free radicals or by the presence of oxygen (Tiwari *et al*., 2008b). Slight changes in pH is usually associated with changes in compounds such as organic acids and polyphenols of the fruit (Sharma *et al*., 2009). Jaeger *et al*. (2010) reported that adverse effects on watermelon quality properties may be due to Maillard reaction occurring during thermosonication at optimum operating conditions. Ercan and Soysal (2013) suggested that a decrease in vitamin content could contribute to the decrease in pH since it acts as a valid criterion for natural pigments and aromatic substances. Kiang *et al*. (2013) associated the decrease in pH with the fact that cavitation causes formation of free radicals which can puncture the cell wall.

Table 14: F and P values of response surface model parameters for yield of pH and TSS of fully – ripe watermelon fruit juice

Source	pH	TSS
Model	0.0037	0.0012
A	*< 0.0001*	*< 0.0001*
B	*0.8229*	*0.2962*
C	*0.7181*	*0.3973*

A is temperature, B is time and C is amplitude (TSS = Total soluble solids)

The fresh sample had 8.41 ˚Brix while treated samples ranged from 7.00 to 8.41 (Appendix A). It was evident that the treatment variables had different effects on the TSS of the juice. There was no-significant ($p < 0.05$) difference at lower temperatures (18 and 25˚C) variables and significant difference ($p < 0.05$) at higher temperatures (35, 45 and 52˚C). In addition, ANOVA results have also shown a quadratic effect of all independent variables, interaction between treatment variables and TSS (Table 14). Interaction is also depicted by response surface plot, which showed that increase in temperature and amplitude had severe effects on overall TSS (Figure 14, b). In agreement with this study, Adekunte *et al*. (2010) and Shaheer *et al*. (2014), observed non-significant in TSS of thermosonicated tomato juice. While Rawson *et al*. (2011a) reported that prolonged treatment time decreased sweetness of the juice.

Lieu and Le (2010) observed that sonication promotes high extractability of sugars from grape juice. Contrary results have been previously recorded by Santhirasegaram *et al*. (2013), their study observed no significant effect of thermosonication on TSS in mango juice. Similarly, this results are in line with the findings of Walkling-Ribeiro *et al*. (2009) and Abid *et al*. (2014) who reported no-significant ($p < 0.05$) effect of treatment with thermosonication on the ˚Brix and pH of orange and apple juice. Guiseppi-Elie *et al*. (2008) explained that, the enzyme glucose

oxidase which catalyses the oxidation of glucose to hydrogen is inactivated by the thermosonication treatment, this inactivation leads to the decrease in TSS of the juice.

In addition, isomerisation of some compounds, enzymatic and non-enzymatic browning have effects on TSS of watermelon juice when exposed to thermal treatment (Sun *et al*., 2010a). Moreover, the decrease in TSS at higher treatment variables may be due to respiration of the process, during this process, more complex materials such as starch, sugars and organic acids are broken down into simple molecules like carbon dioxide and water (Feng *et al*., 2013). Fonteles *et al*. (2012) reported that, decrease in the TSS is due to cell disruption accelerated by the sonication treatment, which releases the intracellular sugar in to the liquid.

Numerical optimisation

Based on the experimental values of the responses, numerical optimisation was carried out to determine the best experimental conditions for minimum processing variables and maximum. Temperature, time and amplitude remained within a range while lightness, redness, yellowness, pH and TSS of watermelon juice were maximised. The optimum condition suggested by the software is presented in Table 15. The numerically optimised data were relatively close to the validated experimental data presented; thereby confirming the validity of the regression models obtained which could adequately predict quality properties of a thermosonicated watermelon fruit juice.

Table 15: Optimum conditions predicted by the response surface methodology

Independent variables			Dependent variables					Desirability
Temp (°C)	Time (min)	Amp (µm)	L*	a*	b*	pH	TSS	Desirability
140	2.00	24.40	28.58	6.48	10.46	5.82	7.63	0.50

5.3.2 Effects of thermosonication on phytochemicals of FULLY - ripe watermelon fruit juice

The lycopene content of fresh watermelon juice at the fully ripe stage before treatment was 6.19 mg/100 ml which is higher than the value reported by Oms-Oliu *et al*. (2009). ANOVA results have shown that, the P-value of the lack of fit was < 0.0001 (Table 16), which implied the lack of fit was significant compared to the pure error. The value of determination coefficient (R^2 = 0.8378) for this model was close to 1, indicating the effective correlation between predicted values and actual ones. Linear effect of all independent variables and lycopene. The

F-value and P-value of the model was 20.67 and < 0.0001, respectively, which suggested the model was significant (Table 16). The coefficients A (temperature) was significant at ($p < 0.05$) and the other coefficients (B = time, C = amplitude) were no-significant ($p > 0.05$). When treatment variables were lower (25°C and 24.4 µm) the yield of lycopene increased from 6.19 to 7.91mg/100 ml and then started to decrease at higher treatment variables (52°C, 42.7 µm). These implies that thermosonication at lowest conditions (25°C and 24.4 µm) has improved lycopene content by 127%. The improvement of the lycopene content may be attributed to the breakdown of cells and molecules during sonication and the properties of instantaneous transfer of acoustic energy into fruit juices.

Table 6: Outputs of model parameters for yield of phytochemicals of fully – ripe watermelon fruit juice

Phytochemical	Source	Sum of squares	Mean square	F Value	P Value	Remarks
Lycopene	Model significant	48.88	16.29	20.67	< 0.0001	Significant
	A	*48.35*	48.35	61.33	*< 0.0001*	
	B	*0.43*	0.43	0.54	*0.4769*	
	C	*0.11*	0.11	0.14	*0.7189*	
	Lack of fit	9.46	0.79	2832.77	*< 0.0001*	*Significant*
β-carotene	Model significant	0.060	0.020	*36.16*	0.0006	Significant
	A	*0.059*	*0.059*	*0.58*	*< 0.0001*	
	B	*468E-004*	*9.468E-004*	*0.040*	*0.4616*	
	C	*6.590E-005*	*6.590E-005*		*0.8443*	
	Lack of fit	*0.020*				Not significant
Ascorbic acid	Model significant	2.02	0.67	16.68	0.0001	Significant
	A	*1.89*	*1.89*	*46.70*	*< 0.0001*	
	B	*0.055*	*0.055*	*1.36*	*0.2661*	
	C	*0.080*	*0.080*	*1.98*	*0.1852*	
	Lack of fit	*0.48*				Not significant
TPC	Model significant	443.52	147.84	22.27	< 0.0001	Significant
	A	*441.97*	*441.97*	*66.56*	*< 0.0001*	
	B	*1.25*	*1.25*	*0.19*	*0.6721*	
	C	*0.30*	*0.30*	*0.046*	*0.8342*	
	Lack of fit	*79.62*	*7.96*	*265.39*	*0.0038*	*Significant*

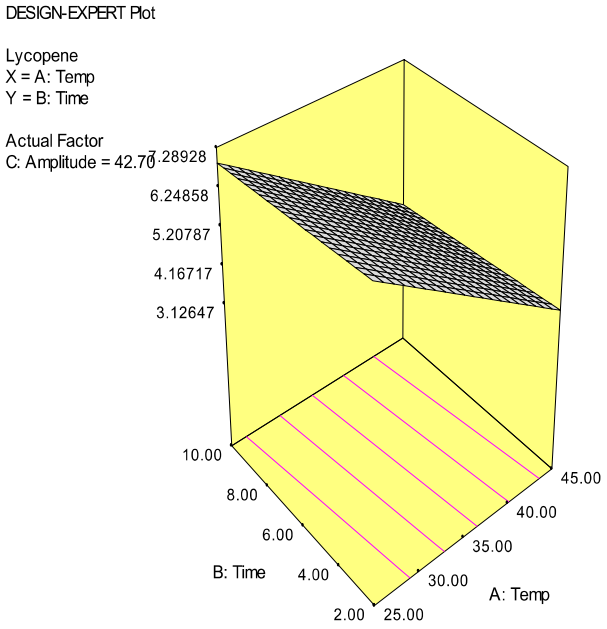
A is temperature, B is time and C is amplitude; TPC = Total polyphenolic content

Higher variables showed to have severe effects on lycopene, at the highest treatment (52°C, 42.7 µm) lycopene decreased from 6.19 mg/100 ml to 3.80 mg/100 ml. These implies that the highest treatment variables have retained about 61% of lycopene. The undesirable decrease

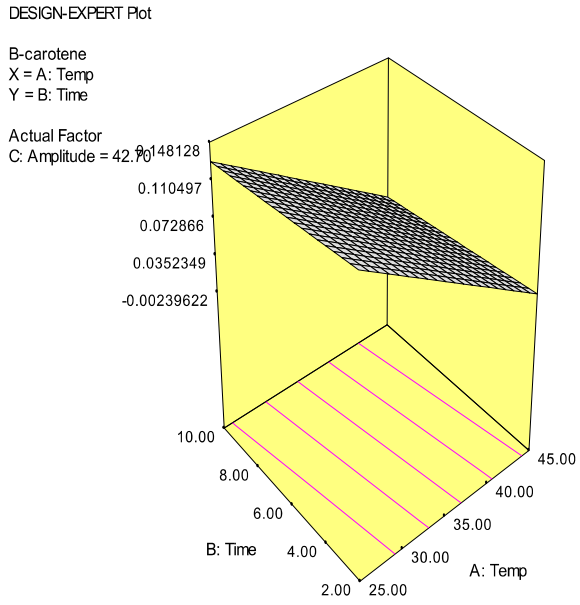
in lycopene at higher conditions is due to isomerisation of trans-lycopene to the cis form as induced by heat combined with ultrasound and oxidation during sonication treatment (Oberoi and Sogi, 2017b). Shi *et al*. (2000) observed a similar trend on tomato processing, their study reported that the lycopene content is influenced by the time-temperature conditions on tomato juice. The value of adjusted determination coefficients (Adj R^2) was also close to 1 (0.7973), which indicated the experimental values could be significantly predicted by the model. Change is also indicated in the mathematical model (Eq. 4) where A is temperature, B is time and C is amplitude.

$$\text{Lycopene} = +12.29982 - 0.18816 \times A - 0.049943 \times B - 4.83786E - 003 \times C \quad \textbf{(Eq. 4)}$$

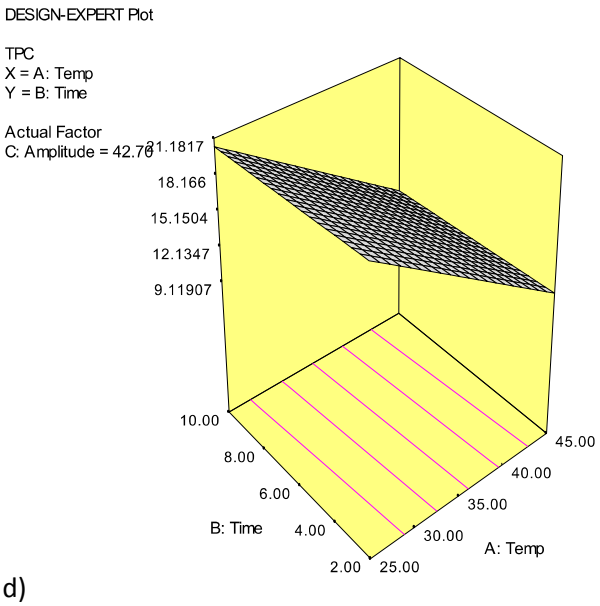
Different treatment variables have different effects on lycopene content (Appendix B), increasing at lower condition while decreasing at higher temperature. Rawson *et al*. (2011a) have reported similar results. Their study has observed a significant decrease of lycopene when watermelon juice was treated at 61 µm, 45°C for 10 min. In addition, Abid *et al*. (2013) reported that higher treatment conditions increase the lycopene content. Interaction is also depicted by response surface plot, which showed that increase in temperature and amplitude had severe effects on overall lycopene content (Figure 15 a). Thermosonication at lower treatment variables (25°C and 24.4 µm) has shown to be effective in retaining important phytochemicals in blackberry and orange (Tiwari *et al*., 2009b), apple (Abid *et al*., 2014). Decrease in lycopene may also be influenced by oxidative injury occurring during thermosonication (Rawson *et al*., 2011a). Kim *et al*. (2014) reported that upon heat treatment, carotenoids including lycopene degrades into volatile compounds such as 2-methyl-2-hepten-6-one, citral and geranyl. Moreover, the decrease in lycopene might also be due to exposure to light during the experiment, since lycopene is an unstable pigment which isomerises under light irradiation and high temperature treatments (Oberoi and Sogi, 2017a). In addition, light penetrates cell walls and release cell contents trapped in the fruit tissues (Singh *et al*., 2015). High-energy cavitation bubbles containing solvent vapour may cause the decrease in lycopene. These bubbles implode near cell walls causing very high local temperatures, pressure increase and cell wall destruction, which eases mass transfer from cell to solvent and enhances loss of important carotenoids (Abid *et al*., 2014). It governs various physical, chemical and biological reactions, such as accelerating chemical reactions, increasing diffusion rates, dispersing aggregates or inactivating enzymes and microorganisms (Rawson *et al*., 2011a).



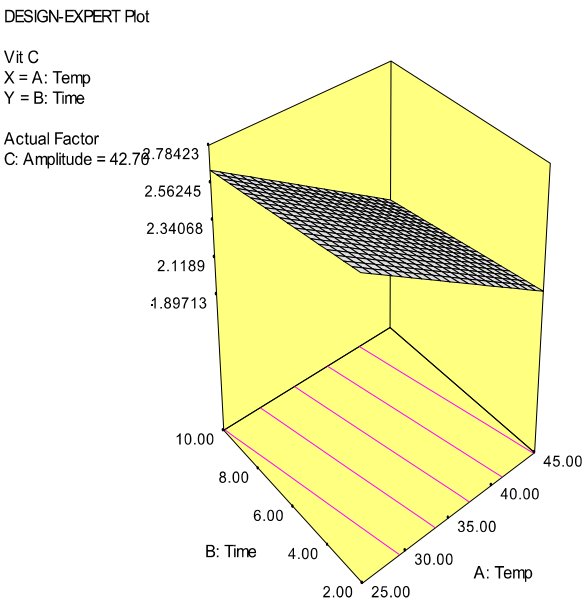
a)
Lycopene



b) ß - Carotene



d)
Total Polyphenolic Content



c) Ascorbic Acid

Figure 15: Response surface plots for phytochemicals; a = lycopene, b =β-carotene, c = Ascorbic acid, d = Total polyphenolic content

Thermosonication at lower treatment variables (25˚C and 24.4 µm) has shown to be effective in retaining important phytochemicals in blackberry and orange (Tiwari *et al*., 2009b), apple (Abid *et al*., 2014). Decrease in lycopene may also be influenced by oxidative injury occurring during thermosonication (Rawson *et al*., 2011a). Kim *et al*. (2014) reported that upon heat treatment, carotenoids including lycopene degrades into volatile compounds such as 2-methyl-2-hepten-6-one, citral and geranyl. Moreover, the decrease in lycopene might also be due to exposure to light during the experiment, since lycopene is an unstable pigment which isomerises under light irradiation and high temperature treatments (Oberoi and Sogi, 2017a). In addition, light penetrates cell walls and release cell contents trapped in the fruit tissues (Singh *et al*., 2015). High-energy cavitation bubbles containing solvent vapour may cause the decrease in lycopene. These bubbles implode near cell walls causing very high local temperatures, pressure increase and cell wall destruction, which eases mass transfer from cell to solvent and enhances loss of important carotenoids (Abid *et al*., 2014). It governs various physical, chemical and biological reactions, such as accelerating chemical reactions, increasing diffusion rates, dispersing aggregates or inactivating enzymes and microorganisms (Rawson *et al*., 2011a).

Fresh watermelon juice had about 0.23 mg/100 g of β-carotene. β-carotene, constantly decreased with the increase of both temperature and amplitude. It reached the maximum when temperature and amplitude were 52˚C and 42.7 µm respectively. A higher retention of about 78% of β-carotene was observed for low processing variables (25˚C and 24.4 µm). While at higher processing variables (52˚C, 42.7 µm) only 0.1 mg/100 g of β-carotene was found in the juice (Appendix B). Thus, thermosonication retained about 44% of β-carotene after treatment. The calculated F-values (12.26) and the P-value (< 0.05) indicated that the model was statistically significant at the 95% of confidence levels (Table 16). Good correlation coefficients were also obtained (R^2 = 0.7540). The plots shown in Figure 15 (b) illustrates the combined effects of temperature, time and amplitude on β-carotene content. According to a regression analysis of the experimental data, the reduced equations that can reliably predict the experimental results were expressed as follows:

$$\beta - carotene = +0.32255 - 6.58341E-003 \times A - 2.35703E-003 \times B - 1.20038E-004 \times C \quad \textbf{(Eq. 5)}$$

The decrease in β-carotene content could be explained by the cell disruption that occurs in the juice during sonication treatment. Thus, the bubbles are released in the liquid medium

increasing the enzyme activity which influences the formation of free radicals (Fernandes *et al*., 2009). In fact, for lower treatment variables (25℃ and 24.4 µm), the rate of cavitation and bubbles released is lower than those released at higher treatment variables. For longer treatment variables the rate of cavitation was faster and caused more cell disruption which resulted in gradual decrease of β-carotene. In agreement with this study, Sun *et al.* (2010b) reported the decrease in β-carotene at higher treatment temperature when analysing effects of thermosonication on watermelon juice. Thus, β-carotene decreases with increase in treatment temperature and time. Most studies reported that lower treatment variables are necessary for the higher retention of β-carotene content during thermosonication (Rawson *et al*., 2011a; Abid *et al*., 2014). One of the interesting results of this study is higher retention of β-carotene content as compared to other methods of processing (Anaya-Esparza *et al*., 2017).

In addition, the decrease in the β-carotene content may be attributed to repeated cycles of compression and decompression called acoustic cavitation produced during sonication (Fonteles *et al*., 2012; Oberoi and Sogi, 2015b). Sun *et al*. (2010b) reported that the bubbles formed during cavitation may collapse less violently, which can accelerate the reduction of this compound. Under high conditions, the bubbles can also disrupt the propagation of ultrasound waves resulting in loss of vital compounds. Cavitation leads to the destruction of cellular structure, therefore natural liquid layers system closes the phase boundaries and consequently stimulates the process of mass transfer (Dolatowski and Stasiak, 2012).

A decrease in β-carotene may also be explained by the formation of peroxide and free radicals at high intensity ultrasound treatment that react undesirably with anthocyanin compounds (Fernandes *et al*., 2009). Free radicals produced by cavitation may degrade –OH by the opening of rings and formation of chalcone mainly due to the temperature rise that occurs during sonication and therefore decrease the quantity of β-carotene (Sadilova *et al.,* 2007; Fernandes *et al*., 2009). In addition, isomerisation of β-carotene is accelerated by pressure and heat treatment, as a result at higher processing variables this antioxidant is decreased (Knockaert *et al.*, 2012). Furthermore, the changes in β-carotene may also be due to the process of homogenisation, this process changes size and structure of rheological properties of the juice during treatment resulting in loss of the antioxidant (Kim *et al*., 2014). The thermosonication treatment was able to retain the β-carotene.

Ascorbic acid (AA) is mostly refered to as vitamin C. Odriozola-Serrano *et al*. (2007); reported that vitamin C is used as the general description for all organic compounds exhibiting biological activity of ascorbic acid. The amount of AA in fresh watermelon was 3.47 mg/ 100 ml, which is slightly lower than the 5.29 mg/ 100 ml reported by Rawson *et.al*. (2011a), this

may be attributed to the difference in cultivar investigated (Tlili *et al*., 2011). There was a decrease of AA throughout the treatment ranging between 1.98 to 2.99 mg/100 g as compared to fresh sample 3.45 mg/100 ml (Appendix B). Thus, when temperature was increased, the yield of AA decreased. The model showed statistical significance at 95% level of confidence according to ANOVA analysis and F-value16.68 while $R^2 = 0.81$. ANOVA results revealed that, the interaction between temperature, amplitude as well as linear effect of time, all had significant effect ($p < 0.05$) on AA values of juice (Table 16). Regression equation of the model is presented below (Eq. 6) where A is temperature, B is time and C is amplitude.

$$AA = +3.92780 - 0.037171 \times A - 0.017961 \times B - 4.17767E - 003 \times C \quad \textbf{(Eq. 6)}$$

The plots shown in Figure (15, c), illustrate the combined effects of temperature, time and amplitude on AA. The decrease was observed to be intensified at high treatment variable (52˚C, 6 min and 42.7 µm) at a significance of $p < 0.05$. At the maximum amplitude and longest processing time, 68% ascorbic acid was retained. Thus, thermosonication is a good treatment to retain considerable amount of ascorbic acid. In accordance, Tiwari *et al*. (2009a) reported that thermosonication reduced about 5% of AA in orange juice; Abid *et al*. (2014) observed a decrease in AA of apple juice, while Adekunte *et al*. (2010) also observed a 32% decrease of AA in tomato juice. In addition, Rawson *et al*. (2011a) who reported that ascorbic acid is an unstable compound, which degrades easily at higher processing variables when processing watermelon juice.

Interestingly, Aguilar *et al.* (2017) observed that the ascorbic acid content was constant at different treatment times when evaluating the ascorbic acid of different juice at different temperatures. However, Cheng *et al*. (2007) and Abid *et al*. (2013) reported contrary results; their studies found that thermosonication increased AA in guava by 88 % and 34% in apple juice. In addition, these authors suggested that the increase might be due to the effective removal of occluded oxygen from the juice which is a critical parameter influencing the stability of AA. The contrary results in literature may be due to the presence of air during sonication since laboratory experimental conditions are not the same.

Abid *et al*. (2014) and Anaya-Esparza *et al*. (2017) reported that the decrease of AA at higher treatment variables might be attributed to severe physical conditions occurring as a result of cavitation collapse of bubbles during sonication treatment. Cavities formed by sonication may

be filled with water vapour and gases dissolved in the juice, such as O_2 and N_2 (Abid *et al*., 2014). Furthermore, it may be due to presence of oxygen and the breaking down of molecules during sonication (Cheng *et al*., 2007). In addition, Rawson *et al*. (2011a) reported that formation of hydroxyl radicals by water sonolysis, which may occur at the gas-liquid interface causing oxidation and several sonochemical reactions occurring simultaneously may lead to decrease in AA.

On the other hand, Tiwari *et al.* (2009b) reported that, AA decreases due to a decrease in other compounds since it has a protective effect on other compounds such as phenols. Sonication can result in enhanced free radical, which leads to oxidation of AA even though it induces degassing by cavitation of air-saturated systems (Aguilar *et al*., 2017). Furthermore, a decrease in AA is associated with the decrease in colour and decrease in pH since it acts as a valid criterion for natural pigments and aromatic substances (Ercan and Soysal, 2013). Therefore, sonication temperature and amplitude are important for retaining AA in the watermelon fruit juice.

There is a close relationship between phenolic compounds and antioxidant activity (Kim *et al*., 2014); they both constitute an important contributor to the antioxidant capacity of watermelons (Bianchi *et al*., 2018). Fresh watermelon juice had a total phenolic content of 25.95 mg GAE/100 ml. The linear effect of processing temperature, amplitude and time had a significant effect ($p < 0.05$) on the total polyphenolic content of the juice (Appendix B). There was a reduction in total polyphenolic content in all the samples analysed. However, the decrease was not severe at lower treatment variables (Table 16). Linear effects (temperature, time and amplitude) and their interaction were significant and have shown 95% of confidence interval $R^2 = 0.8477$ evident from low P-value ($p < 0.05$) and high F-value (Table 16).

Higher treatment conditions had a significant ($p < 0.05$) effect on the total polyphenolic content. As temperature increased from 18 to 52˚C the level decreased significantly ($p < 0.05$) as compared to untreated samples (Appendix B). At the highest amplitude (73.5 µm) TPC was degraded. In addition, amplitude level was found to have a significant ($p < 0.05$) effect on the TPC compared to the untreated samples. Similarly, Rawson *et al*. (2011a) reported that there is a decrease in the total phenolic content of sonicated watermelon juice when the temperature increased from 25 to 45˚C and higher processing times (10 min). The effect of temperature and amplitude were interactive in terms of degradation of TPC as evidenced by response surface plot (Figure 15, D). The relationship between the temperature, time and amplitude is illustrated in Eq. 7.

$$TPC = +35.92301 - 0.56888 \times A - 0.085627 \times B - 8.15161E - 0.003 \times C \quad \text{(Eq. 7)}$$

Fonteles *et al*. (2012) also reported a decrease in the TPC of sonicated cantaloupe juice. On the contrary, Lieu and Le (2010) and Abid *et al*. (2014) observed a 114.3% increase in concentration of TPC on sonicated grape juice and apple juice samples. In a most recent study, Bhat and Goh (2017) observed a significant improvement (from 81.76 up to 89.52 mg GAE/ 100 g) in TPC of strawberry juice treated with high pressure. These contrary results in literature underpins the suggestions that, effect of sonic waves in foods depends on the food matrix besides processing conditions (Fonteles *et al*., 2012). Although thermosonication reduced the level of TPC, this phytochemical activity was not totally degraded, in all assays the retention was in a range of 29.98% to 87.70%.

Fonteles *et al*. (2012) reported that, a decrease in TPC results from cavitational bubbles filled with water vapour and O_2 gas, which may have dissolved the juice during sonication. Their study further indicated that the presence of O_2 and water vapour favours the oxidative decrease of TPC. On the other hand, Abdullah and Chin (2014) stated that, at high heat and vapour pressure there is an increase in viscosity and a weak intensity of bubble collapse, which results in degradation of polyphenols. TPC is highly thermo-sensitive therefore, increase in temperature accelerates its degradation (Saeeduddin *et al*., 2015).

The antioxidant activity of watermelon juice was measured. Antioxidant activity of untreated watermelon juice was 23.90% of DPPH inhibition. Thermosonicated-treated watermelon juice ranged from 31 to 88% (Appendix B). Higher treatment conditions seem to have greater effects on antioxidants activity of the watermelon juice. It was found to be significantly different ($p < 0.05$) compared to the fresh sample. Antioxidant activity was retained when thermosonication treatments were performed at lower temperature and amplitude. These results differ from those reported for tomato and strawberry juices which exhibited an antioxidant activity retention from 50.7 to 100% after a thermosonicated treatment (Odriozola-Serrano *et al*., 2009; Aguiló-Aguayo *et al*., 2010). The decrease in total antioxidant activity of the treated watermelon juice is due to the release of bound phytochemicals from the matrix with thermal processing or the additive effects between treatments (Dewanto *et al*., 2002). The antioxidant activity depends on both concentration and effects between food components.

The total antioxidant activity can be an integrated property of antioxidants present in a complex sample and is often more meaningful to evaluate health beneficial effects when

compared to the bioactive compounds due to the cooperative action of antioxidants (Santos *et al*., 2015). The decrease in antioxidant activity of watermelon may also be associated with formation of novel compounds by Maillard or other reactions (Oms-Oliu *et al*., 2009). Solvent extraction methods applied to obtain antioxidant compounds may have also resulted in a decrease of antioxidants activity (Cömert and Gökmen, 2018). The decrease of antioxidants activity has been attributed to the reaction of these compounds with free radicals mainly whose production is influenced by several factors such as the presence of dissolved gasses (mainly O_2), environmental factors and light hydroxyl (Choudhary *et al*., 2015).

Numerical optimisation

Optimisation was carried out to determine the best experimental conditions for minimum processing variables and maximum (Fonteles *et al*., 2012). Temperature, time and amplitude remained within a range while lycopene, β-carotene, AA and TPC of watermelon juice were maximised. Optimum conditions generated were 125°C for 2 min at an amplitude of 24.4 µm. The resulting predicted responses at these conditions were lycopene 7.4 mg/100 g, 0.15 mg/100 g 2.86 mg/100 g and 21.33 with 0.81 as desirability. 25.0°C for 2.0 min at an amplitude of 25.52 µm, predicated dependent variables is 7.4 mg/100 g for lycopene, 0.15 mg/100 g for β-carotene, 2.86 mg/100 g for AA and 21.32 mg/100 mg/ GAE for TPC and desirability of 0.81. The numerical optimised data were relatively close to the validated experimental data presented; thereby confirming the validity of the regression models obtained which could adequately predict quality properties of a thermosonicated juice. In agreement with these results, Oberoi and Sogi (2017b) who reported that RSM is effective method of predicting treatment variables for watermelon. In addition, Kumar *et al*. (2018) reported the effectiveness of RSM on predicting processing variables of beetroot juice.

5.4 Conclusion

The increasing demand for higher quality fruit juices with absence of additives and preservatives has encouraged the use of viable non-thermal technology such as thermosonication. In this study, the combination of different processing conditions for thermosonication treatment of ripe watermelon juice was confirmed using RSM to determine optimal conditions of preservation of quality properties. The linear effects are significant to effectively retain quality properties. The optimum conditions obtained in this study can serve as a baseline for further studies on improvement of retaining quality properties of watermelon juice. Response surface methodology with a central composite design was successfully employed to investigate and optimise temperature (A), time (B) and amplitude (C)

thermosonication conditions for preserving quality properties of watermelon juice. Lower treatment conditions resulted in lesser decrease as compared to prolonged conditions with special reference to lycopene and redness of the juice. The higher the thermosonication-processing variables, the lower the retention of quality properties in watermelon fruit juice. The most sensitive antioxidant towards thermosonication was β-carotene because a significant ($p < 0.05$) decrease was observed compared to other antioxidants treated at same processing variables. Therefore, the use of thermosonication preservation technology can be an alternative to thermal treatments. The statistical analysis indicates that the proposed quadratic model for all quality properties was adequate ($p < 0.0001$) with satisfactory determination coefficients (R^2) which indicated that the model was significant. This optimisation process provides valuable data, which can be utilised in process design and industrial scale-up operations for the industries, which are involved in processing of watermelon fruit juice for the maximum recovery of quality properties. Although there are many studies relating to thermosonication on a laboratory scale, its application in the beverage processing industries is not sufficiently common. Therefore, future studies should focus on scale-up and standardisation of thermosonication processes for production of watermelon juice which, will reduce the seasonal losses of watermelon fruits in summer.

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSION

6.1 General discussion

Interest in the function and diversity of antioxidants has stimulated research of quantifying and identifying antioxidants present in fruits. This study aimed to quantify the selected quality properties of a watermelon fruit juice. It further investigated the effects of processing on the quality properties. The combination of the CIELAB parameters (L*, a*, b*) was explained by the Chroma (C*) and the hue (h^o) angle of the watermelon fruit juice samples. There was a significant ($p < 0.05$) difference in colour across all sample. This was indicated by the C* and h^o values of the watermelon fruit juice. The C* ranged from 4.32 to 7.35 to with the fully- ripe sample showing the highest intensity of the overall colour (red). The highest h^o values was obtained from the unripe sample, this is mainly due to the cream white colour of the watermelon fruit juice at unripe stage, hence the lightness is high, the decrease in the lightness was influenced by the increased in red colour which became dominant with maturity. The colour of watermelon fruit juice sample cream samples shifted away from cream white towards the red chromaticity, as shown by the hue angle mean values throughout all the stages of maturity. The red colour was the most dominant in the fully ripe watermelon fruit juice. These is further supported by the increase lycopene content of the juice as the red colour is derived from the accumulation of this pigment in the chromoplasts (Bianchi *et al*., 2018). These results concur with those reported by Soteriou *et al.* (2014) when evaluating the physicochemical of the ripe grafted and non-grafted watermelon juice at different maturity stages.

There are many methods used to measure total soluble solids of the fruit juices however since sugar is the major component of soluble solids in fruit, it is easier and quicker and convenient to measure soluble solids content in extracted juice using a refractometer (Guo *et al*., 2013). The TSS were measured as ^{o}Brix (Santos *et al*., 2015). There was significant ($p < 0.05$) difference in TSS throughout maturity stages. There was 82% increase in TSS observed from the fully - ripe sample. Increase in TSS at fully ripe sample is linked to the watermelon ability to accumulate sucrose at this stage due to the high activity of enzymes such as sucrose phosphate synthase and sucrose synthase and the decline in activity of soluble acid invertase (Yativ *et al*., 2010). There was a slight increase in pH at all stages of maturity (4.74, 4.94 and 5.50).

Lycopene content (63%) was observed to be the principal pigment at a fully - ripe stage. On the other hand, β-carotene was available in lower quantities and it was not present in the

unripe sample. The amount of lycopene in the fully ripe watermelon juice was greater than that of tomato reported by Fish *et al*. (2001). This makes watermelon a better dietary source of antioxidant lycopene. In addition, considering that the bioavailability of lycopene in processed tomato is the same as of fresh watermelon (Edwards *et al*., 2003) therefore consumption of watermelon seems to be a healthier option.

Ascorbic acid is an important component of antioxidative defence mechanism in cells and tissues (Soteriou *et al*., 2015). Fresh watermelon juice in this study showed to contain considerable amount of AA at all stages of maturity ranging from 1.3 – 3.47 mg/100 g which corroborates with those reported by Tlili *et al*. (2011) and Rawson *et al*. (2011a). The total phenolic content in watermelon cultivars ranged from 4.87 to 25.95 mg GAE/100 g. Increase in antioxidant activity was also visible at all stages of maturity. Nagal *et a*l. (2012) reported that this increase is correlated with an increase in phenolic compounds and ascorbic acid of the fruit. Because of the presence of antioxidants at all stages of maturity, the consumption of fresh watermelon juice can have potentially beneficial effects on human health (Soteriou *et al*., 2014; Santos *et al*., 2015; Cömert and Gökmen, 2018). Filtration is a necessary step in clarifying the watermelon juice; however, it has been shown to adversely affect the quality properties of the juice significantly ($p < 0.05$), especially in the half - ripe and fully - ripe samples. This shown by the significant ($p < 0.05$) difference in C* and h^o values between the control and filtered samples observed from the half- ripe and fully – ripe samples. These is in accordance with studies done by Perkins-Veazie *et al*. (2004) and Tlili *et al*. (2011). The slight decrease in quality properties after filtration is likely from oxidation, isomerisation, juice leakage and the exposure to oxygen and light during filtration (Bhattacharjee *et al*., 2017b).

The optimisation process provided the valuable data, which was utilised in the process design. RSM was able to predict the processing dependent variables. The linear and quadratic equation that described the inter-relationship between dependent and independent variables were obtained. Quality properties that included colour, pH, TSS, lycopene, β-carotene, AA and total phenols were used as dependent variables while the temperature, time, amplitude were independent variables. Thermosonication is a processing technology that results in the retention of a high percentage of the quality properties of the juice. It has improved the red colour and the lycopene dominance of the juice.

The results obtained from this research showed that, at a milder treatment of 25°C; 24.4 µm, the red colour and lycopene retention was higher. Increase in temperature, amplitude and time has impaired the quality properties of the juice mainly due to the sensitive nature of watermelon juice. Treated juice retained the original colour at lower treatment variables.

Moreover, pH and °Brix decreased gradually at higher treatment variables. In addition, the high treatment variables (52°C; 42.7 µm) were severe on β-carotene content. Previous research has also shown that thermosonication at higher treatment conditions, results in the darkening of the juices and loss of AA, polyphenolic content and antioxidant activity of apple, mango and cantaloupe juice (Tiwari *et al*., 2009b; Fonteles *et al*., 2012; Abid *et al*. 2014). This optimisation process provides the valuable data, which can be utilised in process design and industrial scale-up operations in industries which are interested in processing watermelon.

The extraction solvent (methanol, tetrahydrofuran, hexane and ethanol) used in this study are conventional food grade organic solvents which are efficient for retaining the stability of lycopene and β-carotene unlike when lycopene and β-carotene are extracted with chloroform, methanol, dichloromethane (Oberoi and Sogi, 2017a). Lycopene content and β-carotene content were analysed using HPLC method. Davis *et al*. (2003) indicated that due to its high complexity, which demands high selectivity and sensitivity, the use of HPLC can accurately predicts lycopene and β-carotene content as compared to analysing using other methods such chromatography and titration methods. It is known that ROS accumulated during a normal metabolic process in a human body will result in destruction of cells and organs which finally encourages development of chronic diseases such as heart attacks, cardiovascular diseases, mutation, diabetes, atherosclerosis, and hypertension (Nowicka *et al*., 2019). Since watermelon juice contain lots of antioxidant including lycopene, phenolic compounds, AA and β-carotene, the regular intake in our diet are associated with reduced risk of chronic diseases (Zhu *et al*., 2019).

6.2 General conclusion

Physicochemical and phytochemicals properties of watermelon increase with maturity. This could be due to involvement of numerous genes which are activated at the early stage of maturity and continue to be active through the maturity stage. The red colour of the watermelon juice was accumulated through maturity, it increased from the unripe and was saturated in the fully – ripe sample as indicated by the Chroma (C*) and hue (h^o) values which showed significantly ($p < 0.05$) throughout stages. The highest Chroma values were obtained from fully – ripe (7.85) samples followed by half – ripe (5.88) and the unripe (4.32) samples.

Lycopene is a strong free radical quencher and the present study identified watermelon juice (6.19 mg/100 g) as one of the richest sources at the fully ripe stage. Correlation between

lycopene content and the redness of the juice was observed. Increase in red colour intensity was attributed to the increased synthesis of lycopene (Perkins-Veazie and Collins, 2006). Filtration has proven to be a great tool for clarifying fruit juices. In the filtration operation, all the particles that make juice turbid and cloudy were removed and a completely clear juice was obtained. Phytochemicals segregated more into the filtrate especially as maturity increased, reaching nearly 99% for lycopene specifically. Consequently, a decrease in lycopene content resulted in a decrease in colour and *vice-versa*. Non-thermal filtration provided the best option to treat the juice while retaining its physicochemical and phytochemical properties. Phytochemicals greatly decreased when the juice was subjected to the thermosonication treatment. The decrease of phytochemical following thermosonication was highly temperature dependent. The decrease was severe at higher processing variables (52°C and 42.7 µm). Sweetness is one of the prime quality factors in watermelon fruit, it is related to total soluble solids (TSS). During thermosonication, the total soluble solid content of the watermelon juice was decreased significantly ($p < 0.05$) at higher treatment variables. Based on the results obtained, a serving size of a cup (145 g) from sugar baby cultivar at the half-ripe and fully ripe stage would provide the recommended daily allowance of approximately 4 mg and 7 mg of lycopene per day. However, the stability of thermosonicated juice during storage is not yet known and needs to be investigated, for potential marketing and shipping requirements. Fresh, filtered and treated watermelon juice contains antioxidants that can modulate levels of ROS in both humans and animal models therefore its consumption is beneficial to human health. Lycopene is ore saturated at the fully - ripe stage, therefore the watermelon juice can be used for extraction of lycopene for use in functional food and pharmaceutical industries. Watermelon juice is a drink consisting of natural phytochemicals which contains antioxidant properties, therefore, consumption of watermelon fruit juice will have positive effect on human health.

6.3 Recommendations

From the study, the following recommendations are proposed:

1. The use of mature watermelons in food manufacturing since they contain more phytochemicals than under-mature melons.
2. As watermelon is a seasonal fruit, to make it available all through the year, the juice must be processed in order to store it for longer periods.
3. Further study should be conducted on how to best utilise the filter cakes to prevent waste.
4. A larger scale experiment of thermosonication procedure would be very helpful to evaluate if similar effects are observed when larger quantities of watermelons are used

and try to engineer equipment for close-to-field application (harvesters, rind-flesh separation, low light-oxygen facilities).

5. The influence of processing variables on the overall quality properties of fruit juices be studied in detail in order to determine optimum treatment conditions which do not impair antioxidants.
6. Further effects of thermosonication on nutritional profile of watermelon juice such as amino acid and minerals needs to be studied.
7. Total polyphenols were determined but future studies should also concentrate on quantifying and identifying the polyphenols available in watermelon juice from the unripe stage to the ripe stage.

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Appendix A: Effects of thermosonication on physicochemical properties of fully- ripe watermelon fruit juice

	Independent factors			Dependent factors				
				Colour				
Run	T ˚C	Time min	Amplitude µm	L	a*	b*	pH	˚Brix
0	0	0	0	22.25^{h}	8.95^{e}	17.96^{q}	5.51^{a}	8.41^{i}
1	25	10	24.4	19.22^{e}	11.11^{fg}	12.12^{n}	5.57^{bc}	8.41^{i}
2	45	2	24.4	32.82^{o}	6.17^{bc}	9.15^{c}	6.10^{h}	7.30^{d}
3	35	6	42.7	24.12^{k}	6.98^{cd}	11.01^{i}	5.62^{f}	7.80^{f}
4	25	10	61	19.01^{d}	10.98^{fg}	12.42^{p}	5.56^{b}	8.30^{g}
5	45	10	61	31.55^{n}	4.14^{a}	9.86^{d}	6.30^{j}	7.00^{a}
6	25	2	61	18.98^{c}	11.01^{fg}	11.92^{l}	5.57^{bc}	8.38^{h}
7	35	6	73.5	16.02^{a}	7.01^{cd}	10.89^{g}	5.63^{f}	7.70^{e}
8	45	10	24.4	31.30^{m}	4.57^{a}	9.91^{e}	6.31^{j}	7.20^{c}
9	35	6	42.7	23.11^{j}	6.98^{c}	11.01^{h}	5.62^{f}	7.80^{f}
10	25	2	24.4	20.12^{f}	11.81^{g}	12.40^{o}	5.56^{b}	8.41^{i}
11	35	6	11.9	21.01^{g}	7.38^{d}	12.11^{m}	5.59^{de}	7.80^{f}
12	35	6	42.7	22.31^{i}	6.98^{cd}	11.21^{j}	5.60^{d}	7.80^{f}
13	45	2	61	33.11^{p}	4.23^{a}	9.01^{b}	6.20^{i}	7.30^{d}
14	52	6	42.7	39.08^{q}	3.57^{a}	6.11^{a}	6.40^{k}	7.10^{b}
15	35	13	42.7	25.11^{l}	5.78^{b}	10.21^{f}	5.65^{g}	7.81^{f}
16	18	6	42.7	18.21^{b}	10.20^{f}	11.40^{k}	5.58^{cd}	8.40^{i}

Values are means (n = 3), different letters in the same column are significantly different at $p < 0.05$

Appendix B: Effects of thermosonication on phytochemical properties of fully – ripe watermelon fruit juice

Independent variables				Dependent variables				
				mg/100 g				
Run	Temperature (˚C)	Time (min)	Amplitude (µm)	Lycopene	β-carotene	AA	TPC mg GAE/100 g	Antioxidant activity %DPPH
Control	0	0	0	6.19^{g}	0.23^{g}	3.45^{i}	25.95^{p}	23.90^{k}
1	25	10	24.4	7.91^{hi}	0.18^{ef}	2.99^{h}	22.76^{m}	21.01^{j}
2	45	2	24.4	3.34^{c}	0.02^{ab}	2.01^{b}	8.11^{d}	8.07^{c}
3	35	6	42.7	4.45^{e}	0.03^{bc}	2.12^{d}	14.97^{i}	15.18^{h}
4	25	10	61	7.78^{h}	0.16^{d}	2.65^{f}	22.46^{l}	20.06^{i}
5	45	10	61	3.04^{ab}	0.02^{ab}	1.97^{a}	7.78^{a}	7.52^{b}
6	25	2	61	8.01^{hi}	0.18^{ef}	2.86^{g}	23.76^{n}	19.90^{i}
7	35	6	73.5	4.36^{e}	0.03^{c}	2.08^{c}	14.57f	12.34^{e}
8	45	10	24.4	2.91^{a}	0.02^{bc}	1.98^{a}	7.91^{c}	6.87^{a}
9	35	6	42.7	4.44^{e}	0.03^{bc}	2.12^{d}	14.97^{i}	13.02^{f}
10	25	2	24.4	8.10^{i}	0.19^{f}	3.11^{i}	23.96^{o}	20.02^{i}
11	35	6	11.9	4.74^{f}	0.03^{bc}	2.35^{e}	15.27^{j}	15.11^{h}
12	35	6	42.7	4.44^{e}	0.02^{bc}	2.12^{d}	14.67^{h}	12.34^{e}
13	45	2	61	3.2b^{c}	0.02^{bc}	2.01^{b}	7.88^{b}	7.54^{b}
14	52	6	42.7	3.8^{d}	0.01^{a}	1.98^{a}	9.19^{e}	8.90^{d}
15	35	13	42.7	4.32^{e}	0.03^{bc}	2.11^{d}	14.61^{g}	14.55^{g}
16	18	6	42.7	7.80^{h}	0.17^{e}	2.85^{g}	18.96^{k}	21.03^{j}

Values are means (n = 3), different letters in the same column are significantly different at $p < 0.05$

Appendix C: Conference and publications

List of conference papers

Paper accepted for presentation at INOPTEP Sixth International conference on sustainable postharvest and food technologies in Kladovo, Serbia.

Paper title: Effects of thermosonication on phytochemicals of watermelon (*Citrullus lanatus*) juice using a response surface methodology

List of publications

Maoto, M.M., Beswa, D., and Jideani, A. I. O. (2019). Watermelon as a potential fruit snack. *International Journal of Food Properties*, 22: 1, 355-370, DOI: 10.1080/10942912.2019.1584212