PROCESS OPTIMISATION FOR ENZYMATIC CLARIFICATION OF INDIGENOUS WILD WATERMELON (*CITRULLUS LANATUS*) JUICE

by

MOSELO MAUREEN MAMABOLO

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DECLARATION

Name: Moselo Maureen Mamabolo

Student number: 393934948

Degree: Master of Consumer Science (Full Dissertation)

Title of a thesis: PROCESS OPTIMISATION FOR ENZYMATIC CLARIFICATION OF INDIGENOUS WILD WATERMELON (*CITRULLUS LANATUS*) JUICE

I declare that the above thesis is my own work and that all the sources that I have used or quoted have been indicated and acknowledged using complete references. I further declare that I have not previously submitted this work, or part of it, for any degree or examination in any other higher education institution.

Signature: 

Date: 19 November 2019

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DEDICATION

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ABSTRACT

Tailored wild watermelon (*Citrullus lanatus*) juice clarification process is a fundamental step in improving its appearance and consumer acceptability. The purpose of this research was to investigate the mineral and proximate composition of wild watermelon juice (*Citrullus lanatus*) and to design an optimum processing condition for the enzymatic clarification of the juice. This investigation will help identify the sustainable processing parameters (incubation time, incubation temperature, and enzyme concentration) for ultimate clarification. Wild watermelon juice was treated with pectinase enzyme at different concentrations (0.05 to 0.15 w/w%), incubation temperatures (30 - 50 ^{o}C), and incubation times (60 - 180 min). The different process parameters were utilised with each sample treated individually to determine their effect on selected responses: turbidity, clarity, viscosity, L* value, and brix. It was determined that the incubation temperature was the most crucial factor affecting the physiochemical properties of the juice as it exerted a significant influence on most (turbidity, absorbance, and viscosity) of the clarity attributes of the juice. Incubation time significantly affected turbidity and percent brix, whereas enzyme concentration only significantly affected percent brix of the juice. The optimum conditions for juice clarification were established by the Response Surface Methodology at the following parameters: enzyme concentration 0.15 w/w%, incubation time 60 min, and incubation temperature 60 ^{o} C. The optimum output parameters at the following: turbidity: 14.18 NTU; clarity: 0.04 Abs; colour: 52.30 L value; viscosity: 1.96 cps; brix: 3.08 %. It may be useful to investigate the optimum parameters for other juices.

Keywords: Wild watermelon, Enzyme, Optimisation, clarification, Temperature

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LIST OF ACRONYMS

a_w	Water Activity
CCD	Central Composite Design
CIE	Commission Internationale De L'eclairage
CONC.	Concentration
CPS	Centi Poise
FAO	Food And Agricultural Organisation
FDA	Food And Drug Administration
GRAS	Generally Recognised As Safe
RDA	Recommended Daily Allowance

CHAPTER 1: BACKGROUND

1.1 Introduction

Underutilization of indigenous crops affects the nutrition and health or rural and urban populations, which on the other hand, deprive farmers an opportunity to generate income from their produce (FAO, 2014). Not overlooking other indigenous crops, Tabit et al. (2016) found that some crops can be used not only for food but also for medicinal purposes, because of their phytochemical composition. Wild watermelon (*Citrullus lanatus* subsp. *lanatus*) might have originated from South Africa (Erhirhie & Ekene, 2013, Wang et al., 2016) due to the abundance of the crop in the wild. On the other hand, it is also found in the Kalahari sands of Namibia, Botswana, South-Western Zambia, and Western Zimbabwe (Strauss, 2015). The cultivation of wild watermelon crops and the production of value-added fruit juice from its fruits could contribute to the reduction of poverty, food insecurity, and income generation in many rural communities in Africa (Chivandi et al., 2015).

Among wild watermelon species, there are over 1200 varieties that grow at various conditions worldwide (Campell, 2006). Wild watermelon belongs to the Cucurbit order, Cucurbitaceae family, and genus *Citrullus* subspecies *lanatus*. The most common red sweet fruits are commonly known as the conventional watermelon, and they belong to the subspecies *vulgaris*. Their characteristic red and sweet taste is caused by the accumulation of carotenoid pigment (lycopene) and sucrose during the maturation period of the fruit (Paes et al., 2015). The carotenoid has been found to have antioxidant properties and various health benefits, as stated by Campell (2006). The wild watermelon pulp is usually less sweet and has a pale red colour and in some cases yellow or orange colour, and contains approximately 90% water (National Department of Agriculture, 2014; Paris, 2015). According to Blitz (2017), the sugar content of watermelon correlates with a gene (Lycopene β-cyclase**)** that affects both pigment and colour. In essence, the sweeter the taste of the pulp, the redder it is. Wild watermelons like their

conventional counterparts are rich in vitamin C (antioxidant), Vitamin A (beta carotene), lycopene (antioxidant) and citrulline (an amino acid) (Tola and Ramaswamy 2015; Erhirhie and Ekene 2013).

Fruit juices including that made from watermelon are cloudy by nature and often not desirable without further processing due to the presence of different amounts of polysaccharides (pectin, cellulose, hemicellulose, lignin and starch), protein, tannins, and metals which often hamper filtration processes (Saxena et al., 2014; Sandri et al., 2011). Pectins which are the major component of the primary cell wall and middle lamella of most fruits has been found to be more in wild watermelon as compared to the conventional watermelon and hence can influence the texture and quality of the juice of wild watermelon fruits (Wang et al., 2016: Van der Voosen et al., 2004).

The clarification of wild watermelon juice by enzymatic treatment will ensure viscosity reduction (Castro Dominques et al., 2011), convert sucrose to oligosaccharide products (Johansson et al., 2016) and reduce turbidity with limited polyphenol loss, for quicker filtration (Rinaldi et al., 2013). The clarification processes often include centrifugation, enzymatic treatment and or the application clarifying agents such as gelatin, bentonite, silica solution, and polyvinyl pyrrolidone (Castro Dominques, 2011). Clarification method for juice quality improvement would be a preferred choice as compared to other techniques such as dearation and stabilization. As dearation can usually result in exposing the juice to enzymatic browning if the juice is not protected from the atmosphere in all processing steps or if the air is not replaced by the inert gas adequately. These may result in the destruction of nutrients, modification of flavor and damage of quality. On the other hand stabilization is also an alternative technique, where careful stabiliser choice is very important for better juice quality production. There are a number of stabilisers that are available such as Xanthan gum, where they were found to be than others such as carboxy-methyl cellulose (Akkaracheneeyakorn and

Tinrat, 2015). Various commercial enzymes available for the clarification of wild watermelon juice include protease pectinases, cellulases, and hemicellulases (Aghajanzadeh et al., 2016; Mieszczakowska-frac et al., 2012; and Pinelo et al., 2010).

1.2 Research Problem Statement

The problems this research sought to address are the unexplored nutritional benefits of indigenous crops due to under consumption and the lack of exploitation for commercial purpose.

Threats to malnutrition caused by under consumption of nutritious indigenous crops remain a significant burden to public health worldwide (FAO 2014). Essentially, these are because many of these indigenous crops have received little research attention (Aworh, 2015). The underconsumption can be attributed to poor image and stigmatization caused by negative attitudes towards their usage (Dweba & Mearns, 2011; Malkanthi et al., 2014).

The underutilized indigenous crops are characterized as having drought resistant, heat stress tolerance and nutrient-dense qualities (Modi & Mabhaudhi, 2016; Chivenge et al., 2015). These crops include cereals, legumes, root, and tuber crops, with most of them being the leafy vegetables (Modi & Mabhaudhi, 2016; Muthamilarasan et al., 2019).

Despite technological advances, it has been evident that the global food system has not succeeded in meeting the basic food needs of all citizens. As has been observed, these challenges have not been addressed by technological improvements. As a consequence, the incorporation of indigenous food crops into the food chain system has always been ignored (Mabhaudhi et al., 2019). There is a need to promote the utilization of indigenous food (Aworh 2015) and to enhance local processing of monkey orange from South Africa (Ngadze et al., 2018. There has also been a need for the promotion of indigenous fruit trees through processing and marketing (Hughes & Haq, 2003).

The commercialisation of indigenous food crops is essential, and scientific knowledge on their benefits will support their promotion. In South Africa, indigenous food crops have never been commercialised despite their informal usage within communities to generate income (National Department of Agriculture, 2014). Hence, there is a need for the improvement of markets and quality of indigenous fruit and products by the private sector in the production and commercialisation of indigenous fruit trees (Akinnifesi et al., 2007); Modi & Mabhaudhi, 2016).

1.3 The Motivation for the Study

In order to address the causes of malnutrition in South Africa, there is a need to formulate strategies that will improve the utilization and the commercialisations of indigenous crops. Because wild watermelon has valuable nutritional benefits, the development of this crop will help in eradicating the prevalence of malnutrition and chronic diseases, especially in rural communities. This nutritional information available can be used in product development projects to design other functional foods with improved nutritional benefits.

The processing of wild watermelon into juice will boost the image of the crop, and also enhance consumer acceptance. With more consumer acceptance, the usage of the crop will be increased as it will be able to enter the mainstream market. In turn, it will increase the consumption of the crop.

The better-improved quality of the product will increase the commercialisation of the crop. This will cause the reduction of poverty as farmers will be encouraged to grow and preserve the crop and strengthen food security within the communities as a result.

1.4 Aim and Objectives of the Study

1.4.1 Aim

This research project aimed to establish the optimum process conditions (incubation time, temperature, and enzyme concentration) for enzyme clarification on wild watermelon juice.

1.4.2 Objectives

The research objectives were as follows:

- Investigate the mineral, vitamin C, and proximate composition of wild watermelon juice.
- Investigate the physiochemical properties (turbidity, viscosity, L* value, °brix) of wild watermelon juice.
- To design optimum conditions (incubation time, incubation temperature, and enzyme concentration) for enzymatic clarification of wild watermelon juice.

1.4.3 Research questions

The following research questions are identified.

- What is the mineral composition of crude wild watermelon?
- What are the proximate composition and vitamin C of crude wild watermelon juice?
- What are the physiochemical properties (turbidity, viscosity, L* value, and °brix) of wild watermelon juice?
- What are the optimum process conditions (incubation time, incubation temperature, and enzyme concentration) for wild watermelon juice clarification?

1.5 Dissertation Layout

The study contains six chapters that are organized as follows:

Chapter 1: Introduction: The first chapter is an introduction to the study. It outlines an overview and background for the research. This section also provides the problem statement, the purpose of the study, and elaborates on the blueprint of the dissertation.

Chapter 2: Literature review: The literature review in Chapter 2 outlines an impression of existing literature on enzymatic effect on, physiochemical properties, and sensory analysis of fruit juices. In this chapter, the aim and the objectives, as well as the research questions, will be addressed.

Chapter 3: Research Methodology: In this chapter, the research method and instruments, research area, sampling method, and data collection method were explained, and the ethical principles and limitations were discussed.

Chapter 4: Results: This chapter outline the research outputs from the different research objectives.

Chapter 5: Discussion**:** This chapter discusses the results of all the experiments with one another and published reseach journals.

Chapter 6: Conclusion and Recommendations**:** The outline of the overall conclusions of the study are provided, recommendations for improvements, followed by a list of references.

CHAPTER 2: LITERATURE REVIEW

2.1 The Wild Watermelon Plant

Watermelon belongs to the species *Citrus lanatus*, and is a member of the cucurbit family (Cucubitaceae), which includes squash, pumpkin, cucumber, muskmelon, gourd, and genus Citrullus (Erhirhie & Ekene, 2013). Watermelon species has been divided into three subspecies namely: subsp. *vulgaris*, subsp. *lanatus* and subsp. *mucosospermus*. The most common red sweet fruits, usually known as the convectional watermelon, belong to the subspecies Vulgaris (Diane & Liu, 2007). The growing wild watermelon *(Citrullus lanatus*) plant is therefore shown on Figure 2.1.

Watermelon is a creeping or climbing plant, with herbaceous, firm and strong stems, and with bright yellow flowers, growing up to 3m long. The presence of tendrils that are on the side of the leaves enables them to climb trees or shrubs (Erhirhie & Ekene, 2013). The rind of their fruits consists of two layers; the outer layer called the exocarp which is glossy, striped in shades of green, and the thick inner layer called mesocarp is moist, white and hard. The endocarp, which is the layer that comes after the mesocarp, is the part that is usually eaten (Dane & Liu, 2007).The mature wild water melon (*Citrullus lanatus*) and the sliced fruit are shown on figure 2.2 and 2.3 respectively. Most cultivars of watermelon are monoecious meaning they are both male and female, but many indigenous cultivars are andromonoecious meaning having both the male flowers and bisexual flowers on the same plant (Paris, 2015).

Among the various wild watermelon species available is one commonly known as the Tsamma melon (in Khoisan language) belonging to the subspecies *lanatus* and has been used as a source of water in the Kalahari Desert (Xaba & Croeser, 2012). Another variety that is commonly known as *Cucumis metuliferus* is also named: African horned cucumber or kiwano (texture similar to that of cucumber) or horned melon and is characterised by a yellow-orange skin with a jelly-like flesh with a tart taste (Usman et al., 2010). This variety that is indigenous to

Southern Africa (Namibia, Botswana, South Africa and Swaziland) and central Africa, is used as a nutritional supplement and for medicinal purposes (Aggarwal et al., 2007). Another wild watermelon *Cucumis melo L.* (Musk melon) is a tropical crop that probably originates from Africa with the immature fruits consumed fresh, cooked, or pickled. The mature fruits are consumed canned, used for syrup, jam, juice and the seeds used for medicinal purposes to induce vomiting (Fang & Grumet, 1993). Wild watermelon Citrullus *lanatus var. citroides* (citron watermelon), which is tasteless and colourless is used as a thickening agent in the production of jams, and jellies (Paris, 2015).

Wild watermelon might have originated from South Africa because there is a variety of them growing successfully in the wild in this region (Erhirhie & Ekene, 2013). It is also found in the Kalahari sands of Namibia, Botswana, south-western Zambia, and western Zimbabwe (Strauss, 2015). Egusi, a wild watermelon which is indigenous to western Africa, produces spherical fruits with bitter flesh, but their seed is often used for food (Abbah et al., 2015).

Figure 2. 1 Growing wild watermelon (*Citrullus lanatus*) plant.

Figure 2. 2 The mature wild watermelon (*Citrullus lanatus*) fruit.

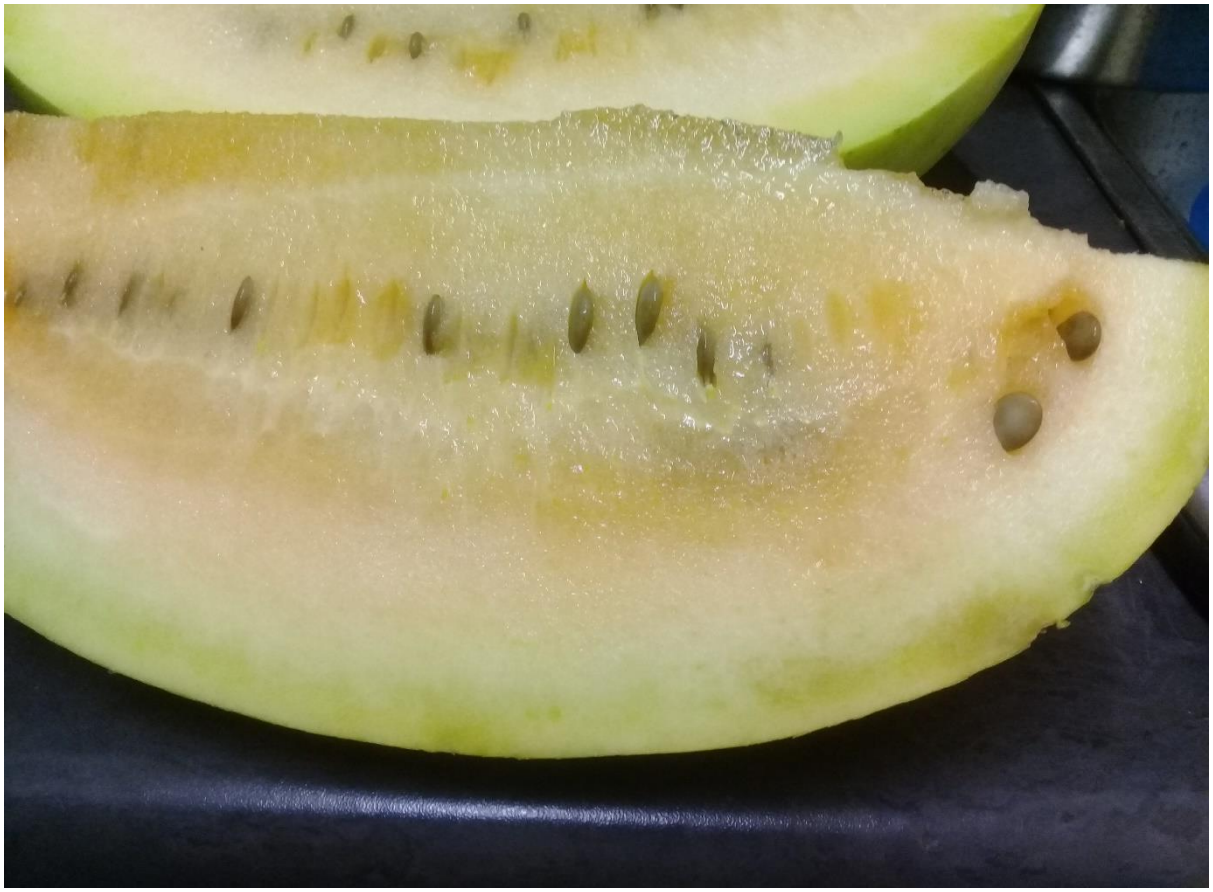

Figure 2. 3 The sliced wild watermelon (*Citrullus lanatus*) fruit.

2.2 The Growth and Cultivation of Watermelon

Wild watermelon grows successfully on soils of low fertility, hence can be easily cultivated by a small-holder farming community with little input cost (Mtumtum, 2012). Wild Watermelons are propagated by seeds which germinate at soil temperatures of ranging from 18 ˚C to 35 ˚C as well as by transplant (Wehner, 2008; Department of Agriculture forestry and fisheries, 2013). Wild watermelons require soil that holds water well and with pH from 5.8–6.6 (Orzolek et al., 2010).

2.3 Watermelon Juice

Fruit juices provide essential vitamins, minerals, and polyphenols that are vital for health (Zeng et al., 2017). With diversifying consumer requirements for innovative product development for nutritional food products, it is a challenge for the food industry (Tiwari, 2018). Additionally, 100% fruit juices are convenient, delicious, and can help individuals meet their daily fruit intake to support a healthy lifestyle. The World Health Organisation promotes the consumption of five fruits and vegetables a day for preventing chronic diseases, such as obesity, diabetes, and cardiovascular disease (Pem & Jeewon, 2015; Tiwari, 2018). According to Zheng et al. (2017), drinking juice is an effective and convenient way of improving fruit and vegetable consumption. Additionally, alternative forms of fruit and vegetable (juices and smoothies) are also essential in providing antioxidant activity to the body that is important for preventing cell damage by free radicals (Tiwari, 2018). Wild watermelon has carotenoids that can be attributed to that benefit (Tola & Ramaswamy, 2015). Fruit juices can be squeezed from the fruit pulp and be mixed with other juices to achieve the recommended fruit intake.

Fresh fruit juices (100%) are usually free of chemical preservatives and other additives. Only in a few cases, for example, apple juice processing, the cloudiness will require the addition of ascorbic acid (vitamin C) for the prevention of browning. Juices commonly appear as the mirror image of the fruit, as it contains primarily all substances which are found in the original ripe and healthy fruit (Zheng et al., 2017). As a consequence, it is the major task of modern food

technology to transfer the valuable fruit components into the juice and to produce stable products by appropriate extraction methods. As stated by Mushtaq (2017), juice extraction entails pressing, squeezing, or screening the liquid part to recover the cellular liquid from fibrous material. The method of extraction implemented will have an effect on the yield, flavour, quality, composition, shelf life and the health benefits of the juice as (Alvarez et al. 2012). Juices have been classified as clarified, concentrates, pulps, purees, and nectars, not excluding syrups (contain 100% juice), nectars (contain 25-95% juice), and ready to drink juices (contain less than 25% juice) (Mushtaq 2017). On the other hand, concentrated juices are obtained by evaporating a significant part of the water content or fraction freezing. Other types of juices have been named: Sport or Isotonic, Nutraceutical, Energy, Herbal, Smart and fun beverages, and functional fruit juice blends as stated by Rodriquez-Roque et al. (2015).

According to Wehner (2008), various juice varieties possess different sugar content due to their fructose and sucrose content. Fructose is generally sweeter than sucrose though in some cultivars of wild watermelon there is approximately 10-14% brix sugar content. The watermelon fruit juices contain vitamin A, vitamin C, vitamin B, amino acid, and carotenoid lycopene (Alam et al., 2013). However, the vitamin C contents in wild watermelon is hardly enough, hence the lack of astringent flavour (Tola & Ramaswamy, 2015). The colour of wild watermelon juice is the same as the colour of the flesh of the wild watermelon fruit due to the presence of carotenoids (lycopene, phytofluene, phytoene, beta- carotene, lutein, and neurosporene), which the quantity thereof depends on the variety (Tola & Ramaswamy, 2015). In some varieties, the juice colour can be yellow, clear, orange, or light pink. In addition, it is found to be less sweet, hence less palatable than the conventional watermelon (Department of Agriculture Fisheries and Forestry, 2013). For this reason, it is necessary to add sugar and acid (usually ascorbic acid) to enhance the taste; however excessive addition may have a detrimental health effect on dental health and obesity (Tiwari, 2018; Zeng et al., 2017).

2.3.1 Spoilage and microbial contamination

Juices shelf life is generally influenced by many factors, including harvesting, processing, and the packing process implemented. Wild watermelon juice is frequently a highly perishable product because of its high water activity ($a_w > 0.95$), and pH close to neutral between 4 and 6 (Pinto et al., 2017). Spoilage of fruit juices can result in undesirable sensory characteristics that are manifested by off-colour, off-odour and slime formation, the formation of surface pellicles, fibrous mats of mould, cloudiness, and off-flavours in juices (Geta, 2015). On the other hand, cloud loss, carbon dioxide ($CO_{2)}$ production, changes in colour, texture, and appearance have also been observed in some juices as stated by Aneja et al. (2014). Freshly squeezed watermelon juice is more vulnerable to spoilage, and when unpasteurised it becomes exposed, which may result in rapid enzymatic, chemical, and physical deterioration (Bates et al., 2001).

According to Dauthy (1995), enzymes that are present inside the fruits can have the following consequences that lead to deterioration: (1) the post-harvest senescence (2) oxidation of phenolic substances by phenolase that may lead to browning (3) sugar- starch conversion in plant tissues by amylases, and lastly (4) post-harvest demethylation of pectic substances that leads to softening of the fruit during ripening.

Chemical changes that occur that lead to deterioration include (1) lipid oxidation and non-enzymatic browning. Lipid oxidation is influenced by light, oxygen concentration, high temperature, the presence of a catalyst, and water activity. The non-enzymatic browning or Maillard reaction may eventually lead to the formation of insoluble brown polymers (Dauthy, 1995).

In watermelon fruit juices, there are enough nutrients that can support the growth of a microorganism (Geta, 2015). Some microorganisms: *Salmonella*, *Eschirichia coli* O157:H7 and *Listeria monocytogenes* (*L. monocytogenes*) is present in most fruit juices, according to Geta (2015); Aneja et al. (2014). Sibanyoni and Tabit (2019) established that among other pathogens, L. *monocytogenes and Staphylococcus aurens (S. aurens*) were the most common

microorganisms that were found to be present in food contact surfaces. Contamination by pathogens such as *Salmonella* and *Escherichia coli* is due to the usage of organic waste fertilizer, water contaminated with faecal material and direct contact of fruits with livestock, wild animals, birds and even postharvest workers (Geta, 2015). Microorganisms can be introduced into fruits through damages incurred during harvesting and during postharvest handling (Qadri et al., 2015). Lactic acid bacteria (LAB), coliforms, moulds, and yeast have been found to be responsible for the spoilage of fruit juices such as watermelon juices utilise the carbohydrate content (Rewat, 2015). Additionally, the LAB produces off-flavours and large amounts of exopolysaccharides that cause slime in some beverages.

2.3.2 Preservatives used in watermelon juice

Traditionally fruit juices have been preserved by the Long Temperature Long Time (LTLT) and High temperature short time (HTST) pasteurisation method, and this method was able to extend the shelf life from six to twelve months as stated by Myer et al., (2016). On the contrary, the LTLT has been established to produce undesirable quality changes during processing. Due to that reason, it resulted in its replacement by the HTST method. Even though thermal pasteurization is the most common method for removing pathogenic microorganisms in fruit juices, it has a negative effect on the organoleptic and nutritional physiochemical characteristics (Mosqueda-Melgar et al., 2008; Myer et al., 2016). Different physical (non- thermal pasteurization) methods including High hydrostatic pressure (HHP), high-pressure homogenisation (HPH), pulsed electric field (PEF), and ultrasound (US) are available, and some are still under research. These methods have been found to provide “fresh like” safe and prolonged shelf life juices (Aneja et al., 2014).

Besides thermal pasteurization, chemical preservation methods are also available, for preserving fruit juices. The most common methods that have been used are potassium sorbate and sodium benzoate. Even though consumer demand for natural products has been on the increase since the 1990s, it has enforced the development of natural antimicrobials such as

bacteriocins, lactoperoxidase, herbs and leaves, spices, chitosan, organic acids, essential oils, and phenolic compounds for possible use in food products (Hintz et al. 2015). They also have been considered as the Generally Recognised As Safe (GRAS) additives.

Wild watermelon juice has been preserved by pasteurization, as the pasteurized juice was observed to have an effect in killing harmful bacteria and extending the shelf life (Geta, 2015). Considering that some spoilage yeast can tolerate acidic environments; acidic addition to the juice alone does not inhibit microbial growth. For this cause, the usage of sodium benzoate, also known as E211, a natural preservative that is also found in apples, cranberries, plums, and prunes, has been implemented. The usage of sodium benzoate as a preverservative has been associated with its ability to react with the present vitamin C to produce a toxic chemical called benzene, (Pylypiw et al., 1997). Therefore, this was seen as a disadvantage in juice processing.

2.4 Enzymes

Generally, enzymes in food processing have the potential to break down the native matrix of the fruit that is composed of structural polysaccharides, such as peptic substances, cellulose, or hemicellulose. Because the cell wall prevents the intracellular liquid from being extracted by maintaining the structure, any mechanism that disrupts the internal structure will result in juice extraction (Garcia, 2017).

Enzymes are proteins (but not all proteins are enzymes) that function as catalysts and speed up chemical reactions by lowering the activation energy. They catalyse the synthesis and breakdown of macromolecules, and with the availability of enzymes most chemical reactions occur in a fraction of a second (Patel et al., 2016). Enzymes target specific substrates and bind to it for chemical reactions, owing to their specificity in reactions (Nelssestuen, 1995). Enzyme specificity are dependent on factors such as temperature, pH, enzymatic concentration, substrate concentration with each enzyme having its specific optimum conditions such as: time and presence of inhibitors (Sulphur dioxide, polyphenols or alcohol) (Garcia, 2017).

2.4.1 Enzyme application in fruit juice processing

2.4.1.1 Pectinase enzyme

Pectinases are classified under three different categories, namely: pectin, pectin acid, and Oligo-D- galacturonate depending on the preferred substrate (Garcia, 2017). These enzymes are used in the food industry to degrade pectin substances in fruit juice (Anvari and Khayati 2014), and these enzymes break down polysaccharide pectin molecules into simpler molecules such as galacturonic acid (Ramos & Malcata, 2011). Pectin molecules predominantly consist of galacturonic acid residues in different proportions with side chains of rhamnose, arabinans, galactans, xylose, and fucose (Aghajanzadeh et al., 2016). Pectin is one of the major components of cloud material in fruit juices that is manifested by the turbid appearance, and pectinase reduces turbidity in fruit juice by degrading the viscous soluble pectin (Cerreti et al., 2016).

In essence, cloud formation is due to the presence of insoluble particles, which mainly constitute pectin substances (Saxena et al. 2014). As stated by Garcia (2017) and supported by Rajdeo et al. (2016), the structure of the particles has been described as a protein core with a positive charge surrounded by a negatively charged pectin molecule, preventing aggregation due to repulsion force. Pectinase may degrade the pectin coat exposing the positive charge, therefore, causing aggregation by electrostatic attraction to adjacent particles. The cloud particles will become larger as a result and cause precipitation. The settled particles can be removed by centrifugation, filtration, or the addition of finings (flocculants) resulting in a clear juice. Modern juice clarification methods include membrane technology and centrifugal decanting (Echavarria et al., 2011).

According to Minoli et al. (2017), pectinase enzymatic treatment is one of the most effective techniques to remove pectin by hydrolysing pectin and stimulating the pectin-protein complex aggregation Pectinase has been found to operate optimally for maximum juice yield at pH-4 and temperature 50^0C, again in apple production it was found to be crucial to leave crushed

apples for 20 -30 minutes for the enzyme inhibitors to be oxidized, and heating the pulp at 30 °C before adding pectinase for effective turbidity reduction (Srivastava and Tyagi 2013).

2.4.1.2 Cellulose enzyme

These enzymes are modular enzymes composed of discrete subunits with independent folding that is specifically called domains or modules (Garcia, 2017). When acting together, the enzymes can hydrolyse cellulose into monosaccharides or oligosaccharides. Their classification is unclear due to their great variety. Frequently they are being classified on their amino acid sequence, functionality, or crystal structure (Bayer et al., 1998). On the other hand, they may be classified focusing on their cleavage ability of the glucosidic bond either internally, or at one of the cellulose, chain ends (Garcia, 2017). Celluloses, together with pectinase and hemicellulose, are called macerating enzymes and are used in extraction and clarification of fruit and vegetable juices. Cellulose enzymes catalyze the bioconversion of cellulose into soluble sugars and glucose (Bhattacharjee, 2017; Sojitra et al., 2016). Combinations of cellulose and pectinase have been used for treating pineapple pulp to produce pineapple juice (Screenath et al., 1994) and to treat apple pomace for juice clarification (Ambroziak, 2010).

2.4.1.3 Protease enzyme

Proteases are a group of enzymes that catalyse hydrolytic reactions responsible for the degradation of protein molecules to peptides and resulting in the production of amino acids (Ramos & Malcata, 2011). The turbidity of fruit juices can be reduced by protease treatment; however, the resultant turbidity increases after cold storage (Pinelo et al., 2010). Proteolytic enzyme treatment in combination with pectinase has been found not to affect the total amount of pectin, proteins, and phenols in pomegranate juice but improved the quality of the juice haze (Cerreti et al., 2016). On the other hand, proteolytic enzyme called Bromelain, which is abundant in pineapple juice was found to be heat sensitive, and the destruction of bromelain by

heat (50°C for 30 min) results in lack of proteolytic activity in pineapple processing (Sew et al., 2014).

2.4.2 Effects of enzymatic treatment on juice properties

Enzymatic treatment of juice does not only affect quality attributes such as improved clarity and increased yield. It has also been found to affect the viscosity, total soluble solids (TSS), pH, turbidity, and phenol content, as stated by Sharma et al. (2017). Reduction in viscosity is therefore caused by the action of pectinase on pectin and protopectin. In juice, there might be three actions that affect viscosity:

1) if the suspended particles consist of soluble pectin particles (e.g. In apple juice), the reduction in viscosity will be evident,

2) if there is an equal amount of soluble and insoluble pectin (e.g., in black currant juice), the viscosity amount will remain unchanged until all insoluble pectin is changed to soluble and then the viscosity will be reduced gradually,

3) if there is more insoluble pectin (as in raw apples), the viscosity will initially rise, when soluble pectin is finished, then the viscosity will start to decrease as pectin is broken down (Garcia, 2017).

Total soluble solids increase with enzymatic treatment. The suspended particles are broken down by enzymes into a smaller and soluble compound which will eventually increase. In essence, TSS and viscosity are reciprocally related (Garcia, 2017). According to a study that was conducted by Joshi et al. (2011), it was established that the TSS of pear, apricot, peach, and plum juice were increased by increasing enzyme concentration and the viscosity was reduced in the same manner. Again it has been reported by the same authors on acidity, total carbohydrates, ascorbic acid, and colour intensity increase after enzymatic application; on the contrary, the pH was slightly lowered. Above all, the effect of the enzyme contributes positively

to the quality of the juice, and also extends the shelf life and enhances the nutritional value (Rinaldi et al. 2013). As a consequence, it has been reported that polyphenol accumulation might be apparent which may cause bitterness in taste (Mieszkowska-Frac et al., 2012).

2.5 Physiochemical Properties of Fruit Juices

2.5.1 Turbidity and proteins in fruit juices

Fruit juices have different percentages of turbidity depending on their physical and chemical characteristics. Turbidity acceptance (usually accepted in orange juice, and not accepted in apple juice) is dependent on the consumer and is usually from the insoluble substances or the cell fragments from the pulp tissues. These components are colloids, and according to Saxena et al. (2014) and Sandri et al. (2011), can vary in size from microscale to larger pulp fragments and are responsible for taste, aroma, and colour characteristics of the juice. Haze and turbidity develop in juice because of aggregation of macromolecules such as protein, starch, and tannins, and juices that are well filtered have particles that are significantly small (0.1μm) in such that they do not form a haze (Mishra & Champaigne, 2009). Moreover, the gravitational forces of the colloids are usually negligible, and their interactions are dominated by short-range forces such as the van der Waals attraction and surface charges. As a result, the inactivity of the dispersed phase is negligible to show random Brownian activity, caused by the velocity transmitted by the collisions with a molecule of the suspended medium (Mishra & Champaigne, 2009).

2.5.2 Antioxidant and sugar content of juices

Fruit juices contain many biologically active antioxidant compounds, especially ascorbic acid and phenols with good anti-inflammatory activities, hence their anticancer and cardio-protective properties, and their effect on age-related diseases (Bartoszek & Polak, 2016). The antioxidant activity of fruit juices is influenced by total polyphenol, total flavonoid, total ortho-diphenol, total tannin, and total anthocyanin contents (Harzallah et al., 2016). Enzymatic browning of fruits is caused by oxidation of phenolic compounds by phenoloxidase which

produces dark pigments and fruits like apples that are rich in polyphenol are highly affected by this reaction (Holderbaum et al., 2010). The colour of pineapple, for example, is stable because it does not have enzymatic browning due to the low polyphenoloxidase activity (Sew et al., 2014).

2.6 Process Optimisation of Enzyme Aided Clarification of Fruit Juices

Optimisation of enzymatic pre-treatment for juice clarification has been reported to be effective in increasing clarity in grape juice (Minoli et al., 2017). The effectiveness of enzymatic pre-treatment has also been accompanied by a decrease in viscosity in fruit juices such as dates, peach, banana, mosambi, pineapple, and kiwi fruit , as reported by Kilkarni et al. (2010), Santin et al. (2008), Lee et al. (2006), Rai et al. (2004), Dawes et al. (1994), and Screenath et al. (1994) respectively. As stated by Abdullah et al. (2007), Lee et al. (2006), Sin et al. (2006), and Minoli et al. (2017), pectin enzyme activity is influenced by the interaction of three parameters namely: (1) incubation time, (2) incubation temperature, and (3) enzyme concentration. Among several studies conducted on the optimization of enzyme aided clarification of fruit juices, the central composite design (CCD) is one of the most popular approaches (Abdullah et al., 2007). It can determine the response surface from the experimental design, therefore transforming the optimization process to be more productive and functional. Response surface methodology (RSM) is the statistical tool that utilizes quantitative data from the fitting experimental design to find and immediately solve multivariate equations (Bajwa et al., 2019). This tool has been widely utilized to explore the optimization process in tropical fruit juice production (Abdullah et al., 2007; Lee et al., 2006; and Sin et al., 2006).

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Enzyme Source

Pectinase powder used for the study contained 1U/mg (1789-50G), and was bought from Sigma Aldrich, South Africa. One (1) U corresponds to the amount of enzyme which liberates 1 microgram galacturonic acid from polygalacturonic acid per milliliter of the reaction mixture per minute at pH 4.0 and maximum temperature 50°C.

3.2 Collection of Wild Watermelon Fruits

Ripened watermelon fruits were obtained and purchased from a farm in the Capricorn District of the Limpopo province, and stored in cold room (4-10°C) to reduce spoilage. The maturity of wild watermelon was determined by testing rind toughness, the surface colour (turned from the surface of the watermelon goes from shiny to dull) and had an oval shape.To test the maturity of the batch, one ripened watermelon broke open when allowed to fall from 50cm above the ground, and it was discarded to avoid contamination. The presence of the dried and brown coloured tendril at the area where the stem joins the main vine confirmed that the watermelon was ripe. Mature and ripe watermelon fruits were also identified with a creamy yellow spot that developed at the section of the watermelon fruit that is in contact with the soil. Ripened fruits used for this research had a bright orange colour and pale red seeds.

3.3 Extraction of watermelon Juice

Fruits were peeled, deseeded, cut into small pulp pieces using a kitchen knife and blended using a blender (Omniblend 111, Durban, South Africa) for 2-3 min at maximum speed until a homogenous fruit pulp was formed. The juice was filtered using cheesecloth and pasteurised by heating it in a water bath (Labcon, Johannesburg, South Africa) at 90°C for 5 minutes. The pasteurised juice was stored in the refrigerator between 0-3°C, and up to 30 litres of crude watermelon juice was processed for further analysis (Abdullah et al., 2007).

3.4 Characterisation of Crude Wild Watermelon Juice

3.4.1 Mineral analysis

Minerals of crude juice were determined ICPE 9000 (Shimadzu, Kyoto, Japan), and the following minerals were analysed: Potassium, Phosphorus, Magnesium, Calcium, Sodium, Iron Selenium, Magnesium, Copper and Zin (Labbe et al., 2016).

For sample digestion, three milliliters (3ml) each of wild watermelon juice samples were placed into the polytetraflouroethene (PTFE) pressure vessels. 5.0ml of 70% Nitric acid (approximately 300 ml were required) and 1.0 ml of 30% hydrogen peroxide (approximately 100ml were required) were added to the vessel and digested under pressure in an automatic Titan MPS microwave sample preparation system (Perkin Elmer, Waltham,USA) digestion system for 10 minutes. The extract was diluted by adding dionised water to 50 ml mark.

For sample analysis, all element (K, P, Mg, Ca, Na, Fe, Se, Mn, Cu, and Zn) contents in wild watermelon juice were determined using inductively coupled plasma atomic emission spectrophotometer (ICPE-9000, Shimadzu, Kyoto, Japan). The results were expressed as mg per g. All measurements were carried out in duplicates.

3.4.2 Vitamin C determination

Vitamin C content of the crude juice was determined by the HPLC (Perkin Elmer,Walthan, USA) procedure (Brause et al. 2003). The standard solutions were prepared as follows: Approximately 5.0 mg of ascorbic acid was dissolved in 100 ml of water (to give 50g/ml concentration). From that 2,4,6 ,and 8ml was diluted with 10 ml of water (to give 10-40g/ml concentration) respectively. To each standard 10mg dithiothreitol (DTT) was added. Always using HPLC water.

The test solutions were prepared as follows: Mobile phase used was KH_2PO_4 (0.5%, w/v), pH 2.5, with dithiothreitol (0.1%, w/v): Approximtely 5.0 g KH_2PO_4 was inserted into 1 L volumetric flask and 950 ml water was added. 1.0 g dithiothreitol was added to the solution

and stirred until it was dissolved, and pH adjusted to 2.5 with concentrated phosphoric acid. Water was added to the 1 L mark, and the solution filtered through 0.45 µm membrane. 1 ml of juice was diluted with 10 ml of water. Then added into the standard solutions.Then 1ml of dithiothritol solution was added.

The instrument was setup according to the following parameters:The detector: 254nm, set flow rate at 0.5ml/min. Water was run through the column for 30 min followed by mobile phase for 1 hour to equilibrate the system. 50 µl of the calibration standards was injected. The peak response was plotted ensuring linear plots. Then 50 µl of the test samples was injected, repeating the samples that were not within the calibration range. Vitamin C concentration was interpolated through the Empower Software (Perkin Elmer, Walthan, USA)

3.4.3 Proximate analysis

3.4.3.1 Determination of total sugars

Total Sugar content was analysed by using HPLC (Perkin Elmer, Walthan, USA). Glucose, fructose and sucrose, were used as standards (Sigma Aldrich, Darmstadt, Germany) (Dymayanti et al. 2012). 2.5 g of glucose, fructose and sucrose were weighed and dissolve in 25ml volumetric flasks each, with acetonitrile and distilled water(50:50) to the mark. They were shaken until dissolved to poduce 10% stock solutions.

The standard solutions were prepared as follows: Glucose, fructose and sucrose standards were prepared at 0.5,1.5,2.5,3.5 and 5.5% in 10ml volumetric flasks. They were mixed with 0.5, 1.5, 2.5, 3.5 and 5.5ml stock solutions with acetonitrile and distilled water (50:50) to the mark respectively and the solutions were mixed thoroughly. 2ml of each homogenised solution was was pipetted from 0.5, 1.5, 2.5, 3.5, 4.5 and 5.5 standard solution to obtain 0.167, 0.5, 0.833, 1.167, 1.5, and 1.833% respectively into a vial.

Mobile phase was prepared as follows: Sodium phosphate (156.01mg), was weighed and mixed with water to the 100ml mark to produce 10Mm sodium phosphate solution. Acenotonitrile

(78ml), Sodium phosphate solution (22ml) were mixed in a 250ml beaker and degassed by using the ultrasonic bath.

The sample solution was prepared as follows: the juice sample (12.5ml) was diluted with acetonitrile and distilled water (50:50) in a 50ml volumetric flask to the mark. The diluted juice was filterd through the 0.45 µm filter paper. Peak identification was based on HPLC retention times compared with those of selected standards. Quantitation was based on the standard external method using calibration curves fitted by linear regression analysis using Statistica 5.0 software (StatSoft, Tulsa, OK). The calibration curves were obtained by plotting peak area (mV/min) versus the amount injected (range covered, 0.2–2 g/100 mL). The carbohydrate content was then expressed as % carbohydrate. All measurements were carried out in duplicates.

3.4.3.2 Determination of fibre content

Fibre content was determined by using the neutral detergent method (Garcia et al. 1997). Approximately 1 ml of wild watermelon was boiled in the solution of Sodium lauryl sulphate at neutral pH for one hour. It was allowed to precipitate for at least 1 hour at room temperature and the precitate decanted through whatman filter no. 54 and then the remaining residue was transferred into the crucibles, and was washed with a small amount of ethanol (78%) . The glass crucibles (with the precipitate) were heated for 1 hour at 525^{o}C adding approximately 1g celite 545 (ignited overnight at 525^{o}C, cooled, and stored at a stoppered container, approximately a total of 5g was required). The crucibles were dried overnight, cooled, and weighed to the nearest 0.1 mg.

W (% total dietary fiber) = (weight of crucible + weight of residue) – weight of crucible/weight of sample x 100. All measurements were carried out in duplicates.

3.4.3.3 Ash determination

Ash was determined by the dry-ashing method using a high-temperature muffle furnace (Lasec, Johannesburg, South Africa). The weight of the crucible and the sample (2.0 g) were taken (in

duplicates) using a balanced scale (Lasec, Johannesburg, South Africa). The crucible with the juice was put in the muffle furnace (Lasec, South Africa) at 600°C for 2-3 hours until the ash was white. White ash indicated the absence of carbon. The crucible was cooled in a desiccator to room temperature. The weight of ash was calculated as:

% Ash (wet basis) = Mass $_{ASH}$ x100/ Mass $_{WET}$ (Sani, 2013).

3.4.3.4 Protein content determination

Protein content was measured by using the Dumas combustion method using Tru Spec N (Leco Corporation, Muchigan, USA) (Saint-Denis & Goupy, 2004) .The equipment was calibrated using EDTA (ethylenediamine tetraacetic acid) (Leco Corporation, Muchigan, USA) , to give 51-61% protein on the display monitor. Approximately 0.2 g of freeze-dried crude juice samples were inserted into the carausel and the analysis initiated. The results were displayed as percentage protein and all measurements were done in duplicates.

3.4.3.5 Fat content determination

The fat content of crude wild watermelon juice was determined by using the Soxhlet extraction method. The weights (W_1) of dried samples (3-4 g) were put in the flask. The extraction involved weighing the extraction flask and adding 85ml petroleum ether (approximately a total of 300 ml was required) and extracting for 4 hours (or 80 cycles). The remaining ether was evaporated on a steam bath. The flask was dried in an oven at 100-102°C for the required time to obtain constant weight (W_2). The flask was cooled in a desiccator. After completion, the extracted fat was weighed and the fat content calculated by the formula:

Fat Percentage (g/100g) = 100(W2-W1)/ Sample weight. All measurements were carried out in duplicates (Jae-min & Seun-Kook, 2015).

3.5 Physiochemical Analysis Wild Watermelon Juice

3.5.1 Turbidity measurement

Turbidity was measured using portable HANNA H1 83414 turbidimeter (Hanna Instruments, Johannesburg, South Africa) (Sin et al., 2006; Abdullah et al., 2007). A sample of extracted wild watermelon juice was placed on the calibrated turbidimeter, and the results were expressed as Nephelometric turbidity units (NTU). All measurements were carried out in duplicates.

3.5.2 Viscosity measurement

The viscosity of wild watermelon juices was determined using Malvern Kinexus rotational rheometer (Malvern Panalytical company., Worcestershire, England, UK.) (Akhtar et al., 2011), using a CP1/60 spindle. The sample was poured into the rheometer cell, which is surrounded by a temperature-controlled vessel, and allowed to equilibrate at 25°C for 5.0 min before the measurement. Viscosity was measured at shear-rates in the range 0.993 – 1.002 s^{-1} using continuous shear, with a 60s delay time and a 60s integration time at each shear rate. Measurements were done in duplicates.

3.5 3 Total soluble solids (TSS)

TSS were determined by using a portable refractometer (Metler Toledo, 30GS, Johannesburg, SouthAfrica). The equipment was first calibrated using distilled water, then the juice TSS was read. The measurements (duplicate) were expressed as % soluble solids (Javarnmardi & Kuborta 2006; Abdullah et al., 2007).

3.5.4 Clarity measurement

Clarity was measured by using Genesys 20 UV-VIS spectrophotometer (Thermofischer Scientific, Johannesburg, South Africa) at 660 nm. The equipment was calibrated using distilled water, and clarity was measured in absorbance (abs) (Sin et al., 2006).

3.5.5 Colour (L* value) measurement

L* value of the juice was measured using portable colorimeter (Konica Minolta Sensing Americas Inc., Johannesburg, South Africa) at room temperature. The CIE (Commission Internationale de L'Eclairage) reference measures system L* value on a numerical scale where brightest white = 100 and black =0. The L* value differences between the samples and the standard (control) was calculated using the resulting calorimetric values according to the formula: ΔL^* = L* sample - L* standard (which was the difference in lightness and darkness (+ = lighter, - = darker) (Sin et al., 2006; Abdullah et al., 2007, & Arsad et al., 2015).

3.6 Enzymatic Treatment of Wild Watermelon Juice

Approximately 500 ml of wild watermelon juice was subjected to enzymatic treatment for each experiment, as shown in Table 1. The range of variables for enzymatic treatment conditions were controlled using a water bath (Labcon shaking water bath), enzyme concentration (approximately 150 g was required), X_1 (0.05- 0.15 w/w%), incubation time X_2 (60-180 min) and incubation temperature used X_3 (30 – 50^{o}C). The choice of these treatment conditions were guided by similar studies previously done on other fruit juices ; carambola juice by Abdullar et al. (2007), sapodilla juice by Sin et al. (2006). The pH of the juice was between 4.8 and 5.0. At the end of enzymatic treatment, the sample was pasteurised by heating in a water bath 90^{o}C for 5 minutes (Abdullah et al., 2007). The treated juices were filtered using Whatman no.1 filter paper, and the filtrate collected for analysis.

Table 3.1 shows experimental design that was implemented in the study. Response Surface Methodology (RSM) was used to generate the experimental designs, statistical analysis, and a regression model with the help of Design Expert 11 Software. The independent variable each could influence the juice physiochemical properties on enzyme activity as stated by Abdullah et al. (2007) and were coded according to the following levels: -1, 0, and +1. Up to 20 different variable combinations were randomly chosen per the CCD (central composite design) configuration for the three selected variables. The coded (x) and actual (X) levels of variables

are shown in Table 3.1. The dependant variables (y) that were measured are (1) turbidity (y_1), (2) clarity (y_2), (3) viscosity (y_3), (4) L* value (y_4), and (4) brix (y_5) of wild watermelon juice. These values were related to the coded variables (xi, i= 1,2,3) by a polynomial function below.

$$y= b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12}x_1x_2+b_{13}x_1x_3+b_{23}x_2x_3+ b_{11} x_1^2 +b_{22} x_2^{\,2} + b_{33} x_3^{\,2}$$

The coefficients of the polynomial were represented by b_0 (constant), b_1, b_2 and b_3 (linear coefficient), b_{11}, b_{22} and b_{33} (quadratic coefficient) and b_{12}, b_{13} *and* b_{23} *(*interactive coefficient*)*. The significance of all terms in the polynomial function was assessed statistically using F-value at a probability (*p*) of less than 0.05 The three dimensional (3D) plots (Figure 4.3 - 4.7) for the dependant variables were produced by keeping one independent variable (temperature =30.00 ^{o} C) constant at the center point and altering the other variables (independent) within the experimental range.

3.7 General Statistical Analysis

The data for mineral, and vitamin C, proximate and physiochemical properties were analysed using varience analysis (ANOVA) using Statistix 10.0 software (Stasost, Tulsa, OK-USA). The data for turbidity, clarity, viscosity, colour and brix were analysed with the aid of a commercial statistical package, R Studio version 3.6.2 (R Studio, Inc. Boston, Mussachusetts-USA).

Table 3. 1 The central composite experimental design employed for enzymatic clarification of wild water melon juice.

Treatment trial	Enzyme concentration (w/w%) X_1 (x_1)	Incubation time (min) X_2(x_2)	Incubation temperature (°C) X_3 (x_3)
1	0.15	60.00	50.00
2	0.10	120.00	40.00
3	0.15	180.00	30.00
4	0.10	60.00	30.00
5	0.05	180.00	50.00
6	0.05	60.00	30.00
7	0.05	180.00	30.00
8	0.10	60.00	50.00
9	0.10	60.00	50.00
10	0.05	60.00	50.00
11	0.15	180.00	50.00
12	0.15	60.00	30.00
13	0.05	180.00	40.00
14	0.15	120.00	40.00
15	0.10	180.00	50.00
16	0.10	120.00	50.00
17	0.10	60.00	40.00
18	0.05	120.00	40.00
19	0.10	180.00	40.00
20	0.10	120.00	30.00

x –represents the coded level of variables; X represents the actual level of variables

CHAPTER 4: RESULTS

4.1 Proximate, mineral and vitamin C composition crude wild watermelon juice

The most abundant mineral in crude wild watermelon juice was potassium (39.300 mg/L). This was followed by magnesium (2.120 mg/L), sodium (1.890 mg/L), and calcium (1.420 mg/L). The iron and phosphorus content of crude wild watermelon juice was 0.487 mg/L and 0.344 mg/L, respectively. Vitamin C was shown to be in a favorable quantity (2.990 mg/L) (Figure 4.1). Regarding the proximate composition, brix (7.285%), total Sugar (4.295 g/100g), and total solid (3.646 g/100g) respectively were the most abundant in crude wild watermelon juice. This was followed by fibre (0.999 g/100g), ash (0.200 g /100g) respectively, and lastly, protein (0.135 g/100g) and fat (0.100 g/100g) respectively (Figure 4.2).

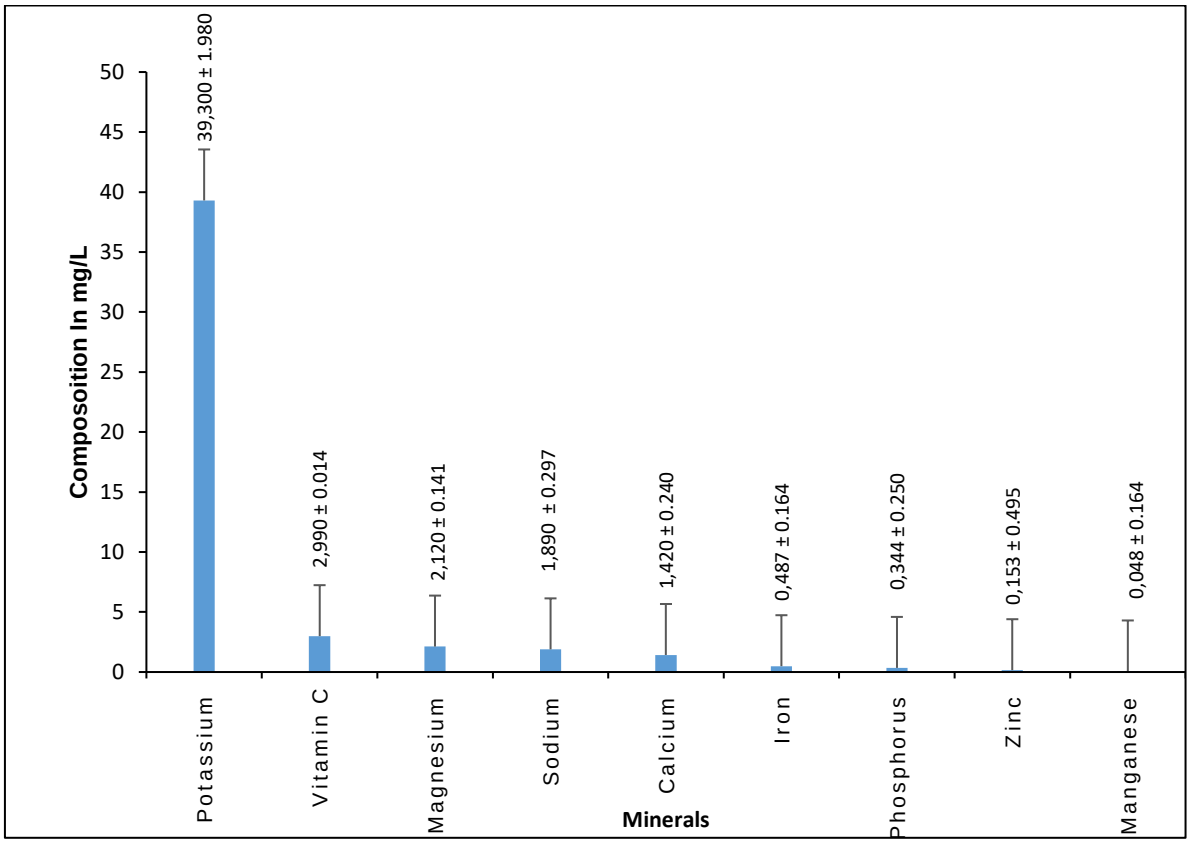

Figure 4. 1 Mineral and vitamin C content of crude wild watermelon juice. Data are represented as mean ± SD.

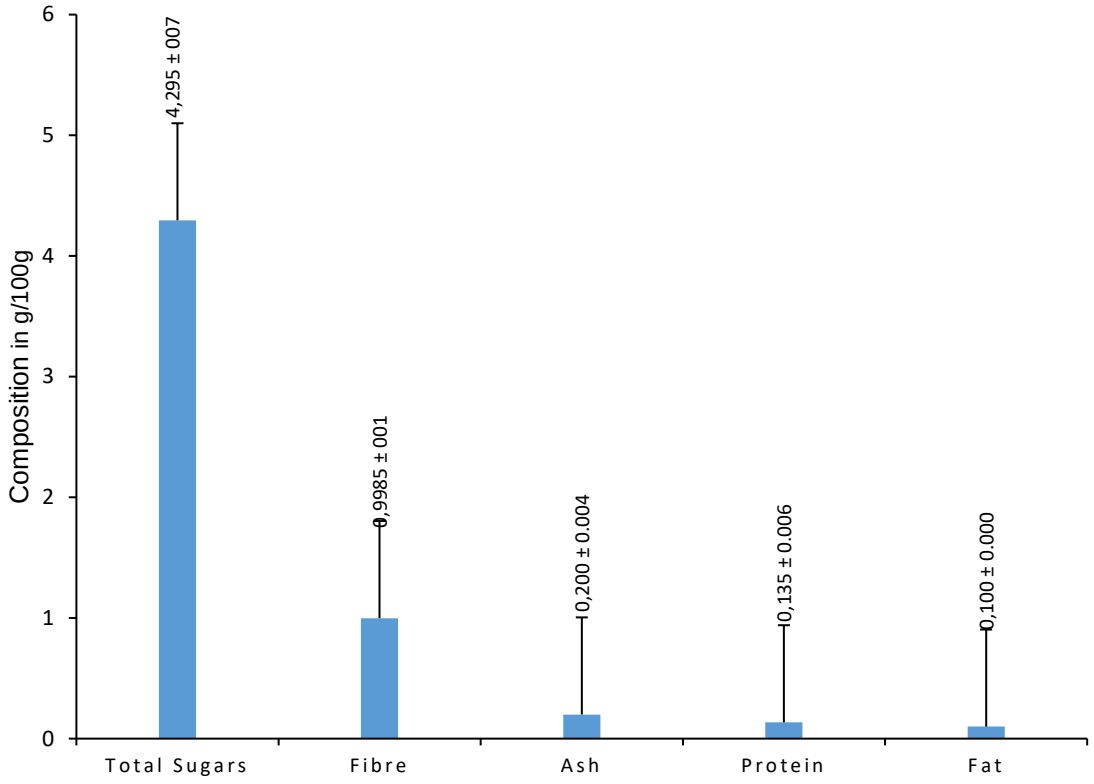

Figure 4. 2 Proximate compositions of crude wild watermelon juice. Data are presented as mean ±SD.

4.2 Turbidity, viscosity, brix, clarity and L* value of crude wild watermelon juice

Regarding the physical characteristics of crude wild watermelon, the mean values were as follows: turbidity was 3208.500 NTU while the viscosity was 62.500 cps. The percent brix was 7.285 %, clarity was 2.059 Abs, and the L* value was 51.61 (Table 4.1).

Table 4. 1 Physiochemical analysis values for crude wild watermelon (Citrullus lanatus) juice.

Parameters	**Values**
Turbidity (NTU)	3208.500 ± 85.91
Viscosity (cps)	62.500 ± 0.354
Brix (%)	7.285 ± 0.004
Clarity (Abs)	2.059 ± 0.012
Colour (L* value)	50.65 ± 1.90

Data are presented as mean ± SD.

4.3 Statistical Analysis

The experimental values for the five response variables (turbidity, clarity, viscosity, L*value, and brix) subjected under different three treatment parameters (enzyme concentration, incubation time, and temperature) have been presented in Table 4.2. Similarly, the p-values, regression coefficients for second-order polynomial equations and results for the linear, quadratic, and interaction for the five response variables have been presented in Table 4.2. The response surface model that was developed was adequate to predict only four variables; namely: turbidity, clarity, viscosity, and as R^2 values were greater than 0.5. The model was not adequate to predict L * value considering that as R^2 values were greater than 0.5, as shown in Table 4.2. The three-dimensional plots for turbidity, clarity, L* value, viscosity, and brix of wild watermelon juice as a function of enzyme concentration and incubation time at 30°C and time at 60 min have been presented in Figure 4.3 to 4.7. The plots were developed by using R Studio version 3.6.2. The contour plot for optimum conditions have been presented in Figure 4.8 and were developed by using Design expert version 11.

4.4 Effect of Treatments on the Turbidity of Wild Watermelon Juice

The enzyme concentration did not significantly ($p>0.05$) influence the turbidity positively in both linear and quadratic terms. The incubation time significantly ($p<0.05$) influenced the turbidity positively in the linear terms but negatively in the quadratic terms (Table 4.2). The incubation temperature negatively influenced the turbidity in the linear but positively in the quadratic term. The interaction effect between the incubation time and incubation temperature significantly ($p<0.05$) affected turbidity negatively. The other quadratic and interactive effects were not significant (Table 4.2). At 30°C, there was an increase in turbidity over the first few minutes, which was followed by a decline (Figure 4.3).

Table 4. 2 Regression coefficients and R^2 values for five response variable for enzymatic clarification of wild watermelon juice.

Regression coefficient	Turbidity (NTU)	Clarity (Abs)	Viscosity (cps)	Colour (L*value)	Brix (%)
β_0	3.1740*	2.076 *	6.2030*	5.1430	4.7170*
β_1	8.4100	0.015	-3.1620	3.0590	1.0140*
β_2	7.6890*	0.0006	0.0331	0.0064	0.0089*
β_3	-8.6950*	-0.0263*	-1.8600*	-0.0785	-0.378
β_{12}	6.188	$-3.583e^{-3}$	$1.042e^{-1}$	$1.223e^{-1}$	$3.125e^{-3}$
β_{13}	$1.012e^{1}$	$4.100e^{-2}$	$6.250e^{1}$	1.191	$5.875e^{-2}$
β_{23}	$-8.563e^{-2}$	$6.319e^{-6}$	$3.472e^{-4}$	$-6.292e^{-4}$	$1.458e^{-5}$
β_{11}	6.3790	2.232	1.4370	-1.4160	1.1880
β_{22}	-0.335*	$2.525e^{-5}$	-0.0002	-0.0001	0.0000*
β_{33}	1.0860*	-3.0 e-4*	0.0230	0.0005	0.0004
R^2	0.8593	0.8122	0.9716	0.2374	0.9395

Subscripts 0= constant, 1= Enzyme concentration, 2=Incubation time, 3= Incubation temperature
*Significant at $p < 0.05$.

Table 4. 3 Regression models obtained of five response variables for enzymatic clarification of wild watermelon juice.

Response variable	Model	Lack of fit p value
Turbidity	$3.17 + 8.41\ \beta_1 + 7.69\ \beta_2 - 8.70\ \beta_3 + 6.19\ \beta_1\beta_3 + 6.4\ \beta_1^2 - 0.34\ \beta_2^2 + 1.1\ \beta_3^2$	<0.0001*
Clarity	$2.076 + 0.02\ \beta_1\ 0.00\ \beta_2 - 0.03\ \beta_3 + 6.19\ \beta_1\beta_3 + 2.23\ \beta_1^2 + 2.53\ \beta_2^2 - 3.0\ e\text{-}^4\ \beta_3^2$	<0.0001*
Viscosity	$6.2 - 3.16\ \beta_1 + 0.03\beta_2 - 1.86\ \beta_3 - 3.58\ \beta_1\beta_3 + 1.43\ \beta_1^2 - 0.2\ e^{-3}\ \beta_2^2 + 0.02\ \beta_3^2$	<0.0001*
L* value	$5.14 + 3.06\ \beta_1 + 0.01\beta_2 - 0.08\ \beta_3 + 0.12\ \beta_1\beta_3 - 1.42\ \beta_1^2 - 0.1e^{-3}\ \beta_2^2 + 5e^{-4}\ \beta_3^2$	0.1245
Brix	$4.72 + 1.01\ \beta_1 + 0.01\ \beta_2 - 0.38\ \beta_3 + 3.13e^{-3}\ \beta_1\beta_3 + 1.19\ \beta_1^2 + 0.4\ \beta_3^2$	<0.0001*

β_1 = Enzyme concentration, β_2=Incubation time, β_3= Incubation temperature
*Significant at $p < 0.05$

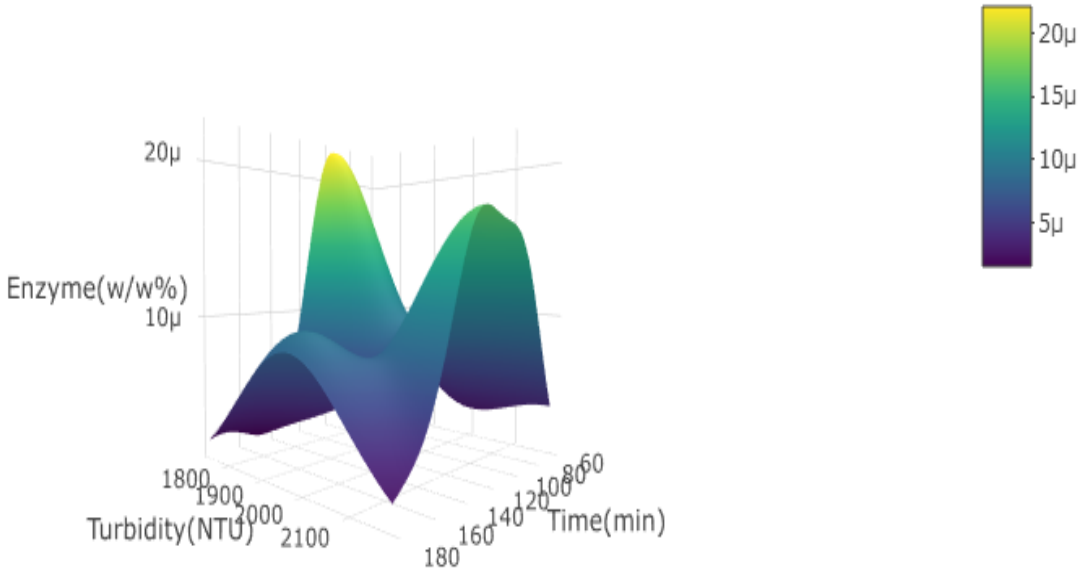

Figure 4. 3 Three dimensional plot for turbidity of wild watermelon juice as a function of enzyme concentration (w/w%) and incubation time at 30°C.

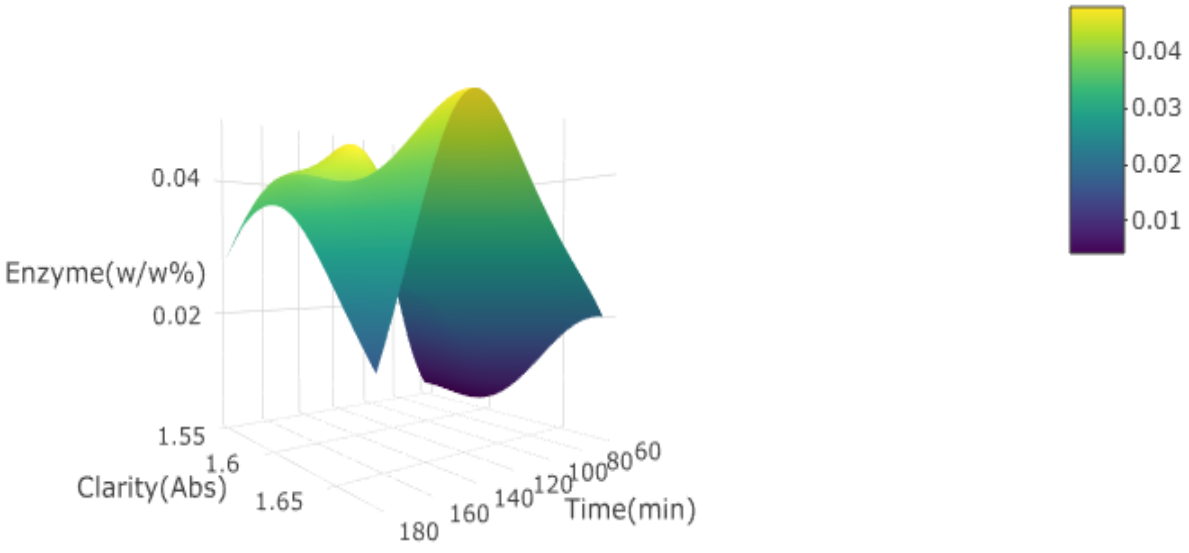

Figure 4. 4 Three dimensional plot for clarity at 660nm of wild watermelon juice as a function of enzyme concentration (w/w%) and incubation time at 30°C.

4.5 Effect of Treatments on the Clarity of Wild Watermelon Juice

The enzyme concentration and incubation time did not significantly ($p>0.05$) influence the clarity positively in both the linear and quadratic terms. The incubation temperature significantly ($p<0.05$) influenced the clarity negatively in the linear and quadratic terms. The interactive effect between enzyme concentration and incubation time significantly ($p<0.05$) influenced the clarity negatively. The other quadratic and interactive effects were not significant (Table 4.2). At 30°C, there was an increase in clarity over the first few minutes, which was followed by a decline (Figure 4.4).

4.6 Effect of Treatment on the Viscosity of Wild Watermelon Juice

The enzyme concentration and incubation temperature significantly ($p>0.05$) influenced the viscosity negatively in the linear terms but only the incubation time significantly ($p<0.05$) influenced viscosity negatively in the linear terms. The other quadratic and interactive effects were not significant (Table 4.2). At 30°C, there was an initial decrease in viscosity, which was followed by an increase in over a few minutes and then a final decline. The viscosity trend was not evident over different enzyme concentrations (Figure 4.5).

4.7 Effect of Treatment on the Lightness Value (L*) of Wild Watermelon Juice

The effect of all treatment (enzyme concentration, incubation temperature, and incubation time) on L* value was not significant ($p>0.05$) in a both linear and quadratic (Table 4.2). At 30°C, there was an initial increase in lightness over a few minutes, followed by a decline at high incubation durations. The lightness seems to be lower at higher enzyme (Figure 4.6).

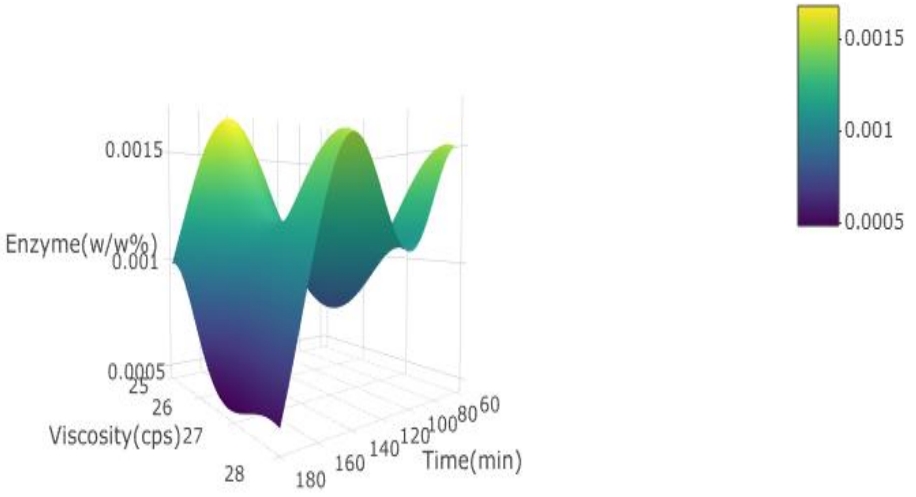

Figure 4. 5 Three dimensional plot for the viscosity of wild watermelon juice as a function of enzyme concentration (w/w%) and incubation time at 30°C.

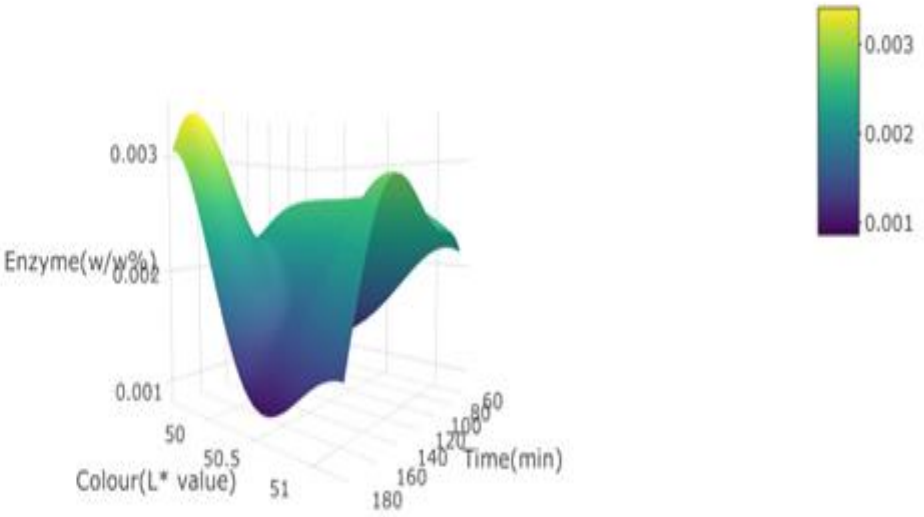

Figure 4. 6 Three dimensional plot for L* value of wild watermelon juice as a function of enzyme concentration (w/w%) and incubation time at 30°C.

4.8 Effect of Enzyme Concentration, Temperature, and Incubation Time on Brix of Wild Watermelon Juice

Both the enzyme concentration and incubation time significantly ($p<0.05$) influenced the brix positively in the linear terms. The incubation temperature did not significantly ($p> 0.05$) influence brix in the linear and quadratic term. The other quadratic and interactive effects were not significant ($p>0.05$) (Table 4.2). At 30 ^{o} C, brix seems to be higher at higher enzyme concentration and at higher incubation times (Figure 4.7).

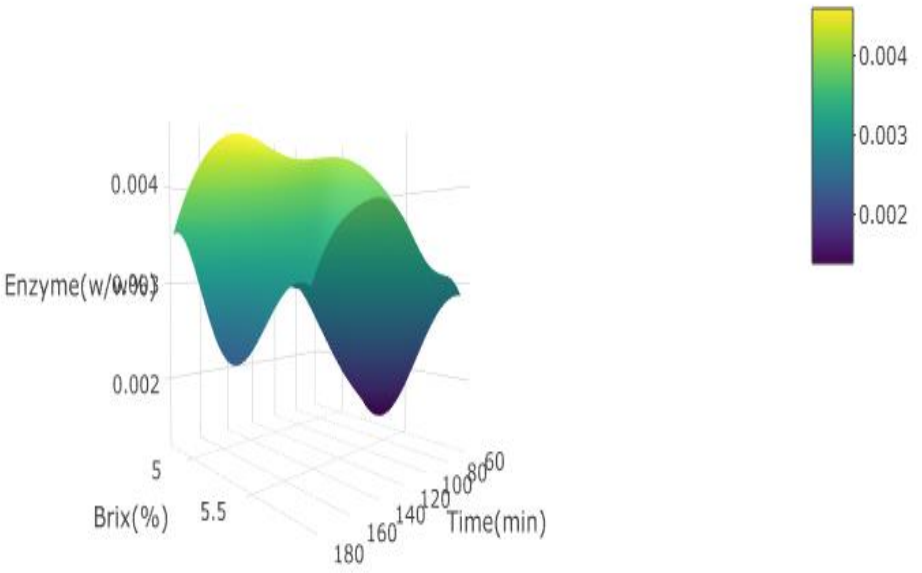

Figure 4. 7 Three dimensional plot for ^{o}Brix of wild watermelon juice as a function of enzyme concentration (w/w%) and incubation time at 30^{o}C.

4.9 Optimisation of Process Parameters

The optimum clarification condition was determined by superimposing the contour plots of all the responses. The final product was considered optimum if the turbidity, absorbance, viscosity, and brix were at the lowest, and L* value was at the highest. Therefore, the criteria implemented for obtaining the optimized parameters were minimum turbidity, minimum absorbance, minimum viscosity, minimum percent brix, and maximum L* value. The superimposed plot (Figure 4.8) showed the optimum output parameter to be as follows: turbidity: 1800 NTU; clarity: 1.55 Abs; L* value: 49.6 L value; viscosity: 25 cps; brix: 4.8%, and optimum parameters to be as follows: enzyme concentration 0.15 w/w%, temperature 30°C, incubation time 60 min, with the desirability value: 0.704.

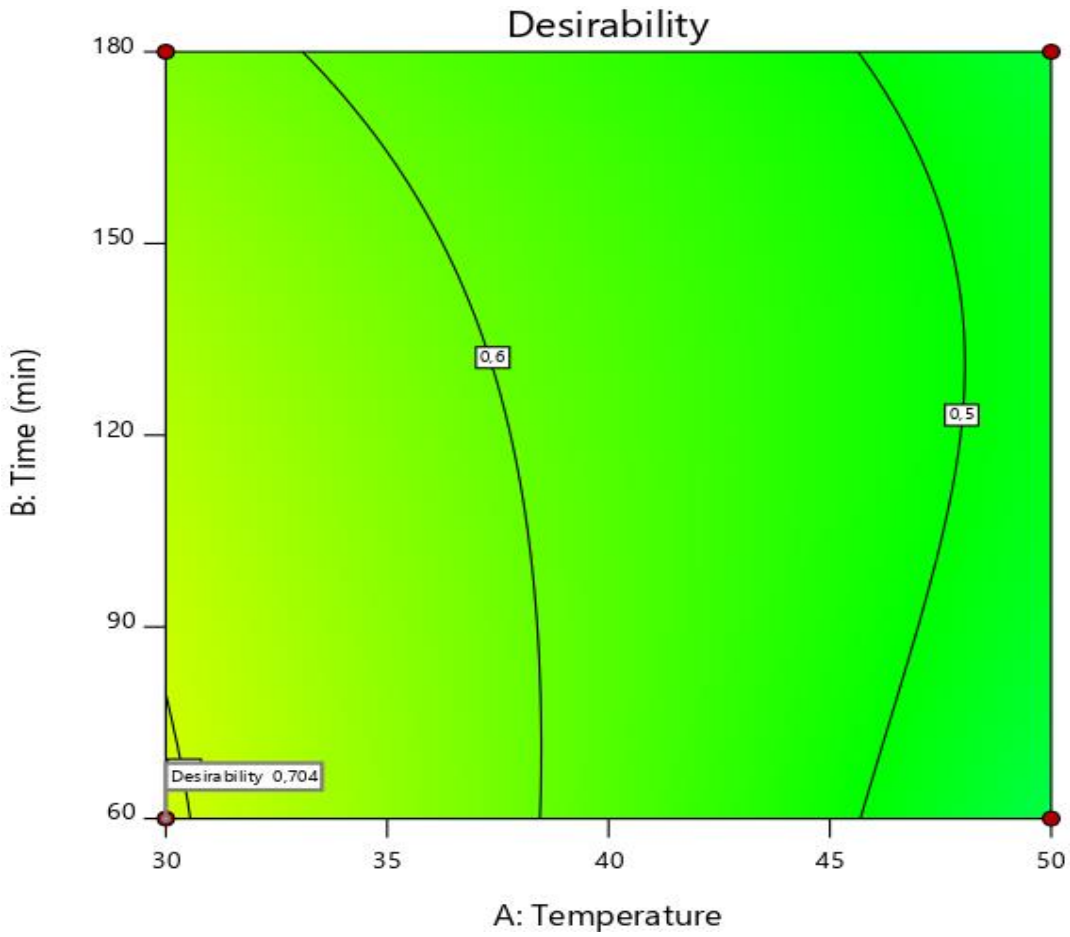

Figure 4. 8 Contour plots for optimum conditions of turbidity, clarity, L* value, viscosity and brix as a function of enzyme concentration and incubation time and incubation temperature at 30°C.

CHAPTER 5: DISCUSSION

5.1 Mineral Composition of Crude Wild Watermelon Juice

The most abundant mineral in the crude wild watermelon juice was potassium (39.300 mg/L), higher than those observed in grapefruit (24.50 mg/L) and lime juice (31.67 mg/L) (Chuku & Chinaka, 2014), but lower than those observed in apple juice (1068 mg/L) (Abid et al., 2014). Potassium is naturally present in fruits in the form of potassium salt of tartaric acid commonly known as potassium tartrate (Savic et al., 2015). The amount of potassium in the wild watermelon and other juices are not sufficient to contribute significantly to the recommended daily allowances per litre in females (2600 mg) and in males (3400 mg) (Stallings et al., 2019). Other macro minerals identified in wild watermelon juice were magnesium (2.2120 mg/L), sodium (1.890 mg/L), and calcium (1.420 mg/L). Adequate intake of minerals such as potassium, sodium, and magnesium helps to regulate blood pressure and reduce the risk of stroke, cardiovascular disease, coronary heart disease in adults (Steffensen et al., 2017). Sodium and potassium are structurally and chemically similar; hence they perform the same functions in cellular metabolism (Maathuis, 2014).

The calcium content of wild watermelon juice was lower than those of other fruits juices such as watermelon juice (3.70 mg/L) (John et al., 2018), orange (5.71 mg/L), apple juice (1.76 mg/L) (Efezino & Kofi, 2016), grape juice (1.615 mg/L), and lime juice (3.524 mg/L) (Chuku & Chinaka, 2014). Eksi and Kirtis (2016), established that sour cherry (35-198 mg/L), orange (5.71 mg/L), and apple juice (1.76 mg/L) were also higher, and like most other juices, they contained more calcium than the recommended daily allowance of 1 mg/L in adults (Food and Nutrition Board, 2005; Stallings et al., 2019). Calcium is an essential mineral that is required for bone mineralisation (Cano et al., 2018). The presence of minerals such as potassium, magnesium, sodium, calcium, iron, and phosphorus implies that the fruit is nutritious and can help fight mineral deficiency (Chuku & Chinaka, 2014).

The heavy metals analysed in wild watermelon juice were zinc (0.153 mg/L), manganese (0.0488 mg/L), and iron (0.487 mg/L). Essential metals must be present at lower concentration because if they are at high concentrations, depending on the group of metal they can become toxic and may cause undesirable health effects (Savic et al., 2015; Anastacio et al., 2018) such as iron poisoning, Parkinson type disease, and reduced levels of high-density lipoprotein (Fraga, 2005).

The zinc content of wild watermelon juice (0.153 mg/L) was comparable with that of contemporary watermelon juice (0.11 mg/L) (John et al., 2018). Higher zinc content was found in orange juice (1.38 mg/L) and apple juice (1.08 mg/L) (Efezino & Kofi, 2016) compared to wild watermelon juice. In support of this evidence higher quantities were also found in orange juice (1.64 -3.18 mg/L) (Savic et al., 2015), citrus burst (1.10 mg/L), passion fruit (1.80 mg/L), and ribenna (0.80 mg/L) juices (Ajai et al., 2014). Zinc is essential, as it is necessary for hundreds of enzyme functions that are responsible for critical metabolic pathways (Ackland & Michalczyk, 2016). The recommended daily allowance for zinc is 8 mg/L in female and 11mg/L in male adults (Institute for health of the national academies, 2011). The wild watermelon juice zinc content is fairly less than the recommended daily allowance.

The manganese content in wild watermelon juice (0.048 mg/L) was lower than those recorded in orange (0.06 mg/L) and apple juices (0.122 mg/L) (Anastacio et al., 2018). Manganese is an essential trace mineral necessary for primary metabolic functions and of phosphotranserase enzymatic activities in the body (Lemos et al., 2009). Manganese recommended daily allowance was found to be 1.8 mg/L in females and 2.3 mg/L in male adults, according to the Institute for health of the national academies (2011).

Iron content of wild watermelon juice was higher than those observed in other fruits such as: sweet orange (0.4 mg/L), lemon (0.45 mg/L) (Chuku & Akani, 2015). On the contrary higher quantities were observed on lime (0.50 mg/L) (Chuku & Akani, 2015), citrus (1.10 ± 0.04 mg/L, passion (1.80 ± 0.03 mg/L) and Ribena (0.80 ± 0.02 mg/L) fruit juice (Ajai et al., 2014). The

recommended daily allowance for iron was found to be 8mg/L in males and 18 mg/L in females (Institute for health of the national academies, 2011). Iron is an essential mineral that is fundamental for various enzyme activities and plays a major role in mitochondrial function. Iron deficiency usually leads to the cause of heart failure (Zhang et al., 2018).

Watermelons, in general, are not a rich source of nutrients (Schaffer & Paris, 2003); nevertheless, the mineral content observed in this study showed that wild watermelon juice is a source of vital nutrients that could supplement the diets of consumers.

5.2 Vitamin C Composition of Crude Wild Watermelon Juice

The wild watermelon juice had a low amount of vitamin C (2.990 mg/L) when compared to conventional watermelon (40.8 mg/L) (Nweze et al., 2015), lemon juice (130.5 mg/L), and orange juice (125.4 mg/L – 127.8 mg/ L) (Chuku & Akani 2015; Tareen et al., 2015). Other fruits juices such as grapefruit (109 mg/L), papaya (93.1 mg/L), and mango (78.4 mg/L) juices have been found to contain higher quantities of vitamin C as compared to wild watermelon juice (Tareen et al., 2015). Vitamin C is an essential antioxidant that plays a role in various enzymatic reactions and the biosynthesis of hormones and collagen metabolism (Lim et al., 2018). Additionally, it is also essential to prevent scurvy in humans and for the prevention of coronary heart disease, stroke, and cancer (Granger & Eck, 2017).

The vitamin C content of wild watermelon juice was substantially low in comparison with other fruits. According to Cardoso et al. (2011), fruits are generally good sources of vitamin C. It has been established by Cardoso et al. (2011) that cultivation methods did not have a direct influence on vitamin C quantity in fruits. Additionally, vitamin C is available in the following active forms: ascorbic acid and the oxidized form, dehydroascorbic acid. The low vitamin C content in wild watermelon might have been influenced by the cultivar of the fruit. Other watermelon cultivars were found to contain vitamin C content in the range of 5.28 – 9.39 mg/L (Johnson et al. 2013) . As stated by Cardoso et al. (2011), the cultivation method did not seem to provide a conclusive influence on vitamin C concentrations in fruits.

5.3 Proximate Composition of Crude Wild Watermelon Juice

The total sugar content of wild watermelon was slightly lower (4.295 g/100g) than that of conventional watermelon (5.22 – 5.29 g/100g) (Sogi et al., 2010) as well as other fruit juices such as orange juice (8.9 g/100g) and grapefruit (8.0 g/100g) (Zhang & Ritonour, 2016). Cultivated fruit juices have been found to be sweeter and more palatable than their wild counterparts (Erclisli & Sagbas, 2017; Ulijaszek et al., 2012) and the sugar profile of cultivativated fruit juices have been found to contain more disaccharide sucrose as opposed to more monosaccharides glucose and fructose in wild fruit juices (Ulijaszek et al., 2012).

The total fibre content of wild watermelon juice was higher (0.9985 g/100g) than those observed in conventional watermelon juices from different regions in Nigeria in which the total fibres values ranged from 0.24 to 0.33 g/100 g (Eziaghighala et al., 2010). Generally, wild fruits juices have been found to contain more fibre than their cultivated counterparts, and this has been partly attributed to the lack of adequate water supply during fruit development (Erclisli & Sagbas, 2017).

Total ash which is a measure of the amount of inorganic residue after the removal of water and organic matter was lower (0.200 g/100g) in wild watermelon that those of different varieties of conventional watermelon with values ranging from 0.78 to 0.86 g/100g (Eziaghighala et al., 2010). A similar result was also observed in pineapple juice (0.22 g/100g), apple juice (0.2207 g/100g), grapefruit juice (0.2642 g/100g), orange juice (0.3335 g/100g), and tangerine juice (0.3651 g/100g) (Corpas et al., 2012). The difference in ash quantities can be attributed to the fact that each fruit has unique mineral contents, as ash composition used to express mineral content of food (Corpas et al., 2012; Morais et al., 2017).

The total protein content of wild watermelon juice was 0.135 g/100g, and this was lower than values (0.48 -0.68 g/100g) obtained for conventional watermelon (Eziaghighala et al. 2010). As highlighted by Guevana-Figueroa et al. (2010) and Kindscher et al. (2018), it was found that wild fruits contained more protein than their cultivated counterparts. The higher protein content

in wild fruits as literature suggests, in comparison to their cultivated counterparts may be attributed to the climatic conditions of the plant and the soil water retaining capacity (Guevana-Figueroa et al., 2010). The soil is the primary source of nutrients to the plant with nitrogen being one of the major components of plant protein (Adamczyk et al., 2010). As it has been confirmed, the indigenous crops are usually adapted to their local climate, soil and are more tolerant to the local environmental stress (Shelef et al., 2017). The wild watermelon that was used for this study did not contain the higher protein quantity as expected. It may be relevant to suggest that the lower protein quantity in the wild watermelon, was caused by lack of adequate nurients in the soil such as nitrogen and others that are necessary for plant protein synthesis.

The fat content of wild watermelon juice was 0.10 g/100g, and this was lower compared to that observed (range 0.25-0.35g/100g) in different varieties of watermelon from Nigeria (Eziaghighala et al., 2010). The lower fat content may have been caused by the soil type where the wild watermelon germinated.

5.4 Turbidity, Viscosity, Brix and L* Value of Crude Wild Watermelon Juice

The turbidity of wild watermelon juice (3208.500 NTU) was higher in comparison to that observed in conventional watermelon (1502 NTU) (Koutchma et al., 2009). The higher turbidity in wild watermelon juice may be attributed to the presence of higher levels of pectin polysaccharides, tannin, and metals suspended in the juice (Ucan et al., 2014; Verma et al., 2018). It has been found that pectin can determine the water holding capacity within the cell (Voragen et al., 2009) because of the presence of hydrophilic (OH^-) groups (Kumar, 2015). In addition, pectin was found to increase plant tissue firmness, by taking part in intracellular adhesion and mechanical resistance of the cell wall. As a consequence, effecting alevated drought resistance and low-temperature resistance within the cell wall of the plant (Lara-Espinoza et al., 2018). Native plants have also been found to be more prone to drought-tolerant and climate change (Shelef et al., 2017). These can be used to substantiate the fact that native plants have more turbidity than conventional plants. Wild watermelon juice with lower

turbidity is desirable to consumers, has a smooth mouthfeel, and clarity (less cloudy) (Kumar, 2015). High turbidity which usually appears as being cloudy in wild watermelon juice, may contribute to poor juice quality appearance to the consumer and may also indicate insufficient processing as well as poor enzyme application (Ashurst et al., 2017).

The viscosity of wild watermelon juice was found to be higher (62.500 cps) than that of conventional watermelon juice (6.72 cps) (Koutchma et al., 2009). The higher viscosity in wild watermelon juice was caused by the availability of pectin polysaccharide (Rai et al., 2004). Pectin has a fiber-like structure that makes the juice to have a higher viscosity. High viscosity value is caused by increased resistance to flow as a result of internal friction between the molecules caused by the cohesive forces that hold molecules together in liquids (Rai et al., 2004; Nawfel, 2017). Furthermore, the high fibre content of wild watermelon (fibre content 0.9985 ± 0.01 g/100g) may contribute to internal friction between the molecules in wild watermelon juice compared to conventional watermelon juice (fibre content 0.24 to 0.33 g/100 g).

The quality of natural juices is usually attributed to its viscosity and better mouthfeel, which is desirable to consumers (Kimball, 1991) and they are also considered to be less artificial (Wlodarska et al., 2016). The higher viscosity which was also found in wild watermelon juice would be considered appealing and can be presumed to be nutritious.

The brix of the crude wild watermelon juice (7.285 %) was less than the conventional watermelon juice from different varieties (9.1- 11.4 %) (Pardo et al., 1997). The percent brix represents the concentration in weight of dissolved solids such as sucrose, fructose, vitamins, minerals, amino acids, proteins, hormones, and other nutrients in a juice (Ugwu et al., 2018). It is estimated that 80 % of the brix is constituted of the natural sugars that give the fruit its flavour and taste (Ugwu et al., 2018). As explained by Xaba and Croeser (2012), wild watermelon is less sweet, has a pale-coloured flesh and has a lower concentration of minerals and vitamins than the conventional watermelon therefore the lower percent brix value thereof seems fitting.

The lower percent brix in wild watermelon juice has contributed to the fact that the fruit is less sweet and the fruit is not receiving much consumer acceptance. Natural Fruit juice with higher sucrose content also was seen to receive more preference by Wlodarska et al. (2016).

Absorbance or clarity also referred to as cloud stability of wild watermelon juice was found to be 2.059 abs (at 660nm) (Saxena et al., 2014). In another study conducted by Bhattacharjee et al. (2017), clarity of conventional watermelon juice measured at 660nm was found to be 2.11 Abs. The two juices (wild watermelon and conventional watermelon) had almost similar clarity.

The L* value of wild watermelon juice was found to be 51.61, and this was darker compared to that observed on watermelon juice (32.60) (Panda, 2013). The L* value of wild watermelon represented less desirable quality attributes. Usually, brightly coloured juices receive more consumer acceptance (Fernandez-Vazquez et al., 2014).

5.5 Effects of Enzymatic Treatment on Wild Watermelon Juice

5.5.1 Statistical analysis

The coefficient of determination, R^2, is defined as the measure of the proportion of variation in the dependent variable that is predicted in the independent variable and is well explained in linear regression models (Zhang & Ritonour, 2016).

As shown on Table 4.2, the model was statistically significant in predicting turbidity (0.86), clarity (0.81), viscosity (0.97), brix (0.94) because the regression coefficients' (R^2) was higher than 0.8. Indicating that the model was a good fit. The model was not statistically signicant in predicting colour (0.24) as regression coefficients' (R^2) was lower than 0.8. When R^2 reached unity the better was the empirical model and the smaller the R^2 value, was the lesser the prediction of the dependent variable in the model in elaborating the variation behaviour (Sin et al., 2006).

5.5.2 Turbidity

The enzyme concentration did not significantly (p <0.05) affect turbidity in both the linear and quadratic terms. Turbidity is a measure the number of particles that are suspended, such as pectin in a liquid (Saxena et al., 2014). The breakdown unit of pectin polysaccharide by pectinase (Ucan et al., 2014) may have been subjected to flocculation and sedimentation as soon as they are produced hence the reason why turbidity was not significantly affected in this study (Saxena et al., 2014). The composition and the structural feature of the hydrolysed pectin particles may be different depending on the juice processing treatment and pH of the juice (Hanine et al., 2016). On the contrary, an increase in enzyme concentration significantly decreased turbidity of pomegranate juice (Cerreti et al., 2016; Hanine et al., 2016) and watermelon juice (Saxena et al., 2014), and this can be attributed to the fact that hydrolysed pectin polysaccharide units were subjected flocculation and sedimentation which is usually accompanied by the reduction of turbidity (Verma et al., (2018).

The incubation time significantly (p<0.05) affected turbidity positively in the linear terms, and this can be attributed to the degradation of the pectin polysaccharide into fragments over time which increase turbidity the of wild watermelon juice at the initial stages (Hanine et al., 2016). At 30°C, there was an increase in turbidity over the first few minutes, which was followed by a decline due to flocculation and sedimentation. The incubation temperature significantly (p<0.05) affected turbidity negatively in the linear term. This can be attributed to the formation of a gel by pectin polysaccharide and related hydrolysed particles which in turn lead to agglomeration and sedimentation hence reducing turbidity with an increase in temperature (Hanine et al., 2016; Grassin & Fauquembergue, 1996). The temperature has also been found to negatively affect the turbidity of watermelon juice and pomegranate juice (Hanine et al., 2016; Saxena et al., 2014).

5.5.3 Clarity

The enzyme concentration and incubation time did not significantly ($p>0.05$) affect clarity positively in both linear and quadratic terms. Like with turbidity, an increase in the enzyme concentration did not affect clarity since a less clear juice would mean it is less turbid (Abdullah et al., 2007). As explained previously, the product of pectin polysaccharide degradation may have been subjected to agglomeration and precipitation as soon as they are produced due to increase in temperature (Hanine et al., 2016; Joshi et al., 2011). Abdullah et al. (2007) profiled a similar effect (an insignificant increase in clarity with the increase in time) in linear terms on carambola juice. The incubation temperature significantly ($p<0.05$) affected clarity negatively in both the linear and quadratic terms, and this can be attributed to the agglomeration and precipitation as a result of gelation of pectin and its breakdown product (Hanine et al., 2016, Grassin & Fauquembergue, 1996).

The fact that the interactive effect between enzyme concentration and incubation time significantly influence the clarity negatively implies that the breakdown of pectin polysaccharide takes place over time, but flocculation, agglomeration, and sedimentation process reduces clarity due to the gelation effect of temperature. This also explains the increase in clarity over the first few minutes, which was followed by a decline at 30 °C. A similar effect was reported by Tapre and Jain (2014) and Abdullah et al. (2007) on banana juice and carambola juice respectively.

5.5.4 Viscosity

The enzyme concentration did not significantly ($p <0.05$) influence the viscosity negatively in the linear terms. The decrease in viscosity may have been influenced by the disintegration of a cohesive network of pectin polysaccharide by pectinase reducing its water holding capacity hence reducing the viscosity of wild watermelon juice (Saxena et al., 2014). The enzyme concentration and incubation time did not significantly ($p>0.05$) affect viscosity positively in the linear terms, and this is because of the breakdown unit of pectin polysaccharide undergoing

flocculation, agglomeration, and sedimentation as soon as they are produced due to the gelation effect of temperature (Sogi et al., 2010)

Agglomeration could be more in wild watermelon juice than the conventional watermelon juice because they grow in the wild and are more drought-resistant and have more water holding capacity than their cultivated counterparts. Additionally, soft fruits contain less pectin than hard fruits; additionally, pectin provides a source of fibre, as explained by Lara Espinoza et al. (2018). If wild fruits contain more pectin, it may imply that more agglomeration will be effected.

5.5.5 Colour (L* value)

The effect of all treatment (enzyme concentration, temperature, and incubation time) on L* value was not significant ($p = 0.01245$) in both the linear and quadratic manner. This may be attributed to the fact that most of the dominant pigments in wild watermelon juice are not the typical substrate of pectinase enzyme. At 30 ^{o}C, there was an initial increase over a few minutes, followed by a decline at high incubation durations. The L* value seems to be lower at higher enzyme concentration (Figure 4.6).

5.5.6 Percent brix

Both enzyme concentration and incubation time significantly ($p< 0.05$) influenced the percent brix positively in the linear terms. An increase in the enzyme concentration over time boosted the action of pectinase on pectin molecules of the juice. This phenomenon is explained by (Saxena et al., 2014), that the increased pectinase action will as a consequence give effect to the degradation of pectin molecule resulting in the release of the dissolved substances such as B vitamins, minerals, and vitamin C. Pectinase action will also cause the increase in soluble sugars content and the breakdown of cellulose on the inside of the cell wall (Rombouts & Pilnik, 1978; Arsad et al., 2015). The degradation and separation of the cell wall eventually affect the release of the nutritional composition of the interior cells (Norjana & Noor Aziah,

2011; Qin et al., 2005). A similar effect was observed by Saxena et al. (2014) on watermelon juice. Ngadze et al. (2018), also recorded an increase in brix percentage after pectinase enzyme exposure on Monkey Orange species (*Strychnos cocculoides*) juice. Additionally, a similar effect was also reported by Joshi et al. (2011) on apricot, pear, peach, and plum juices. The incubation temperature did not significantly ($p > 0.05$) influence percent brix in the linear and quadratic terms.

5.6 Comparing Optimised Response Parameter to that of Untreated Wild Watermelon Juice

The enzymatic treatment of wild watermelon juice resulted in a decrease in turbidity and viscosity. A similar effect was also reported on blueberry, blackcurrant, and raspberries (Bender et al., 2016). This was caused by the breakdown of pectin polysaccharides in juice. The effect of temperature on turbidity reduction was also observed at a higher temperature (90°C), where a profound reduction was demonstrated. There was a reduction in percent brix after enzymatic application. A similar effect was also reported on raspberry juice (Bender et al., 2016).

On the contrary, blackberry and blackcurrant juices showed an increase in percent brix. This may serve to suggest that, soluble solutes are being released into the juice upon enzyme application. The number of soluble solids released depend on the fruit type (Bender et al., 2016). There was a reduction in juice clarity on wild watermelon juice after the enzyme application. A similar effect was also reported by Landbo and Meyer (2004) on blackcurrant juice. Concerning turbidity, clarity provides the absorbed radiation from an incident beam that travels in a straight path, whereas turbidity is the light scattered at 90 degrees from the incident beam (Umsza-Guez et al., 2011). An increase in lightness (L* value) of crude wild watermelon juice was observed. This was in agreement with a finding by Liao et al. (2007) on carrot juice. Lightness was shown to have the same dependence as turbidity and viscosity, as they have the same properties and in a way measure the interference of the sample in an incident ray (Umsza-Guez et al., 2011) (Table 5.1).

Table 5. 1 Comparing optimized parameters of wild watermelon juice to that of untreated juice.

Parameters	**Crude Juice**	**Optimised Parameters**
Turbidity (NTU)	3208.500 ± 1.712	1800 ± 0.001
Viscosity (cps)	62.500 ± 0.707	25 ± 0.5
Brix (%)	7.285 ± 0.007	4.8 ±0.025
Clarity (Abs)	2.059 ± 0.023	1.55 ± 0.03
Colour (L* value)	51.61 ± 0.099	49.6 ± 0.1

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Proximate, mineral and physiochemical analysis of crude wild watermelon juice helped in understanding the chemical properties of wild watermelon juice. The proximate and mineral analyses showed that crude wild watermelon juice contained highest total sugars content (4.295 g/100g) and highest potassium (39.300 mg/L) content. Enzymatic treatment showed that incubation temperature significantly reduced turbidity, viscosity, and clarity of wild watermelon juice and incubation time significantly increased turbidity and brix.

Statistical analysis was conducted by employing the Response Surface Model tool. The optimum process parameters (incubation time, incubation temperature and enzyme concentration) for enzymatic clarification of wild watermelon juice established were found to be useful in helping to undertand juice clarification process. The three dimensional plots for individual response parameters (turbidity, clarity, viscosity, colour and brix) against the independent variables (time and concentration) designed were able to display the relationship between the different variables.

The recommended enzyme clarification conditions was at lower incubation temperature (30^{o} C), lower incubation time (60 min), and higher enzyme concentration (1.5 w/w %). The desirability function of the combined conditions for juice clarification was 0.704. For product development of wild watermelon juices, the optimum parameters would be useful in juice clarification processes in order to obtain the best quality of the juices. It is essential that future studies determine for improved clarification of the juice. It is also required to improve the sensory quality of the juice so that it can be more palable to better consumer acceptance.

REFERENCES

Abbah, O.C., Musa, A.D., Ejembi, D., Omalei, J., Sanni, M., Olajide, J.E. & Ojogbane, B.E. 2015. Medicinal Utility of Egusi Melon (*Citrullus colocynthis L*.). *International Journal of Current Research in Bioscience and Plant Biology*, 2(7):185-191.

Abdullah, A.G.W., Sullaiman, N.M., Auroua, M.K. & Megat Mohd Noor, M.J. 2007. Response surface optimization of conditions for clarification of carambola fruit juice using a commercial enzyme. *Journal of Food Engineering*, 81: 65-71.

Abid, M., Jabbar, S., Wu, T., Hashim, M.M., Hu, B., Lei, S. & Zeng, X. 2014. Sonication enhances polyphenolic compounds, sugars, carotenoids, and mineral elements of apple juice. *Ultrasonics Sonochemistry*, 21: 93-97.

Ackland, M.L. & Michalczyk, A.A. 2016. Zinc and infant nutrition. *Archives of Biochemistry and Biophysics*, 611: 51-57.

Adamczyk, B., Smolander, A., Kitunen, V. & Mirosław Godlewski, M. 2010. Proteins as nitrogen source for plants, A short story about exudation of proteases by plant roots. *Plant Signal Behav,* 5(7): 817–819.

Adepoju, O.T. 2009. Proximate composition and micronutrient potentials of three locally available wild fruits in Nigeria. *African Journal of Agricultural Research,* 4 (9): 887-892.

Aggarwal, B.B., Sundaram, C., Malani, N. & Ichikawa, H. 2007. Curcumin, "the Indian Solid Gold". *Advances in Experimental and Medical Biology*, 595: 1-75.

Aghajanzadeh, S., Ziaiifar, A.M., Kashaninejad, M., Maghsoudlou, Y. & Esmailzadeh, E. 2016. Thermal inactivation kinetic of pectin methylesterase and cloud stability in sour orange juice. *Journal of Food Engineering*, 185: 72-77.

Ajai, A.I., Ochigbo, S.S., Abdallahu, Z. & Anigboro, P.I. 2014. Determination of trace metals and essential minerals in selected fruit juices in Minna, Nigeria. *International Journal of Food Science,* Article ID 462931.

Akhtar, M., Chan, P., Safriani, N., Murray, B. & Clayton, G. 2011. Concentration of apple juice using spinning disc reactor technology. *Journal of Food Processing and Technology,* 2:108.

Akinnifesi, F.K., Ajayi, O.C., Sileshi, G., Kadzere, I. & Akinnifesi, A.I. 2007. *Domesticating and Commercializing Indigenous Fruit and Nut Tree Crops for Food Security and Income Generation in Sub-Saharan Africa*. Department of Agricultural Research and Extension, Ministry of Agriculture, Harare, Zimbabwe. Unpublished.

Akkaracheneeyakorn, S. & Tinrat, S. 2015. Effects of types and amounts of stabilisers on physical and sensory characteristics of cloudy ready-to-drink mulberry fruit juice. *Food Science and Nutrition,* 3(3).

Alam, M.K., Hoque, M.M., Morhed, S., Akter, F. & Sharmin, K.N. 2013. Evaluation of watermelon (Citrus lanatus) juice preserved with chemical preservatives at refrigeration temperature. *J. Sci. Res,* 5(2): 407-414.

Alvarez, R., Carvalho, C.P., Sierra, J., Lara, O., Cardona, D. & Londono-Londono, J. 2012. Citrus juice extraction systems: effect on chemical composition and antioxidant activity of clementine juice. *Journal of Agriculture and Food Chemistry,* 60(3): 774-781.

Ambroziak, W. 2010. Enzymatic liquefaction of apple pomace. *Food Chemistry and Biotechnology*, 74(1081).

Anastacio, M., Dos Santos A.P.M., Aschner, M. & Mateus, L. 2018. Determination of trace metals in fruit juices in the Portuguese market. *Toxicology Reports,* 5: 434-439.

Aneja, K.R., Dhiman, R., Aggarwal, N.K., Kumar, V. & Kaur, M. 2014. Microbes associated with freshly prepared juices of citrus and carrots. *International Journal of Food Science*, article id. 408085.

Anvari, M. & Khayati, G. 2014. The effect of citrus pulp type on pectinase production in solid-state fermentation: Process evaluation and optimization by Taguchi design of experimental (DOE) methodology. *Journal of BioSci. Biotech*, 3(3): 227-233.

Arsad, P., Sukor, R., Wan Ibadullah, W.Z., Mustapha, N.A. & Meor Hussin., A.S. 2015. Effects of enzymatic treatment on physiochemical properties of sugar palm fruit juice. *International Journal on Advanced Science Engineering Information Technology*, 5(5): 308-312.

Ashurst, P.R., Hargritt, R. & Palmer, F. 2017. Fruit juices and fruit nectars'. Soft Drinks and Fruit Juice Problems Solved , 2nd edition. Woodhead Publishing Series in Food Science, Technology and Nutrition.137-144.

Aworh, O.C. 2015. Promoting food security and enhancing Nigeria's small farmer's income through value-added processing of lesser-known and underutilised indigenous fruits and vegetables. *Food Research International*, 76: 986-991.

Bajwa, K., Bishnoi, N.R., Kirrolia, A., Gupta, S.& Selven, S.T. 2019. Response surface methodology as a statistical tool for optimization of physio-biochemical cellular components of microalgae *Chlorella pyrenoidosa* for biodiesel production. *Applied Water Science*, 9 (128).

Bartozek, M. & Polak, J. (2016). A comparison of antioxidative capacities of fruit juices, drinks and nectars, as determined by EPR and UV-vis spectroscopies. *Spectrochim Acta A Mol Biomol Spectrosc*, 153: 546-549.

Bates, R.P., Morris, J.R. & Crandall P.G. 2001. *Principles and Practices of small and medium scale fruit juice processing*. University of Florida, United States: Rome.

Bayer, E.A., Chanzy, H., Lamed, R. & Shoham, Y. 1998. Cellulose, cellulases, and cellulosomes. *Curr. Opin. Struct. Biol*, 8(5): 548-557.

Bemiller J.N. 2019. Pectins. In: Carbohydrate for food scientists. 2nd ed. 303-312. Available from: https://www.sciencedirect.com/topics/food-science/pectin [Accessed on: 15 May 2019].

Bhat, M.K. 2000. Cellulases and related enzymes in biotechnology. *Biotechnology Advances*, 18:355-383.

Bhattacharjee, C., Saxena, V.K. & Dutta, S. 2017. Analysis of fouling and juice quality in crossflow ultrafiltration of watermelon juice. *Food Science and Technology,* 38:71-76.

Bender, C., Killermann, K.V., Rehmann, D. & Weidlich, H.H. 2016. Effect of mash enzyme and heat treatments on the cellular antioxidant activity of blackcurrant (Ribes nigrum), raspberry (Rubus idaus), and blueberry (Vaccinium myrtillus) juices. *Journal of Food*, 15(2): 277-283. doi: 10.1080/19476337.2016.1247914.

Blitz, M. 2017. *The ancient, mysterious history of the watermelo*n. Available from: https://www.foodandwine.com/lifestyle/ancient-history-watermelon?utm_campaign=foodandwineandutm_source=facebook.comandutm_medium=socialandxid=soc_socialflow_facebook_fw. [Accessed on: 06 June 2018].

Brause, A.R., Woollard, D. & Indyk, H.E. (2003). Determination of Total Vitamin C in Fruit Juices and Related Products by Liquid Chromatography: Interlaboratory Study. *Journal of AOAC International*, 86(2), 367-374.

Campell, M. 2006. *Extraction of pectin from watermelon rind*. Unpublished Master of Science Project report. Oklahoma State: University of Oklohama State, School of Science and Bioengineering. University.

Cano, A., Chedraui, P., Goulis, P.G., Lopes, P., Mishra, G., Mueck, A., Senturk, L.M., Simoncinin, T., Stevenson, J.C., Stute, P., Tuomikoski, P., Rees, M. & Lambrronondaki, I. 2018. *Maturitas*, 107: 7-12.

Cardoso, C.P., Tomazini A.P.B., Stringheta, P.C., Ribeino, S.M.R. & Pinherro-Saint'Anna H.M. 2011. Vitamin C and carotenoids in organic and conventional fruits grown in Brazil. *Food Chemistry*, 126(2): 411-416.

Castro Domingues, R.C., Faria Junior, S.B., Silva, R.B., Cardoso, V.L. & Miranda Reis, M.H. 2011. Clarification of passion fruit juice with chitosan: Effects of coagulation process variables and comparison with centrifugation and enzymatic treatments. *Process Biochemistry*, 47: 467-471.

Cerreti, M., Liburdi, K., Benucci, I. & Esti, M. 2016. The effect of pectinase and protease treatment on turbidity and on haze active molecules in pomegranate juice. *Food Science and Technology*, 73: 326-333.

Chivandi, E., Mukonowenzou, N., Nyakudya, T. & Earlwanger, K.H. 2015. Potential of indigenous fruit-bearing trees to curb malnutrition, improve household food security, income and community health in Sub-Saharan Africa: A review. *Food Research International*, 76: 980-985.

Chivenge, P., Mabhaudhi, T., Mudi, A. & Mafongoya, P. 2015. The Potential Role of Neglected and Underutilised Crop Species as Future Crops under Water Scarce Conditions in Sub-Saharan Africa. *International Journal of Environmental Research and Public Health*, 12(6): 5685–5711.

Chuku, E.C. & Akani, N.P. 2015. Determination of proximate composition and microbial contamination of fresh juice from three citrus species. *Journal of Biology and Genetic Research*, 1(8).

Chuku, L.C. & Chinaka, N.C. 2014. Protein and Mineral element levels of fruit juices (citrus spp.) in some Niger Delta areas of Nigeria. *International Journal of Nutrition and Food Sciences*, 3(6-1): 58-60.

Corpas, L., Velciov, A., Rivis, A., Olariu, L., Gravila, C. & Ahmadi, M. 2012. Physico-chemical characterization of some fruits juices from Romanian hypermarket fruit. *Journal of Agroalimentary Processes and Technologies*, 18(1): 95-99.

Damayanti, S., Permana, B. & Chie Weng, C.(2012). Determination of sugar content in fruit juices using high performance liquid chromatography. *Acta Pharmaceutica Indonesia*, 37(4).

Dauthy, M.E. 1995. *Fruit and Vegetable Processing,* Chapter 3. Food and Agriculture Organization of the United Nations, Rome.

Dawes, H, P., Struebi, P. & Keene, J. 1994. *Journal of Food Science,* 59(4): 859-861.

Department Of Agriculture Forestry And Fisheries. 2013. *Bitter Watermelon*. Available from: https://www.daff.gov.za/Daffweb3/Portals/0/Brochures%20and%20Production%20guidelines/Brochure%20Bitter%20watermelon.pdf [Accessed on: 23 June 2016].

Dane, F. & Liu, J. 2007. Diversity and origin of cultivated and citron type watermelon (Citrullus Lanatus). *Genetic Resources and crop evolution*, 54(6):1255-1265.

Drozdz, D., Sirgedaite-Seziene, V. & Pyrzzynska, K. 2017. Mineral Composition of Wild and Cultivated Blueberries. *Biological Trace Element Research,* 181(1).

Dweba, T.P. & Mearns, M.A. 2011. Conserving indigenous knowledge as the key to the current and future use of traditional vegetables. *International Journal of Information Management*, 31: 564-571.

Echavarria, A.P., Torras, C., Pegan, J. & Ibarz, A. (2011). Fruit Juice Processing and Membrane Technology Application. *Food Engineering Reviews*, 3:136-158, doi: 10.1007/s12393-011-9042-8.

Erclisli, S. & Sagbas, H.I. 2017. Wild Edible Fruits: A Rich Source of Biodiversity. *Anadolu. J. of AARI*, 27 (2).

Efezino, A & Kofi, E.A. 2016. *A comparative study of the nutritional quality of freshly extracted juices from organic versus conventional orange and apple fruits.* Glasgow Caledonian University, Scotland.

Eksi, A. & Kirtis, E.B.U. 2016. Determination of the mineral profile and prediction of the fruit content in sour cherry juice. *European Journal of Food Science and Technology*, 4(1): 52-60.

Erhirhie, E.O. & Ekene, N.E. 2013. Medicinal values on Citrus Lanatus (Watermelon): Pharmacological review. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 4(4): 1305-1312.

Eziaghighala, O.Y., Iwe, M.O. & Agiri, A.N. 2010. Proximate and sensory properties of fruit juice produced from varieties of watermelon (Citrullus lanatus). *Nigerian Food Journal*, 28(2).

Fang, G. & Grumet, R. 1993. Transformation in Muskmelon (Cucumis Melo L.). *Biotechnology in agriculture and forestry*, 23:209-214.

Fernandez-Vazquez, R., Hewson, L., Fisk, I., Vila, D.H., Mira, J.H., Vicario, I.M. & Hort, J. 2014. Colour Influences sensory perception and liking of orange juice. *Flavour*, vol.3, no. 1 Available from: https://flavourjournal.biomedcentral.com/articles/10.1186/2044-7248-3-1 [Accessed on: 11 May 2019].

Food and Agriculture Organization of the United Nations. 2014. *The State of Food Insecurity in the World*. Available from: www.fao.org/3/a-i4646e.pdf [Accessed on:25 April 2017].

Food and Nutrition Board; Institute of Medicine. 2005. *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulphate*; The National Academies Press: Washington, DC, USA.

Food and Agriculture Organization, International Fund for Agricultural Development, World Food Program. 2015. *The state of food insecurity in the world 2015. Strengthening the enabling environment for food security and nutrition. Rome*: FAO.

Food and Nutrition Board. 2005. *Dietary Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids.* Available from: https://www.nap.edu/catalog/10490/dietary-reference-intakes-for-energy-carbohydrate-fiber-fat-fatty-acids-cholesterol-protein-and-amino-acids [Accessed on: 27 January 2019].

Fraga, C.G. 2005. Relevance, essentiality, and toxicity of trace elements in human health. Molecular aspects of medicine, 26(1-4): 235-244.

Garcia, C.A. 2017. *Application of enzymes for fruit juice processing*. Fruit juice extraction, composition, quality analysis, Chapter11. Academic Press, Ireland.

Garcia, O.E., Infante, R.B. & Rivera, C.J. (1997). Determination of total, soluble and insoluble dietary fibre in two new varieties of Phaseolus vulgaris L. using chemical and enzymatic gravimetric methods. *Food Chemistry*, 59(1). 171:174.

Geta, K. 2015. Microbiological safety of fruit juices consumed in cafes and restaurants of debre-makros town, north western Ethiopia. *Department of Biology, School of Graduate Studies*, Haramaya University.

Granger, M. & Eck, P. 2017. Chapter Seven - Dietary Vitamin C in Human Health. *Advances in Food Nutrition and Reseach*, 83: 281-310.

Grassin, C. & Fauquembergue, F. 1996. Fruit Juices. In Godfrey T, West S and West S ed(s). *Industrial Enzymology, 2nd Ed.* Stockholm Press: New York.

Guevana-Figueroa, T., Jimenez-Islas, H., Reyes-Escogido M.L., Mortensen, A.G., Laursen, B.B., Lin, L., De Leon Rodriguez, A., Fomsgaard, I.S. & Baba De La Rosa, A.P. 2010. Proximate composition, phenolic acids and flavonoids characterization of commercial and wild nopal (*opuntia spp.). Journal of Food Composition and Analysis*, 23: 525-532.

Hanine, H., Hmid, I., Elothmani, D. & Oukabli, A. 2016. Effects of enzymatic clarification of pomegranate juice by protease and pectinase treatments. *Journal of Bio.Innov*, 4(4): 506-515.

Harzallah, A., Bhouri, A.M., Amri, Z., Soltana, H. & Hammami, M. 2016. Phytochemical content and antioxidant activity of different fruit parts juices of three figs (Ficus carica L.) varieties grown in Tunisia. *Industrial Crops and Products*, 83: 255-267.

Hintz, T., Matthews, K.K. & Di, R. 2015. The Use of Plant Antimicrobial Compounds for Food Preservation. *Biomed Res Int,* 246264.

Holderbaum, D.F., Kon, T., Kudo., T. & Guerra, M.P. 2010. Enzymatic Browning, Polyphenol Oxidase Activity, and Polyphenols in Four Apple Cultivars: Dynamics during Fruit Development. *HortScience*,45:1150-1154.

Hughes, A. & Haq, N. 2003. Promotion of indigenous fruit trees through improved processing and marketing in Asia. T*he International Forestry Review*, 5(2): 176-181.

Institute of Medicine of the National Academies. 2011. Dietary Reference Intakes (DRI) reports. Available from: https://onlinelibrary.wiley.com/doi/pdf/10.1002/9780470775011.app3 [Accessed on:13 March 2019].

Jacob, J.O., Mann, A., Adeshina, O.J. & Ndamitso, M.H. 2016. Nutritional Composition of selected wild fruit from Minna area of Niger State, Nigeria. *International Journal of Nutrition and Food Engineering*, 10(1).

Jae-Min, S. & Seun-Kook, P. 2015. Comparison of fat determination methods depending on fat definition in bakery products. *Food Science and Technology*, 63*(*20): 972-977.

Johansson, S., Diehl, B., Christakopoulos, P., Austin, S. & Vafiadi, C. 2016. Oligisaccharide synthesis in fruit juice concentrates using glucansucrase from lactobacillus reuteri 180. *Food and Bioproducts Processing*, 98: 201-209.

John, P.G., Yusufu, M.I. & Aheman, S.A. 2018. Production and quality evaluation of wine from watermelon juice and ginger extract. *Journal of Human Nutrition and Food Sceince*, 6(1): 1122.

Johnson, J.T., Lennox, J.A., Ujong, U.P., Odey, M.O., Fila, W.O., Edem, P.N & Dasafunjo,K. (2013). Comparative Vitamins Content of Pulp, Seed and Rind of Fresh and Dried Watermelon (Citrullus Lanatus). *International Journal of Science and Technology,* 2(1).

Joshi, V., Parmar, M. & Rana, N. 2011. Purification and characterization of pectinase produced from apple pomace and evaluation of its efficacy in fruit juice extraction and clarification. *Indian Journal of Naturally Produced Resources*, (2)2:189-197.

Javammardi, J. & Kuborta, C. 2006. Variation of lycopene, antioxidant activity, total soluble solids and weight loss of tomato during postharvest storage. *Postharvest Biology and Technology*, 41(2): 151-155.

Kilkarni, S.G., Vijayanand, P. & Shubha, L. 2010. Effect of processing of dates into date juice concentrate and appraisal of its quality characteristics. *Journal of Food Science and Technology*, 47(2):157-161.

Kimball, D. 1991. *Citrus processing quality control and technology*. Springer science + business media, New York.

Kindscher, K., Martin, L., Corbett, S. & Lafond, D. 2018. Nutritional properties of native plants and traditional foods from the central united states. *Ethnology Letters*, 9(2): 214-227.

Koutchma, T.N., Forney, L.J. & Moraru, C.I. 2009. *Ultraviolet Light in Food Technology Principles and Applications*, 62: 18. CRC Press, Taylor and Francis Group.

Kuhnlein, H.V. 2015. *Micronutrient nutrition and traditional food systems of indigenous peoples.* Available from: https://www.researchgate.net/publication/265060630_Micronutrient_nutrition_and_tradi....[Accessed on: 25 April 2017].

Kumar, S. 2015. Role of enzymes in fruit juice processing and its quality enhancement. *Advances in Applied Science Research*, 6 (6): 114-124.

Labbe, M., Ulloa, A.P., Lopez, F., Saenz, C., Pena, A. & Salazar, F. 2016. Characterization of chemical compositions and bioactive compounds in juices from pomegranates ('Wonderful', 'Chaca' and 'Codpa') at different maturity stages. *Chilean Journal of Agricultural Research,* 76(4): 479-486.

Landbo, A.K. & Meyer, A.S. 2004. Effects of different enzymatic maceration treatments on enhancement of anthocyanins and other phenolics in black currant juice. *Innovative Food Science & Emerging Technologies*, 5: 503–513. doi: 10.1016/j.ifset.2004.08.003

Lara-Espinoza, C., Carvajal-Millan, E., Balandran-Quintana, R., Lopez-Franco, Y & Rascon-Chu, A. 2018. Pectin and pectin based composite materials: beyond food texture. *Molecules*, 23(4): 942.

Lee, W.C., Yusof, S., Hamid, N.S.A. & Baharin, B.S. 2006. Optimising conditions for enzymatic clarification of banana juice using response surface methodology (RSM). *Journal of Food Engineering*, 73(1): 55-63.

Lemos, V.A., Novaes, C.G. and Benzerra, M.A. 2009. An automated preconcentration system for the determination of manganese in food samples. *Journal of Food Composition and Analysis*, 22(4):337-342.

Liao, H., Sun, Y., Ni, Y., Liao, X., Hu, X., Wu, J. & Chen, F. 2007. The effect of enzymatic mash treatment, pressing, centrifugation, homogenization, deaeration, sterilization, and storage on carrot juice. *Journal of Food Processing Engineering*, 30(4): 421-435. doi. Org/10.1111/j.1745-4530.2007. 00118.x.

Lim, D.J., Sharme, Y. & Thomson, C.H. 2018. Vitamin C and alcohol: a call to action. *BMJ Nutrition and Health,* Available at: https://nutrition.bmj.com/content/early/2018/12/04/bmjnph-2018-000010 [Accessed 09 September 2109].

Maathuis, F.J.M. 2014. Sodium in plants: perception, signalling, and regulation of sodium fluxes. *Journal of Experimental Botany*, 65 (3): 849-858.

Mabhaudhi, T., Chibarabada, T.P., Chimonyo, V.G.P., Murugani, V.G. & Pereira, L.M. 2019. Mainstreamingng underutilized indigenous and traditional crops into food systems. A South African perspective. *Sustainability*, 11(172); doi:10.3390/su11010172.

Malkanthi, S., Pushpa, H., Karunaratne, A.S., Amuwala, S.D. & Silva P. 2014. Opportunities and Challenges in Cultivating Underutilized Field Crops in Moneragala District of Sri Lanka. *Asian Journal of Agriculture and Rural Development*, 04(01): 96-105.

Mieszczakowska-Frac, M., Markowski, J., Zbrzezniak, M. & Plocharski, W. 2012. Impact of Enzyme on quality of blackcurrant and plum juices. *LWT-Food Science and Technology*, 49: 251-256.

Mishra, C.S.K. & Champaigne, P. 2009. Biotechnology Applications. *I K International Pub*, New Delhi.

Minoli, A. Upul, M. & Ranmalie, M. 2017. Process optimization of pectinase enzyme aided clarification of Israel blue grape juice. *European Journal of Academic Essay*, 4(4):165-174.

Modi, A.T. & Mabhaudhi, T. 2016. *Developing a research agenda for promoting underutilised, indigenous and traditional crops*. School of Agricultural, Earth and Environmental Sciences University of KwaZulu-Natal. Unpublished.

Morais, D.R., Rotta, E.M., Sargi, S.C., Bonate, E.G., Suzuki, R.M., Souza, N.E., Matsushita, M. & Visentainer, J.V. 2017. Proximate composition, mineral contents and fatty acid composition of the different parts and dried peels o tropical fruits cultivated in Brazil. Journal of Brazilian Chemical Society. ISSN 1678-4790.

Mosqueda-Melgar, J., Raybandi-Masillia, R.M. & Martin-Belloso, O. 2008. Non-thermal pasteurization of fruit juices by combining high-intensity pulsed electric fields with natural antimicrobials. *Innovative Food Science and Emerging Technologies*, 9(3): 328-340.

Mouti, M., Savadogo, M.A. & Traore, A.S. 2016. Mineral composition and vitamin A of ten wild fruits consumed in tree departments of Chad. *International Journal of*

Recent Advances in Multidisciplinary Research, 03(08): 1713-1719.

Mtumtum, N.P. 2012. *Performance of wild watermelon (citrullus lanatus L.) in response to population density and mulch*. Unpublished Master of Science project report. Pietermaritzburg: University of Kwazulu Natal, School of Agricultural, Earth and Environmental sciences.

Mushtaq, M. 2017. *Extraction of Fruit Juice*. Fruit juice extraction, composition, quality analysis, Chapter 8. Academic Press, Ireland.

Muthamilarasan, M., Singh, N.K. & Prasad, M. 2019. Multi-omics approaches for strategic improvement of stress tolerance in underutilized crop species: A climate change perspective. *Advances in Genetics*, 103:1-38.

Myer, P.R., Parker, K.R., Kenach, A.T., Zhu, T., Morgan, M.T. & Applegate, B,M. (2016). The effect of a novel low temperature-short time (LTST) process to extend the shelf-life of fluid milk. Springerplus, 5(1):660.

National Department of Agriculture. 2014. *Bitter watermelon*. Available from: http://www.nda.agric.za/docs/Brochures%5CBITTERWATERMELON.pdf [Accessed on: 10 November 2015].

Nawfel, M.B. 2017. Review in causes of viscosity in fluids. *J.Bio. Innov* 6(1): 117-123.

Nelssestuen, G.L. 1995. How Enzymes work, Chapter 2. *Principles of Medical Biology*, 4(1):25-44. doi.org/10.10161/ S1569258206800048..

Ngadze, R.T., Nyanga, L.K., Fogliane, V., Ferracane, R., Troise, A.D. & Linnemann, A.R. 2018. Effect of heat and pectinase maceration on phenolic compounds and physicochemical quality of *Strychnos cocculoides* juice. *PLOS ONE,* 13(8).

Norjana, I. & Noor Aziah, A.A. 2011. Quality attributes of durian (Durio zibethinus Murr) juice after pectinase enzyme treatment. *Int. Food Res J*. 18 (3):1117–1122.

Nweze, C.C., Abdulganiyu, M.G. & Erhabor, O. 2015. Comparative analysis of vitamin C in fresh fruit juice of malus domestica, citrus sinensi, ananas comosus and citrullus lanatus by iodometric titration. *International Journal of Science, Environment and Technology,* 4(1):17 – 22.

Orzolek, M.D., Bogash, S.M. & Harper, J.K. 2010. *Agricultural Alternatives*. The Pennsylvania State University.

Paes, J., Reschke, C. & Antonio, V.L. 2015. Concentration of lycopene in the pulp of papaya (*Carica papaya*) by ultrafiltration on a pilot scale. *Food and Bioproducts Processing,* 96:296-305.

Pardo, J.E., Gomez, R., Tardaguila, J., Amoz, M.. & Varon, R. 1997. Quality evaluation of watermelon varieties (*citrullus vulgaris s*.). *Journal of Food Quality*, 20. Available from: https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1745-4557.1997.tb00495.x [Accessed on: 11 May 2019].

Paris, H.S. 2015. Origin and Emergence of sweet dessert watermelon, *citrus lanatus*. *Annals of Botany,* 116(2):133-148.

Panda, H. 2013. *The Complete Book On Fruits, Vegetables, and Food Processing.* Nijr project consultancy services. Available from: https://books.google.co.za/books/about/The_Complete_Book_on_Fruits_Vegetables_a.html?id=wbg6AQAAQBAJandredir_esc=y[Accessed on: 10 May 2019].

Patel, A.K., Singhania, R.R. & Padyey, A. 2016. Novel enzymatic processes applied to the food industry. *Current Opinion in Food Science,* 7: 64-72.

Pem, D. & Jeewon, R. 2015. Fruit and Vegetable Intake: Benefits and Progress of Nutrition Education Interventions- Narrative Review Article. *Iran Journal of Public Health,* 44(10) :1309-1321.

Pinelo, M., Meyer, A.S. & Zeuner, B. 2010. Juice clarification by protease and pectinase indicates new roles of pectin and protein in cherry juice turbidity. *Food and Bioproducts,* 88 :259-265.

Pinto, C., Moreira, S.A., Fidalgo, L.G., Santos, M.D., Vidal, M., Delgadillo, I. & Saraira, J.A. 2017. Impact of different hyperbaric storage conditions on microbial, physicochemical, and enzymatic parameters of watermelon juice. Food Research International,1-10.

Pylypiw, J.M., Misenti, T. & Mattina, M.I. 1997. Sodium Benzoate and Potassium Sorbate Preservatives in juices and fruits. *The Connecticut experiment station, new haven, bulletin 921*, April 1994.

Qadri, O.S., Yousuf, B., Srivastava, A.K. & Yildiz, F. 2015. Fresh-cut fruits and vegetables: Critical factors influencing microbiology and novel approaches to prevent microbial risks—A review. *Journal of Cogent Food and Agriculture*, 1(1).

Qin, L., Xu, S.Y. & Zhang, W.B. 2005. Effect of enzymatic hydrolysis on the yield of cloudy carrot juice and the effects of hydrocolloids on color and cloud stability during ambient storage. *Journal of Science Food Agriculture*, 85: 505-512.

Rai, P., Majumdar, G.C., Gupta, G.S. & De, S. 2004. Optimizing pectinase usage in pre-treatment of mosambi juice for clarification by response surface methodology. *Journal of Food Engineering,* 64(3): 397-403.

Rajdeo, K., Harin. T., Lavanya, K, & Mattina, M.I. 2016. Immobilization of pectinase on reusable polymer support for clarification of apple juice. *Food and Bioproducts Processing*, 99: 12-19.

Ramos, O.S. & Malcata, F.X. 2011. Food grade enzymes. *Comprehensive biotechnology*, second edition, 3: 555-569.

Rap Publication. 2014. Promotion of Underutilised indigenous food resources for food security and nutrition in Asia and the Pacific. In P. Durst and N. Bayasgalanbat (Eds). *RAP Publication 2014/07.*

Rawson, A., Tiwari, B.K., Patras, A., Brunton, A., Brennan, C., Cullen, P.J. & Donnell, C.O. 2011. Effect of thermosonication on bioactive compounds in watermelon juice. *Food Research Journal*, 22:1168-1173.

Rewat, S. 2015. Food Spoilage: Microorganisms and their prevention. *Asian Journal of plant science and Research*, 5(4,): 47-56.

Rinaldi, M., Caligiani, A., Borgese, R., Palla, G., Bibanti, D. & Massini, R. 2013. The effect of fruit processing and enzyme treatments on pomegranate juice composition, antioxidant activity, and polyphenols content. *Food Science and Technology*, 53: 355-359.

Rodriguez-Rogue, M.J., De Ancos, B.D., Sanchez-Moreno, C., Cano, M.P., Elez-Martinez, P. & Martin-Belloso, O. 2015. Impact of food matrix and processing on the in vitro bioaccessibility of vitamin C, phenolic compounds, and hydrophilic antioxidant activity from fruit juice-based beverages. *Journal of Functional Foods*,14, doi: 10.1016.

Rombouts, F.M. & Pilnik, W. 1978. Enzymes in fruit and vegetable juice technology. *Journal of Process Biochemistry*, 13: 9–13.

Saint-Denis, T. & Goupy, J. 2004. Optimisation of a nitrogen analyser based on the Dumas method. *Analytica Chimica Acta*, 515:191-198.

Sandri, I.G., Fontana, R.C., Barfknecht, D.M. & Da Silveira, M.M. 2011. Clarification of fruit juices by fungal pectinases. *LWT-Food Science and Technology*, 44:2217-2222.

Sani, A.M. 2013. Determination of grape juice concentrate composition. *Nutrition and Food Science,* 43(5).

Santin, M.M., Treichel, H., Valduga, E., Cabral, L.M.C. & Luccio, M.D. 2008. Evaluation of enzymatic treatment of peach juice using response surface methodology. *Journal of Food Agriculture and Environment*, 3:507-512.

Savic, S.R., Petrovic, S.M., Stamenkovic, J.J. and Petronijevic, Z.B. 2015. The presence of minerals in clear orange juices. *Advanced Technologies,* 4(2) 71-78.

Saxena, D., Sabikhi, L., Chakraborty, S.K. & Singh, D. 2014. Process optimization for enzyme aided clarification of watermelon juice. *Journal of Food Science and Technology,* 51, 10: 2490-2498.

Schaffer, A.A. & Paris, H.S. (2003). Melon, squashes, and gourds. *Encyclopedia of Food Sciences and Nutrition,* 2nd edition. Academic Press, USA.

Screenath, H.K., Sudarshan, K.K.R. & Santhanam, K. 1994. Improvement of juice recovery from pine pulp residue using cellulose and pectinases. *Journal of Ferment Bioeng,* 76(6): 486-488.

Sew, C.C., Ghazali, H. M., Martin-Belloso, O. & Noranizan, M.A. 2014. Effects of combining ultraviolet and mild heat treatments on enzymatic activities and total phenolic contents in pineapple juice. *Innovative Food Science and Emerging Technologies*, 26: 511-516.

Sharma, H., Patel, P. & Sugandha, H. 2017. Enzymatic extraction and clarification of juice from various fruits-a review. *Critical Review of Food Science and Nutrition,* 57(6): 1215-1227.

Shelef, O., Weisberg, P.J. & Provenza, F.D. 2017. The value of native plants and local production in an era of global agriculture. *Front Plant Sci.*, 8(2069).

Sibanyoni, J.J. & Tabit, F.T. 2019. An assessment of the hygiene status and incidence of foodborne pathogens on food contact surfaces in the food preparation facilities of schools. *Food Control*, 98: 94-99.

Sin, H., Yusof, S., Sheik Abdul Hamid, N. & Rahman, R.A. 2006. Optimisation of enzymatic clarification of sapodilla juice using response surface methodology. *Journal of Food Engineering*, 73:313-319.

Sogi, D.S. Oberoi, D.P.S. & Malik, S. 2010. Effect of particle size, temperature, and total soluble solids on the rheological properties of watermelon juice: a response surface approach. *International Journal of Food Properties*, 13(16): 1207-1214.

Sojitra, U.V. Nadar, S.S. & Rathod, V.K. 2016. A magnetic tri-enzyme nanobiocatalysts for fruit juice clarification. *Food Chemistry*, 213: 296-305.

Stallings, V.A., Harrison, M. & Oria, M. (eds). 2019. *Dietary reference intakes for sodium and potassium*. National Academies Press, Washington, DC.

Steffensen, I., Frolech, W., Dahl, K.H., Iversen, P.O., Ludvig, L., Lillegeard, I.T. L. & Alexander, J. 2017. *Dietary Reference Intakes for Sodium and Potassium.* National Academies Press, Washington, DC.

Strauss, M. 2015. *The 5,000-Year Secret History of the Watermelon*. Available at: http://news.nationalgeographic.com/2015/08/150821-watermelon-fruit-history-agriculture/[Accessed on:14 September 2016].

Srivastava, S. & Tyagi, S.K. 2013. Effect of Enzymatic Hydrolysis on the Juice Yield from Apple Fruit (Malus Domestica) Pulp. *International Journal of Biotechnology and Bioengineering Research*, 4(4): 299-306.

Tabit, F.T., Komolofe, N.T., Tshilakalange, T.E. & Nyila, M.A. 2016. Phytochemical constituents, antioxidant and antimicrobial activity of selected plants used traditionally as a source of food. *Journal of Medicinal Food*, 19(3).

Tapre, A.R. & Jain, R.K. 2014. Optimization of an enzyme assisted banana pulp clarification process. *International Food Research Journal,* 25(5): 2043-2048.

Tareen, H., Mengal, F., Masood, Z., Mengal, F., Ahmed, S., Bibi, S., Shoaib, S., Sami, U., Mandokhail, F., Riaz, M., Farman, N. & Nawaz, Z. 2015. Determination of vitamin c content in citrus fruits and in non-citrus fruits by titrimetric method, with special reference to their nutritional importance in human diet. *Biological Forum-An International Journal*, 7(2): 367-369.

Tiwari, U. 2018. *Production of Fruit-based Smoothies*. Fruit juice extraction, composition, quality analysis, Chapter14. Academic Press, Ireland.

Tola, Y.B. & Ramaswamy, H.S. 2015. Temperature and high-pressure stability of lycopene and vitamin C of watermelon juice. *African Journal of Food Science*, 9(5):351-358.

Tshikalange, T.E., Modishane, D.C. & Tabit, F.T. 2017. Antimicrobial, antioxidant and cytotoxicity properties of selected wild edible fruits of traditional medicinal plants. *Journal of Herbs, Spices, and Medicinal plants*, 23(1).

Ucan, F., Akyildiz, A. & Agcam, E. 2014. Effects of Different Enzymes and Concentrations in the Production of Clarified Lemon Juice. *Journal of Food Processing*, Article ID 215854.

Ugwu, K.C., Mbajiorgu, C.C., Okwonko, W.I. & Ani, A.O. 2018. Design, fabrication and performance evaluation of a portable hand-held refractometer. *Nigerian Journal of Technology (NIJOTECH),* 37(2):537 – 542.

Ulijaszek, S., Mann, H. & Elton, S. 2012. *Evolving Human Nutrition*. Cambridge University Press.

Umsza-Guez, M.A., Rinaldi, r., Lago-Vanzela, E.S., Martin, N., daSilva, R., Gomes, E & Thomeo, J.C. 2011. Effect of pectinolitic enzymes on the physical properties of caja-manga (Spondias cytherea Sonn.) pulp. *Cienc. Tecnol. Aliment., Campinas*, 31(2): 517-526.

Usman, A., Haruna, M., Itodo, U. & Ofrghor, O.A. 2010. Protein and amino acid composition of watermelon (citrullus lanatus) seeds. *International Journal of Tropical Agriculture and Food Systems,* 4(3).

Van Der Vossen, H.A.M., Denton, O.A. & Tahir, I.M. 2004. Citrus lanatus (Thumb.) matsum. and Nakai. Available at: http://www.prota4u.org/protav8.asp?p=Citrullus+lanatus [Accessed on: 16 May 2016].

Verma, H., Narnoliya, L.K. & Jadaun, J.S. 2018. Pectinase: A Useful Tool in Fruit Processing Industries. *Nutrition and Food Science International Journal Mini Review,* 5(5).

Voragen, A.G.T., Coenen, G.T & Verhoef, R.P. 2009. Pectin, a versatile polysaccharide present in plant cell walls. *Structural Chemistry*, 20(263).

Wang, H., Hu, Z., Long, F., Guo, C., Niu, C. & Yang, Y. 2016. Combined effect of sugar content and pH on the growth of wild strain of *Zygosaccharomyces rouxii* and time for spoilage in concentrated apple juice. *Food Control*, 59:298-305.

Wehner, T.C. 2008. Watermelon. *Vegetables 1 Volume 1 in the Series Handbook of Plant Breeding*. Springer New York.

World Health Organization. 2012. *Guideline: Potassium intake for adults and children.* Geneva World Health Organization. Available at: https://apps.who.int/iris/bitstream/handle/10665/77986/9789241504829_eng.pdf;jsessionid=CA1EE1E7A9E14AB48DFD552C6B875A78?sequence=1 [Accessed on: 02 February 2019].

Wlodarska, K., Pawlak-Lemanska, K., Goreck, T. & Sikorska, E. 2016. Perception of apple juice: a comparison of physicochemical measurements, descriptive analysis, and consumer responses. *Journal of Food Quality* 39: 351-361.

Xaba, P. & Croeser, P. 2012. Tsamma Melon. *Veld and Florora*, march 201. Available from: http://www.botanicalsociety.org.za/ProjectsAndActivities/Useful%20Plants/18%20Tsamma%20Melon%20March%202012.pdf [Accessed on: 30 November 2018].

Zeng, J., Zhou, Y., Li, S., Zhou, P., Xu, D. & Li, H. 2017. Effects and mechanism of fruit and vegetable juices on cardiovascular disease. *International Journal of Molecular Sciences*, 18(3):555.

Zhang, J. & Ritonour, M.A. 2016. Sugar Composition Analysis of Commercial Citrus Juice Product. *Proc.Fla.State Hor.Sci.*,129:178-180.

Zheng, B., Peng, B., Zhang, C., Song, Z. & Ma, R. 2017. Determination of fruit maturity and its prediction model based on the pericarp index of absorbance difference (I_{AD}) for peaches. *Public Library of Science One*, 12(5).

Zhang, H., Zhabyeyev, P., Wang, S. & Oudit, G.Y. 2018. *Role of iron metabolism in heart failure*: From iron deficiency to iron overload. Available from: https://www.sciencedirect.com/science/article/pii/S0925443918303235 [Accessed on: 09 April 2019].

APPENDIX A: ETHICS APPROVAL LETTER

UNISA university of south africa

CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 09/04/2019

NHREC Registration # : REC-170616-051
REC Reference # : 2017/CAES/027
Name : Ms MM Mamabolo
Student #: 39394948

Dear Ms Mamabolo

Decision: Ethics Approval Renewal after Second Review from 01/04/2019 to 31/03/2020

Researcher(s): Ms MM Mamabolo
39394948@mylife.unisa.ac.za

Supervisor (s): Prof FT Tabit
tabitft@unisa.ac.za; 011-471-2080

Working title of research:

The effect of enzymatic treatment on the physicochemical and sensory characteristics of indigenous wild watermelon (*Citrullus lanatus*) fruit juice

Qualification: M Consumer Science

Thank you for the submission of your progress report to the CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is renewed for a one-year period. After one year the researcher is required to submit a progress report, upon which the ethics clearance may be renewed for another year.

Due date for progress report: 31 March 2020

*The **low risk application** was **reviewed** by the CAES Health Research Ethics Committee on 16 February 2017 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

2. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
3. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
4. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
5. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
6. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.
7. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:
The reference number ***2017/CAES/027*** *should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

Prof EL Kempen
Chair of CAES Health REC
E-mail: kempeel@unisa.ac.za
Tel: (011) 471-2241

Prof MJ Linington
Executive Dean : CAES
E-mail: lininmj@unisa.ac.za
Tel: (011) 471-3806

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za