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FACULTE DES SCIENCES
DEPARTEMENT DE BIOCHIMIE
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CENTRE DE RECHERCHE & DE FORMATION DOCTORALE, SCIENCE DE LA VIE, SANTE & ENVIRONNEMENT

REPUBLIC OF CAMEROUN
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UNIVERSITY OF YAOUNDE I
FACULTY OF SCIENCE
DEPARTMENT OF BIOCHEMISTRY
****
GRADUATE PROGRAM FOR LIFE SCIENCES, HEALTH AND THE ENVIRONMENT

Reduction of tomato losses through implementation of simple postharvest techniques using rosemary (Rosmarinus officinalis) essential oil in Bushi, Democratic Republic of the Congo

PhD Thesis
Presented and Defended in the Fulfilment of the Requirements for the degree Doctor of Philosophy in Biochemistry

Par : **SIBOMANA IMANI Caroline**
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Année Académique : 2020

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CERTIFICATE OF CORRECTION

We, the undersigned Professor MOUNDIPA FEWOU Paul (President of the Jury), Professor KANSCI Germain and Associate Professor NGUEFACK Julienne (Examiners of the jury), certify that Mrs. **SIBOMANA IMANI Caroline** (Reg. N° **13T2121**) has made the corrections in conformity with the remarks and suggestions of the members of the Jury of her Doctorate (PhD) thesis in Biochemistry, option Food Science and Nutrition, defended on June 25th, 2020 at 10:00 am in the A3 amphitheatre of the Faculty of Science of the University of Yaoundé I on the theme "**Reduction of tomato losses through implementation of simple postharvest techniques using rosemary (*Rosmarinus officinalis*) essential oil in Bushi, Democratic Republic of the Congo**".

In witness whereof, this certificate is established for her to serve and assert that of right.

Examiners

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DECLARATION OF HONOUR

I, SIBOMANA IMANI CAROLINE (Registration Number 13T2121), PhD student in Food Science and Nutritional Biochemistry option, Biochemistry Department, hereby declare that the research thesis submitted on " **Reduction of tomato losses through implementation of simple postharvest techniques using rosemary (*Rosmarinus officinalis*) essential oil in Bushi, Democratic Republic of the Congo**" is my original work and has not been presented to any university for the award of a degree. Works by other authors that served as source of information have duly been acknowledged by referenced to the authors.

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NOMBRE D'ENSEIGNANTS					
DÉPARTEMENT	**Professeurs**	**Maîtres de Conférences**	**Chargés de Cours**	**Assistants**	**Total**
BCH	08 (01)	13 (09)	14 (06)	03 (02)	**38 (18)**
BPA	13 (01)	09 (06)	19 (06)	05 (02)	**46 (15)**
BPV	06 (00)	10 (02)	09 (06)	07 (01)	**32 (09)**
CI	10 (00)	09 (02)	13 (03)	03 (00)	**35 (05)**
CO	07 (00)	17 (03)	09 (05)	02 (00)	**35 (08)**
IN	02 (00)	01 (00)	14 (00)	09 (01)	**26 (01)**
MA	01 (00)	05 (00)	19 (02)	05 (01)	**30 (03)**
MIB	01 (00)	05 (02)	06 (01)	06 (03)	**18 (06)**
PHY	11 (00)	16 (02)	10 (03)	03 (00)	**40 (05)**
STU	08 (01)	14 (01)	19 (05)	02 (00)	**43 (07)**
TOTAL	**67 (03)**	**99 (27)**	**132 (37)**	**45 (10)**	**343 (77)**

Soit un total de	343
Professeurs	67 (03)
Maîtres de Conférences	99 (27)
Chargés de Cours	132 (37)
Assistants	45 (10)
( ) = Nombre de Femmes	(77)

DEDICATION

Dedicated to

Bill BAHANE LWABANJI, my husband

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SUMMARY

ABSTRACT

Tomato is perishable and its high moisture content makes it susceptible for various postharvest losses. Tomato quality changes continuously after harvesting when fruits start losing quality because of environmental stress and pathogen infection. Thus, the objective of this research was to propose simple postharvest technology using rosemary essential oil (REO) to reduce postharvest decay. This was done by conducting a field survey in order to determine agricultural potential of soil and environmental characteristics of soil used for tomato cultivation in the Bushi region according to the FAO's land suitability classification; and the compilation of cartographic data was done using ArcGIS 10.3 software. Cultivated fruits in the Bushi region were evaluated based on their morphological characters and data were analysed through XLSTAT package. Laboratory experiments on fresh tomato fruits preservation and tomato processing into juice and paste were done in a central composite design (CCD) using REO to extend the products shelf life in combination with chlorine and *Bacillus cereus* spores, respectively. Fruit physical and chemical quality characteristics were evaluated, and statistical analyses were done using XLSTAT and STATISTICA packages for non-linear regression analyses. The data obtained were subjected to Analysis of variance (ANOVA) and significant means were separated by mean differences Tukey-HSD test ($\alpha \leq 0.05$). Harvested tomato fruits at different maturity stages using REO in a randomized complete block design (RCBD) were evaluated for their chemical quality characteristics and shelf life for implementation in the Bushi region. Data were subjected to ANOVA using R 3.5.1, R Studio 1.1.453 software. Significant means were separated by mean differences Tukey-HSD at $\alpha \leq 0.05$. Results indicated that in the Bushi region, soil characteristics and climate data varied from one experimental site to another and that Kabare is theoretically marginally suitable for tomato cultivation compared to Walungu where tomato can be cultivated in moderately and highly suitable areas. During storage, a significant decrease in weight loss (< 10 %) was observed as the REO concentrations increase (500-1000 ppm). The presence of defects was related to the shorter time of immersion in chlorinated water for fruits surface disinfection that immediately impacts on the fruit shelf life. The optimal condition that reduces the percentage defects of fruits was the action of 3 minutes of immersion time in chlorinated water at 200 ppm and high concentration of REO (≥ 500 ppm). Most fruits softened with the increase in fruit colour and prolonged storage time while chlorine and REO concentrations have had a positive influence on the preservation of tomato fresh flavour and on the fruit shelf life extension. High percent reduction (100 %) in *Bacillus cereus* spores was

effective with treatment interactions (cooking time and REO concentrations). In both juice and paste, the percentage reduction of spores depended on the combination of REO with spore concentration and heating time with spore concentration. All treatments in both tomato juice and paste inoculated with 4 log CFU/g have registered variable level of *B. cereus* inhibition (99.7-100 %), except where there was no heating for deactivation (0.79 %). Total polyphenols, Titratable acidity and Vitamin C content were affected under thermal processing of juice and paste. Independently of the treatments, there was an increase in the total polyphenols content (85.56-220 µg/mg) and a decrease in the vitamin C content of tomato paste (0.47-2.93 mg/mg) compared to tomato juice (40-167.22 µg/ml and 14.4-20.43 mg/ml). For storage efficacy, the contamination with a minimum concentration of *Bacillus cereus* spores of 10^4 spores/g was stabilized with treatment at 95°C for 5 minutes in tomato juice and 20 minutes in tomato paste, resulting in a 1 year storage extension. The application of REO alone permitted to extend the shelf life of fresh tomato fruits for preservation in the Bushi region. Soluble solids content in tomato varied significantly ($P \leq 0.05$) in fruits of different maturity stages. Vitamin C content was high in fruits of early maturity stages and the shelf life in harvested tomato fruits was evaluated at 14 days of storage, in respect to the percentage of shrivelled fruits. The developed technology for implementation was found to be suitable in the presence of REO by increasing tomato's shelf life.

Keywords: Fruit quality, Postharvest losses, Production, Rosemary essential oil, Shelf life, Tomato

RESUME

La tomate est une denrée périssable et sa forte teneur en humidité la prédispose à diverses pertes post-récolte. La qualité de la tomate change continuellement après la récolte lorsque les fruits commencent à perdre leur qualité en raison du stress environnemental et de l'infection par des agents pathogènes. L'objectif de cette recherche était donc de proposer une technologie post-récolte simple utilisant l'huile essentielle de romarin (HER) pour réduire la pourriture post-récolte. Ceci a été réalisé par une enquête sur terrain afin de déterminer le potentiel agricole suivant les caractéristiques de l'environnement et du sol pour la culture de tomates dans la région du Bushi, conformément à la classification de l'aptitude des sols de la FAO; et la compilation des données cartographiques a été réalisée à l'aide du logiciel ArcGIS 10.3. Les fruits cultivés dans la région du Bushi ont également été évalués sur la base de leurs caractères morphologiques et les données ont été analysées dans le logiciel XLSTAT. Deux expériences au laboratoire ont été réalisées dans un modèle composite centré (CCD) : d'une part, la conservation des fruits de tomates fraîches avec comme facteurs en étude la HER, le chlore et le temps d'immersion ; et d'autre part, la transformation de la tomate en jus et en purée toujours avec l'utilisation de la HER combinée aux différentes concentrations en spores de *Bacillus cereus* et le temps d'appertisation, comme facteurs en étude. Les caractéristiques physiques et chimiques du fruit ont été évaluées et des analyses statistiques ont été effectuées à l'aide des progiciels XLSTAT et STATISTICA pour les analyses de régression non linéaire. Les données obtenues ont été soumises à une analyse de variance (ANOVA) et les moyennes significatives ont été séparées par des différences moyennes suivant le test Tukey-HSD ($\alpha \leq 0,05$). Les fruits de tomate récoltés à différents stades de maturité et l'utilisation de la HER ont été évalués dans un dispositif en blocs complets randomisés pour leurs caractéristiques chimiques et durée de conservation en vue de leur mise en œuvre dans la région du Bushi. Les données ont été soumises à une analyse de variance et analysées dans le logiciel R 3.5.1, R Studio 1.1.453. Les moyennes significatives ont été séparées par les différences moyennes (Tukey-HSD à $\alpha \leq 0,05$). Les résultats indiquent que, dans la région du Bushi, les caractéristiques du sol et les données climatiques varient d'un site expérimental à l'autre et que Kabare convient peu à la culture de la tomate par rapport à Walungu où la tomate peut être cultivée dans des zones moyennement et hautement adaptées. Pendant la conservation, une diminution significative de perte de poids (< 10 %) des fruits lors du stockage a été observée avec l'augmentation des concentrations en HER (500-1000 ppm). La présence de défauts était liée au temps d'immersion plus court dans l'eau chlorée pour la désinfection de la

surface des fruits, lequel a immédiatement raccourci la durée de conservation. La condition optimale permettant de réduire le pourcentage de défauts sur les fruits est celle de la combinaison d'un temps d'immersion de 3 minutes dans de l'eau chlorée à une concentration de 200 ppm et de niveaux de concentration élevés de HER (≥ 500 ppm). La plupart de fruits se sont ramollis suite à la durée prolongée de conservation et la maturation des fruits (mûrissement), alors que les concentrations de HER et de chlore ont eu une influence positive sur la préservation de la saveur fraîche de la tomate et sur la prolongation de la durée de conservation. La réduction en spores de *B. cereus* s'est avérée efficace (100 %) suite à l'interaction des facteurs (temps d'appertisation, concentrations en HER et en spores de *B. cereus*). Dans le jus comme dans la purée, le pourcentage de réduction des spores dépendait de la combinaison de HER et de la concentration en spores de *B. cereus* ainsi que du temps d'appertisation et de la concentration en spores. Tous les produits (jus et purée) inoculés avec 4 log UFC/g ont enregistré un niveau variable (99.97-100 %) de réduction de *B. cereus*, sauf en l'absence de temps d'appertisation pour la désactivation de spores (0.79 %). Les polyphénols totaux, l'acidité titrable et la teneur en vitamine C ont été affectés par le traitement thermique du jus et de la purée. Il y a eu une augmentation de la teneur en polyphénols totaux (85.56-220 µg/mg) et une diminution de la teneur en vitamine C (0.47-2.93 mg/mg) dans la purée comparativement au jus de tomate (40-167.22 µg/ml et 14.4-20.43 mg/ml), indépendamment des traitements. Pour assurer l'efficacité de stockage des produits transformés pour une période de conservation d'1 an, la contamination par une concentration minimale de spores de *B. cereus* de 10^4 ufc/g a été stabilisée par un traitement thermique de 95°C pendant 5 minutes pour le jus et 20 minutes pour la purée. L'application de HER seule a permis de prolonger la durée de conservation des fruits frais de tomate dans la région du Bushi. La teneur en sucres solubles des fruits de tomate variait de manière significative ($P \leq 0.05$) dans les fruits de différents stades de maturité et la teneur en vitamine C était élevée dans les fruits des premiers stades de maturité. La durée de conservation dans les fruits de tomates récoltés a été évaluée à 14 jours de stockage. La technologie développée pour la mise en œuvre s'est avérée appropriée en présence de HER en augmentant la durée de vie de la tomate.

Mots clés: Conservation, Huile essentielle de romarin, Pertes post-récolte, Production, Qualité des fruits, Tomate

ACRONYMS

1-MCP	: 1-methyl-cyclopropene
ATP	: Adenosine Triphosphate
BESUP	: Bureau de l'Enseignement Supérieur et Universitaire Protestant
CAID	: Cellule d'Analyses des Indicateurs de Développement
CCD	: Central Composite Design
CEC	: Cation Exchange Capacity
CFU	: Colony Forming Unit
CORAF/ WECARD	: Conseil ouest et centre africain pour la recherche et le développement / West and Central African Council for Agricultural Research and Development
DRC	: Democratic Republic of the Congo
DTM	: Digital Terrain Model Database
EFSA	: European Food Safety Authority
FAO	: Food and Agricultural Organisation
GIS	: Geographic Information Systems
IITA	: International Institute of Tropical Agriculture
IPAPEL	: Inspection Provinciale de l'Agriculture, Pêche et Elevage
IDRC	: International Development Research Center
MAP	: Modified atmosphere packaging
MCE	: Multicriteria Evaluation
MPN	: Most Probable Number
NZFSA	: New Zealand Food Safety Authority
NASA	: National Aeronautics and Space Administration
OECD	: Organisation for Economic Cooperation and Development
PHL	: Postharvest losses
REO	: Rosemary Essential Oil
SFCG	: Search For Common Ground
SOTERCAF	: Soil and Terrain for Central Africa
TA	: Titratable Acidity
TSS	: Total Soluble Solids
UEA	: Université Evangélique en Afrique
USDA	: United States Department of Agriculture

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

INTRODUCTION

The number of people to be fed is increasing; over 60% of people suffer from hunger, many of them being in developing countries (33 % in Africa and 16 % in Asia). In addition, the world is experiencing climate variability (FAO, 2010) with consequences on food production. The socio-economic and food importance of fresh products are very considerable for many people in developing countries such as the Democratic Republic of the Congo (DRC). Fresh products (fruits and vegetables) are very important sources of vitamins and minerals and have been part of human diets and source of income for many years, although their availability on the market is conditioned by many social and environmental factors. Their production has been increasing worldwide over many years, partly in response to population growth but also due to rising living standards (Wills, 2007). Increased consumption of a variety of fruits and vegetables on a daily basis is highly recommended because of associated health benefits (FAO, 2010).

Fresh produce is highly perishable with estimates suggesting a postharvest loss of 30 to 50 % (Atanda *et al*., 2011). Losses in fruits and vegetables can vary from an estimated 10-50 % depending on the commodity in tropical countries (Mohapatra *et al*., 2014). The quality of fresh produce is governed by many factors which combined effect determines the rate of deterioration and spoilage (Ahmad and Siddiqui, 2015). These factors (including environmental conditions) are very important in the occurrence and severity of plant diseases. However, the same conditions that favour plants are the favourite for soil borne pathogens and very important in the occurrence and severity of plant diseases (Alhussaen, 2012).

Fresh produce can become microbiologically contaminated at any point along the farm-to-table food chain (USFDA, 1998). Losses due to postharvest spoilage or pathological decay are a result either of latent infections in the field that become active, following harvest or of cross-contamination during harvest, cleaning, storage and distribution (Barth *et al*., 2009). Therefore, the reduction of postharvest loss is a complementary mean for increasing production and food availability to the growing world population, ensuring that sufficient food, both in quantity and in quality is available to every human in our planet (Kader, 1986; Buntong *et al.,* 2013).

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetables worldwide. It has a limited shelf life at ambient conditions and is highly perishable. Being a relatively short duration and high yielding crop, tomato is economically attractive and the area under cultivation is continually increasing (Shankara *et al*., 2005; Babalola *et al*., 2010; Elbadrawy and Sello, 2011; Georgé *et al*., 2011; Emana *et al*., 2017). Tomato is an excellent source of

many nutrients and secondary metabolites that are important for human health (Giovanelli and Paradise, 2002; Davoodi *et al*., 2007; Hadizadeh *et al*., 2009). Research efforts have helped to increase tomato production; unfortunately, there were no similar efforts to minimize the postharvest losses and extend its shelf life (Nasrin *et al*., 2008). However, world losses in the tomato yield have been greatly referred to soil and air borne pathogens (Nowsheen, 2015) and, much of the production is being lost during the postharvest due to inadequate storage conditions (Vinha *et al*., 2013; Luna-Guevara *et al*., 2014). These losses are most often caused by microbial infection, physiological breakdown due to natural ripening processes and environmental conditions such as heat and drought (Sanyaolu, 2016).

Therefore, the need for food protection which intends to prevent contamination and spoilage of foods cannot be under emphasized. Washing procedures applied to fresh produce before packaging (with chlorine) have the potential to reduce contamination from the surface of the product (Nasrin *et al*., 2008; Barth *et al*., 2009). However, the active chlorine is readily degraded by organic matter (Tomás-Callejas *et al*., 2012) and the high turbidity of water reduces the efficiency of chlorine disinfection (Jiang, 2016).

A new approach to the control of postharvest pathogens while maintaining fruit quality has been implemented by the use of natural plant extracts. Plant essential oils have the potential to replace the synthetic fungicides in the management of postharvest diseases of fruit and vegetables (Vitoratos *et al*., 2013). Furthermore, essential oils are environmentally friendly, degradable, and cheaper than chemical preservatives (Willis, 2013). Spices and herbal essential oils are used by the food industry as natural agents for extending the shelf life of foods (Kamdem *et al*., 2007; Eissa *et al*., 2008; Essia *et al*., 2014; Shimaa *et al*., 2014). Apart from antimicrobial activities, spices are believed to have medicinal value and have desirable determinative influences on the overall organoleptic analysis when used (Adedeji and Ade-Omowaye, 2013).

More than 1300 plant species are known to be potential sources of antimicrobial components but only some of them have been studied scientifically (Jasenka *et al*., 2010). These include oregano (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.), lemon (*Citrus limon* L.), marjoram (*Origanum majorana* L.), rosemary (*Rosmarinus officinalis* L.), garlic (*Allium sativum*), ginger (*Zingiber officinale*), coriander (*Coriandrum sativum*), peppermint (*Mentha piperita*), sage (*Salvia officinalis*), cinnamon (*Cinnamomun zeylanicum*), clove (*Eugenia caryopyllata*), lavander (*Lavandula hybrida*), etc.

Tomato is a staple vegetable fruit. In South-Kivu, tomato yield was estimated from 58,928 tons/ha in 2015 to 129,053 tons/ha in 2017 (IPAPEL, 2018). Postharvest losses of this product contribute to the country's high dependence on vegetable imports estimated at about 70% on average (Buntong *et al*., 2013). At our knowledge, there is no documentation on data regarding the soil surveys before tomato farming in the Bushi region. Another constraint to food production is the absence of farm storage facilities and proper packing houses. The result is that tomato is being marketed immediately after harvesting without primary processing and adequate packaging to avoid its rapid deterioration. Due to perishability, the farmers are disadvantaged and compelled to get rid of their produce at reduced prices (Adubofuor *et al*., 2010). This situation forces them to sell off their products and then buy the same products at very high prices (Mathooko and Nabawanuka, 2003). To increase the preservation of tomato fruits, it is important to target pre and postharvest factors that influence its perishable nature. In fact, preharvest conditions like soil composition and environmental conditions affect the tomato growth process and finally the nutritional quality, mineral content and disease resistance. Soils tend to be vary greatly in chemical and physical properties even within fields that appear uniform, and this variability brings interest in soil sampling analyses. The overall role of postharvest technology is to devise methods by which deterioration of produce is restricted as much as possible during the period between harvest and end use, and to ensure that maximum market value for the produce is achieved.

Based on these informations and statement, our research question was: "can a simple postharvest treatment based on the use of rosemary essential oil (REO) end to the extension of shelf life for fresh fruits and transformed tomato products in the Bushi region"? The hypothesis to this research was that the use of REO can help to reduce tomato postharvest losses and extend its shelf life. The general objective of this study was to propose simple postharvest technology using REO to reduce postharvest decay. The specific objectives for this research thesis were to:

1. Conduct survey on soil characteristics of the potential areas for tomato production and characterize tomato varieties in the Bushi region (Democratic Republic of the Congo)
2. Develop conservation techniques using a natural preservative and evaluate its effects on the fresh-like aspect of tomato fruits and products
3. Implement on the field one of the developed techniques in the Bushi region (DRC)

CHAPTER 1:
LITERATURE REVIEW

CHAPTER ONE
LITERATURE REVIEW

I.1. IMPORTANCE OF TOMATO

I.1.1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetables worldwide. Tomato production accounts for about 4.8 million hectares of harvested land area globally with an estimated production of 162 million tonnes (FAOSTAT, 2014). Tomato has become an important cash and industrial crop in many parts of the world (Babalola *et al*., 2010) because of its short duration for cultivation and high yield; and the area under tomato cultivation is increasing daily (Shankara *et al*., 2005). Tomato fruits are one of the most frequently consumed vegetables in the world, since it contributes to a healthy well-balanced diet which is rich in vitamins such as vitamin A, B, C and E; carbohydrates such as fructose and glucose; minerals which include phosphorous, sodium, potassium, calcium, magnesium and trace elements like iron, copper, zinc and dietary fibers (Dominguez *et al*., 2012; Ugwu *et al*., 2014). It can be eaten fresh or in several processed forms and is used for culinary purposes and in the production of fruit drinks. Fresh tomatoes and other processed tomato products make a significant contribution to human nutrition, owing to the concentration and availability of several nutrients in these products and to their widespread consumption (Sibomana *et al*., 2013). The quality and nutritional value of freshly produced tomato fruits is affected by pre and postharvest diseases, improper handling and other conditions (Kader, 1986).

I.1.2. Postharvest losses in tomato

Postharvest losses (PHL) refer to the losses (qualitative and quantitative) that occur along the food supply chain, from the farm gate through, till it gets on the table of the final consumer (Aulakh and Regmi, 2015; Emana *et al*., 2017). Fresh horticultural products are highly perishable with estimates suggesting a postharvest loss of 30 to 50 % in fruits and vegetables (Atanda *et al*., 2011). According to Emana *et al*. (2017), postharvest losses of vegetables such as tomato are attributed, in some cases, to socioeconomic and institutional factors, inadequate marketing information and support systems, inappropriate transportation facilities, unfavourable government policies, inability to implement regulations and legislations, lack of appropriate tools and equipments, lack of technical know-how and poor maintenance culture for existing facilities and infrastructure. Postharvest losses for horticultural produce are, however, difficult to measure; they can arise from a number of causes.

Generally, quality and duration of shelf life of fruit and vegetables are affected by the combined effect of preharvest and postharvest treatments. Tomatoes are prone to rapid quality losses after harvest, due largely to the stage of ripeness at which they are harvested. Postharvest losses in tomatoes can be as high as 25-42 % globally (Arah *et al*., 2015), but this is much higher in developing countries (Ebimieowei *et al*., 2013). Postharvest management starts with preharvest managements. According to Kader (2005), there are numerous factors affecting postharvest losses, from the soil in which the crop is grown to the handling of produce when it reaches the shop. Preharvest factors (environmental conditions, cultural practices, cultivars, pesticides, fertilizers, etc.) influence overall fruit quality and suitability for storage by modifying physiology, chemical composition, and morphology of fruits.

I.1.2.1. Environmental factors

Preharvest environmental conditions such as water, temperature, light intensity, soil fertility, and CO_2 concentration all affect tomato postharvest quality (Jiang, 2016). Temperature management is essential for maintaining the quality of postharvested fruits and vegetables. According to López Camelo and Gómez (2004) as cited by Cherono (2016), temperature influences colour development by stimulating plastid development at T° > 12°C and < 30°C. It also has been reported that a lower temperature can cause chilling or freezing injury to the produce, decrease metabolic rates and reduce deterioration (Shalluf, 2010). Light and temperature may influence the ripening index of tomato; screening light inhibits β-carotene synthesis while increased exposure to light increases β-carotene synthesis (Dumas *et al.*, 2003).

Adequate soil moisture during preharvest periods is essential for the maintenance of postharvest quality. Tomato plants need a controlled supply of water throughout the growing period for optimal quality and higher yield. Tomatoes are very sensitive to water deficits during and immediately after transplanting, at flowering and during fruit development (Sibomana *et al*., 2013). It has been reported that soil type can affect sensory attributes of freshly harvested produce. Fallik *et al*. (2009) reported a number of researches where clay soil appeared to have some advantages over sandy loam soil in producing cantaloupe fruits with superior sensory quality attributes; and that the type of soil growth medium has influenced tomato firmness.

I.1.2.2. Cultural practices

The increase in yield of tomato needs appropriate techniques that minimize postharvest loss. Cultural practices such as nutrient, water supply and harvesting methods are also claimed to be factors influencing tomato quality after harvest (Meaza *et al*., 2009). Sibomana *et al*. (2013)

found that the decrease in tomato plant growth and yield could be attributed to the effects water stress on the physiology of the crop (chlorophyll content, leaf relative water content and vegetative growth). Irrigation is also a very important preharvest factor that affects quality and sensory attributes of fresh produce (Fallik *et al*., 2009). Deficit irrigation regulates tomato fruit quality; water deficit stress during the growing season can affect the size of the fruit and lead to soft or dehydrated fruit that is more prone to damage and decay during storage (Sibomana *et al.*, 2015).

Application of mineral fertilizers, especially of nitrogen, affects the chemical composition of vegetables including tomato. It has been reported that adequate supply of potassium fertilizer in tomato production improves fruit colour; insufficient supply of potassium in soilless tomato production result in ripening disorders; and calcium application in tomato production has a positive effect on the prevention of some plant diseases (Meaza *et al*., 2009; Arah *et al*., 2015).

Cultivar selection may be an important factor to consider in tomato production because of the genetic diversity. According to Jiang (2016), many genes affect fruit colour by controlling the quantity and type of pigments. Cultivars differ in size, colour, texture, and flavour as well as storage potential and there are characterised by different quality parameters making some more desirable to the producers and consumers than others. The quality potential of fruit is dependent on the cultivar type (Getinet *et al*., 2011). Arah *et al*. (2015) recommended that pruning (which is controlling the number of flowers, fruits, or fruit trusses in tomatoes) is an effective way of reducing the competition between fruits.

Tomato ripeness at harvest also affects subsequent fruit quality. Being a climacteric fruit, tomato can be harvested at different stages during maturity. Each stage at harvest has its own postharvest attribute that the fruit will exhibit. Maturity stage at harvest is very important to composition and quality of tomatoes, being a very determinant factor for different postharvest quality attributes of tomato fruit such as soluble solid, sugar, acidity, pH, colour and firmness (Tilahun, 2013). In table I, a description of a different classes of ripeness of tomato is presented.

Table I: Ripeness classes of tomatoes

Class	Description*
Immature-green	The surface is completely light to dark green. There is no jelly-like material in any of the locules, and the seeds are cut upon slicing the fruit with a sharp knife.
Mature green	Seeds are fully developed and are not cut upon slicing of the fruit. Jelly-like material is formed in at least one of the locules. This is the minimum stage of harvest maturity.
Breaker	Tomatoes at this stage are characterised by a definite break in the colour from green to tannish-yellow, pink or red on not more than 10 % of the surface.
Turning	More than 10 % but not more than 30 % of the surface, in the aggregate, shows a definite change in colour from green to tannish-yellow, pink, red or a combination thereof.
Pink	More than 30 % but not more than 60 % of the surface, in the aggregate, shows pink or red colour.
Light-red	More than 60 % of the surface, in the aggregate, shows pinkish red or red colour, provided that not more than 90 % of the surface is red colour.
Red	More than 90 % of the surface, in the aggregate, shows red colour.

*All percentages refer to both colour distribution and intensity.

Source: Rees *et al*. (2012).

I.1.2.3. Pests and diseases

Fungi are the most important and prevalent pathogens that infect a wide range of host plants, causing destruction and economic loss in tomato either in the field, storage or transportation. Several soil borne fungi that attack tomato plants causing wilt diseases and root rot include *Fusarium sp., Verticillium dahliae* and *Rhizoctonia solani.* These fungi are limiting factors for production of good quality and high quantity tomato fruits. A significant proportion of postharvest losses are also due to postharvest diseases caused by fungi and bacteria (Nowsheen, 2015). The extent of postharvest damage due to spoilage fungi is reportedly dependent on tomato variety. *Phytophthora capsici* causes late blight and other infections, resulting to wilting in tomato crops. *Alternaria solani* causes early blight; *Septoria lycopersici* is responsible for *Septoria* leaf spot in tomato; *Fusarium oxysporum* cause *Fusarium* wilt (Ebimieowei *et al*., 2013). Anthracnose causes rotting of ripe fruit; spots enlarge, become more sunken and develop concentric rings. These spots can run together and large rotten areas may appear. Ripe tomato fruits infected with blackmold fungus (*Alternaria alternata*) show superficial lesions (top) and large deep lesions (bottom).

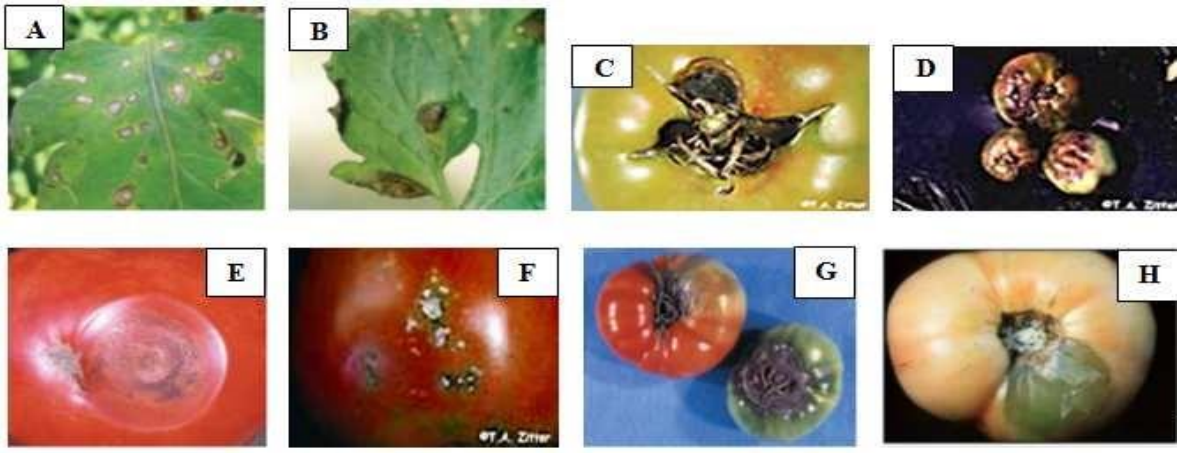


Figure 1: Some postharvest tomato diseases key identification

(A) Septoria leaf spot lesions (B) Early blight lesions on tomato leaves (C) Early blight on tomato fruit (D) Late blight (E) Anthracnose fruit rot (F) Blackmold (G) Graymold (H) Bacteria soft rot lesion

Source: Kennelly (2009) ; Bartz *et al*. (2013).

Literature presented by Sibomana *et al*. (2016) indicates that irrigation water quality influences the microbial load in harvested produce. High levels of coliform bacteria were found in fresh produce irrigated using untreated water, and significant incidences on contamination by fecal coliforms were detected on tomatoes irrigated with treated waste water.

The use of chemical compounds can have a positive effect upon vegetable yield but may result in secondary effects on shelf life, affecting appearance and nutritional value. Chemical fungicides are widely used for the control of preharvest fungal diseases. However, they may induce changes in quality characteristics, accelerating or delaying senescence (Domínguez *et al*., 2012). In recent years, there has been growing interest in the application of plant-derived substances in agriculture. These natural products isolated from plants appear to be one among the alternatives to the use of pesticides in order to obtain healthy crops and more environmentally sustainable crop production systems (Galani *et al.*, 2013; Sturchio *et al.*, 2014). Natural plant products and their analogues have been found as important sources of agricultural bio-pesticide which serve as antimicrobial properties of plant extracts (Tijjani *et al.*, 2014). The use of plants extracts in reducing preharvest tomato microbial infection have been proposed. An experiment on greenhouse tomato with essential oils of clove, thyme and rosemary was conducted by La Torre *et al.* (2016) in the control of *Fusarium* wilt disease; the essential oils provided antifungal activity compared with control treatments. Dakole *et al.* (2016) have worked on 13 Cameroonian plants against *Fusarium oxysporum f. sp lycopersici*

and *Phytophtora infestans* of tomato, and have found that all the tested essential oils exhibited an antifungal activity against both pathogens.

I.1.2.4. Physiological disorders

Physiological disorders (blossom-end rot, sunscald, puffiness, cracking) are also to be considered as important factors since they affect the fruit quality in the field and cause extensive losses in tomato production. Fruit cracking not only reduces fruit appeal and marketing, but also increase fruit susceptibility to decay and shorten shelf life, depending on the cultivar and environmental conditions. Tomatoes with defects may provide entry to pathogens and should be separated from good-quality fruit. It has been reported that lower grades of tomato were found to develop higher incidence of decay (Rees *et al*., 2012).

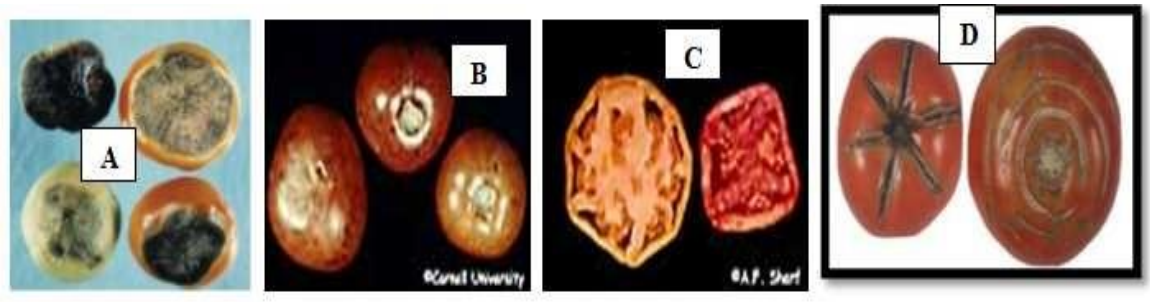


Figure 2: Some of the tomato physiological disorders

(A) Blossom end rot (B) Sunscald (C) Puffiness irregular fruit shape and empty locules (D) Radial and concentric growth cracks

Source: Kennelly (2009); Wikipedia (2017).

I.1.2.5. Land conditions impact on plant preharvest and postharvest resistance to diseases

The quality of soil macronutrients and micronutrients is generally transferred to the plant. Hence, a healthy soil produces a healthy plant and fruits. The correlation between soil nutriments and plants or fruits nutriments on one part, and between compositions of plants resistant to diseases compared to non-resistant ones on the other part, has permitted to understand the impact of nutrients on plant resistance to diseases. This resistance to diseases obtained during preharvest conditions is also important to reduce postharvest susceptibility for infection. The main macronutrients usually controlled in vegetable production are nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulphur (S) and magnesium (Mg).

Nitrogen is absorbed in plants in either a reduced form or an oxidized form and are internally reduced to amino acids prior to utilization by cells (Havlin *et al*., 2009). No matter the importance of this macronutrient, its impact on disease control is still contradictory in literature

(Gupta *et al*., 2017). Nonetheless, there is an inverse relationship between soil nitrogen concentration and fruit Total Soluble Solids (TSS), while Total Acids (TA) reduce with soil nitrogen concentration (Elamin and Al-Wehaibi, 2005; Parisi *et al*., 2006; Benard *et al*., 2009). On the contrary, Wang *et al*. (2007) observed that TSS and TA increased with soil nitrogen increase, indicating that this relationship may be genotype specific.

Regarding phosphorus, its importance in the metabolism is enormous. It is a building block of Adenosine Triphosphate (ATP) and is included in many important compounds. It is used as fungicide, bactericide and nematicide in plants disease control (Gupta *et al*., 2017). It has been demonstrated that application of P can increase resistance to *Fusarium* in tomato and some other crops (Kiraly 1976). When it comes to potassium, it has been demonstrated that its deficiency induces the reduction of cell wall diameters, the weakening of stalks and stems and excessive accumulation of nitrogen. These factors increase the probability of fungal, bacterial and viral infections (Marschner, 1995). Hence, adequate plant level of potassium can induce disease resistance in vegetable (Dordas, 2008). In fact, having reviewed 2449 references, Perrenoud (1990) concluded that K can decrease the incidence of fungal diseases by 70 %, bacterial diseases by 69 % and viral diseases by 41 %.

According to Marschner (1995), the presence of calcium in plants actively increase the resistance to pathogens, specially its ability to produce enzymes to dissolve the middle lamella. Calcium deficiency increases metabolic leakage and hence pathogen infection susceptibility (Spann and Schumann, 2010). Moreover, postharvest disorders like decay and fruit ripening delay are associated to low pre and postharvest application of Ca (Hernandez-Munoz *et al*., 2006; Lara *et al*., 2004). On the other hand, Garcia *et al*. (1996) found that Ca can prevent fruit softening and delay TSS during storage. Moreover, fruit glucose correlates negatively with soil calcium content, but magnesium and sulphate positively affect fructose level in tomato (Gravel *et al*., 2006).

Magnesium is known to be central in photosynthesis. Its deficiency usually enrich product with sucrose and amino acids, which are attractive nutrients for pathogens (Huber and Jones, 2013). Regarding Zinc, its deficiency can lead to an increased membrane leakage of low molecular weight compounds that may improve living conditions of pathogens (Huber *et al*., 2012). According to Heckman *et al*. (2003), Manganese can be useful in controlling a number of pathogenic diseases in plants. Alternatively, Copper deficiency can induce low plant cell rigidity and alter the membrane's lipid structure (Broadley *et al*., 2012).

I.1.2.6. Harvest and storage

After harvest, quality cannot be improved, only maintained. Postharvest losses in quality and quantity are related to immaturity at harvest, inadequate initial quality control, incidence and severity of physical damage, exposure to improper temperatures and delays between harvest and consumption (Kader, 1986); are encountered along the chain in the handling, storage, transportation and processing. Physical damage to tomatoes may occur during harvesting as well as by the action of various pests (rodents, insects, birds) and can lead to increased microbial spoilage and the potential transmission of food borne pathogens (EFSA, 2014). Loss of quality can be due to physiological and compositional changes that alter the appearance, taste or texture and make the produce less aesthetically desirable to end users (Wills, 2007).

Temperature greatly influences the rate of respiration of fruits and vegetables, and is undoubtedly one of the most important factors in maintaining postharvest quality of tomato fruits. Storage temperature of 10-15°C and 85-95 % of relative humidity could extend the postharvest life of fruits (Žnidarčič *et al*., 2010). Optimum storage conditions depend on the maturity stage of tomatoes. Literature indicates that high temperatures (38°C) inhibit lycopene production while low chilling temperatures inhibit both lycopene production and fruit ripening; cold storage has a negative effect on fruit aroma and consequently on its organoleptic quality. Rapid cooling to at least 12.5°C immediately after harvest and packing is important to remove heat, retard ripening, and prolong storage and shelf life; it also reduces water loss and decay incidence (Rees *et al*., 2012).

The microbial load associated with tomatoes during storage plays an important role on quality deterioration. The susceptibility of tomato to microbial colonization is due to its differential chemical composition such as high level of sugar, low pH (4.9-6.5) and its high water activity ($p > 0.99$). According to Duffy (2003), this could be due to varietal characteristics, climate influence, geographical and seasonal variations, various internal and external sources of contamination and agro-technical procedures which include the use of contaminated irrigation water, use of animal and human wastes as fertilizer, improper handling and storage.

I.1.3. Postharvest treatments in tomato

Once the produce is harvested, postharvest handling practices do not improve the quality attained to the field, they only slow the rate at which physiological and pathological deterioration occurs (Fallik *et al*., 2009). Harvested products are metabolically active, undergoing ripening and senescence processes that must be controlled to prolong postharvest

quality. Inadequate management of these processes can result in major losses in nutritional and quality attributes, outbreaks of food borne pathogens and financial loss for all players along the supply chain, from growers to consumers (Mahajan *et al*., 2014).

I.1.3.1. Surface disinfection

The quality and safety of fresh produce depend very much on their microbiological flora and the storage conditions that determine their shelf life (Seymour, 2003). Tomatoes may have been contaminated through contact with soil amendments, irrigated and/or sanitized pesticide water. After harvest, tomatoes are usually washed (with no regard for water quality) to remove dust and other foreign materials, prior to packaging. According to a USDA-Economic Research Service study in 1995, 18.9 billion pounds of fresh fruits and vegetables are lost annually due to spoilage (Barth *et al*., 2009). Underhill and Kumar (2015) reported that postharvest loss is primarily due to postharvest pathogens. Therefore, one of the major strategies in reducing food spoilage is to reduce microbial contamination by ensuring sanitization of fruits and vegetable surfaces (Magashi and Bukar, 2007).

Washing sanitizers and washing aids for fruits and vegetables include chlorine, organic acids, ozone and peroxone, chlorine dioxide, hydrogen peroxide, trisodium phosphate and surfactants. Each type of disinfectant has its own efficacy in killing microorganisms, which is dependent upon the nature of the microbial cells as well as the characteristics of fruit and vegetable tissues (Seymour, 2003). Different chemical washing agents have been studied to determine their efficacy in the inactivation of pathogenic bacteria on vegetables. Chlorine compounds have been used successfully for controlling postharvest vegetable decay by reducing bacterial inoculum during washing (Segall, 1968). Chlorine is still the most widely and commonly used disinfectant due to its efficacy, cost-effectiveness ratio and simple use (Das, 2002; Petri *et al*., 2015). The effectiveness of chlorine increases as pH is reduced from pH 11 to pH 8, but at lower pH, chlorine becomes unstable (Ogawa and Manji, 1984). However, chlorination has no residual effect, and therefore tomatoes exposed to pathogens after treatment remain susceptible to re-infection (Sawyer, 1978 cited by Rees *et al*., 2012).

Sodium hypochlorite (NaOCl) is a clear, slightly yellowish solution with a characteristic odour. It is used on a large scale (agriculture, food, chemical, pharmaceutical and waste disposal industries). When NaOCl dissolves in water, 2 substances which play a role in for oxidation and disinfection are formed; these are hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) as indicated in this equation:

$$NaOCl + H_2O \longleftrightarrow NaOH + HCl \longleftrightarrow Na^+ + OH^- + H^+ + OCl^- \quad \text{Eq 1}$$

Table II: Overview of different sanitizers used as decontaminants in tomato disinfection

Sanitizers	Concentration	References
Chlorine solution	200 ppm	Nasrin *et al*., 2008
Sodium hypochlorite (NaOCl)	50 ppm	Segall, 1968
	1.05%	Hong and Gross, 1998
	200 ppm	Ayala-Zavala *et al*., 2008
	150 ppm	Cantwell *et al*., 2009
Calcium chloride ($CaCl_2$)	4%, 8%, and 12%	Tessema, 2013
	1%, 2%, and 3%	Mujtaba and Masud, 2014
	0%, 2%, and 6%	Arthur *et al*., 2015
- Calcium chloride ($CaCl_2$) - Potassium metabisulphite (KMS) - Sodium chloride (NaCl)	- 1 g/100 g - 0.2 g/100 g - 7 g/100 g	Davoodi *et al*., 2007
- Calcium chloride ($CaCl_2$) - Acetic acid	- 2% - 5%	Gharezi *et al*., 2012
Essential oils (thyme, cinnamon, clove)	0.5%	Jiang, 2016

The effectiveness of chlorination is pH and temperature dependent (Das, 2002). Chlorine treatment effectiveness also depends greatly on the length of time the product is in contact with the disinfectant. It has been suggested that 50-200 ppm free chlorine is necessary to destroy vegetative bacterial and fungal cells in commercial fruit and vegetable washing (Bachman and Earles, 2000; Seymour, 2003).

I.1.3.2. Methods applied in delaying tomato ripeness

Major changes in the texture of tomatoes occurs during ripening and is mainly associated with softening that considerably influences postharvest quality. The ripening of fruits is complex and genetically programmed process that results in several changes such as texture, aroma, colour and flavour of the fruit (Mwendwa, 2016).

Tomato is a climacteric fruit and therefore its ripening is accompanied by a peak in respiration and a concomitant burst of ethylene. Ethylene production is promoted by stresses (chilling injury and wounding), and this stress-induced ethylene can enhance fruit ripening. Ethylene is a phytohormone that initiates and accelerates ripening, produces softening and degradation of chlorophylls, and inevitably leads to deterioration of fresh or minimally processed fruits and vegetables that shorten their postharvest life (Wassim *et al.*, 2011; Prasad and Kochhar, 2014). Tomato fruits produce moderate amounts of ethylene at 1 to 10 μL Kg^{-1} h^{-1} at 20°C and are sensitive to ethylene exposure. Ethylene levels as low as 0.5 μL L^{-1} is sufficient to trigger ripening and other associated metabolic processes (Mwendwa, 2016).

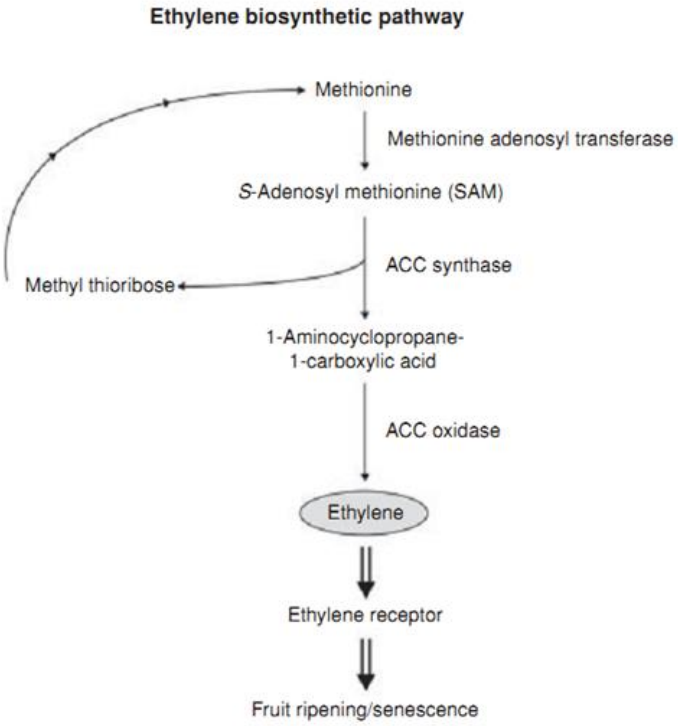


Figure 3: Summary of ethylene biosynthesis and action during fruit ripening

Source: Gopinadhan *et al*. (2012).

According to Fallik *et al*. (2009), exposure to 1-methyl-cyclopropene (1-MCP) which inhibits ethylene action, is an alternative method of slowing ripening of fruits or vegetables after harvest. Literature indicates that 1- MCP is an active organic compound that interacts with the ethylene receptors and prevents ethylene effects (Leibovitz, 2003). Commodities are exposed to 1-MCP in its gaseous form, which is typically applied in minute concentration as a fumigant in closed chambers at 20-25°C for 12-24 hour period. Sabir *et al*. (2012) reported a number of researches where tomato fruits treated with 1-MCP indicate lower respiration rate, ethylene production and weight loss, slower rates of lycopene accumulation, external colour development and ripening index, and prolonged postharvest life than untreated fruits.

It also has been reported that postharvest calcium chloride application reduces respiration, decreases ethylene production, and delays senescence in fresh produce such as tomatoes (Arah *et al*., 2015). Sound waves are thought to regulate a variety of plant hormones and genes. In the findings of Kim *et al*. (2015), sound wave can be used as an external stimulus to delay the ripening of tomato fruit. Tomato fruit treated with sound waves of 1 KHz exhibited delayed ripening as compared with the non-treated fruit at 5 or 7 days after treatment, most of the tomato fruit transitioned to the red ripening stage by 14 days after treatment. Pinheiro *et al*. (2015) have also reported that ultrasounds treatment was found to be effective in delaying

colour development and texture losses, preserving sensorial quality of whole tomato with increase of total phenolic content and microbial load reduction.

I.1.3.3. Use of packaging to delay postharvest quality loss

Packaging generally helps to protect and retain the quality of fresh horticultural produce and reduces damage during transport. It has been used to extend the storage life through the inhibition of physiological deterioration and reducing weight loss of many fresh fruits and vegetables. In general, packaging material will not only hold the food substance, but will also protect it from contamination. However, the type or properties of these packaging materials may influence the product quality (Mekonnen, 2017). Tomatoes are packed in a variety of packages, depending on the type of fruit, maturity or ripeness stage, and type of market and market requirements. The package should be sufficiently strong and adequately designed for sufficient ventilation, depending on the air circulation system employed in storage or during transit (Rees *et al*., 2012).

Packaging can create modified gas atmospheres around the product which slows down the respiratory activity of fruits including tomatoes. Modified atmosphere packaging (MAP) refers to the development of a modified atmosphere around the product through the use of packages constructed of semi-permeable polymeric film or with restricted diffusion through one or more pores (Kader *et al*., 1989). MAP is referred to as 'passive' if the atmosphere in the package is allowed to slowly establish itself by product respiration; 'active' MAP refers to more rapid atmosphere establishment achieved by flushing the package with nitrogen or a gas mixture near the expected equilibrium atmosphere.

I.1.4. Processing of tomatoes

According to Klein (1987), fruits and vegetables undergo varying degrees of handling, storage and processing before they are consumed. Minimal processing encompasses postharvest handling and storage, including pre-cooling, refrigeration, controlled and modified atmosphere storage, irradiation, and preparation for consumption (e.g. peeling, slicing, shredding). The processing of food commodities generally implies the transformation of the perishable raw commodity to value added product that has greater shelf life and is closer to being table ready (Chin,1997 as cited by Kaushik *et al*., 2009).

Tomato processing enables the highly perishable fresh produce to become available at reasonable prices during off season and low production periods. Tomato can be processed into a variety of products, including whole peeled fruit and diced tomatoes, as well as juice, ketchup and puree (Temesgen *et al*., 2011). It has been suggested that, although cultivar is

probably the most important factor affecting the quality of processed tomato products, other major parameters to be considered are tomato maturity and ripeness, growing location and climate, and processing conditions (Garcia and Barrett, 2006).

Food processing techniques implies the set of methods and techniques used to transform raw ingredients into food or to transform food into other forms for consumption by humans or animals either in the home or by the food processing industry (Kaushik *et al*., 2009). Processing of tomatoes leads to some nutrient losses as indicated in Table III.

Table III: Composition of fresh tomato and tomato products (100g)

	Product					
	Tomato	**Juice**	**Paste**	**Ketchup**	**Chili sauce**	**Purée**
Water (%)	93.5	93.6	75	68.6	68	87
Carbohydrate (g)	4.7	4.3	18.6	25.4	24.8	39
Protein (g)	1.1	0.9	3.4	2.0	2.5	1.7
Fat (g)	0.2	0.1	0.4	0.4	0.3	0.2
Ash (g)	0.5	1.1	2.6	3.6	4.4	2.2
Ascorbic acid (mg)	23	16	49	15	16	33
Vitamin A (IU)	900	800	3300	1900	1400	1600
Food energy (calories)	22	19	82	106	104	39

Source: Thakur *et al*. (1996).

There are many processing methods of tomato fruits (Figure 4), the most important being drying (to produce dried tomatoes or a powder) and concentration (to a paste or purée). It is recommended that for each of the processes, the tomato should be ripe, red, firm to soft, free of mould growth. In figure 4, some flow charts of tomato processing are presented.

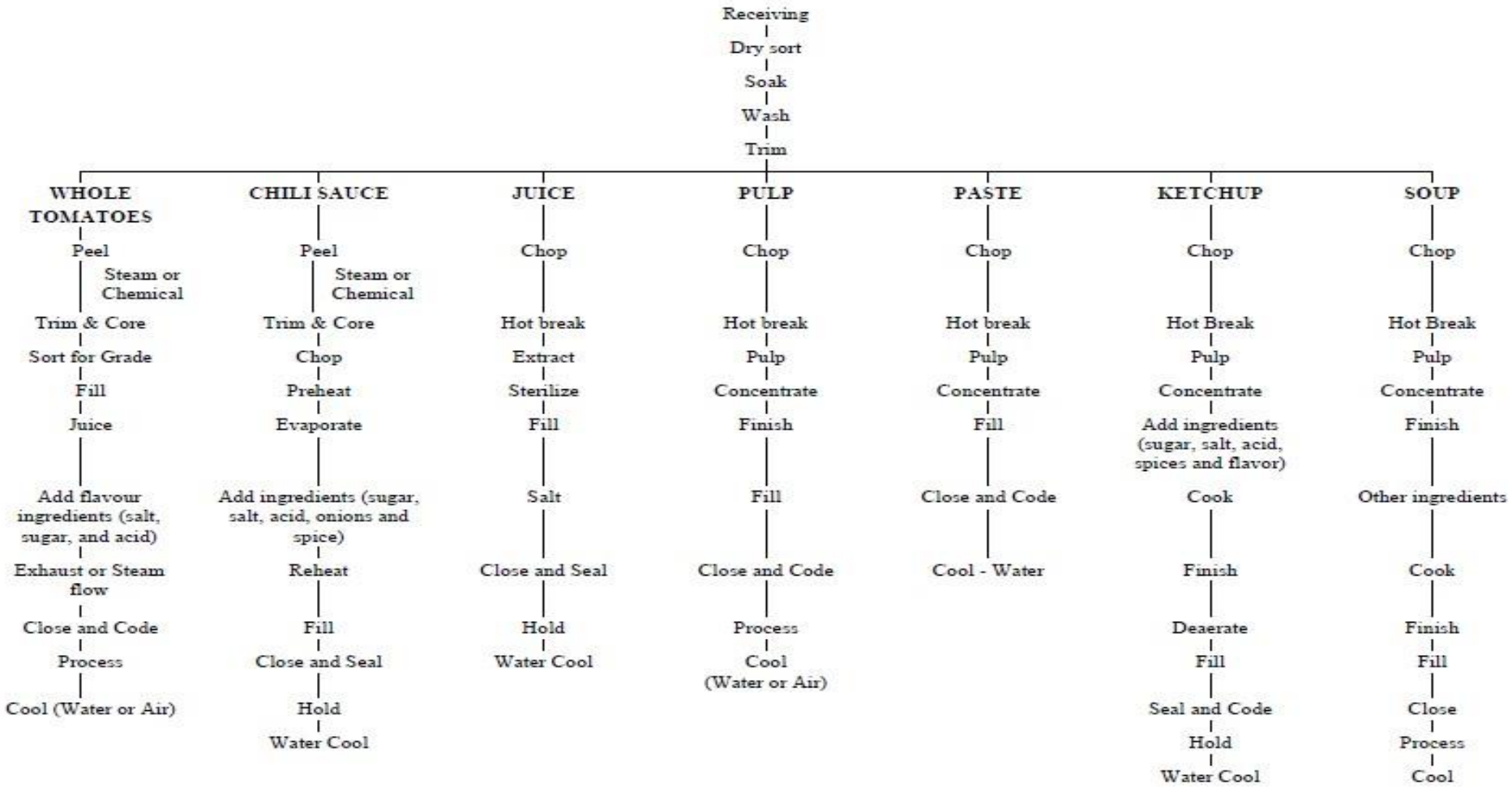


Figure 4: Tomato processing flow chart

Source: (Gould, 1992) referenced by OECD (2008).

I.1.4.1. Use of thermal treatments in tomato processing

Thermal processing is one of the most common forms of food preservation because it efficiently reduces microbial population and destroys natural enzymes. The use of heat through thermal processing operations, which among others includes pasteurization, sterilization, drying and evaporation, is still a common practice of the food industries in order to guarantee the microbiological safety of their products (Pereira and Vicente, 2010).

It is widely believed that processing results in significant losses of nutrients (Klein, 1987; Dewanto *et al*., 2002); and fresh produce being perceived as healthier than frozen or canned. The largest portion of tomato is thermally processed and concentrated into tomato paste (Wilkerson *et al*., 2013), yet the nutritional value of tomato products is a topic attracting much attention, particularly regarding the effects resulting from food processing and storage treatments (Capanoglu *et al*., 2010).

I.1.4.2. Effects of processing on nutrients' availability

During treatment, several additional changes can occur to affect the appearance, composition, nutritional value, and sensory properties in terms of colour, texture, and flavour of the product. The effect of processing on the antioxidant level of tomatoes has previously been reviewed, and the exact level of losses or even gains of antioxidants have been reported to differ widely according to the type or variety of the tomatoes used, fruit ripeness, agricultural treatments, conditions such as temperature, time of treatment, presence of oxygen or light, and methods of processing (Mayeaux *et al.*, 2006; Capanoglu *et al*., 2010; Rees *et al*., 2012; Wilkerson *et al*., 2013).

Although it is known that longer processing times may be associated with increased nutrient losses, researchers reported that bioavailability of some nutrients, antioxidant activity and other nutritional properties of tomato products may be improved by heat treatment. Thermal processing enhances the nutritional value of tomatoes by increasing the content of bio-accessible lycopene content and total antioxidant activity (Dewanto *et al*., 2002; Bicanic *et al*., 2010; Ishiwu *et al.*, 2014). However, Takeoka *et al*. (2001) have reported that thermal processing of tomatoes into paste can result in a decrease in lycopene concentration of 9-28 %; losses in antioxidant activity associated with decreases in lycopene concentration during processing might be accompanied by increases in antioxidant activity of other components, particularly polyphenolics. Mayeaux *et al.* (2006) have reported that lycopene is not stable during long heating times and rapidly decomposed at a heating temperature of 150^{o}C and above. On the other hand, Mohammed and Malami (2013) reported that lycopene in tomato

appears to be a relatively stable compound during food processing, and Ishiwu *et al.* (2014) attested that thermal processing makes the lycopene more available in processed tomato. It has also been reported that lycopene content significantly increased when tomatoes were heated in an oil bath at 100^{o}C for 2 hours (Graziani *et al*., 2003). It is thought that the increase in bio-accessible lycopene content is primarily due to the increased release of phytochemicals from the matrix to make it more accessible in the extraction (Dewanto *et al*., 2002).

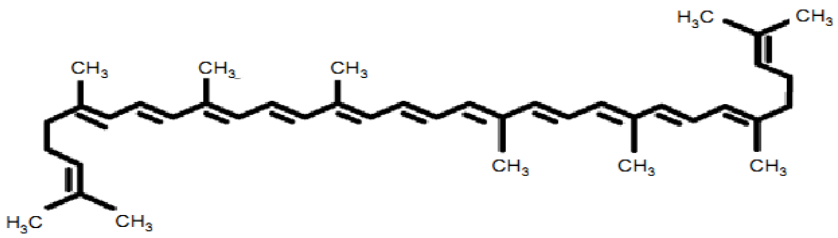


Figure 5: Molecular formula of lycopene

Source: Robert and Mildred (2008)

An increase in carotenoids by thermal processing has also been attributed to enzymatic degradation which causes weakening in protein-carotenoid aggregates, even though the extractability of carotenoids may vary according to the ripening stage, firmness, and genotype of the fruit (Capanoglu *et al*., 2010). According to Dewanto *et al*. (2002), thermal processing may release more bound phenolic acids from the breakdown of cellular constituents, and the heat-processed tomatoes might retain their total phenolics, flavonoids, and total antioxidant activity.

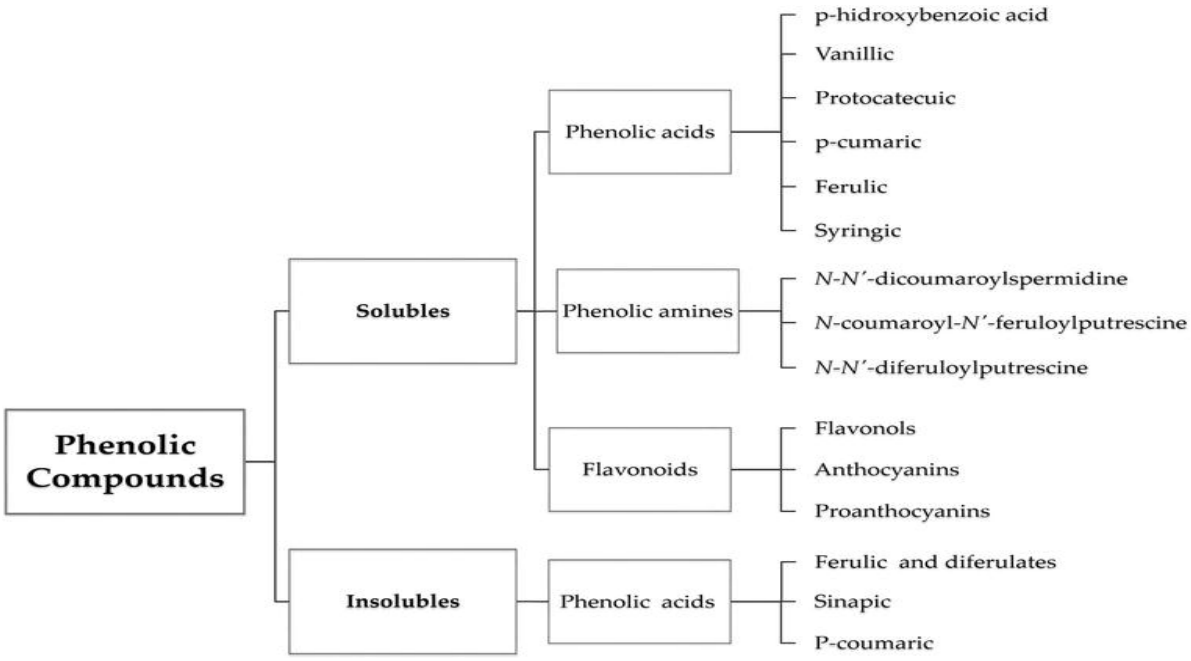


Figure 6: Phenolic compounds

Changes in the antioxidant activity of tomato products are complex and depend on the specific compounds being studied; the increase in antioxidant activity may also be partially explained by the production of new antioxidants during processing, particularly polyphenolics (Takeoka *et al*., 2001).

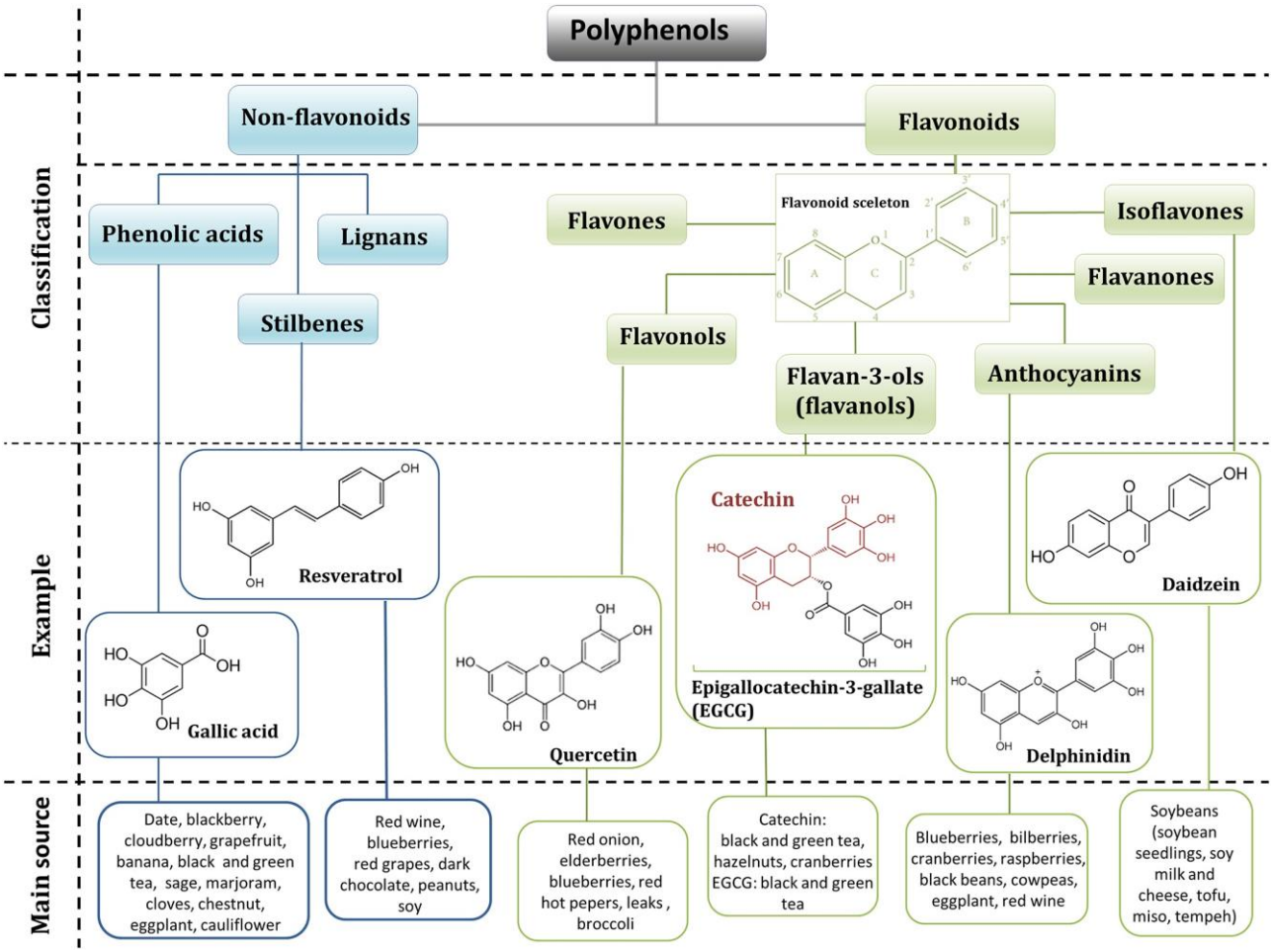


Figure 7: Structures of Polyphenols

The vitamin C (ascorbic acid) content of fresh tomatoes depends on the variety and the cultivation conditions. Many researchers have reported a decrease in the vitamin C content with increased heating time (Takeoka *et al*., 2001; Dewanto *et al*., 2002). Literature indicates that vitamin C is destroyed mainly due to oxidation reactions and the heat applied in the presence of air (Capanoglu *et al*., 2010), because heat is known to speed the oxidation process of ascorbic acid. Loss of vitamin C occurs primarily by chemical degradation that involves oxidation of ascorbic acid to dehydroascorbic acid (DHAA), followed by hydrolysis to 2,3-diketogulonic acid and further polymerization to form other nutritionally inactive products (Gregory, 1996 as cited by Dewanto *et al*., 2002).Losses have also been attributed to a number of enzymes: ascorbic acid oxidase, peroxidase, cytochrome oxidase and polyphenol oxidase (Klein, 1987).

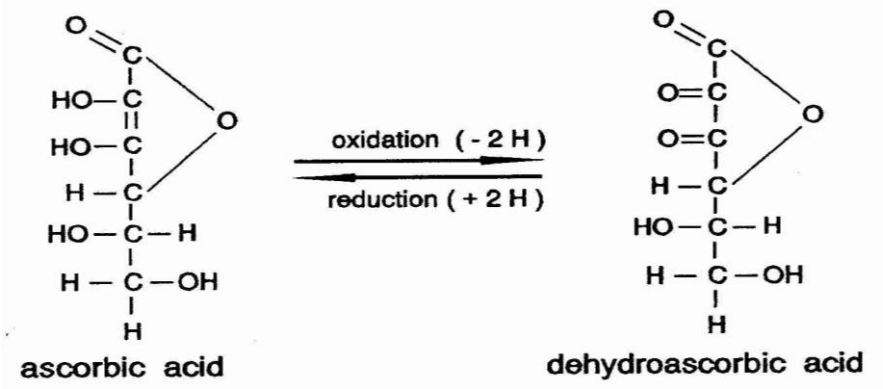


Figure 8: Reversible 2-electron oxidation/reduction of Ascorbic acid to dehydroascorbic acid

Among the parameters analysed for the assessment of tomato quality, pH is very important because acidity influences the thermal processing conditions required for producing safe products. Tomato pH is dependent on several factors, including cultivar, maturity stage, cultural practices as well as growing location and seasonal variations (Gould, 1992).

Although the pH of mature tomatoes may exceed 4.6, tomato products are generally classified as acid foods (pH < 4.6), which require moderate conditions of processing to control microbial spoilage and enzyme inactivation (Garcia and Barrett, 2006).

I.2. ROSEMARY (*Rosmarinus officinalis L.*)

I.2.1. Introduction

Rosemary (*Rosmarinus officinalis* L.) is an aromatic, medicinal and condiment plant that belongs to the Lamiaceae (Labiatae) family (Boutekedjiret *et al.*, 2003; Tavassoli *et al.*, 2011). The plant, a small evergreen shrub, grows to a height up to 2 m, and has linear leathery leaves that are sharply pointed, deep green with revolute margins, and are whitish beneath. Its flowers are pale to mid-blue, 10-12 mm long, and borne in small lateral clusters (Katerinopoulos *et al.*, 2005). They bloom almost throughout the year.

Rosemary, a woody and perennial herb with fragrant, grows principally in the Mediterranean region and is well known around the world as a common spice for culinary purposes (fresh and dried rosemary leaves, whole or ground, are used as seasonings for soups, stews, sausages, meat, fish and poultry) and folklore medicinal uses (Boutekedjiret *et al.*, 2003; Rožman and Jeršek, 2009; Hać-Szymańczuk *et al.*, 2011; Chahboun *et al.*, 2014; Sahraei *et al.*, 2014).

Rosemary plant Rosemary leaves Rosemary flowers

Figure 9: Rosemary (*Rosemarinus officinalis*)

Source: Leplat (2017)

I.2.2. Rosemary essential oil composition

Natural extracts obtained from plants have been widely used since many centuries ago. Most of the times, natural extracts owe their biologic activity to the synergism between their different compounds, as their activity is lesser than when these substances are together (Calvo *et al*., 2011). Herbal benefits are known to be many; they contain unique antioxidants, essential oils, vitamins, phyto-sterols and many other plants derived nutrient substances. Essential oils (EO) are a complex mixture of natural, volatile, and aromatic compounds synthesized by aromatic plants that have been often used in traditional medicine. An essential oil is a concentrated, hydrophobic liquid extracted from a plant, and containing volatile aroma compounds in high concentrations (Ferdeş and Ungureanu, 2012; Willis and Saidman, 2013). Essential oils are natural antimicrobials with promising potential applications in the food and pharmaceutical industry for the control of pathogenic bacteria (Rahaman and Kang, 2009, cited by Ribeiro *et al*., 2013).

The essential oil composition of rosemary has been investigated and reported in literature of several areas, mainly in the Mediterranean region. Studies in the Balkan and Northern Mediterranean regions include analyses of Greek, Yugoslavian, Bulgarian, Hungarian, Portuguese, Spanish, French, and Italian oils (Katerinopoulos *et al*., 2005; Sienkiewicz *et al*., 2013; Sahraei *et al*., 2014); various compositions have been described in terms of the major constituents and growing regions (Jamshidi *et al*., 2009; Chahboun *et al*., 2014), and methods of extraction (Boutekedjiret *et al*., 2003; Abdeldaiem *et al*., 2009). This herb is intensively grown in South Africa and is being introduced in many other countries.

Many compounds have been isolated from rosemary, including flavones, diterpenes, steroids, and triterpenes. The leaves of rosemary contain 0.5-2.5 % of volatile oil. Natural polyphenols found in the leaves have potential therapeutic benefits, because of their potent antioxidant activity and their anticarcinogenic and antiviral properties (Savoini *et al*., 2003 as cited by Sahraei *et al*., 2014). It has been reported that the rosemary antioxidant properties are attributed to its diterpenoids, lavonoids, triterpenoids and phenolic acids constituents (Mounchid *et al*., 2004).

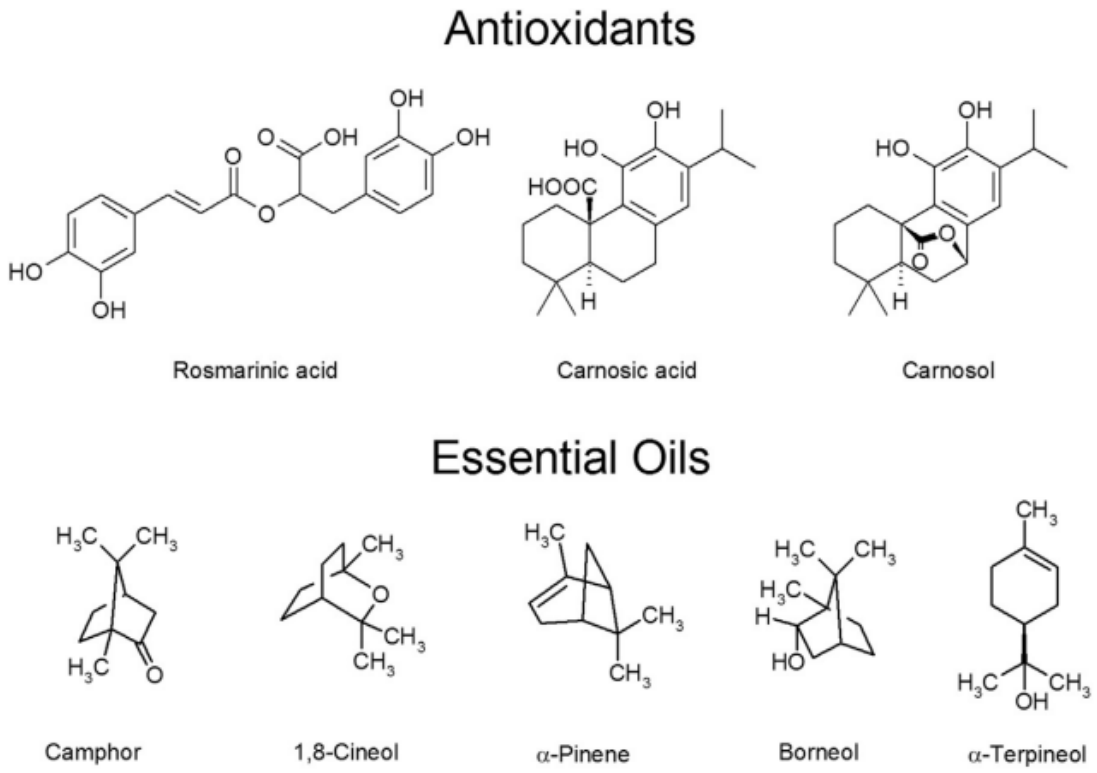


Figure 10: Chemical structures of the antioxidants present in rosemary leaves, subdivided into antioxidants and essential oils

Source: Wollinger *et al*. (2016).

According to Sienkiewicz *et al*. (2013), the essential oil from *Rosmarinus officinalis* L. contains 37 components (Table IV), the main ones being 1,8 cineole (46.4 %), camphor (11.4 %), α-pinene (11.0 %), β-pinene (9.2 %) and camphene (5.2 %).

Table IV: Chemical composition of the Rosemary oil

	Compound	%	RI
1	Tricyclene	0.2	919
2	α-Thujene	0.1	923
3	**α-Pinene**	**11.0**	**932**
4	**Camphene**	**5.2**	**944**
5	Sabinene	0.1	966
6	**β-Pinene**	**9.2**	**971**
7	**Myrcene**	**1.2**	**983**
8	α-Phellandrene	0.2	997
9	Car-3-ene	0.1	1005
10	α-Terpinolene	0.1	1010
11	*p*-Cymene	1.3	1017
12	**1,8-Cineole**	**46.4**	**1027**
13	**Limonene**	**1.0**	**1027**
14	γ-Terpinene	1.0	1050
15	*trans*-Sabinene	Tr	1054
16	Terpinolene	0.2	1079
17	Linalool	0.5	1087
18	α-Campholenol	Tr	1096
19	*endo*-Fenchol	Tr	1102
20	**Camphor**	**11.4**	**1124**
21	**Borneol**	**3.1**	**1152**
22	Terpinen-4-ol	0.4	1163
23	**α-Terpineol**	**1.8**	**1175**
24	**Bornyl acetate**	**1.0**	**1269**
25	α-Cubebene	Tr	1349
26	α-Ylangene	Tr	1372
27	α-Copaene	0.1	1377
28	Longifolene	0.1	1407
29	**β-Caryophyllene**	**3.5**	**1421**
30	α-Humulene	0.4	1452
31	γ-Muurolene	0.1	1471
32	α-Selinene	Tr	1492
33	α-Muurolene	Tr	1494
34	γ-Cadinene	Tr	1506
35	*trans*-Calamenene	Tr	1511
36	δ-Cadinene	0.1	1514
37	β-Caryophyllene oxide	0.1	1571

RI-Retention Index; tr < 0.05%

Source: Sienkiewicz *et al*. (2013).

I.2.3. Rosemary essential oil properties

Rosemary essential oil (REO) is a colourless or pale yellow liquid, with characteristic odour of the plant. Rosemary is a prospective plant culture in the world; it is in the middle of interest as a preservative due to its antioxidative characteristics: it contains a wide variety of volatile and aromatic components and it is used in the medicinal phytology, pharmaceutical, food and cosmetic industries (Boutekedjiret *et al*., 2003; Mounchid *et al*., 2004; Abd-Alla *et al*., 2009; Jamshidi *et al*., 2009; Hać-Szymańczuk *et al*., 2011; Naser *et al*., 2011; Lima *et al*., 2013).

It has been reported that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria (Moreno *et al*., 2006; Sienkiewicz *et al*., 2013). Literature indicates that the main compounds responsible for the antimicrobial activity are α-pinene, bornyl acetate, camphor and 1, 8-cineole (Genena *et al*., 2008).

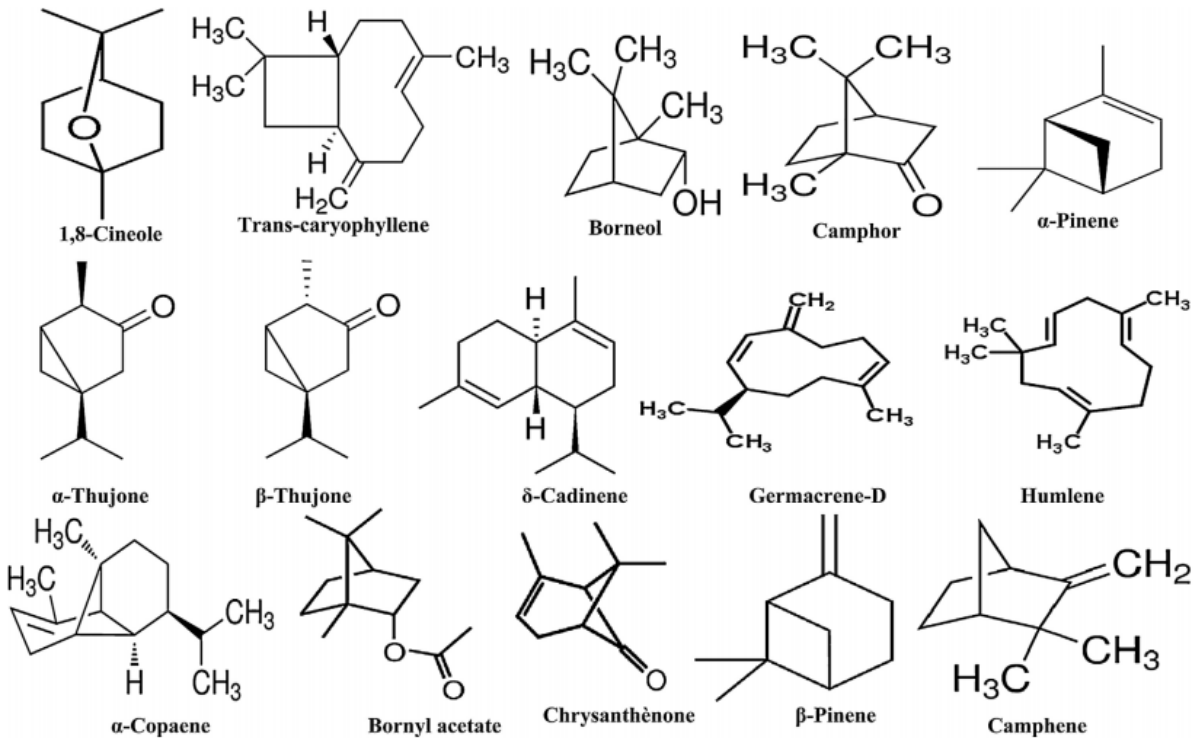


Figure 11: Structures of chemical components of Rosemary Essential Oil (REO)
Source: Selmi *et al.* (2017).

Celiktas *et al*. (2005) have reported a number of studies that have focussed on the biological and antimicrobial properties of the REO and their main constituents. Bacterial strains especially susceptible to the activity of essential rosemary oils include: *Enterococcus*

faecalis, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Klebsiella pneumoniae* (Hać-Szymańczuk *et al*., 2011). Due to its antioxidant and antimicrobial activity, REO is capable to extend the shelf life of food products and maintain their quality during storage (Rašković *et al.* 2014).

I.2.4. Mechanism of action of essential oils

In recent years, essential oils have attracted lots of scientific interests because they exhibit a wide spectrum of bioactivities, such as antibacterial, antifungal, antiviral, antioxidant, and insecticidal activities (Li *et al*., 2019). More than 3000 essential oils have been identified and despite their use in traditional medicine, a very small fraction of about 10 % is approved for use in pharmaceuticals, cosmetics and food products (Lopez-Romero *et al*., 2015). Factors determining the activity of essential oils are composition, functional groups present in active components, and their synergistic interactions (Chouhan *et al*., 2017).

The antimicrobial mechanism of action varies with the type of essential oil or the strain of the microorganism used. Essential oils as well as their major components are having a variety of targets on the bacterial cell where exhibiting their action. Various reports indicate that essential oils can act by degrading the cell wall or can damage the cytoplasmic membrane, causing cytoplasm coagulation. Their detrimental effect on membrane proteins increase membrane permeability and ends up to leakage of the cell contents. However, the mode of essential oils action depends mostly on their chemical composition, and their activity against bacteria is not attributed on a single mechanism but is the result of numerous reactions concerning the bacterial cell (Alexopoulos *et al*., 2017).

Nieto *et al.* (2018) reported that the inhibitory effect of rosemary is the result of the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol. These interact with the cell membrane, causing changes in genetic material and nutrients, altering the transport of electrons, leakage of cellular components and production changes in fatty acid. In addition, it also produced an interaction with the membrane of proteins that produced the loss of membrane functionality and its structure. Extensive documentation on the antimicrobial properties of rosemary essential oils and their constituents has been carried out by several researchers (Table V).

Table V: Literature review on the use of Rosemary Essential Oil (REO) and extracts

Activities	Microorganisms	Food product	References
Pest control	Thrips		Katerinopoulos *et al*., 2005
Food additive		Sheep	Sahraei *et al*., 2014
Food preservative		Lamb patties	Baker *et al*., 2013
Food preservative		Potato chips	Lalas & Dourtoglou**,** 2003
Food preservative		Fish	Makri, 2013
Antifungal	*C. albicans*		Genena *et al*., 2008
Antifungal	*F. oxysporum*		La Torre *et al*., 2016
Antibacterial	*E. coli*		Sienkiewicz *et al*., 2013
Antibacterial	*P. aeroginosa*	Chicken	Petrová *et al*., 2013
Antimicrobial	*E. coli*	Cheese	Ribeiro *et al*., 2013
Antibacterial	*S. aureus, B. cereus E. coli, P. aeruginosa*		Genena *et al*., 2008
Antimicrobial	*A. flavus, A. ochraceus NRRL 3174*	Apple juice	Eissa *et al*., 2008
Antibacterial, Food preservative and Flavouring	mesophilic aerobic microorganisms, psychrophilic bacteria, coliforms, enterococci	Pork meat batter	Hać-Szymańczuk *et al*., 2011
Antibacterial, Antioxidant	*S. epidermidis, S. aureus, B. megaterium, E. coli, P. aeroginosa, P. hydrophila*		Abdeldaiem *et al*., 2009
Antibacterial, Histopathological	*E. coli, P. Vulgaris, S. enteritidis* strains on mice		Mounchid *et al*., 2004

I.3. *BACILLUS* SPECIES

I.3.1. Classification

The genus *Bacillus* is the largest genus within the Bacillaceae family, presently consisting of at least 226 species most of which are saprophytes, widely distributed in the environment and commonly isolated from soil, air, water, plants and animals (IDF, 2016). The genus *Bacillus* is divided into six subgroups based on spore morphology (Shangkuan *et al*., 2000; Mehrdad, 2007). According to IDF (2016), *Bacillus cereus sensu lato* (or *B. cereus* group) consists of eight formally recognized species: *B. anthracis, B. pseudomycoides, B. mycoides, B. thuringiensis, B. weihenstephanensis, B. cytotoxicus, B. toyonensis* and *B. cereus sensu stricto* (the *B. cereus* species). Most of these species are very difficult to distinguish, even

with 16S rDNA sequencing. However, Shangkuan *et al*. (2000) have found that all species of the *B. cereus* group are closely related. They reported the findings of Henderson *et al*. (1995) that, there are relatively few tests to distinguish the isolates of *B. anthracis* from those of *B. cereus*. They can only be differentiated on the basis of mutable characteristics such as colony morphology, penicillin and gamma phage susceptibility, motility, lack of haemolysis, and elaboration of certain virulence factors.

Table VI: Criteria to differentiate among four closely related *Bacillus spp*.

Species	Colony	Motile	Haemolysis	Susceptibility to Penicillin	Parasporal Body	Virulent to Mice
B. cereus	White	Yes	Yes	No	No	No
B. anthracis	White	No	No	Yes	No	Yes
B. mycoides	Rhizoid	No	No	No	No	No
B. thuringiensis	White/ Grey	Yes	Yes	No	Yes	No

Source: Mehrdad (2007).

I.3.2. Description of *Bacillus cereus*

B. cereus is a large, motile (flagellated), gram-positive, facultative aerobic, sporeforming, rod shaped bacterium (Kannapha, 2006; Mehrdad, 2007; Daryaei *et al.,* 2013; Montanhini and Bersot, 2013), a major cause of food poisoning reported from many countries in the world (Mahendra *et al*., 2014). Literature indicates that one of the most distinct features of *Bacillus cereus* is its ability to produce heat resistant spores (Rodríguez-Lozano *et al*., 2010; El-Nour and Hammad, 2013). As a result of sporulation, resistance to wet heat, dry heat, radiation, desiccation, extreme pH, chemicals, enzymes, and high pressure is greatly enhanced. *Bacillus cereus* is widespread in nature and readily found in soil, where it adopts a saprophytic life cycle; germinating, growing and sporulating in this environment.

I.3.3. Growth and survival characteristics of *Bacillus cereus*

Bacillus cereus growth is optimal in the presence of oxygen but can occur under anaerobic conditions. The *Bacillus cereus* group are ubiquitous and abundant as spores in the soil. Being a soil resident, *B. cereus* is part of the microbiota of plant raw materials, attached as vegetative cells or spores (Mahendra *et al*., 2014). Many researchers have indicated that one of the most distinct features of *Bacillus cereus* is its ability to produce heat resistant spores. This resistance enables the bacterium to survive commercial food pasteurization and cooking at ambient pressure. Several environmental conditions influence bacterial heat resistance. In

addition to the heating temperature, it has been recognized that pH and water activities of the heating and recovery medium are the main factors that affect the apparent heat resistance of bacteria (Leguérinel *et al.*, 2004). According to Mahendra *et al*. (2014), low pH (below 4.5) and reduction in water activity (aw) (below 0.92) would inhibit *B. cereus*.

Table VII: Growth and survival characteristics of *B. cereus*

Growth	Survival
Temperature • Optimum 30-40°C • Range 4-55°C, emetic strains have a minimum of 10°C • Maximum toxin production at 20-25°C, toxin production range 10-40°C **pH** • Optimum 6-7 • Range 4.5-9.5 **Atmosphere** • Facultative anaerobe • Oxygen required for production of emetic toxin **Minimum Water Activity (aw)** • With NaCl >0.93 and <0.95 aw • With glycerol 0.93 aw	**Temperature** • Spores more resistant to dry than moist heat, and are also more resistant in oily foods. Cooking at or below 100°C may allow spore survival • Emetic toxins remain active after 150 min at 100°C (pH values 8.7 to 10.6) **pH** • Generally vegetative cells decline rapidly in stomach acid, however some may survive depending on food and level of stomach acidity • Spores are resistant to gastric acidity (between pH 1 and pH 5.2) • Emetic toxin stable between pH 2 and pH 9 **Water Activity** - Spores survive long periods in dry foods e.g. population unchanged after 48 weeks in cereal (aw 0.27-0.28)

Source: New Zealand Food Safety Authority (NZFSA, 2010).

Bacillus cereus is responsible of two types of food poisoning, causing two types of illnesses in humans, mainly diarrhoeal syndrome and emetic illness. The emetic syndrome is caused by cereulide, a heat- and pH-stable peptide toxin. Consumption of food contaminated with this toxin may lead to emesis between 30 min and 5 h after ingestion. The diarrhoeal syndrome is caused by enterotoxins that are produced during growth of *B. cereus* in the small intestine (Mehrdad, 2007; Daryaei *et al.*, 2013; Mahendra *et al*., 2014).

I.3.4. Prevention and control

Bacterial spores are more resistant to heat and other preservative treatments than the vegetative cells. Inactivation of bacterial spores requires high temperature and long heating time. Although spore dormancy and associated resistance are very stable, these properties are easily lost during inactivation (Brooks, 2013; Luu *et al*., 2015). Spores of *B. cereus* are heat

and radiation resistant, causing a big problem in food processing because of the high temperature or irradiation dose needed to inactivate them (El-Nour and Hammad, 2013). Heat resistance of *B. cereus* spores can be modified by the pH (Fernandez *et al.*, 2002). The pH value during heating can increase, decrease or remain constant, depending on the buffer system (Stoeckel *et al*., 2014). According to Leguérinel and Mafart (2001), low pH reduces spore resistance. *B. cereus* has been recovered from a wide range of food types; its presence in processed foods results from contamination of raw materials and the subsequent resistance of spores to thermal and other manufacturing processes (Houška *et al.*, 2007). During the cooling process, spores may germinate, enabling *B. cereus* to multiply in the food and/or produce high levels of the emetic toxin cereulide.

The number of spores in processed foods must be kept as low as possible by proper cleaning and disinfection of equipments. The decimal reduction time (D-value) of *B. cereus* spores can range from 2.2 to 5.5 min at 100ºC to above 80 min at 80ºC. Combined pressure–heat treatment provides a possible alternative to conventional heat treatment to inactivate bacterial spores while minimizing the adverse thermal effects on the product quality attributes (Daryaei *et al.*, 2013). According to Haberbeck *et al*. (2012), literature suggests a positive interaction for thermochemical treatments involving heat and natural preservatives, such as essential oils and their major molecules. EFSA (2005) has reported that food additives such as nisin produce the inactivation of *Bacillus cereus* vegetative cells while others like carvacrol have a very little effect.

I.4. IMPORTANT GAPS FROM LITERATURE REVIEW

Tomato is one of the most important consumed vegetable crop: its quality preservation and shelf life extension are a continuous challenge. Tomato losses are predominant between field and market; however, the biggest losses are observed after harvest. The reduction of postharvest losses can increase its year-round market availability. Research efforts have helped to increase tomato production; unfortunately, little is known on the influence of cultivation conditions on tomato quality and there is little information on the correlation between soil quality composition and tomato crop productivity.

In recent years, considerable interest has developed on the preservation of foods by the use of essential oils. Among these, rosemary essential oil has shown a strong antimicrobial action against spoilage and pathogenic microorganisms. Even though its use has been demonstrated in animal products, there is lack of information on its potential use in vegetables and vegetable products.

CHAPTER II: MATERIAL AND METHODS

CHAPTER TWO
MATERIAL AND METHODS

II.1. SURVEY ON TOMATO POTENTIAL PRODUCTIVE AREAS AND CHARACTERISATION OF TOMATO

II.1.1. Experimental site

The BUSHI region is an administrative zone that includes 2 territories of the South-Kivu Province in the Eastern Democratic Republic of Congo (CAID, 2017). Kabare territory (1,96 sq Km; 2°30'S-28°48'E) counts 197,100 ha of total area, of which 54% (82,000 ha) are arable land and 45% for pasture, while Walungu (1,8 sq Km; 2°38'S-28°40'E) has 172,700 ha of total area, of which 49% (93,000 ha) of arable land and 51 % for pasture (Kinghombe, 2003; SCG, 2014).

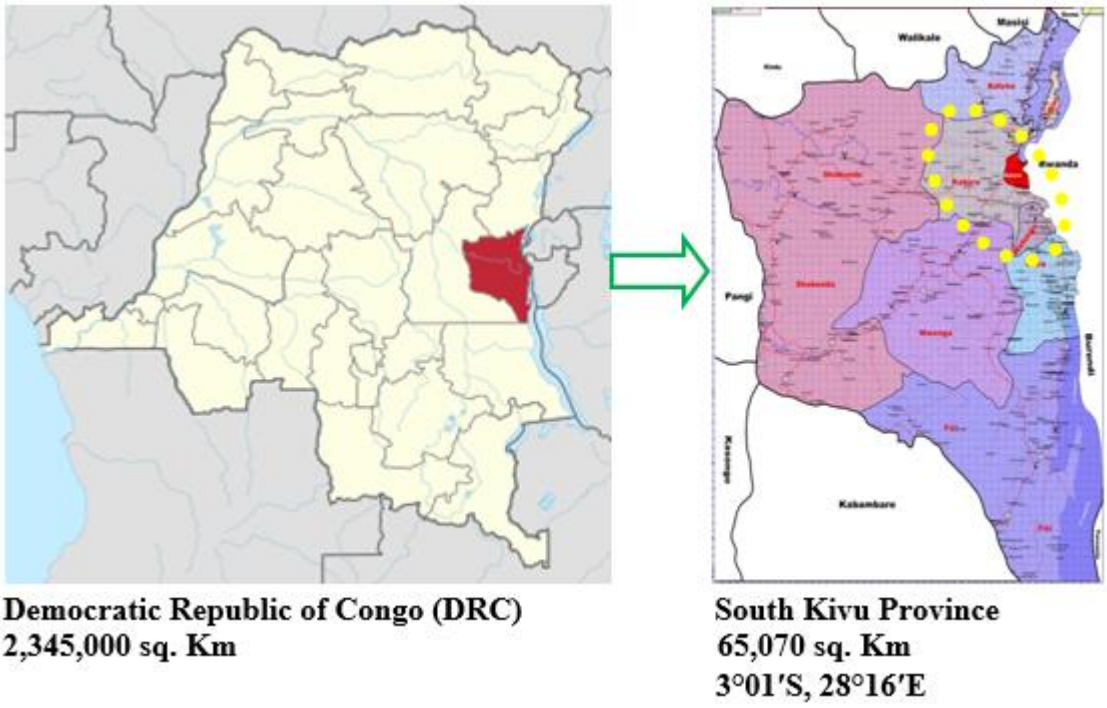


Figure 12: Geographical map of the study area

II.1.2. Suitability assessment and potential productive areas

Land suitability refers to the ability of a portion of land to tolerate the production of crops in a sustainable way (Boitt *et al*., 2015). Crop land suitability is determined based on soil properties, climatic, topographic and current land use factors. FAO classifies agricultural potential according to soil and environmental characteristics into four classes (Table VIII). Suitability classification for tomato is based on the FAO's land suitability classification found in Table IX (Jayasinghe and Machida, 2008).

Table VIII: FAO's Land Suitability classification

Classification	Description
S1	Highly suitable
S2	Moderately suitable
S3	Marginally suitable
NS	Not suitable

Table IX: Crop requirements criteria for tomato

Soil properties	Ranks			
	1	**2**	**3**	**4**
	Highly suitable	**Moderately suitable**	**Marginally suitable**	**Not suitable**
Soil pH	> 5.8 and < 7.5	> 7.5 and < 8	> 8 and < 9	Other values
Texture	Sandy clay loam/ sandy loam/ loam	Clay loam	Loamy sand/ sandy clay	Clay/ sand
Drainage	Well drainage	Moderately drainage	Poor drainage	Very poor drainage
Organic C (%)	> 5	> 3 and < 5	> 2 and < 3	< 2
CEC ($cmol_c kg^{-1}$)	> 15	> 10 and < 15	> 5 and < 10	< 5
Available P (ppm)	> 15	> 10 and < 15	> 5 and < 10	< 5
Elevation (m)	> 1000 and < 2500	> 2500 and < 3000	> 3000 and < 3500	Other values
Temperature (°C)	> 18 and < 24	> 24 and < 25	> 25 and < 26	Other values
Rainfall (mm)	> 1500 and < 2000	> 1000 and < 1500	> 2000 and < 2500	> 2500

To analyse soil suitability for tomato cultivation, the approach applied in this study required compiling cartographic data (topographic, land cover and climatic map). ArcGIS 10.3 software was used for this purpose. As part of our research, we have relied on the integration of Geographic Information Systems (GIS) and Multicriteria Evaluation (MCE) to highlight spatial recompositions and particularly, potential areas for implementation of tomato culture in the study area according to figure 13.

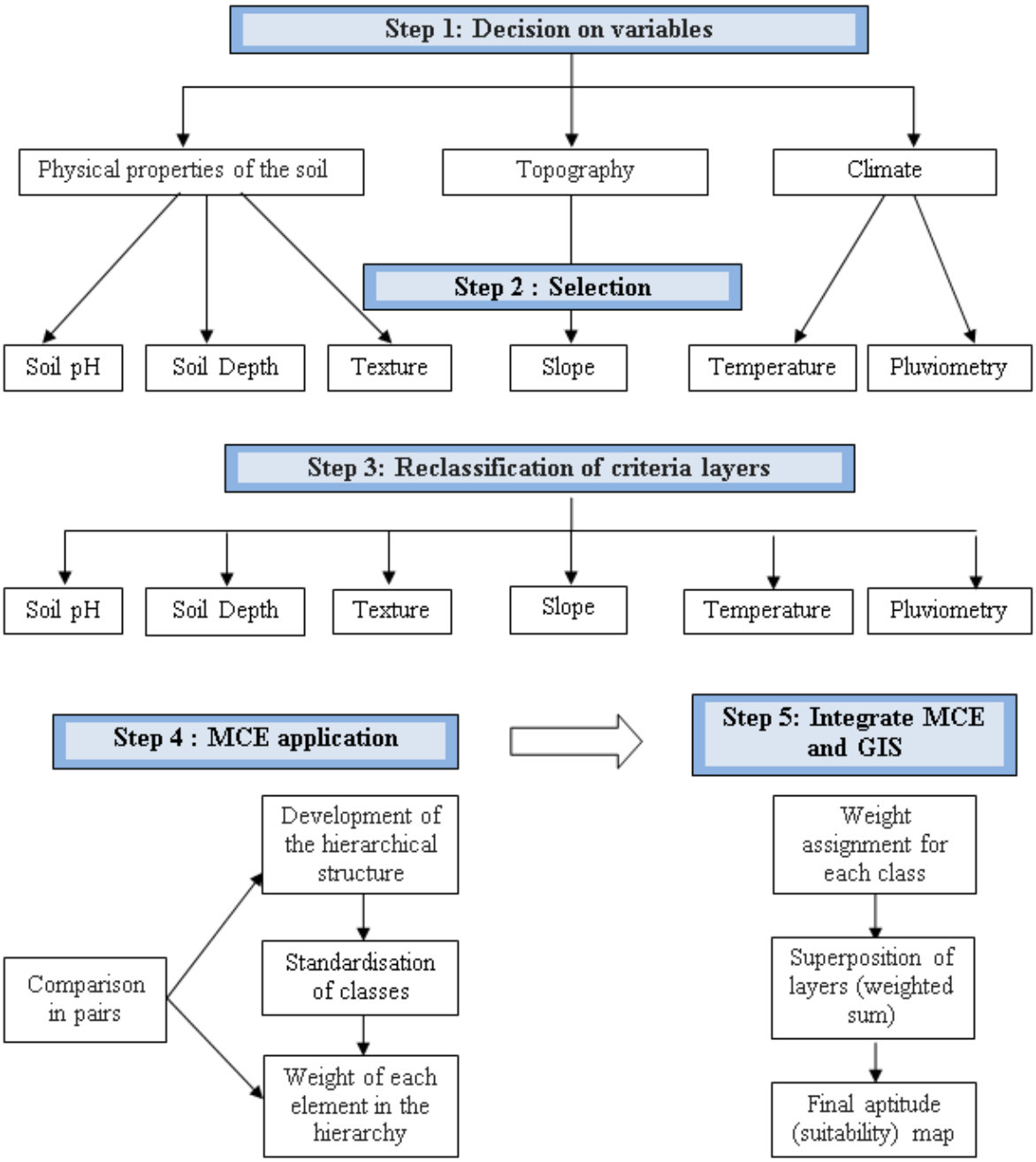


Figure 13: Chart model of land suitability assessment for tomato

II.1.2.1. Data collection

Survey sampling ran from October to December 2016 in Kabare and Walungu territories. Data were collected through a simple questionnaire on tomato growing and production conditions. Soil samples were taken in the middle of the farm at 30 cm of depth, and soil samples analyses (pH, Total N, Organic C, Available P, Exchangeable K, Exchangeable Ca and Exchangeable Mg) were done according to the methods described by Haluschak (2006).

The sample size was 384, according to the Cochran's sample size formula:

$$n = Z^2 pq / e^2 \quad \text{Eq 2}$$

Where:

n = sample size

Z = 1.96 of a 95% confidence interval

p = estimated proportion of the population (0.5)

q = 1-p

e = margin of error (5%)

II.1.2.2. Sources of data used for suitability tomato map production

Table X: Different data sources in developing the tomato land suitability map

Variables	Year	Source
Soil data	2016	Soil database from SOTERCAF (Soil and Terrain for Central Africa) by www.isric.org
Topographic map	2016	The Digital Terrain Model Database (DTM) by the following link: www.diva-gis.org
Temperature and rainfall	2016	NASA (National Aeronautics and Space Administration)
Board of tomato production chain	2016	2015 report of IPAPEL / Bukavu

Table XI: Climatic data for different locations

ID station	Name	Longitude	Latitude	Year	T°C Mean	Pressure Mean
1	Bukavu	28,8430284	-2,512301	2012-2017	19,01	1508
2	Goma	29,2204548	-1,658501	2012-2017	19,49	1306
3	Kalehe	28,9048234	-2,096872	2012-2017	16,95	1640
4	Kamanyola	29,0052561	-2,740512	2012-2017	23,52	1094
5	Kavumu	28,7955841	-2,299802	2012-2017	18,8	1696
7	Minova	29,021261	-1,707663	2012-2017	19,96	1508
8	Uvira	29,1448792	-3,372883	2012-2017	26,22	1112

II.1.2.3. Weight assignment

A weight is a percentage that indicates the importance of the layer in the analysis. A relative importance for each layer was defined for the analysis. For the study on tomato, we used the analysis of soil properties, climate, temperature, and water availability. Thus, different weights were assigned to following criteria as given in Table XII.

Table XII: Weight criteria assignment for tomato

Criteria	Weight
Soil texture	2
Soil CEC	1
Soil pH	1
Organic carbon	2
Temperature	1
Rainfall	1
Drainage	1
Elevation	1

II.1.2.4. Grouping of criteria (overlay)

All the factors mentioned above have been weighed and aggregated to obtain a decisional map. Once the assessment criteria were done, they were combined to come up with a composite decision on the optimum ability for growing tomatoes. This operation is called multicritera evaluation (MCE) or aggregation of criteria (Figure 14 a). The method by complete grouping allows the compensation between criteria; a low fitness factor for one area may be offset by another with a high degree of fitness because the importance of each factor is determined by the score assigned to it. The most common technique is the weighted average, which fully integrates all the criteria considered into one. It consists in multiplying each factor-layer by its weighting coefficient and then adding these results to produce an aptitude index located on a scale. This approach was used individually for all decision factors, to produce an index of ability to grow tomatoes. Once these decision factors are evaluated, a combination of the weighted layers was performed after assigning each weighting decision factor (Figure 14 b).

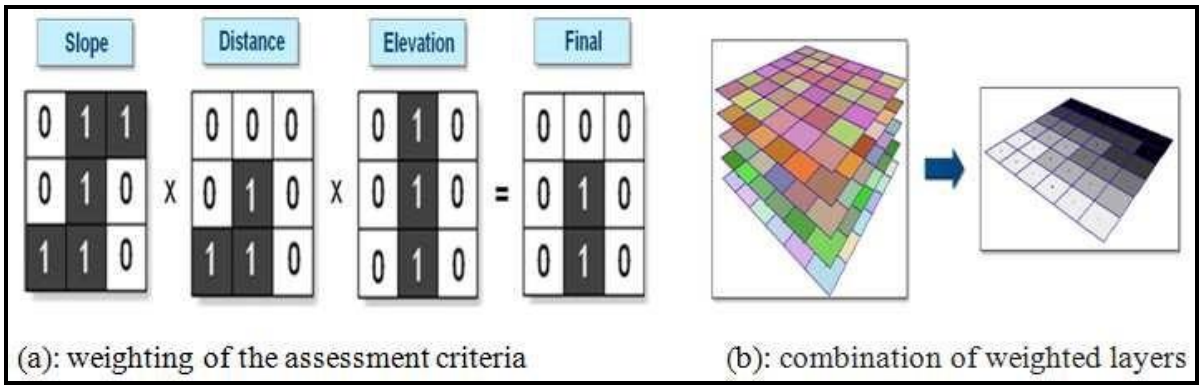


Figure 14: Multicriteria Evaluation (MCE)

II.1.3. Tomato fruit characterization

Tomatoes exhibit great differences in morphological characters. Fruit characters such as fresh weight, longitudinal and transverse diameters, number of locules, dry matter, sugar content and shelf life were determined through measurements (Campos de Melo *et al*., 2015). Five fruits were evaluated for each parameter, and the average value was used for analyses. The shape was expressed as an index number, where a round fruit is rated at approximately 1.00, an ovate type about 0.75, and an oblate fruit varies from 1.15 upwards. The index number is given by the shape index in the following ratio (Lindstrom, 1927):

$$\text{Shape index} = \frac{\text{equatorial diameter}}{\text{polar diameter}} \quad \text{Eq 3}$$

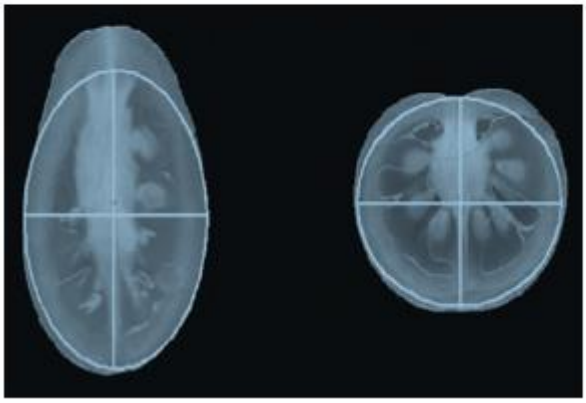


Figure 15: Fruit shape index internal

Source: Gonzalo *et al.* (2009).

II.2. LABORATORY EXPERIMENTS FOR POSTHARVEST FRESH FRUIT PRESERVATION AND TRANSFORMATION

II.2.1. Raw material selection

Tomatoes (*Rio Grande cv*. at red light stage) were procured from a local producer in one of the local markets in Yaoundé, then immediately transported to the Microbiology Laboratory (University of Yaoundé I, Cameroon).

II.2.2. Rosemary Essential Oil (REO)

Rosemary essential oil was bought on the market (Rayons Verts, Yaoundé). It was obtained through steam distillation from flowers and stems of the *Rosmarinus officinalis Linnaeus* plant and delivered by Reynauld & Fils (France). The lot of essential oil was declared pure, without any mixture or dilution by the producer.

The choice in using REO was not only justified by the simple use of rosemary leaves in flavouring local dishes (spice) but also by its antioxidant and antimicrobial activity to extend the shelf life of food products and maintain their quality during storage (Rašković *et al.* 2014).

II.2.3. Chlorine disinfectant

The chlorine disinfectant (La Croix Bleech) was bought in a supermarket as the aqueous solution of Sodium hypochlorite diluted, with 3.8% of active chlorine.

II.2.4. Fresh tomato fruits postharvest preservation using REO and Chlorine

II.2.4.1. Experimental design and treatments

Three treatment factors namely Chlorine concentration (F_1), Immersion time (F_2) and REO concentration (F_3) were considered at 5 levels (-2, -1, 0, 1, 2) combined in a central composite experimental design (CCD). Regarding the fresh tomato preservation, F_1, F_2 and F_3 were considered at the following different concentrations:

Table XIII: Treatments distribution for fresh tomato

Factors \ levels	**-2**	**-1**	**0**	**1**	**2**
F_1: Chlorine concentration (ppm)	0	100	200	300	400
F_2: Immersion time (minutes)	0	1.5	3	4.5	6
F_3: Rosemary essential oil (ppm)	0	200	500	700	1000

Table XIV: Central Composite Design (CCD) used for fresh fruits preservation

Coded values in the CCD ⟺ **Corresponding real values**

Condition	F1 (ppm)	F2 (min)	F3 (ppm)
1	-1	-1	-1
2	-1	1	1
3	1	-1	1
4	1	1	-1
5	0	0	0
6	-1	-1	1
7	-1	1	-1
8	1	-1	-1
9	1	1	1
10	0	0	0
11	-2	0	0
12	2	0	0
13	0	-2	0
14	0	2	0
15	0	0	-2
16	0	0	2
17	0	0	0
18	2	-2	2
19	2	2	2
20	-2	-2	-2

Condition	NaOCl (ppm)	Time (min)	REO (ppm)
1	100	1.5	200
2	100	4.5	700
3	300	1.5	700
4	300	4.5	200
5	200	3	500
6	100	1.5	700
7	100	4.5	200
8	300	1.5	200
9	300	4.5	700
10	200	3	500
11	0	3	500
12	400	3	500
13	200	0	500
14	200	6	500
15	200	3	0
16	200	3	1000
17	200	3	500
18	400	0	1000
19	400	6	1000
20	0	0	0

Tomato fruits (*Rio Grande cv*. at red light stage) were procured from a local producer, then sorted. Ten fruits per treatments were retained according to the experimental CCD. Chlorine concentrations were prepared from an aqueous solution of sodium hypochlorite diluted with 3.8% of active chlorine. From this, calculations were made considering the following formula:

$$percentage\ (\%) = \frac{Weight\ (g)}{Volume\ (ml)} \quad \text{Eq 4}$$

As 3.8% active chlorine were converted to 3.8 g a. Cl into 100 ml,

3800 mg ⟶ 100 ml

400 ppm ⟺ 400 x 10 / 380 = 10.53 ml volume of solution at 3.8 % to prepare 1L at 400 ppm. From this, solution of concentration 100, 200, 300 and 400 ppm were prepared using distilled water.

A solution of 5 % ethanol was prepared from a 96% ethanol solution. This solution was used to prepare 200, 500, 700 and 1000 ppm of REO by adding 200 µl/L, 500 µl/L, 700 µl/L and 1000 µl/L of pure REO in 1 litre ethanol 5%.

II.2.4.2. Tomato treatments

Tomato fruits of uniform shape and colour and free from visible microbial infection were selected, and dipped in a hypochlorite solution and left to drip of on an absorbent paper, then exposed to different concentrations of REO released by spray. The spray was done in order to cover all the surface of the tomatoes. Fruits were kept in boxes at ambient temperature, varying between 28-30°C. During storage, observations were taken at 2 days interval for 22 days, except for the weight loss.

II.2.4.3. Data recording and analyses

- **Tomato fruit weight loss**

Tomato fruits were weighed (before applying any of the treatments) using electronic digital balance at the beginning and the end of storage experiment. The loss in weight was determined by the following formula and expressed as percentage (Gharezi *et al*., 2012):

$$\text{Weight loss } (\%) = \frac{\text{Initial fruit weight-Final fruit weight}}{\text{Initial fruit weight}} \text{X } 100 \qquad \text{Eq 5}$$

- **Tomato fruit quality**

Tomato fruit quality was determined by visual assessment, and the quality characters (appearance, colour, texture and flavour) were assessed by 10 trained panellists, aged between 20 and 40 years on a 5 point hedonic scale. The evolution of the assessed characteristics of the fruit were assessed and the scores used to calculate a storage index (weighted average of the score) for each parameter. The formula proposed was:

$$Is = \frac{[\sum(SCOREi \; X \; DAYi)]}{(\textbf{Maximum score X} \sum^{\square} DAYi)} \qquad \text{Eq 6}$$

Where:

Is = storage index

$SCORE_i$ = evaluation of a certain criteria on a certain day_i

Maximum score = highest evaluation possible for that criteria, and

$\sum DAY_i$ = sum of the numbers of storage days at each analysis

The Is varies from “0” total quality preserved to “1” total quality lost.

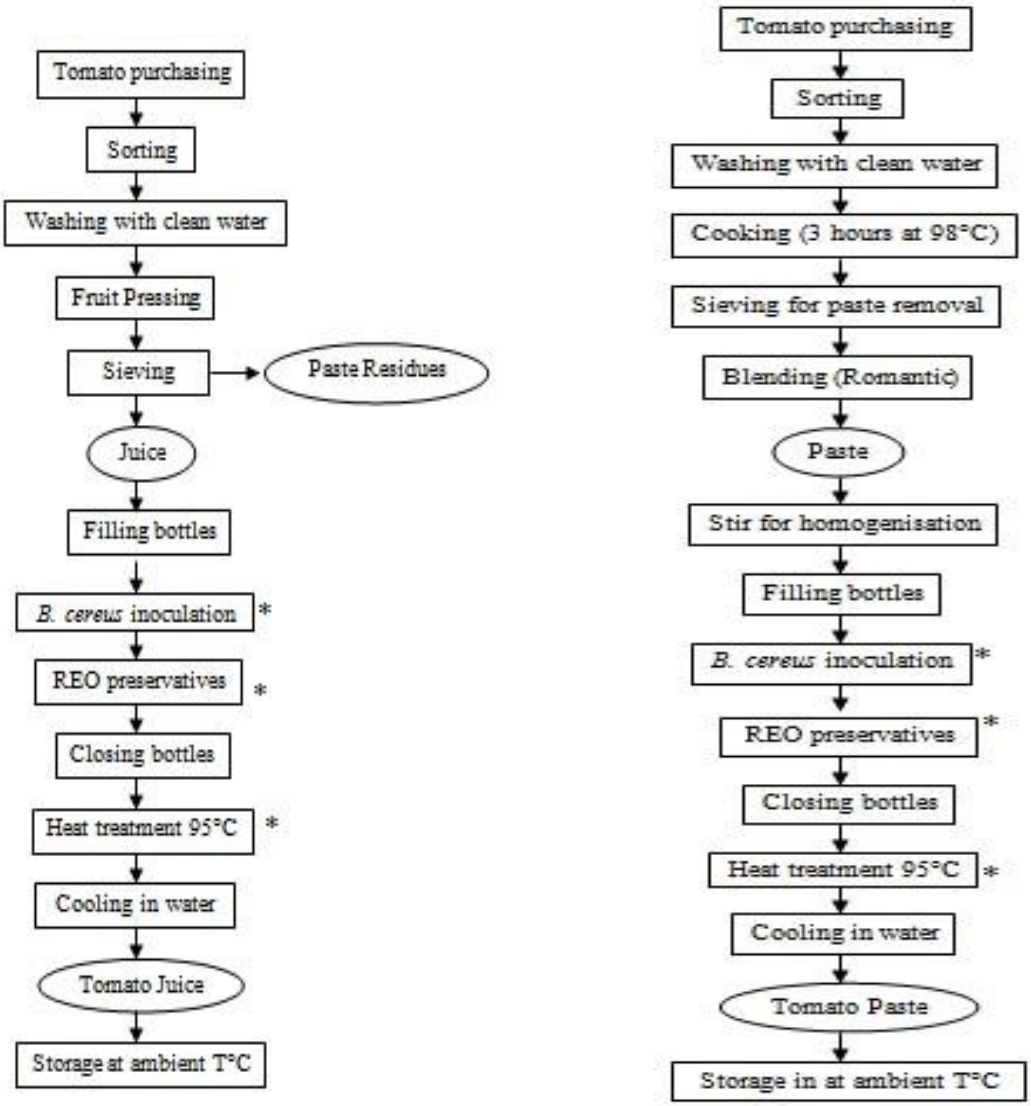
Table XV: Evaluation of tomato quality- Rating scale

Scale	Colour Development	Defects presence	Firmness	Flavour
1	Green	Absence (0%)	Very firm	Stink
2	Turning	Very poor (10-25%)	Slightly firm	Poor
3	Pink	Poor (30-45%)	Shrivel	Mild
4	Light red	Strong (50-65%)	Soft	Good
5	Full red	Very strong (70-100%)	Squashy	Excellent

The colour development was assessed using a tomato colour chart as proposed by Abdullah *et al.* (2004). The effect of the treatments on the shelf life was determined with respect to the control after 22 days of storage.

II.2.5. Use of REO for the production of tomato juice and paste

Tomato fruits (*Rio Grande cv.*) were sorted and washed with tap-water to remove dirt and soil before processing into juice (Figure 16 a) and paste (Figure 16 b).



(a) **(b)**

* *According to the experimental plan*

Figure 16: Flow chart of processing tomato juice (a) and tomato paste (b)

Figure 17: Tomato juice and paste after processing

II.2.5.1. Experimental design and treatments

Three treatment factors namely *Bacillus cereus* concentration deliberately inoculated (F_1), Cooking time (F_2) and REO concentration (F_3) were considered at 5 levels (-2, -1, 0, 1, 2) each in a central composite experimental design (CCD). For tomato juice, following are the treatments combinations in the CCD design:

Table XVI: Treatments distribution for tomato juice

Factors \ levels	**-2**	**-1**	**0**	**1**	**2**
F_1: *Bacillus cereus* (log)	0	2	4	6	8
F_2: Cooking time (minutes)	0	5	10	20	30
F_3: Rosemary essential oil (ppm)	0	50	100	150	200

Table XVII: Central Composite Design (CCD) experimental design used for tomato juice

Coded values in the CCD ⟺ **Corresponding real values**

Condition	**F_1 (log)**	**F_2 (min)**	**F_3 (ppm)**
1	-1	-1	-1
2	-1	1	1
3	1	-1	1
4	1	1	-1
5	0	0	0
6	-1	-1	1
7	-1	1	-1
8	1	-1	-1
9	1	1	1
10	0	0	0
11	-2	0	0
12	2	0	0
13	0	-2	0
14	0	2	0
15	0	0	-2
16	0	0	2
17	0	0	0
18	2	-2	2
19	2	2	2
20	-2	-2	-2

Condition	***B. cereus* (log)**	**Time (min)**	**REO (ppm)**
1	2	5	50
2	2	20	150
3	6	5	150
4	6	20	50
5	4	10	100
6	2	5	150
7	2	20	200
8	6	5	50
9	6	20	150
10	4	10	100
11	0.1	10	100
12	8	10	100
13	4	0	100
14	4	30	100
15	4	10	0
16	4	10	200
17	4	10	100
18	8	0	200
19	8	30	200
20	0.1	0	0

For tomato paste, following are the treatments combinations in the CCD design:

Table XVIII: Treatments distribution for tomato paste

Factors \ levels	**-2**	**-1**	**0**	**1**	**2**
F_1: *Bacillus cereus* (log)	0	2	4	6	8
F_2: Cooking time (minutes)	0	10	20	30	40
F_3: Rosemary essential oil (ppm)	0	50	100	150	200

Table XIX: Central Composite Design (CCD) experimental design for tomato paste

Coded values in the CCD ⟺ **Corresponding real values**

Condition	**F_1 (log)**	**F_2 (min)**	**F_3 (ppm)**
1	-1	-1	-1
2	-1	1	1
3	1	-1	1
4	1	1	-1
5	0	0	0
6	-1	-1	1
7	-1	1	-1
8	1	-1	-1
9	1	1	1
10	0	0	0
11	-2	0	0
12	2	0	0
13	0	-2	0
14	0	2	0
15	0	0	-2
16	0	0	2
17	0	0	0
18	2	-2	2
19	2	2	2
20	-2	-2	-2

Condition	***B. cereus* (log)**	**Time (min)**	**REO (ppm)**
1	2	10	50
2	2	30	150
3	6	10	150
4	6	30	50
5	4	20	100
6	2	10	150
7	2	30	200
8	6	10	50
9	6	30	150
10	4	20	100
11	0.1	20	100
12	8	20	100
13	4	0	100
14	4	40	100
15	4	20	0
16	4	20	200
17	4	20	100
18	8	0	200
19	8	40	200
20	0.1	0	0

II.2.5.2. Preparation of *Bacillus cereus* spores

To induce sporulation of vegetative cells of *B. cereus* (ATCC 11966), the procedures described by Etoa *et al*. (2017) were used. After the revivification process, *B. cereus* strain was cultured in nutrient broth and incubated at 37°C for 24 hours. Later, 10^3 cells/ml of the inoculum were sporulated on nutrient agar supplemented with salts (0.5 g of dissodic phosphate, 0.1 g of calcium chloride, 0.04 g of manganese sulphate), then incubated for 7 days at 37°C. After these days, spores were harvested by flooding the plates with distilled

water. For the obtained suspensions, ethanol (50 %) was added to have a mixture in the proportions, and then incubated at 4°C for 12 hours to eliminate vegetative cells. Purification of spores was performed by several centrifugations at 4000 g/15 min at 4°C and stored at 4°C for 1 month before use.

II.2.5.3. Production of tomato juice and paste

Four tomato baskets were purchased in Yaoundé market. Ripe fruits were selected and rinsed with water. Tomatoes were processed into tomato juice and paste as demonstrated in Figures 18 and 19 respectively. Initially, 20 Kg of fresh tomatoes were processed into 12 Kg of tomato paste after 3 hours of cooking on charcoal stove. Processed products were put in glass bottles of 125 ml, and then filled up to 100 ml representing 80 % of the total volume (Figure 16). At 95ºC, juice and paste treatments were assessed following the CCD design.

**The numbers represent steps followed in the tomato juice production*

Figure 18: Processing steps of tomatoes into juice

**The numbers represent steps followed in the tomato paste production*

Figure 19: Processing steps of tomatoes into paste

II.2.5.4. Data recording and analyses

❖ **Enumeration of residual spores in paste and juice**

Juice or paste treated according to the experimental CCD plan were diluted using tubes containing 9 ml of nutrient broth. Dilution were performed using a three repetition most probable number protocol and ranged from 1 to 10^{-5} dilutions. After incubation of the diluted samples at 37°C for 24 hours, observations of the number of tubes presenting *B. cereus* growth per replicate were recorded. The generated code was used to calculate the MPN cells/g or ml using a MPN Excel add-in provided by Jarvis *et al*. (2010).

❖ **Total polyphenols**

Total polyphenols content were determined using a modified Folin-Ciocalteu colorimetric method (Dewanto *et al*., 2002; Sumathy *et al.*, 2013). After incubation at room temperature for 90 minutes, the absorbance was read at 760 nm using a UV-VIS Spectrophotometer. Gallic acid was used as the standard, and results were expressed as µg of Gallic acid equivalents (µg GAE) per ml of tomato juice or per mg of tomato paste.

❖ **Titratable acidity**

Titratable acidity was obtained by titrating 5 ml of tomato extracts with an alkaline solution (0.1 N NaOH) using phenolphthalein indicator up to pH 8.1. The appearance of light pink colour was marked as the end point. Percentage of titratable acidity (TA) was calculated as follows (Monash Scientific, 2003):

$$TA\ (g/l) = \frac{T \times M \times 0.75}{V \times 10 \times 0.1} \quad \text{Eq 7}$$

Where,

T= Titre (ml) of 0.1 M NaOH

M= Molarity (M) of 0.1M NaOH

V= Volume (ml) of sample

Results were expressed as grams of citric acid per 100 g of tomato products (Majidi *et al*., 2011; Gharezi *et al*., 2012; Tessema, 2013).

❖ **Vitamin C concentration**

Vitamin C in tomato juice and paste was determined by the indicator method (2, 6 dichloroindophenol titration method). The vitamin C contents of fruit juices were reported in mg/100 ml (Garcia-Alonso *et al*., 2009; Leahu *et al*., 2013).

❖ **Shelf life of tomato juice and paste**

In order to analyse the data for shelf life recorded for one year, a storage efficacy of the process was calculated using the following formula, developed in the preliminary lab works:

$$Shelf\ life = \frac{[\sum (number\ of\ good\ samples\ on\ DAYi\ X\ DAYi)]}{(Maximum\ sample\ repetition\ X\ \sum DAYi)} \quad \text{Eq 8}$$

The value ranges from 0 to 1: 0 being a condition where all repetitions got spoiled on the first day of storage, and 1, a process condition that have assured all sample repetitions stable until 366 days (one year).

❖ **pH of tomato juice and paste**

Initial pH values were measured by using electronic pH meter (Hanna) (Leahu *et al*., 2013).

II.3. IMPLEMENTATION OF ONE OF THE PRESERVATION TECHNIQUES IN THE BUSHI REGION

II.3.1. Constraints

In the assessment of the suitability of preservation techniques in the study area, only the preservation of fresh fruits was done, this due to different constraints in the region among which the absence of packaging and storage facilities, difficulty of having thermal treatments devices and the cultural apprehension of chlorine which is believed to be an hospital product.

Tomato fruits (*cv Roma VF*) were harvested in a local farm at different harvesting stages and conveyed to the laboratory for physical and chemical analyses. The use of tomato fruits from *Roma cultivar* was justified by its abundance in the farms by the time of conducting our research, and its quality characteristics are quite similar to the fruits from *Rio grandi cultivar*.

II.3.2. Experimental Design

The experimental design in a randomized complete block design (RCBD) was conducted in the Biotechnology Laboratory of UEA (DRC) in the period of July- August 2017. The main factor was Dose (REO concentration) and harvesting stages being the sub factors, replicated three times (Figure 20).

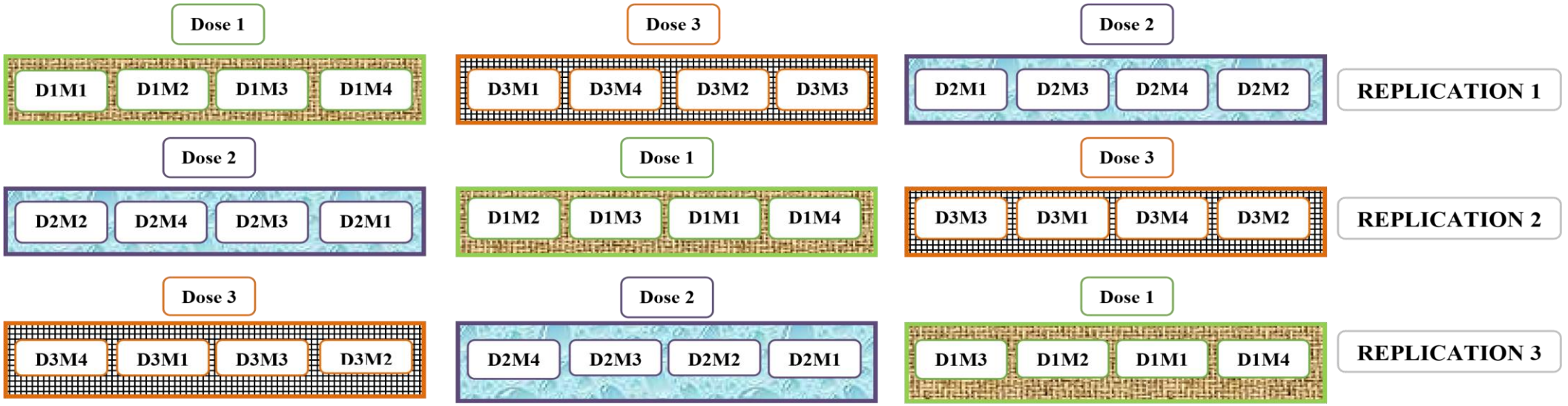


Figure 20: Randomized complete block design (RCBD)

FACTOR LEVELS

MATURITY STAGES	REO DOSES
M1= Maturity 1 (Breaker)	D1 = 0 ppm
M2= Maturity 2 (Pink)	D2 = 200 ppm
M3= Maturity 3 (Light red)	D3 = 500 ppm
M4= Maturity 4 (Red)	

Harvesting stages were determined according to the colour chart as shown in Figure 21 (Abdullah *et al*., 2004).

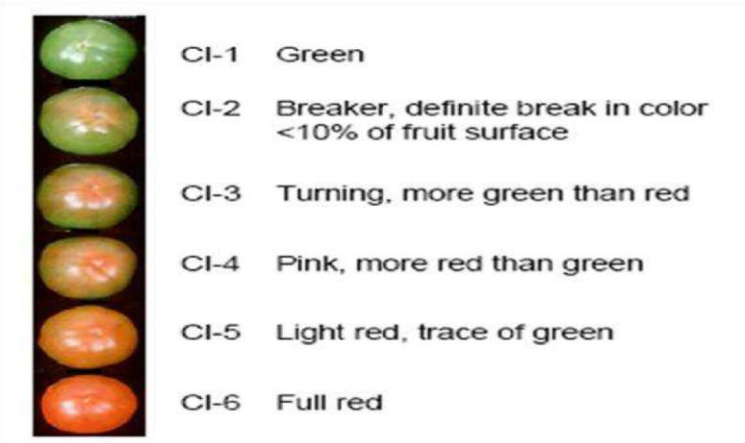


Figure 21: Tomato colour chart corresponding to stages of fruit ripeness

After harvest, tomato fruits were packaged in gallons and conveyed to the university's laboratory for experimentation. Fruits were sorted out and the selected ones were dipped in tap water since for cultural reasons chlorine could not be used, and left to drip before being kept in carton boxes corresponding to each treatment. Thirty tomato fruits were considered for each treatment, and REO dose (as described previously) was sprayed on fruits in bulk.

II.3.3. Data recording and analyses

Data were taken on parameters such as total soluble solids, titratable acidity, Vitamin C content, fruit colour development and fruit shelf life. Titratable acidity, Vitamin C, and colour of these analyses were performed as indicated previously.

- **Total soluble solids (TSS)**

The Total soluble solids content was measured using a hand-held refractometer [Model ZRGB- 20 ATC (Brix, 0-32%)]. Determination was done by calculating the average TSS for the 2 fruits per treatment for each replicate. The final value was obtained by determining the average of the replicates for each treatment.

- **Fruit Shelf life**

The fruit shelf life was calculated by counting the days required for them to attain the last stage of ripening, beginning of rotting of fruits and remained still acceptable for marketing (Nirupama *et al*., 2010; Rahman *et al*., 2010).

II.2. STATISTICAL ANALYSES

To assess soil suitability for tomato cultivation, the approach applied in this study required compiling cartographic data (topographic, land cover and climatic map). ArcGIS 10.3 software was used for this purpose. For tomato fruit characterization, data were analysed using XLSTAT package for descriptive analyses: position (mean) and variability (coefficient of variation) measurements.

For fresh fruits preservation using chlorine and REO concentrations, and evaluation of tomato quality products (juice and paste), statistical analyses were done using XLSTAT and STATISTICA packages for non-linear regression analyses. The data obtained were subjected to Analysis of variance (ANOVA) and significant means were separated by mean differences Tukey-HSD test ($\alpha \leq 0.05$).

For the implementation of one of the developed preservation techniques in the Bushi region, data obtained were subjected to Analysis of Variance (ANOVA) using R 3.5.1, R Studio 1.1.453 software. Significant means were separated by mean differences Tukey-HSD at $\alpha \leq 0.05$.

CHAPTER III: RESULTS AND DISCUSSION

CHAPTER THREE
RESULTS AND DISCUSSION

III.1. RESULTS

III.1.1. Survey on tomato potential productive areas and characterisation of tomato produced

III.1.1.1. Tomato land suitability cartography

In order to assess if enough tomato could be produced in this region, land suitability was assed. Soil samples were collected and analysed for determination of soil properties. Results on chemical characteristics are given in the Table XX.

Table XX: Soil analyses data of representative study area

Territory	Villages	pH in water	% Total Nitrogen	% org. Carbon	Available P (mg P/Kg)	Exchangeable K (cmolc/ Kg soil)	Exchangeable Ca (cmolc/ Kg soil)	Exchangeable Mg (cmolc/Kg soil)
Kabare	Mugogo	7.47	0.23	1.85	47.18	0.84	3.75	1.67
	Buhinda	5.61	0.22	1.63	22.56	0.1	6.13	1.24
	Shonga 1	5.48	0.02	0.60	14.87	0.24	2.52	1.42
	Mulengeza 1	5.72	0.08	1.76	20.26	0.7	4.81	1.87
	Shonga 2	5.84	0.08	1.52	21.79	0.81	3.91	1.15
	Kankule	5.65	0.32	3.28	3.59	0.21	7.77	2.62
	Muguru	6.32	0.11	1.89	49.74	0.68	3.71	1.58
	Ciraba	6.09	0.20	1.99	136.28	0.18	3.26	1.3
	Buhandahanda	5.59	0.17	1.78	31.67	0.67	2.91	0.95
	Mulengeza 2	7.04	0.26	2.47	20.78	2.09	6.53	1.99
	Value range	**5.48-7.47**	**0.02-0.32**	**0.60-3.28**	**3.59-136.28**	**0.10-2.09**	**2.52-7.77**	**0.95-2.62**
	CV	**0.11**	**0.53**	**0.35**	**0.97**	**0.85**	**0.36**	**0.29**
Walungu	Bugano	7.52	0.08	0.98	211.28	0.85	3.54	1.19
	Kirire	8.39	0.04	0.46	3.46	0.19	8.38	3.23
	Kamanyola	6.26	0.24	2.28	29.23	0.13	7.84	2.52
	Kambara	6.12	0.11	1.10	6.54	0.30	3.28	1.34
	Majengo	6.65	0.13	1.6	7.69	0.80	4.06	1.28
	Value range	**6.12-8.39**	**0.04-0.24**	**0.46-2.28**	**3.46-211.28**	**0.13-0.85**	**3.28-8.38**	**1.19-3.23**
	CV	**0.12**	**0.57**	**0.48**	**1.56**	**0.68**	**0.41**	**0.43**

Results in Table XX give a general view of soil chemical characteristics in the Bushi region. Criteria like Total Nitrogen (%), Available P (mg/Kg) and Exchangeable K (cmol/Kg) mostly indicate variations in the 2 territories with regards to each parameter considered separately. Soil pH varies from slightly acidic (5.48) in Shonga 1 (Kabare) to moderately alkaline (8.39) in Kirire (Walungu). Slight variations in the concentration of Total Nitrogen (%) are observed in the 2 territories, with the highest value in Kabare being of 0.324 (Kankule), while in Walungu is found to be 0.24 (Kamanyola). Regarding the Organic carbon (%), the highest value (3.28%) was recorded in Kankule (Kabare), while 2.28% was found in Kamanyola (Walungu), and the lowest values were 0.46% (Kirire) and 0.6% (Shonga 1) in Walungu and Kabare respectively. From our results, we observed high variability in the concentration of Available P (mg/Kg) among and within the 2 territories. The highest value (211.28 mg/Kg) in Bugano and the lowest value (3.46 mg/Kg) in Kirire are recorded in Walungu territory. On the other hand, 136.28 mg/Kg was found in Ciraba while 3.59 mg/Kg was recorded in Kankule, both in Kabare territory. High variations are also observed in Exchangeable K (cmol/Kg). The highest value (2.09) was recorded in Mulengeza 2 and the lowest value (0.1) in Buhinda, both villages found in Kabare territory; while in Walungu territory, Bugano has recorded the highest value (0.85) and Kamanyola the lowest value (0.13). Results on Exchangeable Ca (cmol/Kg) indicate high value (8.38) in Kirire (Walungu) and lowest value (2.52) in Shonga 1 (Kabare). The highest value (3.23 cmol/Kg) on Exchangeable Mg was recorded in Kirire (Walungu) and Buhandahanda (Kabare) has recorded the lowest value (0.95 cmol/Kg).

Apart from soil chemical characteristics necessary as requirements for tomato cultivation, available data on other criteria like soil texture, temperature, rainfall and elevation in the 2 territories are given in Table XXI.

Table XXI: Other parameters for land suitability classification

Criteria	KABARE	WALUNGU	Classification
Soil texture	Clayey	Sandy clay/ clay	Moderately/ highly suitable
Temperature (°C)	19.5	18.6	Highly suitable (> 18 and < 24)
Rainfall (mm)	1521	1620	Highly suitable (> 1500 and < 2000)
Elevation (m)	1535	1765	Highly suitable (> 1000 and < 2500)

All data presented in Table XX and XXI were used to generate a land suitability map for tomato cultivation. This table shows that for tomato growth, the climate is highly suitable. The evaluation of physical land suitability was carried out by matching crop requirements

and land characteristics. According to FAO's classification, 4 classes were obtained for the land suitability for tomato crop in the Bushi region (Figure 22).

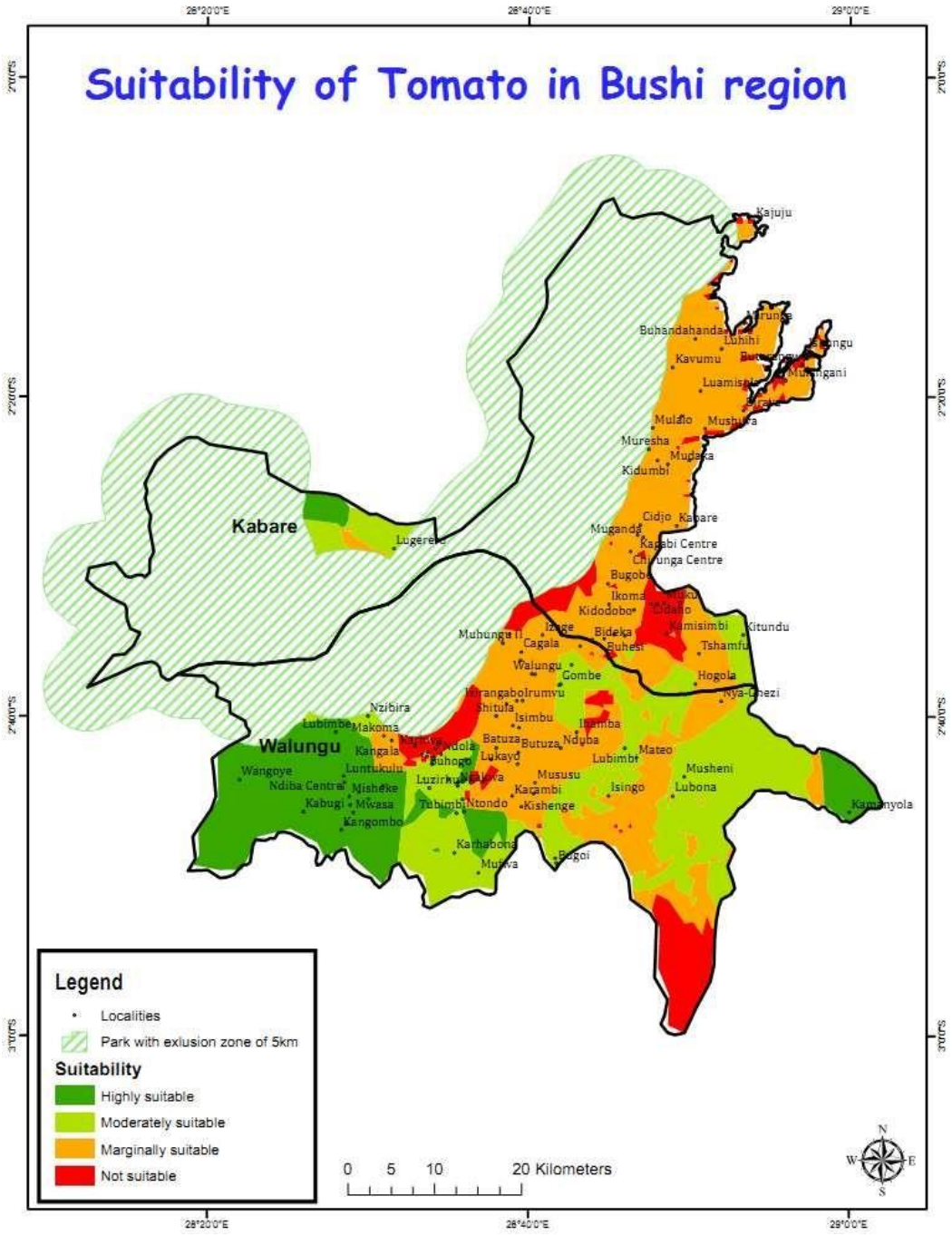


Figure 22: Land suitability map for tomato in the Bushi region

It is clearly indicated that there are no human activities allowed in the restricted zone (Kahuzi-Biega National Park), which covers the ¾ of Kabare and ¼ of Walungu territories. From Figure 22, Kabare is marginally suitable for tomato cultivation compared to Walungu where tomato can be cultivated in moderately and highly suitable areas.

III.1.1.2. Tomato fruit characterization

Table XXII indicates tomato cultivars found in the 2 territories: Kabare and Walungu.

Table XXII: Cultivated varieties in the Bushi region

Variety	Kabare	Walungu	Khi²	p-value
Karhahanyuzwe	0	16	7.82	< **0.0001**
Marglobe	0	5		
Marmande	35	0		
Sorwatome	133	66		
Total	**168**	**87**		

The most cultivated variety in both territories is *Sorwatome*. Other varieties are *Marmande* (Kabare), *Karhahanyuzwe* and *Marglobe* (Walungu). The 2 territories are different in terms of the variability of the cultivars. Some physical characteristics of these varieties were evaluated. Table XXIII presents the results obtained.

Table XXIII: Physical characteristics of tomato fruits

Variety	FW (g)	TD (cm)	LD (cm)	Shape	LOC	DM (%)	SC (%)	Shelf (week)
Marglobe	46.7	4.24	3.44	Oblate	3	7.3	5	2-3
Marmande	179.25	6.45	5.8	Round	3-5	-	-	-
Karhahanyuzwe L	231.25	7.8	6.35	Oblate	> 5	5.9	5	2-3
Karhahanyuzwe M	138.9	6.42	5.36	Oblate	4-5			
Karhahanyuzwe S	76.4	4.88	4.74	Round	3-4			
Sorwatome L	135.9	6	6.68	Ovate	4	7.67	5.5	3-4
Sorwatome M	78.7	4.82	5.4	Ovate	3			
Sorwatome S	45.9	3.62	5.2	Ovate	2			
CV (%)	**0. 53**	**0.22**	**0.17**		**0.25**	**0.11**	**0.05**	

L*- Large;* ***M****- Medium;* ***S****- Small;* ***CV****- Coefficient of Variation;* ***FW****- Fresh weight;* ***TD****- Transversal Diameter;* ***LD****- Longitudinal Diameter;* ***LOC****- number of locules;* ***DM****- Dry Matter;* ***SC****- Sugar Content;* ***Shelf****- Shelf life*

This survey revealed variation in fruit morphology and the quality characteristics of tomato among cultivars. Tomato had a wide variation in fruit weight among the different cultivars. The mean weight of the fruit ranged from 45.90 g to 231.25 g; the highest being in *Karhahanyuzwe L* (231.25 g), whereas the lowest was found in *Sorwatome S* (45.9 g). The fruit shape extended from ovate (*Sorwatome L, Sorwatome M* and *Sorwatome S*), round

(*Marmande* and *Karhahanyuzwe S*) to oblate (*Marglobe*, *Karhahanyuzwe L* and *Karhahanyuzwe S*). There are also variation in locule number (determined visually) of different cultivars, varying from 2 to 5 and over 5. The internal structure of the tomato fruit is important. Our results indicate that the high dry matter content was found in the *Sorwatome L* (7.67 %), followed by *Marglobe* (7.3 %), then *Karhahanyuzwe L* (5.9 %). Relationships among physical attributes were determined and a high positive correlation was found between fresh weight, transversal diameter and locule numbers (Table XXIV).

Table XXIV: Pearson correlation coefficient matrix for physical parameters

Variables	FW (g)	TD (mm)	LD (mm)	LOC	DM(%)	SC (%)
FW (g)	1	**0.98**	0.72	**0.95**	-0.76	-0.02
TD (mm)		1	0.67	**0.98**	-0.75	-0.01
LD (mm)			1	0.58	-0.23	0.58
LOC				1	-0.75	0.00
DM (%)					1	0.66
SC (%)						1

FW*- Fresh weight;* ***TD****- Transversal Diameter;* ***LD****- Longitudinal Diameter;* ***LOC****- number of locules;* ***DM****- Dry Matter;* ***SC****- Sugar Content*

Table XXIV indicates positive correlation between fruit weight (FW), transversal diameter (TD) (0.98) and locule number (LD) (0.95).

III.1.2. Fresh and transformed tomato preservation using REO

III.1.2.1. Tomato fresh fruits preservation

External parameters (visual appearance) were used to evaluate the fruits during preservation. These included colour development, presence of defects, firmness, flavour, weight loss and shelf life. The experiment was conducted by applying different amount of NaOCl and REO at different immersion times.

Table XXV shows the storage indices with regards to fruit colour development, presence of defects, fruit firmness and fruit flavour.

Table XXV: Storage indices of tomato fruit quality parameters treated at different immersion times with different concentrations of NaOCl and REO

Treatments	NaOCl (ppm)	Time (min)	REO (ppm)	Colour index (Ci)			Defects index (Di)			Firmness index (Fi)			Flavour index (Fl)		
				Ci 1*	Ci 10*	Ci 22*	Di 1	Di 10	Di 22	Fi 1	Fi 10	Fi 22	Fl 1	Fl 10	Fl 22
1	100	1.5	200	0.80	0.98	1.00	0.20	0.45	0.63	0.20	0.38	0.55	1.00	0.19	0.54
2	100	4.5	700	0.80	0.99	1.00	0.20	0.20	0.23	0.20	0.42	0.56	0. 80	0.20	0.60
3	300	1.5	700	0.80	0.99	1.00	0.20	0.45	0.56	0.20	0.38	0.55	0.80	0.20	0.46
4	300	4.5	200	0.80	0.99	1.00	0.20	0.38	0.40	0.20	0.50	0.58	0.80	0.20	0.46
5	200	3	500	1.00	1.00	1.00	0.20	0.38	0.40	0.20	0.42	0.56	0.80	0.20	0.60
6	100	1.5	700	0.80	0.99	1.00	0.20	0.45	0.56	0.20	0.45	0.56	0.80	0.26	0.40
7	100	4.5	200	0.80	0.99	1.00	0.20	0.35	0.39	0.20	0.32	0.53	0.80	0.20	0.60
8	300	1.5	200	0.80	0.99	1.00	0.20	0.45	0.56	0.20	0.45	0.56	0.80	0.26	0.32
9	300	4.5	700	0.80	0.99	1.00	0.20	0.20	0.20	0.20	0.35	0.54	0.80	0.20	0.60
10	200	3	500	1.00	1.00	1.00	0.20	0.20	0.20	0.20	0.35	0.54	0.80	0.20	0.60
11	0	3	500	0.80	0.95	0.99	0.20	0.35	0.39	0.20	0.35	0.54	0.80	0.20	0.60
12	400	3	500	0.80	0.99	1.00	0.20	0.20	0.34	0.20	0.35	0.54	1.00	0.19	0.46
13	200	0	500	0.80	0.99	1.00	0.20	0.38	0.40	0.20	0.47	0.57	1.00	0.19	0.45
14	200	6	500	0.80	0.98	1.00	0.20	0.20	0.34	0.20	0.20	0.51	0.80	0.20	0.60
15	200	3	0	0.80	0.99	1.00	0.20	0.35	0.39	0.20	0.38	0,55	0.80	0.20	0.46
16	200	3	1000	0.80	0.99	1.00	0.20	0.20	0.20	0.20	0.45	0.56	0.80	0.20	0.60
17	200	3	500	0.80	0.99	1.00	0.20	0.20	0.20	0.20	0.47	0.57	0.80	0.20	0.60
18	400	0	1000	0.80	0.99	1.00	0.20	0.32	0.53	0.20	0.47	0.57	0.80	0.26	0.32
19	400	6	1000	0.80	0.99	1.00	0.20	0.20	0.35	0.20	0.35	0.54	1.00	0.19	0.60
20	0	0	0	0.80	0.95	0.99	0.20	0.35	0.42	0.20	0.38	0.55	1.00	0.19	0.57

** the code followed by the number that refers to the day of storage*

III.1.2.1.1. Colour Development

Results in Table XXV indicated that all tomatoes turned to full red colour independently of the treatments. Initially, all fruits were light red in colour; the full red colour was quite effective from day 10 of storage.

III.1.2.1.2. Presence of defects

Significant differences were observed between treatments at different days of storage in terms of pathogen infection. Results (Table XXV) showed that after 10 days of storage, some fruits started showing symptoms of pathogen infection, thus affecting the fruit quality. At the end of the storage period, most fruits from the treatments had defects, though at different levels: < 25 % for treatments T2, T9, T10, T16 and T17; 25 – 50 % for T4, T5, T7, T11, T12, T13, T15, T19 and T20 and > 50 % for treatments T1, T3, T6, T8 and T18.

III.1.2.1.3. Fruit firmness

Results (Table XXV) indicated significant differences in treatments at 10 days of storage, where fruits from T14 were slightly firmer compared to other treatments with regards to day 1 of storage. After 22 days of storage, all the treated fruits were softened.

III.1.2.1.4. Fruit flavour

Significant differences resulted among the treatments (Table XXV). A regression analysis for flavour prediction was obtained in order to explain the effect of different variables on the tomato flavour. According to this equation which was obtained with R^2= 0.64 and SSE= 0.12:

Flavour score at 22 days = 0.436+ 0.007*[HE] + 0.0024*[CL]*[t] - 0.0031* [t]*[HE] Eq 9

Where, HE refers to REO; CL refers to NaOCl, and t to immersion time

It can be observed that for treatment for 4.5 min, increase in CL increases Flavour while it is not the case with HE (Figure 23).

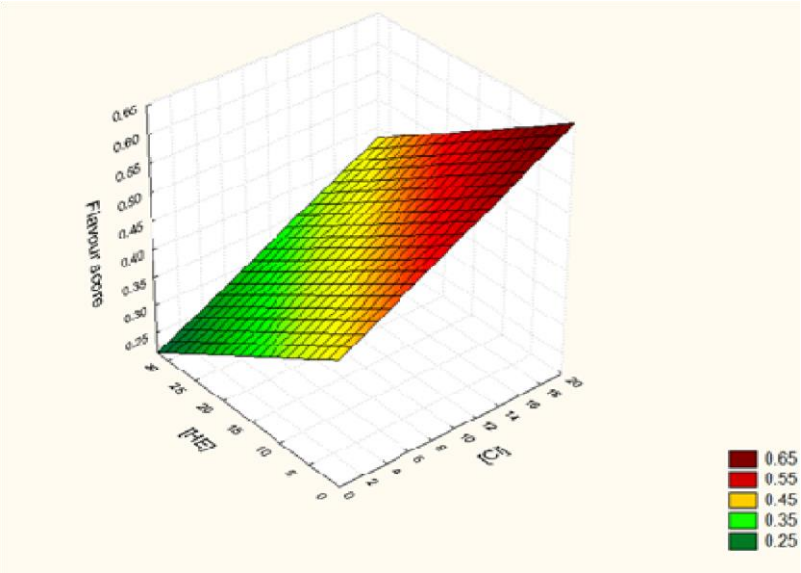


Figure 23: Influence of chlorine and REO at 4.5 min. treatment on the tomato flavour

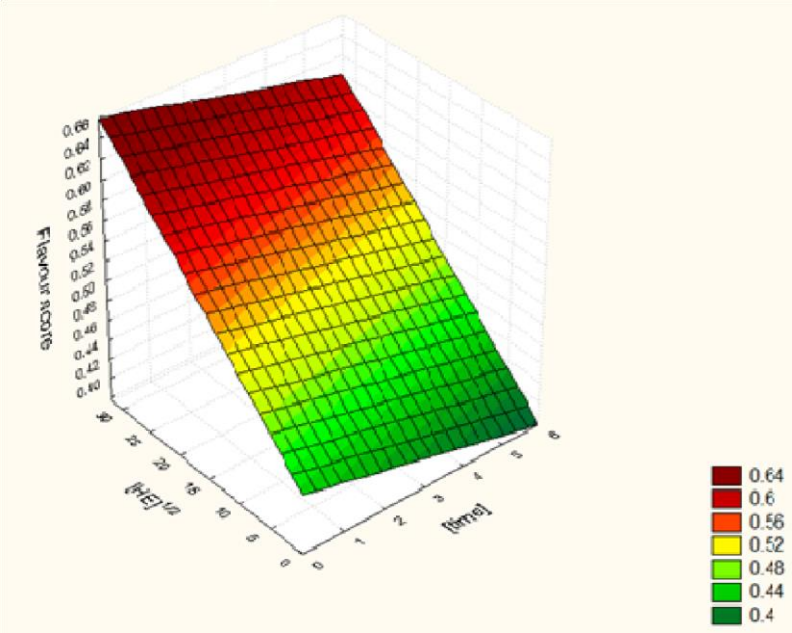


Figure 24: Influence of immersion time and REO when chlorine is used at 200 ppm on the flavour of fresh tomatoes

Results from Figures 23 and 24 indicate that the higher the concentrations of chlorine and REO, the higher the tomato flavour is preserved. It can be observed that at fixed NaOCl concentrations, the increase of REO induced low flavour scores. Moreover, at fixed REO, increase of NaOCl induced an increase of flavour appreciation after 22 days of storage. High flavour values were observed for low REO and high NaOCl. As indicated in Figure 24, at fixed times, increase in REO increased flavour appreciation after 22 days of storage while immersion time increase was not favourable to flavour.

III.1.2.1.5. Fruit weight loss

Results on physiological weight loss at the final day of storage are indicated in Figure 25.

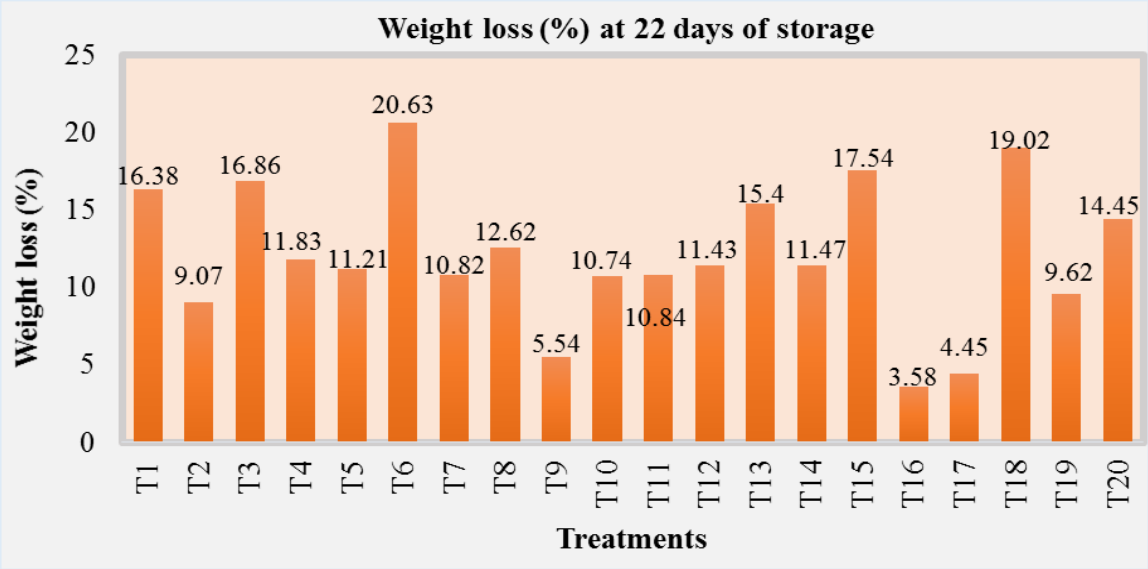


Figure 25: Percentage of fruit weight loss after 22 days of storage

It is indicated from Figure 25 that the lowest percentage (3.57 %) in weight loss was observed with T16 (200 ppm NaOCl, 3 min., 1000 ppm REO), whereas the highest (20.63 %) was with T6 (100 ppm NaOCl, 1.5 min., 700 ppm REO). There was significant fruit weight loss among the treatments after 22 days of storage at ambient temperature among different treatments.

Table XXVI: ANOVA for percentage in fruit weight loss

Source	Df	SS	MS	F	Pr > F
Treatments	16	346.497	21.656	15.428	0.022
Error	3	4.211	1.404		
Total	19	350.708			

(α=0.05)

This indicates that the treatments significantly affected the weight loss.

III.1.2.1.6. Fruits shelf life

Although some fruits had shown the presence of defects all along the period of the experiment, the shelf life associated to a treatment was determined on the bases of percentage of fruits having non acceptable features. Based on this assumption, two groups of fruits from two different treatments were considered different in shelf life at 22nd day of experiment if they had different percentage of spoiled fruits. The groups with less spoiled fruits were having a longer shelf life (at 22 days).

Table XXVII: Percentage of spoiled fruits during storage

Treatments	NaOCl (ppm)	Time (min)	REO (ppm)	Day 10	Day 22
1	100	1.5	200	25	50
2	100	4.5	700	0	25
3	300	1.5	700	20	40
4	300	4.5	200	0	22
5	200	3	500	10	10
6	100	1.5	700	14.29	42.86
7	100	4.5	200	10	20
8	300	1.5	200	25	37.5
9	300	4.5	700	0	25
10	200	3	500	0	0
11	0	3	500	11.11	22.22
12	400	3	500	10	30
13	200	0	500	10	10
14	200	6	500	0	10
15	200	3	0	0	10
16	200	3	1000	0	0
17	200	3	500	0	0
18	400	0	1000	20	30
19	400	6	1000	0	10
20	0	0	0	10	30

Results (Table XXVII) indicated that at 10 days of storage, treatments T2, T4, T9, T10, T14, T15, T16, T17 and T19 had fruits that did not show any sign of decay. All other treatments had 10-25 % of decayed fruits. At the last day of the storage period, only treatments T10, T16 and T7 maintained the fresh aspects of fruit (0 % of spoiled fruits), while treatments T5 and T13 had maintained fruits in the same aspect as from 10 day of storage (10 % of spoiled fruits). At 22 days of storage, all treatments that had had less than 3 minutes immersion in chlorinated water had recorded more than 30 % spoiled fruits. The fruit shelf life was increased in most of the treatments as compared to the control (T20) regarding the harvesting stage (light red).

Figure 26: Some samples of pictures taken during the preservation of fresh tomatoes for postharvest qualities and shelf life evaluation

III.1.2.2. Use of REO for the production of tomato juice and paste

Two types of tomato products were experimented using REO to prolong shelf life, namely tomato juice and tomato paste. The shelf life was challenged by artificial inoculation of *B. cereus* spores and treatment with varied REO concentration and time of appertisation.

III.1.2.2.1. Tomato juice

❖ **pH value readings**

The tomato juice had a mean pH value of 4.63 ± 0.05 before applying any treatment according to the experimental design.

❖ **Bacterial inhibition**

Artificial inoculated tomato juice were produced as indicated in the material and methods. After the appertisation, the percentage of microbial reduction was assessed before the beginning of storage at ambient temperature. These results are presented in Table XXVIII.

Table XXVIII: Bacterial load reduction in tomato juice treated at 95°C for varied time and concentrations in *B. cereus* and REO

Treatments	*B. cereus* (log CFU/ml)	Time (min)	REO (ppm)	MPN/ml	% reduction
J1	2	5	50	38.01	61.99
J2	2	20	150	3.00	97.00
J3	6	5	150	18.00	99.99
J4	6	20	50	9.16	99.99
J5	4	10	100	0.00	100.00
J6	2	5	150	3.57	96.43
J7	2	20	200	3.00	97.00
J8	6	5	50	21.00	99.99
J9	6	20	150	146.63	99.99
J10	4	10	100	0.00	100.00
J11	0.1	10	100	0.00	100.00
J12	8	10	100	920.00	99.99
J13	4	0	100	0.00	100.00
J14	4	30	100	0.00	100.00
J15	4	10	0	3.00	99.97
J16	4	10	200	3.00	99.97
J17	4	10	100	0.00	100.00
J18	8	0	200	43653877.62	56.35
J19	8	30	200	198.25	100.00
J20	0.1	0	0	0.00	100.00

These results were analysed using a non-linear regression in order to assess the relations between factors and the percentage reduction. The percentage reduction of *B. cereus* in juice is given in the below equation with R^2= 0.32 and SSE= 1351.

% Reduction = 93.5 + 0.1542*[*B. cereus*]*[Time] - 0.0158*[*B. cereus*]*[REO] Eq 10

It indicates that interaction between treatments (*B. cereus* concentration, cooking time and REO concentration) was responsible for the spore reduction. Maximum reduction (100 %) of *B. cereus* was observed in J5, J10, J11, J13, J14, J17 J19 and J20 where there was a concentration of 10^4 *B. cereus*/ml, cooking time (10 minutes) and 100 ppm of REO. 99.99 % reduction of *B. cereus* were observed in J3, J4, J8, J9 and J12 treatments with 10^6 *B. cereus*/ml concentration, 5-20 minutes of cooking time and 50-150 ppm of REO. 99.97 % reduction of *B. cereus* were observed in J15 and J16 treatments with 10^4 *B. cereus*/ml concentration, 10 minutes of cooking time and 0-200 ppm of REO. 97 % reduction of *B. cereus* were observed in treatments (J2 and J7) with 10^2 *B. cereus*/ml concentration, 20 minutes of cooking time and 150-200 ppm REO concentration, while 96.43 % reduction of *B. cereus* were recorded in J6 (10^2 *B. cereus*/ml, 5 minutes of cooking time and 150 ppm of

REO). 61.99 % reduction of *B. cereus* were observed in J1 treatment with reduced *B. cereus* (10^2 *B. cereus*/g), REO concentration (50 ppm) and cooking time (5 minutes) whereas in treatment J18 with high concentration in *B. cereus* (10^8 *B. cereus*/ml) and REO (200 ppm) and 0 minutes of cooking time, only 56.35 % reduction of *B. cereus* were recorded.

❖ **Total polyphenols**

Results on Total Polyphenols in tomato juice are given in Figure 27:

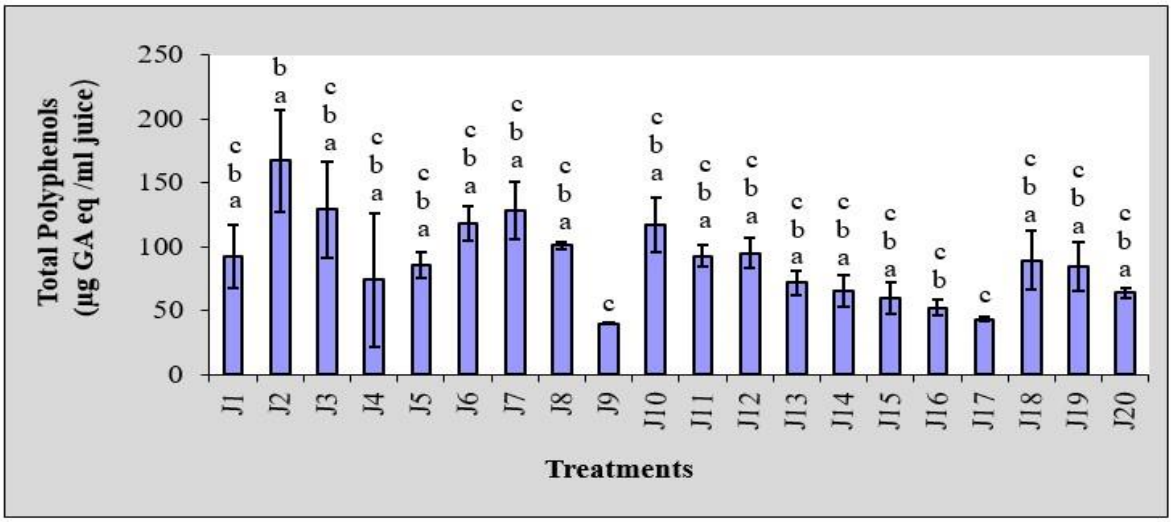


Figure 27: Levels of total polyphenols in tomato juice treatments

Results presented in Figure 27 indicate significant differences in treatments with regards to the concentration of total polyphenols (µg/ml juice). Three different groups were formed statistically ($p < 0.05$), the higher concentration was recorded in J2 (167.22 µg/ml juice), whereas the lower concentration was observed in J9 (40 µg/ml juice).

❖ **Titratable acidity**

Results on Titratable acidity in tomato juice are given in Figure 28:

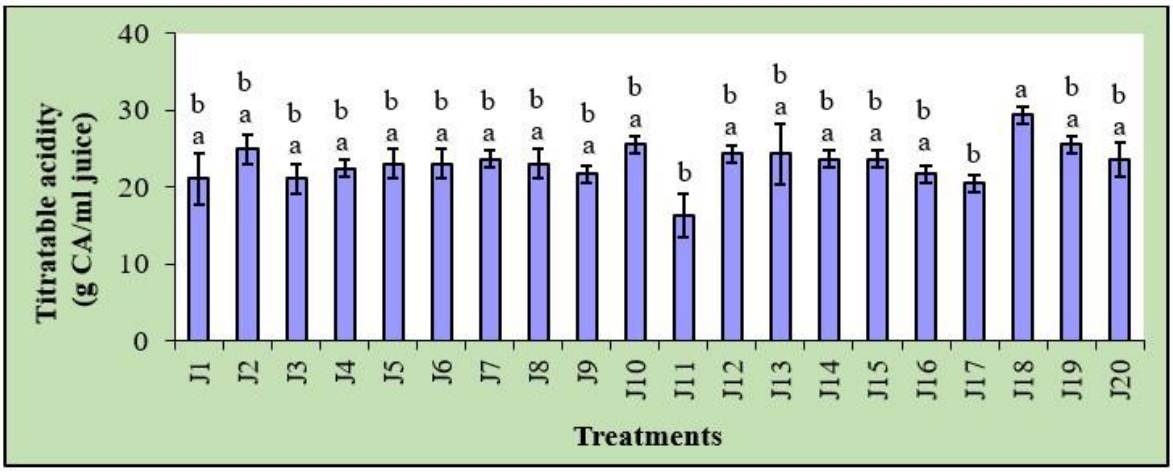


Figure 28: Levels of Titratable acidity in tomato juice treatments

There were significant differences on levels of titratable acidity between J11 (16.32 g/100 ml juice) and J17 (20.48 g/100 ml juice), and the highest value was found in J18 (29.44 g/100 ml juice). All the other treatments were similar.

❖ **Vitamin C content**

Results on Vitamin C content in tomato juice are given in Figure 29:

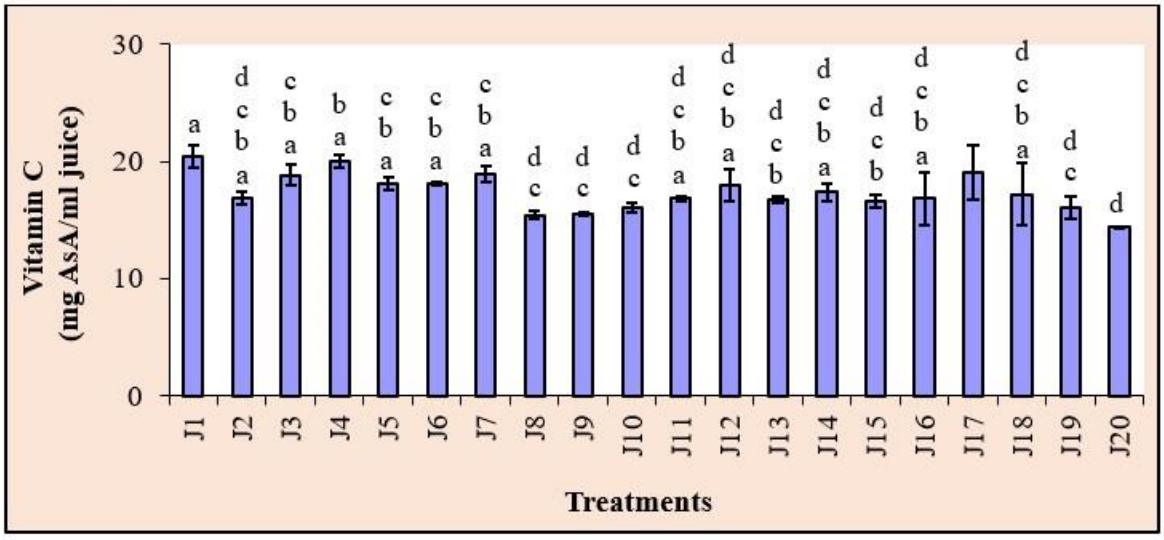


Figure 29: Levels of Vitamin C content in tomato juice treatments

Significant differences were recorded among juice treatments, where 4 groups were formed (Appendix 7). However, the highest concentration was found in J1 (20.43 mg/ml) while the lowest concentration was found in J20 (14.40 mg/ml).

❖ **Shelf life of tomato juice**

Results on the efficacy evaluation of the process on one-year basis of the conservation of tomato juice artificially challenged with *Bacillus cereus* inoculation are given in Table XXIX.

Table XXIX : Treatment efficacy for the achievement of one year conservation of tomato juice as subjected to *B. cereus* contamination, REO concentration and time of appertisation

Juice Treatments	*B. cereus* (log CFU/g)	Time (min)	REO (ppm)	Juice Storage efficacy	Shelf life (months)
J1	2	5	50	**1.00**	**12**
J2	2	20	150	**1.00**	**12**
J3	6	5	150	**1.00**	**12**
J4	6	20	50	**0.79**	**9.6**
J5	4	10	100	**1.00**	**12**
J6	2	5	150	**1.00**	**12**
J7	2	20	200	**0.79**	**9.5**
J8	6	5	50	**0.93**	**11.2**
J9	6	20	150	**0.75**	**9**
J10	4	10	100	**0.75**	**9**
J11	0.1	10	100	**0.54**	**6.5**
J12	8	10	100	**0.75**	**9**
J13	4	0	100	**0.50**	**6**
J14	4	30	100	**0.54**	**6.5**
J15	4	10	0	**0.75**	**9**
J16	4	10	200	**0.77**	**9.2**
J17	4	10	100	**0.79**	**9.5**
J18	8	0	200	**0.79**	**9.5**
J19	8	30	200	**0.79**	**9.5**
J20	0.1	0	0	**0.54**	**6.5**

The overall results of the observation during the year of conservation of juices is presented in Appendix 11. From these data, the shelf life conferred by the treatments were calculated using the proposed formula (Eq 8) of Material and Methods. From the results presented on the storage efficacy of the process on a shelf life period of 1 year, it can be seen that quite a number of treatments that had initial spore load of 2 *B. cereus* log combined with different REO concentrations and appertisation had a prolonged shelf life. These products were still with no sign of bacterial growth after 1 year of storage at ambient temperature. It can be observed that the interaction between REO and thermal treatments is not very straight forward. In fact, when using 200 ppm of REO, the same level of inactivation was observed without treatment (J18) and after 20 minutes (J4) at 95°C.

III.1.2.2.2. Tomato paste

❖ **pH value readings**

The tomato paste had a mean pH value of 4.7 ± 0.05, before applying any treatment according to the experimental design.

❖ **Bacterial inhibition**

Results on heat treatment for inactivation and enumeration of survivors of *B. cereus* ATCC 11966 in tomato paste are presented in Table XXX.

Table XXX: Bacterial load reduction in tomato paste treated at 95ºC for varied time and concentrations in *B. cereus* and REO

Treatments	*B. cereus* (log CFU/g)	Time (min)	REO (ppm)	MPN/ml	% reduction
P1	2	10	50	1.0025	99.95
P2	2	30	150	1.0025	99.95
P3	6	10	150	1.0025	99.98
P4	6	30	50	92	67.27
P5	4	20	100	1.0025	99.97
P6	2	10	150	1.0025	99.95
P7	2	30	200	1.0025	99.95
P8	6	10	50	21	77.96
P9	6	30	150	9.177039	83.95
P10	4	20	100	0.0000	100.00
P11	0.1	20	100	1.0025	98.92
P12	8	20	100	930000	100.00
P13	4	0	100	9.30E+03	0.79
P14	4	40	100	1.0025	99.97
P15	4	20	0	1.0025	99.97
P16	4	20	200	1.0025	99.97
P17	4	20	100	1.0025	99.97
P18	8	0	200	2.4E+08	0.10
P19	8	40	200	42	79.71
P20	0.1	0	0	23	0.00*

** affected because an increase in cells was observed.*

It can be observed that high values of treatment time and REO were not favourable to *B. cereus* spore reduction. The combination of a time of 20 minutes and REO of 100 ppm seems the best. 100% reduction were noted in P10 and P12, where there were 20 minutes of cooking time and 100 ppm of REO. 99.98 % (P3) were recorded where there was high *B. cereus* (10^6

B. cereus/g), cooking time (20 minutes) and REO concentration (150 ppm). 99.97 % were recorded in all treatments with 10^4 *B. cereus*/g and cooking time varying from 20-40 minutes (P5, P10, P14, P15, P16 and P17). 83.95 % in treatment (P9) with high *B. cereus* (10^6 *B. cereus*/g), cooking time (30 minutes) and REO concentration (150 ppm). 79.71 % in treatment (P19) with maximum concentration of *B. cereus* (10^8 *B. cereus*/g), REO (200 ppm) and cooking time (40 minutes); 77.96 % (P8) where *B. cereus* (10^6 *B. cereus*/g), cooking time (10 minutes) and REO concentration (50 ppm); 67.27 % (P4) where *B. cereus* (10^6 *B. cereus*/g), cooking time (30 minutes) and REO concentration (50 ppm). 0 % percentage reduction of *B. cereus* were found in treatments P13 (0.79 %) and P18 (0.1 %) with 0 minute of cooking time and *B. cereus* (10^4 and 10^8 *B. cereus*/g) and REO concentration (100 and 200 ppm) respectively. P20 had an increase in *B. cereus* number and hence, no reduction.

In order to assess the relation between factors and the percentage reduction, a non-linear regression was performed:

% Reduction = 92.422 + 0.179*[REO]*[*B. cereus*] - 0.428*[*B. cereus*]*[Time] Eq 11

[R^2= 0.35 and SSE= 62.9]

It can be observed that the combination of *B. cereus* concentration with time and REO concentration affected significantly the percentage reduction of the strain. However, it can be said that further significant parameters should be found as the model only explains 35 % of the data variability.

❖ **Total polyphenols**

Results on Total Polyphenols in tomato paste are given in Figure 30 presented below:

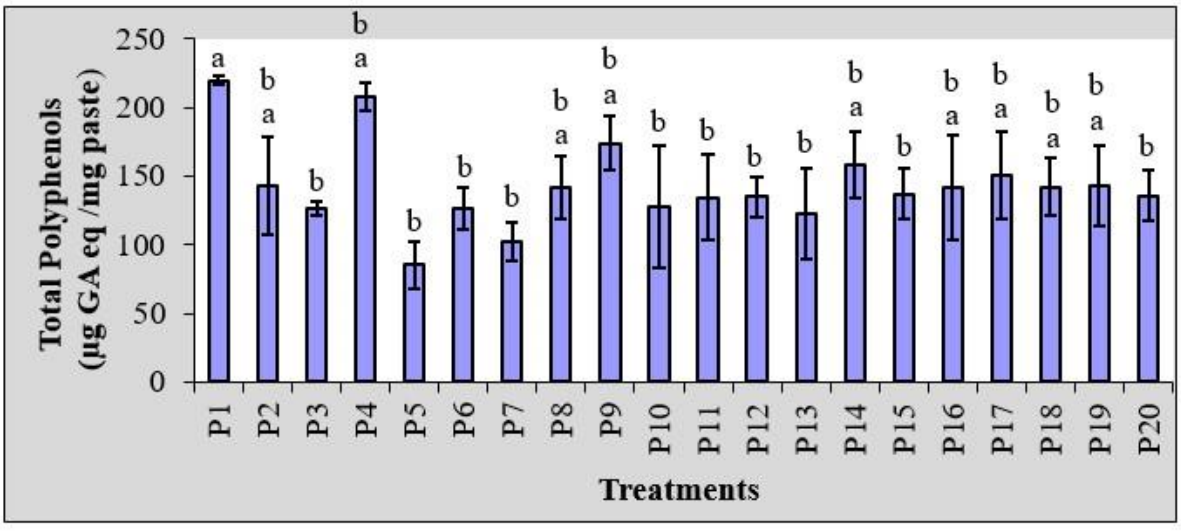


Figure 30: Levels of total polyphenols in tomato paste treatments

In tomato paste, the highest concentrations of total polyphenols (µg/mg paste) was recorded in P1 (220 µg/mg paste) and the lowest concentration in P5 (85.56 µg/mg paste). Significant differences ($p < 0.05$) were observed in most treatments and 2 groups were formed from the mean comparison.

❖ **Titratable acidity**

Results on Titratable acidity in tomato paste are given in Figure 31 presented below:

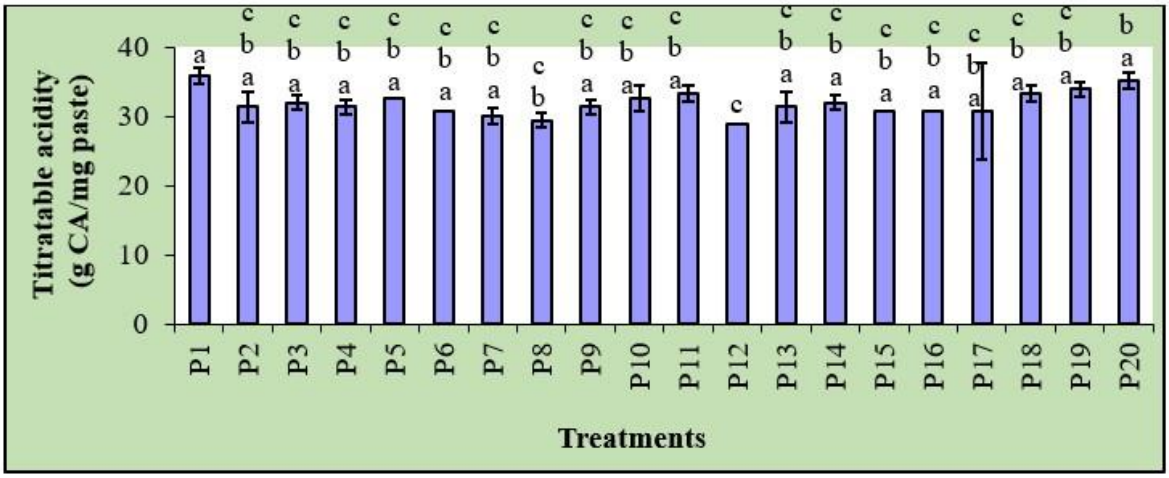


Figure 31: Levels of Titratable acidity in tomato paste treatments

Results from the above figure indicate significant differences ($p < 0.05$) in the concentration of titratable acidity among treatments. 3 groups were formed with the highest value found in P1 (35.84 g/mg paste) and the lowest value being in P12 (28.8 g/mg paste).

❖ **Vitamin C content**

Results on Vitamin C content in tomato paste are given in Figure 32 presented below:

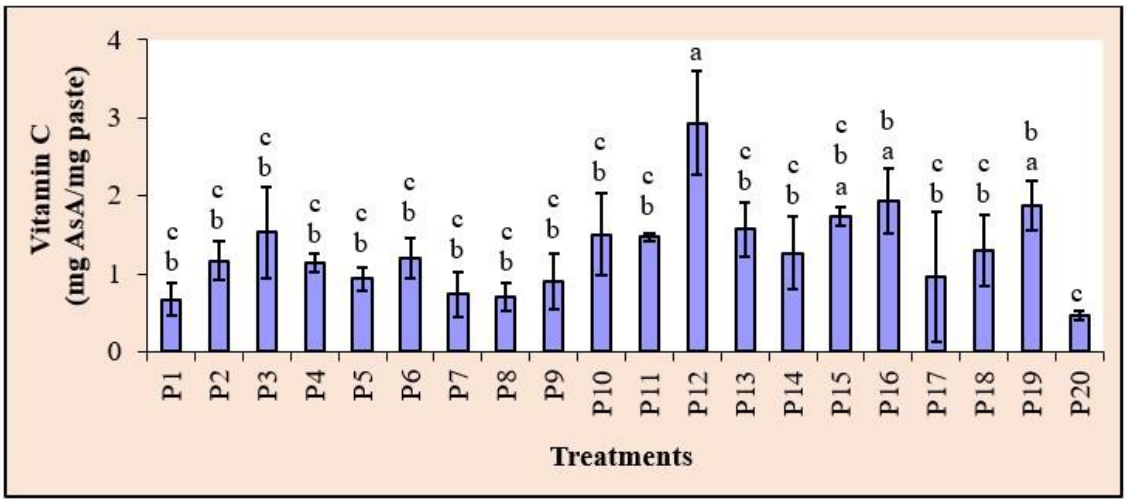


Figure 32: Levels of Vitamin C in tomato paste treatments

The concentration of vitamin C in tomato paste has been reduced and significant differences ($p < 0.05$) were found among treatments with the highest concentration being in P12 (2.93 mg/mg paste) and the lowest found in P20 (0.47 mg/mg paste).

❖ **Shelf life of tomato paste**

Results on the efficacy evaluation of the process on one-year basis of the conservation of tomato paste artificially challenged with *B. cereus* inoculation are given in the below Table:

Table XXXI: Treatment efficacy for the achievement of one year conservation of tomato paste as subjected by *B. cereus* contamination, REO concentration and time of appertisation

Treatments	*B. cereus* (log CFU/g)	Time (min)	REO (ppm)	Paste Storage efficacy	Shelf life (months)
P1	2	10	50	**0.84**	**10.1**
P2	2	30	150	**0.79**	**9.5**
P3	6	10	150	**0.50**	**6**
P4	6	30	50	**0.54**	**6.5**
P5	4	20	100	**1.00**	**12**
P6	2	10	150	**1.00**	**12**
P7	2	30	200	**0.59**	**7.1**
P8	6	10	50	**0.59**	**7.1**
P9	6	30	150	**0.54**	**6.5**
P10	4	20	100	**1.00**	**12**
P11	0.1	20	100	**0.75**	**9**
P12	8	20	100	**0.79**	**9.5**
P13	4	0	100	**0.17**	**2**
P14	4	40	100	**0.54**	**6.5**
P15	4	20	0	**1.00**	**12**
P16	4	20	200	**1.00**	**12**
P17	4	20	100	**0.84**	**10.1**
P18	8	0	200	**0.03**	**0.4**
P19	8	40	200	**0.66**	**7.9**
P20	0.1	0	0	**0.84**	**10.1**

The overall results of the observation during the year of conservation of pastes is presented in Appendix 12. From these data, the shelf life conferred by the treatments were calculated using the proposed formula (Eq 8) of Material and Methods. From the results presented on the storage efficacy to evaluate the shelf life of tomato paste in a period of 1 year, it can be seen that quite a number of treatments that had initial spore load of 4 *B. cereus* log and pasteurization time of 20 minutes combined with different REO concentrations had a shelf life of at least 1 year. Moreover, when the inoculated load was 2 log, a treatment time of 10 minutes and REO of 50 ppm, a 1-year of shelf life was assured. It can also be observed that

tomato paste with 20 minutes appertisation and no REO concentration (P15) also demonstrated a 1-year of storage level. Treatment times higher than 30 minutes in combination of REO were not in favour of the storage efficacy. The use of REO alone gave lower storage times inversely proportional to the microbial initial load.

III.1.3. Implementation of one of the developed preservation techniques in the Bushi region

The present economical conditions in the Bushi region imparting the availability of energy orientated us on the implementation of the fresh fruits preservation. It is important to state here that the best solution based on our results would have been the juice and paste production. With respect to Eq 8, the use of REO alone can still assure an increase of fresh product's shelf life. In fact, taking into consideration [Chlorine] = 0, Eq 9 becomes

Flavour score at 22 days = 0.436+ 0.007*[HE] + 0 - 0.0031* [t]*[HE] Eq 12

And for [HE] ≤ 500 ppm, positive effects of REO alone have been observed. This permitted to overcome the resistance of population to use chlorine.

III.1.3.1. Colour development

Results on colour development of tomato fruits during the assessment of REO preservation potential on tomatoes in the Bushi region are given in Figure 33:

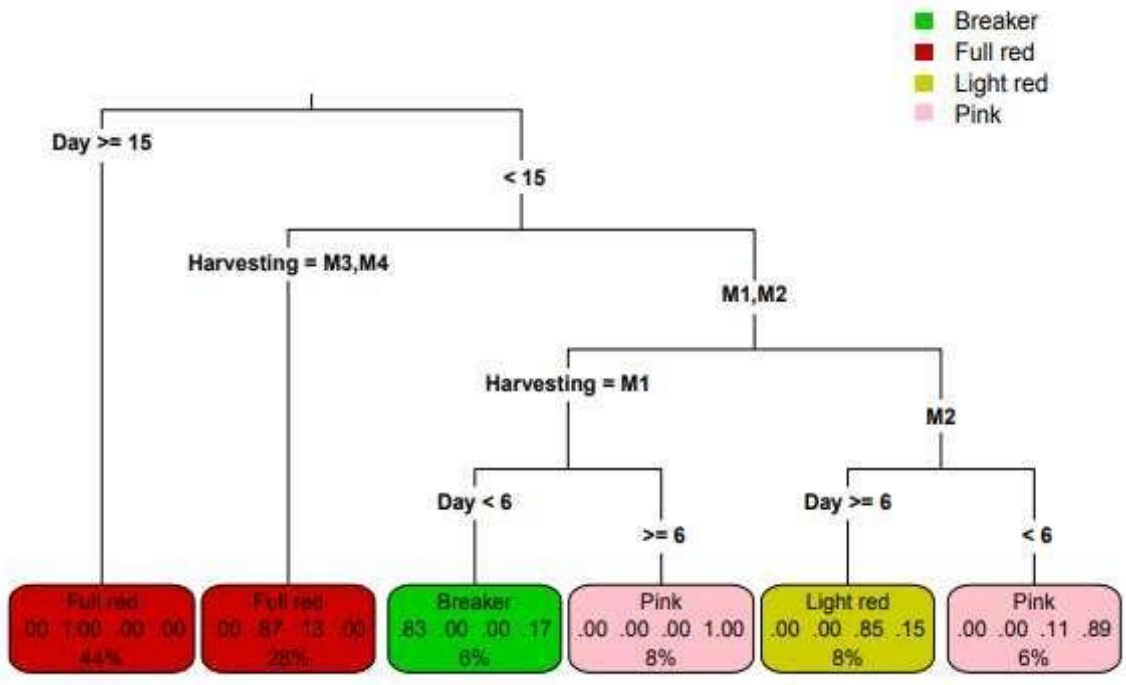
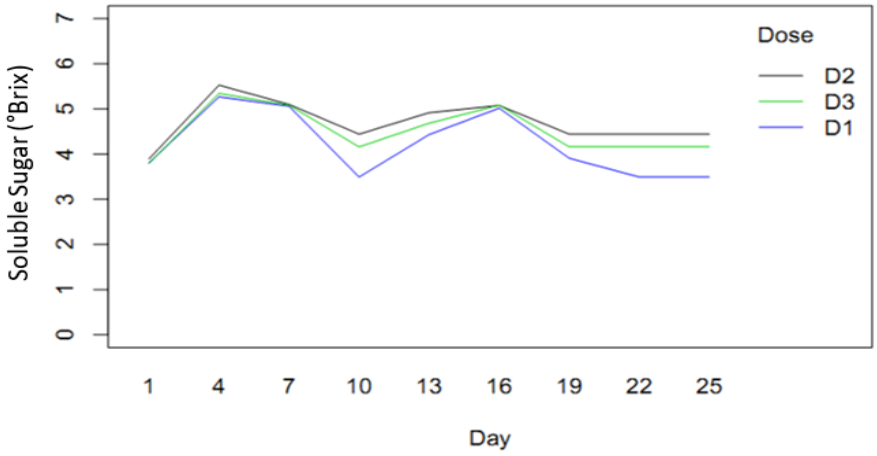


Figure 33: Dendogram grouping fruit colours during preservation

Figure 33 indicated that at 15 days of storage, all fruits harvested at different maturity stages had reached the full red colour, though the progression towards full red colour was done depending on the maturity stage. The doses of REO had no influence on the tomato colour development.

III.1.3.2. Soluble Sugar content

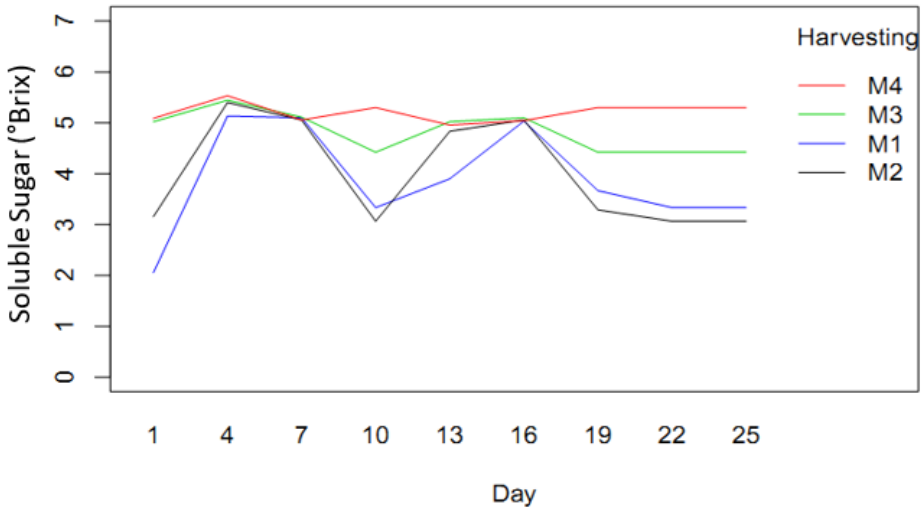
Results on the influence of REO doses and maturity stages on tomato fruits soluble sugar are given in Figures 34 and 35:



D1= 0 ppm, D2= 200 ppm, D3= 500 ppm

Figure 34: Influence of REO doses on soluble sugar of tomato fruits during storage

Results indicated that there was gradual increase in soluble sugar contents at day 4, 13 and 16 of storage in all the treatments with regards to the doses of REO. The decrease in soluble sugars was observed at day 7, 10, 19, 22 and 25 of storage. The highest values were recorded in treatments with D2 REO application (200 ppm), and the lowest values in D1 (0 ppm).



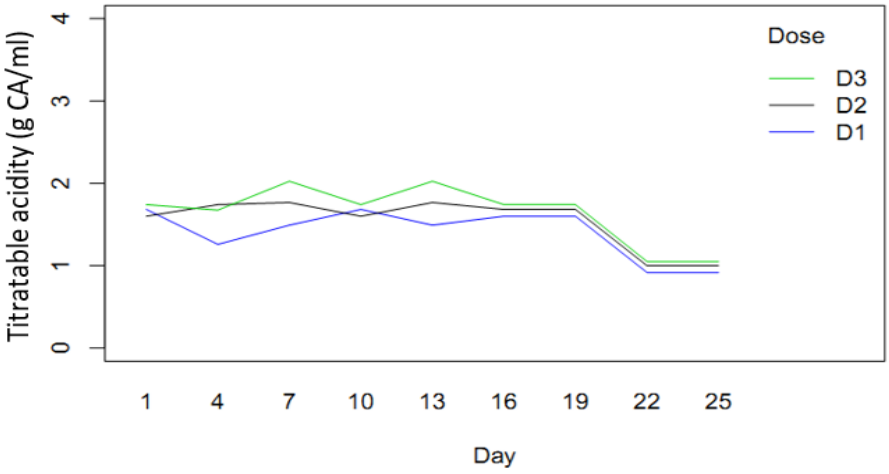
M1= Breaker, M2= Pink, M3= Light red, M4= Full Red

Figure 35: Influence of maturity stages on soluble sugar of tomato during storage

Higher value in soluble sugar content (5.5° Brix) was found in M4 (Full red) at day 4 of storage, with little variations till the end day of storage. The lowest value (2.1° Brix) in soluble sugar content was observed in M1 (Breaker) at day 1 of storage, followed by decrease in sugar content at day 10, 19 and 22 of storage. The Pink stage (M2) had decreased in sugar content from the 4th, 7th, 10th, 19th and 22nd day of storage, lower than all other treatments.

III.1.3.3. Titratable acidity

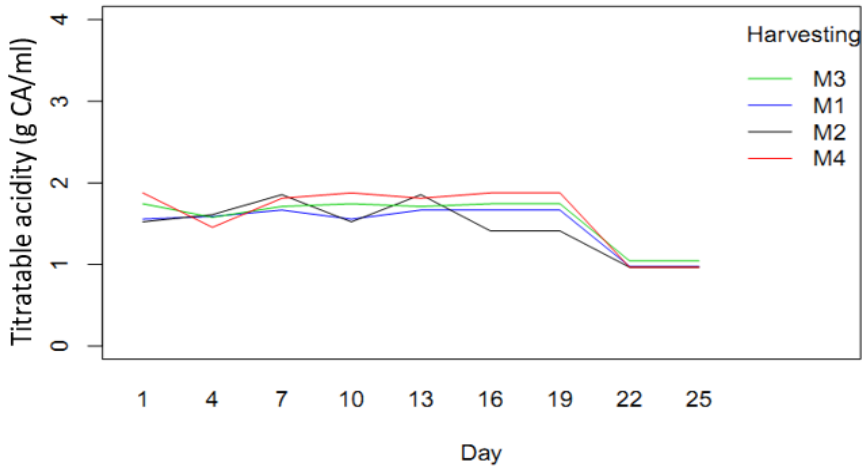
Results on the influence of REO doses and maturity stages on tomato fruits titratable acidity are given in Figures 36 and 37:



D1= 0 ppm, D2= 200 ppm, D3= 500 ppm

Figure 36: Influence of REO doses on titratable acidity of tomato fruits during storage

Results in Figure 36 indicated higher values of titratable acidity in D3 treatments and lower values in D1 treatments. There was decrease in Titratable acidity at the end day of storage in all treatments.



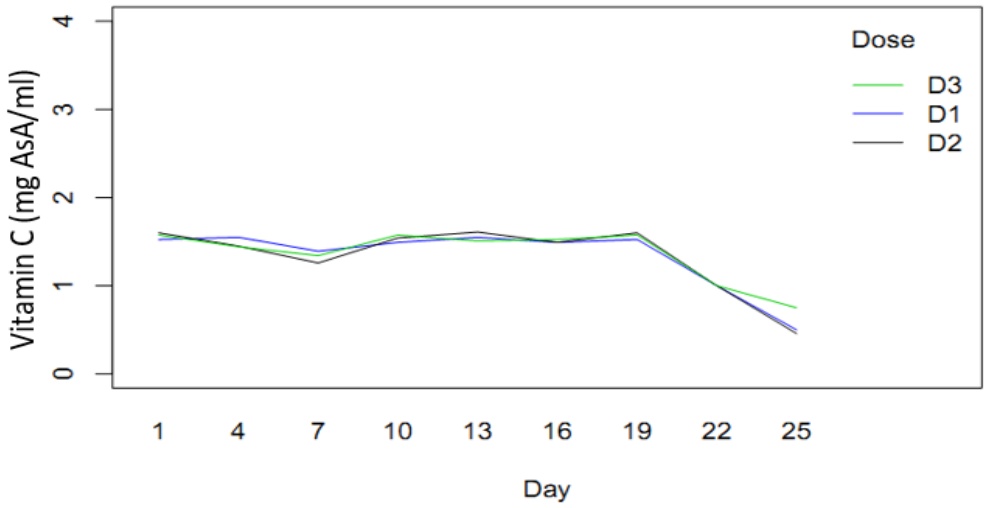
M1= Breaker, M2= Pink, M3= Light red, M4= Full Red

Figure 37: Influence of maturity stages on titratable acidity of tomato during storage

It can be seen that changes of titratable acidity during storage were relatively small in M1 and M3 treatments than in M2 and M4 treatments. Variations in the content of titratable acidity are observed in all treatments up to Day 19, after what there was a drastic decrease.

III.1.3.4. Vitamin C

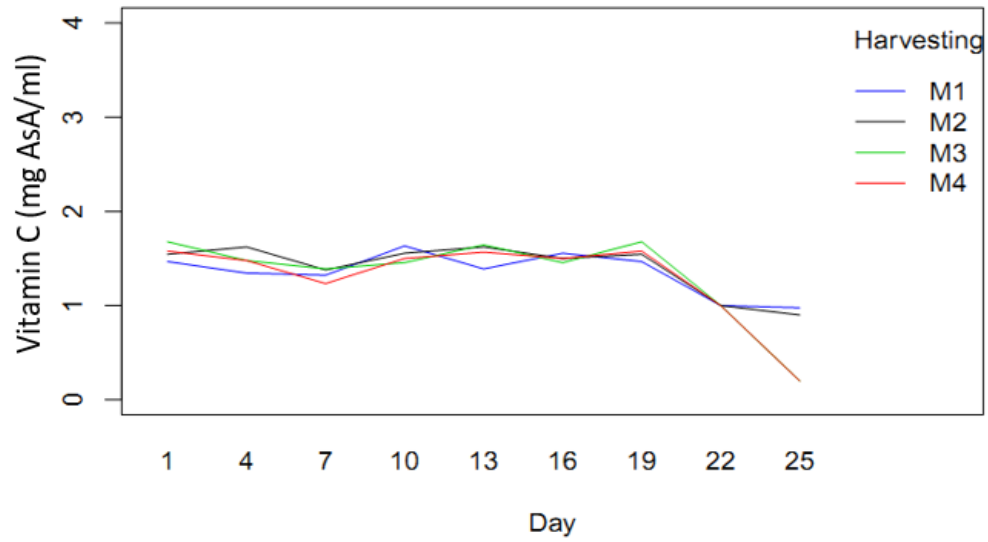
Results on the influence of REO doses and maturity stages on vitamin C content of tomato fruits are given in Figures 38 and 39:



D1= 0 ppm, D2= 200 ppm, D3= 500 ppm

Figure 38: Influence of REO doses on the Vitamin C content of tomato during storage

Ascorbic acid content did not vary significantly among REO dose applications (Figure 38). Slight variations in vitamin C content were observed until it dropped from day 19 of storage.



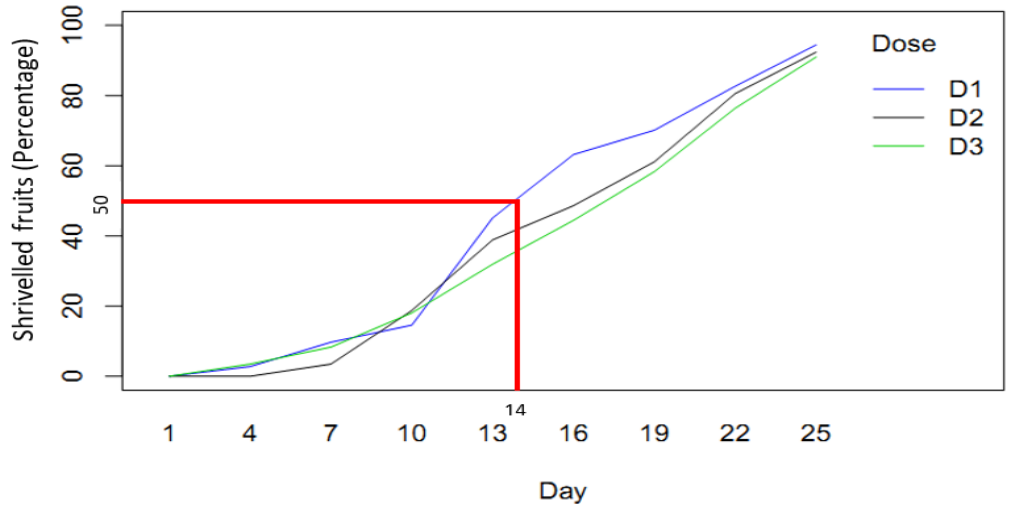
M1= Breaker, M2= Pink, M3= Light red, M4= Full Red

Figure 39: Influence of maturity stages on Vitamin C content of tomato during storage

No significant differences were observed among treatments with regards to harvesting stages, except on the last day of storage where differences were observed. The ascorbic acid content of fruits from M1 (0.98 mg/100 ml) and M2 (0.90 mg/ml) was different from fruits of M3 and M4 (0.20 mg/ml).

III.1.3.5. Shelf life

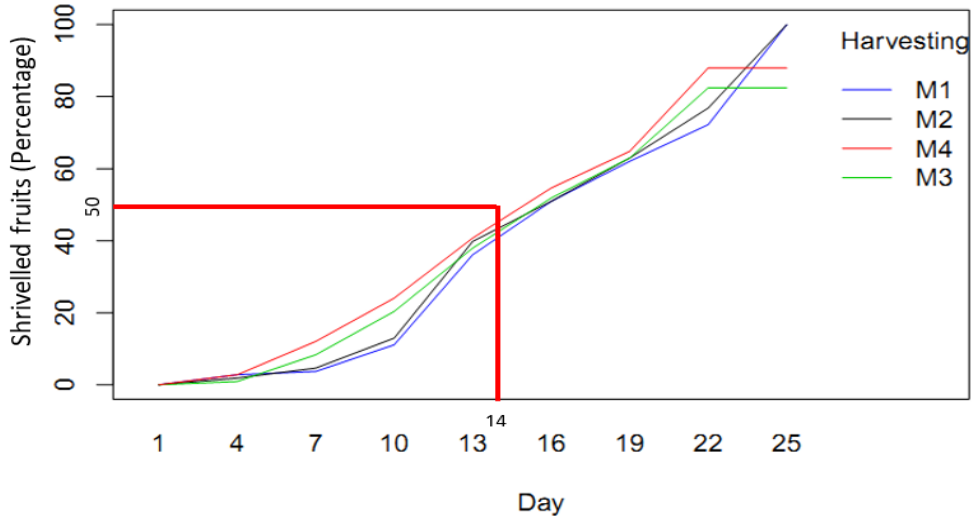
Results on the influence of REO doses and maturity stages on shelf life of tomato fruits are given in Figures 40 and 41:



D1= 0 ppm, D2= 200 ppm, D3= 500 ppm

Figure 40: Influence of REO doses on Shelf life of tomato during storage

Results in Figure 40 indicated that the percentage in shrivelled fruits is lower in treatments with increased REO although this was statistically significant between 13 and 22 days of storage.



M1= Breaker, M2= Pink, M3= Light red, M4= Full Red

Figure 41: Influence of maturity stages on Shelf life of tomato during storage

There were no significant differences among treatments (Figure 41). However, the shelf life was determined when the fruits started showing signs of shrivelling and decay. In this study, most treatments had attained 50 % of shrivelled fruits at 16 days of storage. Fruits from Breaker (M1) and Pink (M2) maturity stages had 50.93 % of wilting (shrivelling), while fruits from Light red (M3) and Full red (M4) stages had registered respectively 51.85 % and 54.63 % of wilting.

III.2. DISCUSSION

II.2.1. Land suitability and nutrients availability

The land suitability survey for tomato production in the Bushi region (Kabare and Walungu territories) has been evaluated. Generally, tomato is a moderately tolerant crop to a wide pH range. A pH of 5.5-6.8 is preferred, though tomato plants will do well in more acidic soils with adequate nutrient supply and availability (Upendra *et al*., 2003). 8 villages out of 10 in Kabare territory and 3 villages out of 5 in Walungu territory were within this range. In tomato, it is reported that chlorophyll indices significantly increase with increasing pH, and the use of soils with pH 6 plays an important role in floral and fruit set (Maasumeh, 2014). Soil pH is important because it influences most soil chemical processes that can affect nutrient availability, dictates the availability of elements that are toxic to plants and affects microbial activity, particularly biological processes affecting decomposition rates of organic matter, which in turn affect nutrient availability and plant growth (Botta, 2015).

Nitrogen levels in soils fluctuate widely, greatly depending on biological activity, seasonal conditions and rainfall (Botta, 2015). Literature on recommended levels of nutrients for tomatoes presented by Upendra *et al*. (2003) has indicated that desirable N levels range between 50-100 mg/Kg soil. However, our results have indicated excessive levels of Total N [0.021-3.24% (210-3240 mg/Kg)] in both Kabare and Walungu territories, probably due to regular amendments in the form of manures (fertilizers) to increase soil fertility for other important crops (maize, beans, leaf vegetables ...). Unfortunately, high N level in the soil promotes excessive vegetative growth which can delay the setting and maturity of tomato fruits, thereby reducing tomato production. Likewise, N deficiency in the soil results in stunted spindly growth and yellowing of leaves at the base of tomato plant, therefore decreasing the production of number of fruits, fruit size, storage quality, colour, and taste of tomato (Upendra *et al*., 2003). Nevertheless, Passam *et al*. (2007) have reported that the supply of reduced nitrogen forms such as ammonium or organic nitrogen to tomato results in

an improved fruit flavour. Regarding the impact of nitrogen on plant susceptibility to diseases, literature contains contradictory information (Gupta *et al*., 2017). Soil organic carbon is the basis of soil fertility: it releases nutrients for plant growth, promotes the structure, biological and physical health of soil. There were many variations (0.46-3.284%) in terms of organic carbon in Kabare and Walungu territories. It is reported that the levels of carbon stocks are highly variable, depending on land use, soil type and climate (Kong *et al*., 2005). Farming systems that utilize best management practices hold promise for sequestering soil carbon, which has the potential to enhance agricultural sustainability, reduce negative environmental impacts and attenuate anthropogenic carbon dioxide emissions. Since the reconstruction of organic carbon stock in the soil takes several decades (ADEME, 2015), there are so many changes which, if not taken into account, could affect soil quality and lower soil carbon stocks.

In tomato, the desirable recommended P nutrient levels are ranged between 60-70 mg/Kg (Upendra *et al*., 2003). Our results have indicated high variability in the concentration of Available P (mg/Kg) among and within the Kabare and Walungu territories. Nevertheless, it has been reported that excess level of P in the soil is less harmful to tomato; however, it can reduce the availability of some micronutrients, such as Fe, Zn, Mn and Cu by decreasing their solubility in the soil and translocation within the plant. This absence of translocation may impact the plant and tomato resistance to pathogen infection. In fact, Zn deficiency can lead to an increase in membrane leakage of low-molecular-weight compounds that may improve living conditions for pathogens (Huber *et al*., 2012). According to Heckman *et al*. (2003), manganese can be useful in controlling a number of pathogenic diseases in plants. On the other hand, Cu deficiency can induce low plant cell rigidity and alter cell membrane lipid structure (Broadley *et al*., 2012). Deficiency in phosphorus results in stunted growth of tomatoes with thin stems and green colour on the upper surface of leaves (Upendra *et al*., 2003). It has been demonstrated that good level of P can increase tomato resistance to *Fusarium* and some other crops (Kiraly, 1976). Regarding the exchangeable cations (K, Ca and Mg), it has been reported that soils with higher cation exchange capacity (CEC) are generally considered more fertile, able to support higher production levels and can retain larger amounts of nutrients against losses *via* leaching through the soil profile. However, a CEC > 12 cmol/Kg would be considered moderate and means that a soil has a good capacity to retain nutrients for plant growth (Botta, 2015). Unfortunately, our findings have indicated lower values than the recommended levels, probably due to the effects of erosion and excessive land use, since lack of arable spaces has important implications on farming soil

capacity. Knowing the importance of K, Ca and Mg in the suitability of plants in general and tomato in particular to resist disease and postharvest physiological decay (Lara *et al*., 2004; Dordas, 2008; Spann and Schumann 2010; Huber and Jones, 2013), specific soil management decisions should be taken to correct this CEC level.

According to Upendra *et al*. (2003), potassium helps in vigorous growth of tomato and stimulates in early flowering and setting of fruits, thereby increasing the number and production of tomatoes per plant. Excess K level in the soil can have hardly any direct effect on tomatoes but it can reduce the availability of Mg in the soil. It has been reported that potassium nutrition can affect the quality of tomato fruit; low levels of K supply in tomato are associated with ripening disorders (Passam *et al*., 2007), and shortened internodes. An adequate supply of K (600-700 mg/Kg) improves fruit colour and sensory quality of tomato (titratable acidity, citric and malic acids concentrations, total solids, sugars and carotene), thereby improving its storage quality (Upendra *et al*., 2003). Calcium plays a key role in the quality of tomato fruit and is also needed by tomato in large amount because of its higher concentration in the plant components. Ca deficiency (< 1000 mg/Kg) is to be avoided mainly due to its impact on the occurrence of the "blossom end rot" (physiological disorder). An enhanced supply of Ca may reduce the incidence of another physiological disorder that leads to a deterioration in fruit quality, "shoulder check crack" (Upendra *et al*., 2003; Passam *et al*., 2007). Magnesium is not directly involved in the fruit quality of tomato. However, an increase of the Mg supply above the standard recommended levels (350-700 mg/Kg) may considerably increase the incidence of "blossom end rot", and severe Mg deficiency negatively impact on the size and overall appearance of the fruit. Mg deficiency may not affect fruit production unless the problem is severe. This can be observed in sandy soils, soils high in K level and in soils with poor structure or drainage (Upendra *et al*., 2003). This deficiency can also cause an attractive environment for pathogen by increasing the availability of sucrose and amino acids (Huber and Jones, 2013).

The benefits of soil cultivation are to improve the soil physical condition; soil in its natural state rarely provides the most favourable physical conditions for crop growth. Soil texture indicates much about the possible limitations to crop production in a given soil (Catriona *et al*., 1999). According to Elsheikh *et al*. (2013), the principle purpose of agriculture land suitability evaluation is to predict the potential and limitation of the land for crop production, this because, continuous utilization of agriculture land has caused much more destruction than provide the resources. Land suitability evaluation is done on agronomic aspects as well as

management requirements for soil fertility, irrigation, tillage practices, conservation measures and costs of land improvements (Nethononda *et al*., 2014).

The land suitability map for tomato production in Bushi has given out 4 classes. It does not imply that farmers in the unsuitable areas cannot grow tomatoes, rather it gives information on the environmental conditions and its impacts that may arise with the varying climate conditions, and helps to plan and manage agricultural activities for better yields and products' quality. Soil fertility management is a great challenge in the Bushi region, mainly due to the overuse of cultivable lands, not only for agriculture purposes but for other activities (mineral extraction) and constraints (mountain regions, natural risks, climate change, rural migration, poverty status ...). Cultivated areas are deficient: less than 1 ha is used by small scale farmers, from generation to generation, in all seasons for different crops with significant impacts on soil fertility (IPAPEL, 2010). In the Eastern DRC, many are the causes of overexploitation of lands: population growth and political factors such as rebellion wars and insecurity in the region, where many people are forced to leave their homes and lands, looking for security in towns and leaving behind them plunder and total destruction. These situations affect immediately the agricultural sector since the majority of households live on intensive small-scale subsistence farming where cultural practices are traditional.

Thus, according to Nethononda *et al*. (2014), land suitability evaluation in developing countries requires simple dynamic land suitability evaluation guidelines that cater for the needs of different and specific land uses and recognize the interaction between land use and land users. In this light, Boitt *et al*. (2015) reinforce that suitability assessment is an important phenomena for a region or a country to engage in more rational planning and optimizing resource use for the present and in the future. The present study has not only assessed the soil suitability for tomato production, but has made theoretical correlations between the soil nutrients availability and disease resistance. It may hence be observed that the classification obtained ranging from highly suitable to not suitable could correspond to the need of preservation techniques in order to catch up with pre-harvest quality defects. In orther words, not suitable lands may basically need more preservation techniques than highly suitable ones, although in order to reduce postharvest losses all productions should be preserved.

II.2.2. Characterization of cultivated tomato fruits in Bushi

Cultivated tomato fruits characteristics vary among the Bushi region. The shape and size of tomato fruit are defining features that distinguish one variety from another; shape depends on the appearance of the fruits from their side, while size is an easily measurable trait that

depends on the weight of the fruits as well on their diameter and length from top to bottom. Houghtaling (1935) reported that, in tomatoes, the ultimate size of the fruit is determined very early in its development, and that shape differences develop by differential growth rates between polar and equatorial dimensions. Researchers have established that the diversity of tomato fruit shape and weight is due mainly to mutations of genes, and that tomato varieties differ in capacity in relation to their genetic characteristics (Tanksley, 2004; Avdeyev *et al*., 2014). High and positive correlation has been demonstrated between shape and size of tomato fruits (Lindstrom, 1927). Gonzalo *et al*. (2009) also reported significant positive correlation between fruit weight and locules number, together with other important traits (pericarp, septum, placenta area, lobedness degree).

Several studies showed that the dry matter concentration of tomato varies during the growing season. According to Klunklin and Savage (2017), most of the dry matter contents in tomatoes are made up from dietary fibre and carbohydrates, which are mainly fructose and glucose. It is well known that tomato contains about 94 % water that generally increases between the green-mature and ripe stages (Abou-Aziz, 1968). The quantity of water present in the fruit is an essential factor for its quality since it determines the concentration of different elements such as dry matter, sugars and acids (Guichard *et al*., 2001). Regarding the storage life (expressed in weeks), the results indicated variations among the cultivated varieties. This could be due to the nature and characteristics related to each variety, as well as growing conditions (Ponjičan *et al*., 2012).

II.2.3. Preservation of fresh fruits

Preservation of fresh tomatoes in combinations of REO and chlorine has improved postharvest qualities of fruits in this study. Colour change is often used as an index of the degree of ripeness, and provides primary information about physiological condition of the fruits (Sabir *et al*., 2012). After harvest, ripening continues and tomatoes can become overripe very rapidly due to maturation (Batu, 2004; Žnidarčič *et al.*, 2010). According to Munhuweyi (2012), ambient temperature conditions provide the most conducive environment for tomato ripening. Ripening processes are associated with increasing lycopene content that varies considerably between cultivars, stages of maturity and growing condition (Darrigues *et al*., 2008; Shalluf, 2010; Sibomana *et al*., 2015).

Quality appearance of tomatoes is greatly influenced by the presence and magnitude of defects (Kader, 1986). Fruit lose quality after harvest because of environmental stress and pathogen infection (Lai *et al*., 2011). Disinfectants such as chlorine are used at very high

concentrations in order to attain a rapid rate of killing microorganisms. Chlorine is a powerful antimicrobial substance due to its potential oxidizing capacity (Virto *et al*., 2005). Pinheiro *et al*. (2013) have reported that the antimicrobial effectiveness of hypochlorite solution depends on temperature, its concentration, treatment time and state of pathogenic micro-organism's growth. The mechanisms of action of chlorine on microorganisms have been widely investigated. According to Virto *et al*. (2005), chlorine is generally considered to be a nonselective oxidant which reacts avidly with a variety of cellular components and affects metabolic processes. The cytoplasmic membrane has been proposed to be a possible key target involved in bacterial inactivation by chlorine, since alterations in its permeability after chlorination have frequently been described. Maris (1995) has reported that chlorine dioxide acts on the permeability of the external membrane of *E. coli* through a primary lethal phenomenon which consists in a substantial leakage of K^+ ions; such leakage does not occur for macromolecules.

Our results have shown that the very high percentage (> 50%) of defects presence could probably be related to the reduced time of immersion (0-1.5 minutes) in chlorinated water for fruits surface disinfection. Hong and Gross (1998) have stated that sodium hypochlorite treatment has a pronounced effect on the texture of tomato tissue after treatment, this effect may be very low in our work since the concentrations used were far beyond those of these authors. The lowest defect percentage observed might have resulted from the combination of both treatments (immersion time in chlorinated water and rosemary essential oil treatment). From our results, a minimum chlorine concentration of 200 ppm and at least 500 ppm of REO can assure no defect after 22 days of storage. The presence of defects on tomato fruits could probably be the consequences of microbial growth during storage period associated to others uncontrolled factors (internal or external). According to Bartz *et al.* (2013), certain pathogens, including bacteria and viruses, may survive on or in fresh tomato fruit. The movement of living bacteria or fungal structures into fruit tissues is known as internalization and leads to a situation that cannot be corrected. It has also been mentioned that active growth of a pathogen may also occur in food from improper storage as a result of passive transfer of pathogens to food (Celiktas *et al*., 2005).

As concerning fruit firmness, it generally decreases with prolonged storage (Žnidarčič and Požrl, 2006; Tabaestani *et al*., 2013), and with advance in maturity stage of tomato fruit (Tilahun, 2013). Arthur *et al*. (2015) stated that decrease in firmness in tomatoes might be attributed to the fast weakening of their cell walls, since there is a weakening of middle

lamellae during ripening process that may explain the softening. It has been reported that maturation causes a slight softening in tomato (Batu, 2004), and that flesh softening of tomato fruit is usually the result of fruit ripening which is accompanied by a burst in ethylene production (Lai *et al.*, 2011). A research done by Kashmire and Kader (1978) as reported by Mutari and Debbie (2011) indicated that the softening of tomatoes is either the result of the cells losing water and becoming less turgid or the breakdown of the cell walls as a result of physical damage. According to Luna-Guevara *et al*. (2014), during the ripening and storage period, the loss of firmness in tomatoes is the actions of different enzymes like cellulase, pectinesterase and polygalacturonase (which is most implicated in tomato softening) on cell wall, media lamella and plasmatic membrane. The breakdown of these large polymers into smaller water-soluble components during ripening leads to fruit softening as observed during the breakdown of pectin in tomato (El-Ramady *et al*., 2015). Firmness has been associated with increased susceptibility to infection by fungal pathogens and consequent reduction in fruit quality (Nirupama *et al*., 2010).

Regarding the tomato fruit flavour, high tomato flavour means flavour associated to freshly harvested product. It has been noted that flavour of tomato results mainly from a combination of volatile compounds for aroma and of sugars and acids for taste (Barrett *et al.*, 2010; Aoun *et al*., 2013). Our results showed that REO and the combination of REO and chlorine with time of immersion impacted the level of Flavour score after 22 days of storage. Sodium hypochlorite concentrations higher than 2600 ppm can induce tomato surface damage by causing electrolyte leakages, changes in cell membrane fluidity and even chilling like injury (Hong and Gross, 1998). The highest concentration used in this work was 400 ppm. It may be hypothesised that the increase leakage could have contributed in liberating the flavour while high level of REO will tend to dominate it. Several studies report aroma composition by cultivars, stages of ripeness, different culture conditions, and treatments suggesting that these parameters influence the aroma composition of tomato (Messina *et al*., 2012).

Concerning the loss in weight of tomato fruits, generally it increases progressively during their storage and continues till the fruit attains fully ripened stage (Moneruzzaman *et al*., 2009; Nirupama *et al*., 2010). It is reported that weight loss of above 5% is a limiting factor for the postharvest life of many fresh fruits and vegetables (Dilmacunal *et al*., 2011; Niño-Medina *et al.*, 2013). At the time when the fresh product loses 5-10% of its fresh weight, it begins to wilt and becomes unusable (Pinheiro *et al*., 2013; Arthur *et al*., 2015). After 22 days of storage, conditions with weight loss between 5-10% were mainly those with time of

dipping ≥ 4.5 minutes and REO concentration ≥ 700 ppm. This indicate that dipping in chlorine and treatment with REO proved to be useful in reducing the decay rate. Fruits lose weight when their metabolic rate increases (Mutari and Debbie, 2011). Reduction in weight loss is probably due to the effects of the coating as a semi-permeable barrier against O_2, CO_2, moisture and solute movement, thereby reducing respiration, water loss and oxidation reaction rates (Abd-Alla *et al*., 2009; Dilmacunal *et al*., 2011; Gharezi *et al*., 2012; Tabaestani *et al*., 2013). The weight loss of fresh tomatoes is primarily due to transpiration and respiration which can lead to wilting and shrivelling (Žnidarčič and Požrl, 2006; Gharezi *et al*., 2012; Mujtaba and Masud, 2014). Arthur *et al*. (2015) have reported the findings of Ullah (2009) where dipping tomatoes for longer time in calcium chloride ($CaCl_2$) solution might retard respiration and transpiration rate which are the major cause of weight loss.

The shelf life is a period of time which starts from harvesting and extends up to the start of rotting of fruits (Mondal, 2000 as cited by Nasrin *et al*., 2008). Postharvest shelf life and quality of tomatoes are significantly affected by the cultivar, ripening stage and storage temperature (Sabir *et al*., 2012). Although some fruits had shown the presence of defects all along the period of the experiment, the shelf life was determined on the bases of percentage of fruit having non acceptable features. After harvest, ripening continues and tomatoes can become overripe very rapidly, resulting in restricted shelf life; this probably because, according to Nasrin *et al.* (2008), shelf life of tomato can be extended at ambient temperature up to 17 days without excessive deterioration in quality by treating the fruits with chlorine, and packaging in perforated polyethylene bags. Similar results were found by Nirupama *et al*. (2010) where tomato fruits were treated with gibberellic acid, calcium chloride and salicylic acid. It is also well known that, being a climacteric and a perishable vegetable, tomatoes have a very short life span, usually 2-3 weeks (Dilmacunal *et al*., 2011; Pinheiro *et al*., 2013) when harvested at mature green stage. The fruits used in this experiment were in a higher stage than the mature green stage, notwithstanding some combinations mainly characterized by a time of dipping = 3 minutes and REO ≥ 500 ppm allowed a 0% defect after 22 days of storage. Nikos *et al*. (2011) observed that tomato pre-treated with oregano essential oil before ambient air storage prevented fruit decay and induced higher glucose, fructose, lycopene and ascorbic acid content compared to the control experiment. It has been reported that in tropical regions, the main factor associated with tomato shelf life is increased respiration rate which results in faster fruit ripening and deterioration of fruit quality (Sinha *et al*., 2014). Thus, Atanda *et al*. (2011) suggested the recommendations made by Shewfelt (1986), to maintain a commodity at its optimal temperature, relative humidity and environmental conditions, since the shelf life of

commodity is dependent on its initial quality, its storage stability, the external conditions and the handling methods. The most performing conditions could be a good balance between microbial inactivation and tomato structure degradation by chlorine during dipping.

II.2.4. Processing of fresh fruits into juice and paste

Processing tomato fruits into juice and paste, using REO as a preservative and challenging these products with *B. cereus* spores allowed to assess shelf life. It is indicated that inactivation of bacterial spores requires high temperature and long heating time. According to Jantová and Lukáová (2001), the destruction of spores is effective at 135°C; however, temperatures of 115°C at 60 minutes and 120°C at 20 minutes are sufficient for the destruction of *B. cereus* spores. According to Houška *et al*. (2007), it is well known that the spores of *B. cereus* are heat resistant and that boiling in aqueous environment kills them only after a longer time than is the time of boiling necessary for reaching the culinary acceptable state. A research done by Brooks (2013) on packaged fruit juices has indicated that the viable spore subpopulations observed at different temperatures might be due to high moisture content of fruit juices that might have offered some protection against thermal inactivation of bacterial endospores. Thus, he suggested that high temperatures (> 95°C) may be required to inactivate bacterial spores in fruit juices within 30 minutes. Despite the fact that inactivation of bacterial spores requires high temperature and long heating time, food containing > 10^4 *B. cereus*/g is not safe for the consumption, as the real infectious dose vary from about 10^5–10^8 viable cell or spores/g (Valik *et al*., 2003). Our results indicated that all treatments in both tomato juice and paste inoculated with 4 log CFU/g have registered variable level of *B. cereus* reduction, except where there was no heating for deactivation. Mahendra *et al.* (2014) have suggested that the number of spores in processed foods must be kept as low as possible. This reduction is a combined effect of heat and REO. It is reported that, besides the likely safety of REO when used topically, the risk of development of bacterial resistance to it is low because of the multicomponent nature of essential oils (Oliveira Lima *et al*., 2013). However, the small amounts of minor components might also contribute the antimicrobial activity of the essential oils. According to Selim (2011), volatile oils would reduce bacterial contamination without affecting organoleptic properties, even added in small quantity to foodstuffs. It has been reported that rosemary extracts at a level of 0.06-1 % inhibit the growth of Gram-positive pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus* (Hać-Szymańczuk *et al*., 2011). According to the equations obtained by regression and expressing the spore percentage reduction, it can be observed that no direct effect of the

independent variables was observed. In both juice and paste, the percentage reduction of spores depended on the combination of REO with spore concentration and heating time with spore concentration. It is important to note that these equations only explained a maximum of 35% of the variability of the data, indicating that other important factors explaining this reduction are still to be identified. As hypothesis, the pH, various organic acids and ascorbic acid content may also contribute in the spore inactivation in combination to heat. In tomato, it is determined by its organic acid content with citric acid being the most abundant (Kumar, 2016).

pH is very important because the acidity influences the thermal processing conditions required for producing safe products (Garcia and Barrett, 2006; Siddiqui and Singh, 2015). Microorganisms, including yeasts, moulds and bacteria are sensitive to a food's pH. Very low or high pH values will prevent microbial growth, and low pH reduces spore resistance (Leguérinel and Mafart, 2001). Thakur *et al*. (1996) reported that when processing tomatoes, the pH should be lower than 4.4 to avoid potential spoilage with thermophilic organisms. The pH of our juice and paste were 4.63 and 4.7, respectively. This little discrepancy with the recommended levels was compensated with the use of essential oil during thermal treatments. Tomatoes are high acid foods, thus, require less thermal treatments than foods classified as low-acid foods (pH > 4.6). Peng *et al*. (2012) also have reported that the equilibrium pH of tomatoes varies from 4.0 to 4.7, depending on the fruit variety and ripeness. Our results indicated that these pH values are not suitable for preservation because they are higher than the pH 4.5 that is the limit to prevent easy growth of most pathogens and toxin production. When preserving at industrial level, it is best to treat these products as low-acid foods or add an acidifying agent to lower the pH well below the critical value of 4.6. In an industrial process, the use of citric acid will help convert this. Being in a context of formulation for local community application, a choice was made to maintain the pH as it is, and to use other natural barriers for microbial growth.

Our results indicated an increase in the total polyphenols content of tomato paste compared to tomato juice, this independently of the treatments (*B. cereus* spores, REO, cooking time). These results are in accordance with the findings of Odriozola-Serrano *et al*. (2009) reported by Peng *et al*. (2012) where there was a decrease in the phenolic concentration of pasteurized tomato juice. There is a number of studies where the increase in total phenolics content upon heat processing treatment might have produced changes in the extractability of phenolics due to the disruption of the plant cell wall (Peleg *et al*., 1991 as cited by Capanoglu *et al*., 2010).

Thermal processing has been shown to release more bound phenolics due to the breakdown of cellular constituents (Dewanto *et al*., 2002; Capanoglu *et al*., 2010; Skrutvold, 2014). According to Rivero *et al*. (2001), thermal stress induces the accumulation of phenolics in the plant by activating their biosynthesis as well as inhibiting their oxidation. This can explain better the increase in total polyphenols content observed in tomato paste after being subjected to processing techniques, it also underwent high cooking time for pasteurization.

As for the titratable acidity, it has been reported that titratable acidity and pH are two important quality attributes of processing tomatoes, though acid concentration and pH are not always inversely related. According to Siddiqui and Singh (2015), increase in titratable acidity of tomato products may be due to acids produced by *Bacillus coagulans*, *Clostridium butyricum* and because of phenolic compounds produced by *Bacillus coagulans*. Some findings have been reported by Chattopadhyay *et al*. (2013) that minimum acidity requirement for processing tomato should be 0.40 % as the processed product from low acid tomato may be affected by *Bacillus coagulans*. It is reported that processing conditions and temperature of processing affect the acid composition of tomato products. It was found that hot-processed juice had lower titratable acidity than cold processed juice, differences that could be attributed to the activity of the pectolytic enzymes in the cold-break juice which produce acidic breakdown products from pectin; and high acidity was recorded in tomato products processed at 64.4°C and 77.2°C compared to those processed at 100°C and 104.4°C. The average acidity of processing tomatoes is about 0.35 % expressed as citric acid (Thakur *et al*., 1996).

Concerning the vitamin C content in tomato products, our results are ranged in the same findings of many researchers who have found that the vitamin C content in tomato varies between 7 and 30 mg/100 g of fresh matter (depending on the variety and growing conditions); and through processing methods, it degrades systematically because of its high sensitivity to heat and light (Capanoglu *et al*., 2010; Boumendjel *et al*., 2012; Boubidi and Boutebba, 2013; Siddiqui and Singh, 2015). Literature has demonstrated significant losses in ascorbic acid during tomato processing. Georgé *et al*. (2011) reported that loss of vitamin C in different tomato products increase with heating time and number of processing steps. Our results are consistent with data from the literature, where numerous examples can be found of vitamin C degradation during thermal processing of tomato products. In other studies, around half of the vitamin C was lost during thermal processing of tomatoes; and up to 90 % degradation of vitamin C after pasteurization of tomato purée has been observed. Again, during processing of tomato paste, there was 54.6 % of loss of ascorbic acid where 38 % of

the original ascorbic acid (AsA) content of tomato was lost during hot-break extraction. On thermal processing, the ascorbic acid and total vitamin C contents of tomato juice decreased to 38 % and 42 % (Garcia-Alonso *et al*., 2009; Adubofuor *et al*., 2010; Capanoglu *et al*., 2010; Siddiqui and Singh, 2015).

Tomato juice typically has a commercial shelf life of 12 months (Garcia-Alonso *et al.*, 2009), and concentrated tomato paste is typically stored for 1 year or more (Anthon and Barrett, 2010). Microbiological safety and stability should always be a priority for determining the product's shelf life which is influenced by a number of factors, including raw material quality, product formulation (pH, water activity), hygiene during manufacturing, scheduled heat or other preservation treatments, cooling methods applied to products, type of package, storage temperature and relevant hurdles. It has been reported that active components of spices at low concentrations may interact synergistically with other factors to increase preservative effect. The findings of Eissa *et al*. (2008) have demonstrated that the use of volatile oil extracts (lemon grass, clove and rosemary) has been an effective method of quality improvement and shelf life extension in apple juice, stored at 4 °C.

II.2.5. Implementation of tomato fruits preservation technique in Bushi

Preserving fresh tomato fruits in the Bushi region using REO is an alternative way in reducing losses due to spoilage since the main constraints to processing tomato fruits are the lack of processing facilities and electricity. The simple preservation technique developed for fresh tomato fruits was implemented in the Bushi region with different harvesting (maturity) stages. According to Moneruzzaman *et al*. (2009), suitable harvesting stage of fruit (maturity) and optimum ripening conditions to have the best quality and longer storage of tomato has not completely been recognized for developing countries. Mature tomatoes pass through several stages of ripeness, which are characterized by changes in colour and firmness. Zoltan and Lajos (2010) have considered that the ripening process of tomatoes is well characterized by the colour evolution of fruit surface. It has been reported that ripening process progresses more rapidly within the tomato fruit than its external manifestation (Adegoroye *et al*., 1988). In the case of this study, tomatoes were harvested at different maturity stages and kept until they were not marketable. Batu (2004) estimated that tomatoes which reached the red colour stage might have had a long overall storage time or might have stayed on the vine too long. He found that the colour was not completely pink at the pink stage of maturity whereas tomatoes were predominantly green in colour at green and breaker stages and predominantly red in colour at the light red and red stages. In tomato, as well as other climacteric fruits, the

plant hormone ethylene is required for normal fruit ripening and thus considered a trigger of a wide range of physical, physiological and biochemical changes (Misbaudeen *et al*., 2012).

As for the soluble solid content, observations were made that soluble solids content in tomato varied significantly in fruits of different maturity. The highest quantity of total sugar was recorded in full ripened tomatoes while the lowest quantity was in tomatoes harvested at breaker stage (up to 16 days) and pink stage (from 19 to 25 days of storage); similar results with lower solid contents in fruits harvested at mature green stage were observed by Moneruzzaman *et al*. (2009). Harvesting later promotes higher sugar accumulation in riper fruits (Arah *et al*., 2015). As a general rule, fruit soluble solids content values increase with ripening and slightly decrease with senescence (Antunes *et al*., 2013), causing carbohydrate respiratory losses. Ripening contributes to the breakdown of pectin substances into more simple sugars thereby increasing the total soluble sugar (Munhuweyi, 2012). In general, soluble solids commonly range from 4-6 °Brix in the different tomato fruits (Klunklin and Savage, 2017). The higher sugar content observed in fruits harvested at later stage of ripening, increasing with the growth of the fruit until reaching its maximum with the development of colouration and diminishing with the advance of maturation, indicate that tomatoes allowed to self-ripe on the vine at field condition (Misbaudeen *et al*., 2012).

Our results indicated variations in the content of titratable acidity up to 19 days of storage, after what it decreased drastically. Kader *et al*. (1977) have reported no consistent changes after the increasing in titratable acidity from the green to the green-yellow stage. Several investigators have reported that acidity in tomatoes increases during development and reaches the maximum at the breaker stage, then decrease with further ripening. According to Arah *et al*. (2015), the acidity of tomatoes is highest at the pink stage of maturity with a rapid decrease as the fruit ripens. There is an inverse relationship between the total soluble solids and titratable acidity: where the value of sugar content increases, that for the acidity decreases (Sibomana *et al*., 2015). During storage, the titratable acidity was reduced slightly, irrespective of different treatments. Nirupama *et al*. (2010) have reported that the change in total titratable acids during storage is mainly due to the metabolic activities of living tissues during which depletion of organic acids takes place.

As for the Vitamin C content, the increase in ascorbic acid content is thought to be indication that the fruit is still in ripening process (Nirupama *et al.* 2010). However, the ascorbic acid begins to degrade immediately after harvest; and as reported Rickman *et al*. (2007), ascorbic acid degrades steadily during prolonged storage. According to Mekonnen (2017), the vitamin

C content of tomatoes may vary between 39-263 mg/100 g, depending on variety and growing conditions.

Finally, several investigators have studied the shelf life of tomato fruits. As climacteric fruit, tomatoes are prone to rapid quality losses after harvest. These losses depend significantly on the ripening stage at harvest (Sibomana *et al*., 2016). According to Batu (2004), ripening continues after harvest, and tomatoes can become overripe very rapidly, which can result in loss of quality and restricted shelf life. Žnidarčič and Požrl (2006) have reported that tomato can be stored at ambient temperature for a period of up to 7 days. For Nasrin *et al*. (2008), shelf life of tomato can be extended at ambient temperature up to 17 days without excessive deterioration in quality by treating the fruits with chlorine, and packaging in perforated polyethylene bags. As for us, the shelf life expressed by the percentage of shrivelled fruits was lower in the presence of REO than in its absence, notwithstanding the absence of NaOCl in the dipping solution due to non-acceptability for population in this region. The shelf life of tomatoes is relatively short due to different postharvest physiological, physical and chemical changes that occurs during storage (Lai *et al*., 2011; Mekonnen, 2017). Moneruzzaman *et al.* (2009) reported that the shelf life of all tomato cultivars under investigation is longest when harvested at green mature stage. However, our findings have indicated that tomato fruits can be kept in storage up to 22 days when harvested at Light red and Full red stages, probably due to the exposure to REO before storage.

CONCLUSION AND PERSPECTIVES

CONCLUSION

The goal of this study was to propose simple postharvest technology using REO to reduce tomato postharvest decay in the Bushi region. Results from this research indicate that:

- In the Bushi region, Kabare territory is marginally suitable for tomato cultivation compared to Walungu territory where tomato can be cultivated in moderately and highly suitable areas. Soil characteristics (pH, texture, organic carbon, available P) and climate data vary from one territory to another, and this with predictable agricultural consequences for tomato production. This study revealed a number of cultivated varieties, different in fruit morphology and other important quality characteristics (fruit weight, shape, dry matter, locule number, sugar content, shelf life). The most cultivated variety in both territories was found to be *Sorwatome*, appreciated for its characteristics (shape, sugar content, and prolonged shelf life).

- Independently of the treatments, tomato colour change was totally effective at 10 days of storage. The presence of defects observed on the fruits (after 10 days of storage) was related to the shorter time of immersion in chlorinated water for fruits surface disinfection. The optimal condition that reduces the percentage defects in fruits is that combining 3 minutes of immersion time in chlorinated water at 200 ppm and high levels of REO ($\geq$ 500 ppm). Reduction in fruit weight loss was effective in treatments with REO application. It was established from this study that chlorine and REO concentrations had a positive influence on the preservation of tomato fresh flavour. The percentage reduction in *Bacillus cereus* was effective where there were treatment interactions between appertisation and REO applications. However, the longer the heating time, the higher the percentage reduction (100%) both in tomato juice and paste products. There was increase in total polyphenols content of tomato paste compared to tomato juice. The loss of vitamin C content observed in different tomato products increased with heating time and number of processing steps. For storage efficacy, the contamination with a minimum concentration of *Bacillus cereus* spores of 10^4 spores/g was stabilized with treatment at 95°C for 5 minutes in tomato juice and 20 minutes in tomato paste, resulting in a 1 year shelf life.

- The application of REO alone allowed to extend the shelf life of fresh fruits for preservation in the Bushi region. There were nutrient variations (soluble sugar content and titratable acidity) among fruits harvested at different maturity stages. The developed

technology was found to be suitable after observing that the wilting ratio was lower in the presence of REO than in the absence, notwithstanding the absence of NaOCl.

Recommendations

Based on the results obtained for the land suitability assessment for healthy tomato production in the Bushi region and characterization of tomato species, we recommend that researchers and research centres should share information on results of their investigations to raise awareness on potential soil productivity.

Regarding the fresh fruit preservation techniques using REO and chlorination, and processing techniques of tomato fruits, we recommend that a program for technology transfer should be launched in order to help the farmers in the use of proposed preservative methods.

Perspectives

Despite numerous studies on tomato crop, the problem of postharvest losses is challenging. There is still need in handling the postharvest biology because the magnitude of losses differs with respect to the field of study. Maintaining tomato's year-round availability on the market by controlling the postharvest biology is proved to be taken care of. Thus, in the near future we intend to:

1. Implement juice and paste production in the Bushi region
2. Test some other essential oils from different plant species from the 2 territories
3. Test spores other than *Bacillus cereus*
4. Evaluate the nutritional quality after a long period of storage
5. Evaluate the influence of maturity stages on the results of the processes

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APPENDICES

APPENDICES

Appendix 1: Mean separation of NaOCl and immersion time for the % in weight loss

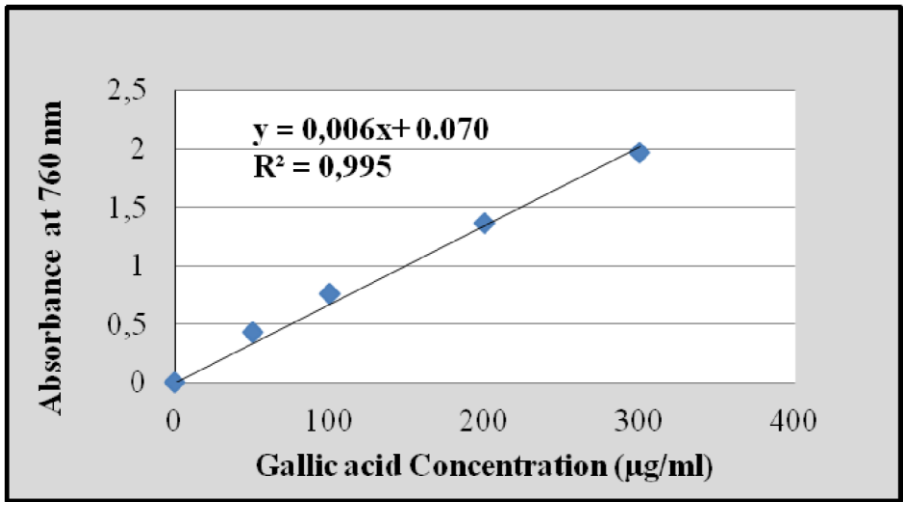
Factors	Mean	Standard error	Groups				
NaOCl400*min0	26.977	1.616	a				
NaOCl400*min6	17.582	1.616		b			
NaOCl100*min1.5	16.997	1.039		b			
NaOCl200*min0	16.471	1.294		b			
NaOCl300*min1.5	13.230	1.039		b	c		
NaOCl200*min6	12.538	1.294		b	c	d	
NaOCl400*min3	12.502	1.294		b	c	d	
NaOCl0*min3	11.905	1.294		b	c	d	
NaOCl200*min3	11.536	0.604			c	d	
NaOCl0*min0	8.441	1.616			c	d	e
NaOCl100*min4.5	8.436	1.039				d	e
NaOCl300*min4.5	7.174	1.039					e

Appendix 2: Mean separation of NaOCl and REO for the % in weight loss

Factors	Mean	Standard error	Groups			
NaOCl200*REO0	20.986	1.452	a			
NaOCl400*REO500	19.414	1.378	a			
NaOCl00*REO0	16.925	1.428	a	b		
NaOCl200*REO500	13.908	0.820		b	c	
NaOCl100*REO700	12.658	1.142		b	c	
NaOCl400*REO1000	12.524	0.881		b	c	
NaOCl100*REO200	11.407	1.142		b	c	d
NaOCl300*REO200	10.032	1.142		b	c	d
NaOCl0*REO500	9.847	1.523			c	d
NaOCl300*REO700	9.005	1.142			c	d
NaOCl200*REO1000	7.018	1.317				d

Appendix 3: Mean separation of immersion time and REO for the % in weight loss

Factors	Mean	Standard error	Groups			
min1.5*REO700	23.143	1.283	a			
min0*REO0	20.374	1.246	a	b		
min1.5*REO200	18.898	1.283	a	b	c	
min4.5*REO200	15.721	1.283	a	b	c	d
min3*REO0	15.059	1.584	a	b	c	d
min0*REO500	13.296	0.996	a	b	c	d
min4.5*REO700	11.701	0.897		b	c	d
min6*REO500	11.701	0.897		b	c	d
min3*REO500	7.981	0.837			c	d
min0*REO1000	6.406	1.517			c	d
min6*REO1000	4.811	1.086				d
min3*REO1000	1.091	1.805				d



Appendix 4: Standard calibration curve of Gallic acid for total polyphenols determination

Appendix 5: Significant differences in levels of total Polyphenols (TP) in juice treatments

Juice Treatments	TP- Mean	1	2	3
J9	40.000	****		
J17	43.333	****		
J16	52.223	****	****	
J15	59.443	****	****	****
J20	63.887	****	****	****
J14	65.557	****	****	****
J13	71.667	****	****	****
J4	73.887	****	****	****
J19	84.443	****	****	****
J5	85.667	****	****	****
J18	89.447	****	****	****
J1	92.223	****	****	****
J11	92.777	****	****	****
J12	95.000	****	****	****
J8	100.830	****	****	****
J10	117.220	****	****	****
J6	117.780	****	****	****
J7	128.333	****	****	****
J3	128.890	****	****	****
J2	167.223		****	****

($p<0.05$)

Appendix 6: Significant differences in Titratable acidity content (TA) of juice treatments

Juice Treatments	TA- Mean	1	2
J11	16.32	****	
J17	20.480	****	
J3	21.120	****	****
J1	21.120	****	****
J9	21.760	****	****
J16	21.760	****	****
J4	22.400	****	****
J8	23.040	****	****
J6	23.040	****	****
J5	23.040	****	****
J7	23.680	****	****
J15	23.680	****	****
J20	23.680	****	****
J14	23.680	****	****
J13	24.320	****	****
J12	24.320	****	****
J2	24.960	****	****
J19	25.600	****	****
J10	25.600	****	****
J18	29.440		****

($p<0.05$)

Appendix 7: Significant differences in Vitamin C content of juice treatments

Juice Treatments	Vit. C- Mean	1	2	3	4
J20	14.400	****			
J8	15.433	****	****		
J9	15.500	****	****		
J10	16.033	****	****		
J19	16.033	****	****		
J15	16.600	****	****	****	
J13	16.767	****	****	****	
J2	16.833	****	****	****	****
J16	16.833	****	****	****	****
J11	16.867	****	****	****	****
J18	17.200	****	****	****	****
J14	17.367	****	****	****	****
J12	18.000	****	****	****	****
J5	18.100		****	****	****
J6	18.133		****	****	****
J3	18.833		****	****	****
J7	18.933		****	****	****
J17	19.067		****	****	****
J4	20.000			****	****
J1	20.433				****

($p<0.05$)

Appendix 8: Significant differences in total polyphenols content of tomato paste treatments

Paste Treatments	TP- Mean	1	2
P5	85.557	****	
P7	102.223	****	
P13	122.777	****	
P3	126.670	****	
P6	126.670	****	
P10	127.777	****	
P11	134.447	****	
P12	135.000	****	
P20	135.557	****	
P15	137.223	****	
P16	141.667	****	****
P8	141.890	****	****
P18	142.223	****	****
P2	142.780	****	****
P19	142.780	****	****
P17	150.553	****	****
P14	157.777	****	****
P9	173.890	****	****
P4	208.223	****	****
P1	220.000		****

($p<0.05$)

Appendix 9: Significant differences in titratable acidity content of paste treatments

Paste Treatments	TA- Mean	1	2	3
P12	28.800	****		
P8	29.440	****	****	
P7	30.080	****	****	****
P16	30.720	****	****	****
P15	30.720	****	****	****
P6	30.720	****	****	****
P17	30.720	****	****	****
P9	31.360	****	****	****
P13	31.360	****	****	****
P2	31.360	****	****	****
P4	31.360	****	****	****
P3	32.000	****	****	****
P14	32.000	****	****	****
P5	32.640	****	****	****
P10	32.640	****	****	****
P11	33.280	****	****	****
P18	33.280	****	****	****
P19	33.920	****	****	****
P20	35.200		****	****
P1	35.840			****

($p<0.05$)

Appendix 10: Significant differences in Vitamin C content of paste treatments

Paste Treatments	Vit. C- Mean	1	2	3
P20	0.467	****		
P1	0.667	****	****	
P8	0.700	****	****	
P7	0.733	****	****	
P9	0.900	****	****	
P5	0.933	****	****	
P17	0.967	****	****	
P4	1.133	****	****	
P2	1.167	****	****	
P6	1.200	****	****	
P14	1.267	****	****	
P18	1.300	****	****	
P11	1.467	****	****	
P10	1.500	****	****	
P3	1.533	****	****	
P13	1.567	****	****	
P15	1.733	****	****	****
P19	1.867		****	****
P16	1.933		****	****
P12	2.933			****

($p<0.05$)

Appendix 11: State of defective bottles of tomato juice recorded for 1 year of storage

	19th May 2015 Day 0 of storage	**Evolution of Defective bottles**					**Storage shelf life**
Juice Treatments	**Number of bottles**	**22nd July-15**	**26st Aug-15**	**22nd Sept-15**	**23rd Oct-15**	**22nd June-16**	**months**
J1	4	0	0	0	0	0	12
J2	4	0	0	0	0	0	12
J3	4	0	0	0	0	0	12
J4	4	0	0	0	1	1	9.6
J5	4	0	0	0	0	0	12
J6	4	0	0	0	0	0	12
J7	4	0	0	0	1	1	9.6
J8	4	0	0	1	1	1	11.2
J9	4	1	1	1	1	1	9
J10	4	1	1	1	1	1	9
J11	4	1	1	1	2	2	6.5
J12	4	1	1	1	1	1	9
J13	4	1	2	2	2	2	6
J14	4	0	1	1	2	2	6.5
J15	4	1	1	1	1	1	9.2
J16	4	0	0	1	1	1	9.6
J17	4	0	0	0	1	1	9.6
J18	4	0	0	0	1	1	9
J19	4	0	0	0	1	1	9.6
J20	4	1	1	1	2	2	6.5

Appendix 12: State of defective bottles of tomato paste recorded for 1 year of storage

	19th May 2015	**Evolution of Defective bottles**					**Storage shelf life**
Paste Treatments	**Number of bottles**	**22nd July-15**	**26st Aug-15**	**22nd Sept-15**	**23rd Oct-15**	**22nd June-16**	**months**
P1	4	0	0	0	0	1	10.1
P2	4	0	0	0	1	1	9.5
P3	4	0	2	2	2	2	6
P4	4	1	1	1	2	2	6.5
P5	4	0	0	0	0	0	12
P6	4	0	0	0	0	0	12
P7	4	1	1	1	1	2	7.1
P8	4	0	0	0	2	2	7.1
P9	4	0	1	1	2	2	6.5
P10	4	0	0	0	0	0	12
P11	4	1	1	1	1	1	9
P12	4	0	0	0	1	1	9.5
P13	4	1	2	2	2	4	2
P14	4	0	1	1	2	2	6.5
P15	4	0	0	0	0	0	12
P16	4	0	0	0	0	0	12
P17	4	0	0	0	0	1	10.1
P18	4	1	2	4	4	4	0.4
P19	4	0	0	2	3	3	7.9
P20	4	0	0	0	0	1	10.1

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FICHE TECHNIQUE

Nom du produit : ROMARIN HE AT 032
ORIGINE : TUNISIE

NUMEROTATION
N° CAS :8000-25-7 CAS US : 84604-14-8 N° EINECS :283-291-9 N° FEMA : 2992 N° FDA :182.20 N°CoE : 406

DESCRIPTION

L'huile essentielle de Romarin est obtenue par distillation à la vapeur d'eau des rameaux et des sommités fleuries du Rosmarinus Officinalis Linnaeus de la famille des Labies

Note olfactive : fraîche camphrée
Couleur et apparence : jaune pâle, liquide

PROPRIETES TECHNIQUES

Densité relative à 20 °C, d_{20}^{20} :	0,907 - 0,920	(Densimètre électronique DMS4100M Anton Paar)
Indice de réfraction à 20°C :	1,464 - 1,470	(Réfractomètre électronique PTR2 INDEX INSTRUMENTS Ltd)
Angle de rotation optique :	-2 / +5	(Polarimètre électronique AA-10R)
Point éclair (donnée standard) :	45 °C	(Coupelle fermée – ASTM D6450)
Solubilité :	soluble dans l'ethanol	

Caractéristiques chromatographiques (CPG) :

alpha pinene	: 9 - 14%
Beta pinene	: 4 - 9 %
Limonene	: 1,5 - 4%
Cineol	: 38 - 55%
Camphre	: 5 - 15%
Acetate bornyl	: 0,1 - 1,5%
Methyl eugenol	: < 0,5%

CONSERVATION ET STOCKAGE

En emballage d'origine, hermétiquement fermé, à température ambiante, à l'abri de la lumière et de l'humidité.
La durée de conservation n'est pas valable en dehors des conditions indiquées.
Si le stockage dépasse 18 mois, la qualité doit être réévaluée avant utilisation

MANIPULATION

Selon les pratiques IFRA CODE

RECOMMANDATIONS POUR L'UTILISATION

Usages divers

MISE EN GARDE

Produit concentré pour usage industriel. Eviter le contact avec les yeux et les muqueuses.
Ne pas boire.

Date : 03/04/2018

Michel RIQUIER, Responsable Technique **Karima MKIOUDANE, Assistante Qualité**

LIST OF PUBLICATIONS

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1. Sibomana Imani Caroline, Sado Kamdem Sylvain Leroy, Mashugalusa Nachigera Gustave and Ngang Jean-Justin Essia (2018). **Influence of chlorine and rosemary essential oil postharvest pre-treatments on quality parameters of fresh tomatoes during storage.** Journal of Postharvest Technology, 06 (2): 57-68.

2. C. Imani Sibomana, S. L. Sado Kamdem, G. Mushagalusa Nachigera and J. J. Essia Ngang (2019). **Nutritional quality and shelf life of processed tomato juice and paste using *Rosmarinus officinalis* essential oil combined with low heat treatment in challenged conditions with *Bacillus cereus* spores**. Journal of Advances in Microbiology (JAMB), 19 (3): 1-13.

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Journal of Postharvest Technology
2018, **06**(2): 57-68
http://www.jpht.info

RESEARCH ARTICLE

Influence of chlorine and rosemary essential oil postharvest pre-treatments on quality parameters of fresh tomatoes during storage

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ABSTRACT

Tomato is perishable and its high moisture content makes it susceptible for various postharvest losses. Tomato quality changes continuously after harvesting, when fruits start lose quality because of environmental stress and pathogen infection. The study determined the effects of chlorine and rosemary essential oil (REO) on the quality parameters and shelf life of fresh tomatoes during storage at ambient temperature. Tomatoes of uniform shape and colour and free from pathogens were selected. Treatments were applied in a central composite design where chlorine (100, 200, 300 and 400 ppm of NaClO as hypochlorite of sodium), immersion time (1.5, 3, 4.5 and 6 minutes) and REO (200, 500, 700 and 1000 ppm) were factors considered. Data were analysed through XLSTAT and STATISTICA. Results indicate that significant decrease in weight loss was observed where there was increase in REO concentrations. All tomatoes converted towards full red independently of the factors from D10 of storage. Presence of defects was observed with different intensity in treatments. Most fruits softened with the increase in fruit colour and storage time. Chlorine and REO concentrations had an influence on the tomato flavour. There was extended shelf life of fruits since they were harvest at later stage for storage (light red).

Keywords: Chlorine, Fruit quality, Rosemary essential oil, Shelf fife, Tomato

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INTRODUCTION

Tomato (*Solanum lycopersicum* Mill.) is one of the most consumed foods throughout the world (Méndez et al., 2011). It is a horticultural crop of great interest, having good nutritional value, medicinal properties and economic importance of being widely consumed either fresh or processed in products (juice, soup, paste, puree, ketchup, sauce and salsa); unfortunately, it is highly perishable and has limited shelf life at ambient conditions (Davoodi et al., 2007); which is about 48 hours under tropical conditions (Arah et al., 2016). Spoilage of tomatoes is a continuing problem, although significant improvements have been made in packaging and refrigeration. Softening and ripening during storage and distribution of tomatoes can be a major problem because it may increase their susceptibility to damage (Batu, 2004; Ndirangu et al., 2017). The use of 1-MCP at low concentrations has been shown to slow down many of the metabolic activities associated with the ripening process such as colour change, cell wall breakdown, and respiration rates making it a useful technique in extending storage life of fruits (Arah

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et al., 2016), but when used at high concentrations, it increased their decay (Cai et al., 2006). Fruits lose quality after harvest because of environmental stress and pathogen infection (Lai et al., 2011). Lipid oxidation and bacterial contamination are the main factors that determine food quality loss and shelf life reduction (Rozman and Jersek, 2009). The presence of spoilage organisms in fruits and vegetables is a result of contamination of their surfaces; therefore, there is need to reduce contamination by ensuring sanitization of fruits and vegetable surfaces (Potter and Hotchkiss, 1993 as cited by Magashi and Bukar, 2007). Different chemical washing agents have been studied to determine their efficacy in the inactivation of pathogenic bacteria on vegetables (Petri et al., 2011; Arah et al., 2016). It has been reported that fruits and vegetables are washed in chlorine or potassium permanganate before packaging in order to reduce micro flora, especially bacteria from the produce (Giraldo et al.,1977 as cited by Nasrin et al. 2008; Bartz et al., 2013). Chlorine is the most commonly used sanitizer due to its efficacy, cost-effectiveness ratio and simple use (Petri et al., 2011). Washing raw produce with water containing sodium hypochlorite (NaOCl) is the most commonly used method for removing pathogens from the surfaces of vegetables (Povratanak et al., 2015; El-Ramady et al., 2015; Arah et al., 2016). Chlorine water is achieved by adding 200 ppm sodium hypochlorite in clean water (Povratanak et al., 2015).

There has been increasing interest to replace synthetic preservatives with natural, effective and nontoxic compounds (Marija and Nevena, 2009; Oliveira et al., 2013). In the search for natural preservatives, much attention has been directed to herbs and spices (Govaris et al., 2010). Essential oils from are known to possess biological activity, notably antibacterial, antifungal, and antioxidant properties (Celikel and Kavas, 2008). A number of essential oils components that are considered to present no risk to the health of the consumer have been registered for use as flavourings in foodstuffs by the European Commission (Hamedo and Abdelmigid, 2009). Rosemary (*Rosmarinus officinalis* L.) is widely used as a culinary herb for its desirable flavour throughout the world. It is reported that rosemary has been widely accepted as one of the spices with the highest antioxidant activity and rosemary essential oil is also used as an antibacterial, antifungal and anticancer agent (Peng et al., 2005; Genena et al., 2007; Rozman and Jersek, 2009).

An increase in the storage life and improvement of tomato fruit quality is really desirable and the extending the shelf life of tomatoes is very important for domestic and export markets. Thus, the objective of this study was to determine the effects of a rosemary essential oil (REO) combined to chlorine, on the fresh-like aspect, shelf life and quality of tomato fruits.

MATERIALS AND METHODS

Raw materials Selection

Tomatoes (*Rio Grande* cv. at red light stage) were procured from a local producer and immediately transported to the Microbiology Laboratory (University of Yaoundé I, Cameroon). Rosemary essential oil was bought on the market. It was produced from flowers and stems of the plant and delivered by Reynauld et Fils (France). The lot essential oil was declared pure, without any mixture or dilution by the producer.

Treatments

Tomato fruits of uniform shape and colour and free from visible microbial infection were selected, and dipped in a hypochlorite solution (100, 200, 300 and 400 ppm of NaOCl as sodium hypochlorite) at different times (1.5, 3, 4.5 and 6 minutes). Fruits were then left to drip for on an absorbent paper and exposed to different concentrations of Rosemary essential oil (REO) released by spray (200, 500, 700 and 1000 ppm) according to a central composite design (CCD) (Table 1). The spray was

done in order to cover all the surface of the tomatoes. In all, 20 treatments were performed according to the experimental design and kept in boxes at ambient temperature, varying between 28-30°C.

TABLE 1. Central Composite Design (CCD)

Central Composite Design (CCD)				Corresponding values			
Treatments	***F_1 (ppm)**	**F_2 (min)**	**F_3 (ppm)**	**Treatments**	**NaOCl (ppm)**	**Time (min)**	**REO (ppm)**
1	-1	-1	-1	1	100	1.5	200
2	-1	1	1	2	100	4.5	700
3	1	-1	1	3	300	1.5	700
4	1	1	-1	4	300	4.5	200
5	0	0	0	5	200	3	500
6	-1	-1	1	6	100	1.5	700
7	-1	1	-1	7	100	4.5	200
8	1	-1	-1	8	300	1.5	200
9	1	1	1	9	300	4.5	700
10	0	0	0	10	200	3	500
11	-2	0	0	11	0	3	500
12	2	0	0	12	400	3	500
13	0	-2	0	13	200	0	500
14	0	2	0	14	200	6	500
15	0	0	-2	15	200	3	0
16	0	0	2	16	200	3	1000
17	0	0	0	17	200	3	500
18	2	-2	2	18	400	0	1000
19	2	2	2	19	400	6	1000
20	-2	-2	-2	20	0	0	0

Observations

During storage, observations were taken after 2 days interval for 22 days, except for the weight loss. Tomato fruit quality was determined by visual assessment, and the quality characters (appearance, colour, texture, and flavour) were assessed by 10 trained panellists, aged between 20 and 40 years on a 5 point hedonic scale. Storage indices for each parameter were analyzed using the following formula:

$$Is = \frac{[\sum(SCOREi\ X\ DAYi)]}{(\text{Maximum score X}\sum^{\square} DAYi)} \quad ,$$

Where by:

Is is the storage index
SCOREi is the evaluation of a certain criteria on a certain dayi
Maximum score is the highest evaluation possible for that criteria, and
∑DAYi is the sum of the numbers of days at each analysis

The colour development was assessed using a tomato colour chart as proposed by Abdullah et al. (2004). The effect of the treatments on the shelf life was determined with respect to the control, after 22 days of storage. Selected fruits were weighed before applying the treatments using electronic digital balance at the beginning and the end of storage experiment. The loss in weight was determined by the following formula and expressed as percentage (Gharezi et al., 2012):

$$\text{Weight loss (\%)} = \frac{\text{Initial fruit weight} - \text{Final fruit weight}}{\text{Initial fruit weight}} \times 100$$

Statistical Analyses

A central composite design was used with 20 treatments. Statistical analyses were performed using XLSTAT and STATISTICA packages. Results and Regression analysis were statistically evaluated by variance analysis (ANOVA) and statistical differences between the mean of the groups was determined by Tukey test ($\alpha \leq 0.05$).

RESULTS AND DISCUSSION

Evaluation of fruit quality parameters

Table 2 shows the storage indices with regards to fruit colour development, presence of defects, fruit firmness and fruit flavour.

Colour development

All tomatoes converted towards full red independently of the factors (Table 2). Initially, all fruits were light red in colour; the full red colour was quite effective from day 10 of storage. Colour in tomato is the most important external characteristic to assess ripeness and postharvest life. Red colour is the result of chlorophyll degradation as well as synthesis of lycopene and other carotenoids (Radzevicius et al., 2008; Tigist et al., 2015; Dari et al., 2018). The increment in colour index might be an indication of the development of deep red colour in tomato. After harvest, ripening continues and tomatoes can become overripe very rapidly (Batu, 2004). It has been reported that colour development depends on a number of factors including temperature (which influences colour uniformity of tomatoes), maturity stage and the storage duration (Grierson and Kader, 1986 and Tijskens and Evelo, 1994 as cited by Tigist et al. 2015). Ripening processes are associated with increasing lycopene content that varies considerably between cultivars, stages of maturity and growing condition (Shalluf, 2010).

Defects presence

Fruit lose quality after harvest because of environmental stress and pathogen infection (Lai et al., 2011). From the results (Table 2), statistical analyses show significant differences in terms of pathogen infection in all treatments at different days of storage. Results (Table 2) show that after 10 days of storage, some fruits started showing signs of pathogen infection, thus affecting the fruit quality. At the end of the storage period, most fruits from the treatments had defects, though at different levels: < 25% for treatments T2, T9, T10, T16 and T17; 25 - 50% for T4, T5, T7, T11, T12, T13, T15, T19 and T20 and > 50% for treatments T1, T3, T6, T8 and T18. Results show that the very high percentage of defects presence (> 50%) could probably be related to the reduced time of immersion (0-1.5 minutes) in chlorinated water for fruits surface disinfection. It is known from literature that not only higher concentration of chlorine can increase its effectiveness of killing microorganisms but also that its bactericidal activity increases with longer exposure time. Also, Hong and Gross (1998) state that sodium hypochlorite treatment has a pronounced effect on texture of tomato tissue after treatment; this in explanation to the lower percentage of defects presence (42%) observed in the control treatment (T20). The lowest percentage (< 25%) observed might have resulted from the combination of both treatments: immersion time (3- 4.5 minutes) and rosemary essential oil

concentrations (500-1000 ppm). The presence of defects on tomato fruits could probably be the consequences of microbial growth during storage period associated to others uncontrolled factors (internal or external). According to Bartz et al. (2013), certain pathogens, including bacteria and viruses, may survive on or in fresh tomato fruit. The movement of living bacteria or fungal structures into fruit tissues is known as internalization and leads to a situation that cannot be corrected. It has also been mentioned that active growth of a pathogen may also occur in food from improper storage as a result of passive transfer of pathogens to food (Celiktas et al., 2007).

Table 2. Storage indices of tomato fruit quality parameters

Treatments	NaOCl	Time	REO	Colour index (Ci)			Defects index (Di)			Firmness index (Fi)			Flavour index (Fl)		
	(ppm)	(min)	(ppm)	Ci 1*	Ci 10*	Ci 22*	Di 1	Di 10	Di 22	Fi 1	Fi 10	Fi 22	Fl 1	Fl 10	Fl 22
1	100	1.5	200	0.80	0.98	1.00	0.20	0.45	0.63	0.20	0.38	0.55	1.00	0.19	0.54
2	100	4.5	700	0.80	0.99	1.00	0.20	0.20	0.23	0.20	0.42	0.56	0.80	0.20	0.60
3	300	1.5	700	0.80	0.99	1.00	0.20	0.45	0.56	0.20	0.38	0.55	0.80	0.20	0.46
4	300	4.5	200	0.80	0.99	1.00	0.20	0.38	0.40	0.20	0.50	0.58	0.80	0.20	0.46
5	200	3	500	1.00	1.00	1.00	0.20	0.38	0.40	0.20	0.42	0.56	0.80	0.20	0.60
6	100	1.5	700	0.80	0.99	1.00	0.20	0.45	0,56	0.20	0.45	0.56	0.80	0.26	0.40
7	100	4.5	200	0.80	0.99	1.00	0.20	0.35	0.39	0.20	0.32	0.53	0.80	0.20	0.60
8	300	1.5	200	0.80	0.99	1.00	0.20	0.45	0.56	0.20	0.45	0.56	0.80	0.26	0.32
9	300	4.5	700	0.80	0.99	1.00	0.20	0.20	0.20	0.20	0.35	0.54	0.80	0.20	0.60
10	200	3	500	1.00	1.00	1.00	0.20	0.20	0.20	0.20	0.35	0.54	0.80	0.20	0.60
11	0	3	500	0.80	0.95	0.99	0.20	0.35	0.39	0.20	0.35	0.54	0.80	0.20	0.60
12	400	3	500	0.80	0.99	1.00	0.20	0.20	0.34	0.20	0.35	0.54	1.00	0.19	0.46
13	200	0	500	0.80	0.99	1.00	0.20	0.38	0.40	0.20	0.47	0.57	1.00	0.19	0.45
14	200	6	500	0.80	0.98	1.00	0.20	0.20	0.34	0.20	0.20	0.51	0.80	0.20	0.60
15	200	3	0	0.80	0.99	1.00	0.20	0.35	0.39	0.20	0.38	0,55	0.80	0.20	0.46
16	200	3	1000	0.80	0.99	1.00	0.20	0.20	0.20	0.20	0.45	0.56	0.80	0.20	0.60
17	200	3	500	0.80	0.99	1.00	0.20	0.20	0.20	0.20	0.47	0.57	0.80	0.20	0.60
18	400	0	1000	0.80	0.99	1.00	0.20	0.32	0.53	0.20	0.47	0.57	0.80	0.26	0.32
19	400	6	1000	0.80	0.99	1.00	0.20	0.20	0.35	0.20	0.35	0.54	1.00	0.19	0.60
20	0	0	0	0r.80	0.95	0.99	0.20	0.35	0.42	0.20	0.38	0.55	1.00	0.19	0.57

** the number followed by the indices refers to the day of storage*

Fruit Firmness

Generally, the fruit firmness decreases with increase in the storage period (Znidarcic and Pozrl, 2006; Tabaestani et al., 2013). Results (Table 2) indicate significant differences in treatments at 10 days of storage, where fruits from T14 were slightly firmer compared to other treatments with regards to day 1 of storage. Reaching 22 days of storage, all treatments were softened. It has been reported that maturation causes a slight softening in tomato (Batu, 2004), and that flesh softening of tomato fruit is usually the result of fruit ripening which is accompanied by a burst in ethylene production (Lai et al., 2011). According to Luna-Guevara et al. (2014), during the ripening and storage period, the loss of firmness in tomatoes is the actions of different enzymes like cellulose, pectinesterase and polygalacturonase (which is the principal responsible of softening in tomato) on cell wall, media lamella and plasmatic membrane. The breakdown of these large polymers into smaller water-soluble components during ripening leads to fruit softening as observed during the breakdown of pectin in tomato (El-Ramady et al., 2015).

Table 3. Percentage of spoiled fruits during storage

Treatments	NaOCl (ppm)	Time (min)	REO (ppm)	Day 10	Day 22
1	100	1.5	200	25	50
2	100	4.5	700	0	25
3	300	1.5	700	20	40
4	300	4.5	200	0	22
5	200	3	500	10	10
6	100	1.5	700	14.29	42.86
7	100	4.5	200	10	20
8	300	1.5	200	25	37.5
9	300	4.5	700	0	25
10	200	3	500	0	0
11	0	3	500	11.11	22.22
12	400	3	500	10	30
13	200	0	500	10	10
14	200	6	500	0	10
15	200	3	0	0	10
16	200	3	1000	0	0
17	200	3	500	0	0
18	400	0	1000	20	30
19	400	6	1000	0	10
20	0	0	0	10	30

Fruit Shelf life

The shelf life (expressed in days) is a period of time which starts from harvesting and extends up to the start of rotting of fruits (Mondal, 2000 as cited by Nasrin et al., 2008). Although some fruits had shown the presence of defects all along the period of the experiment, the shelf life was determined when the fruits started decaying. Our results (Table 3) indicate that at 10 days of storage, treatments T2, T4, T9, T10, T14,T15, T16, T17 and T19 had fruits that did not show any sign of decay; and all other treatments had 10-25% of decayed fruits. This observation could be the right effect of immersion time of fruits in chlorinated water together with high concentration of REO; most treatments that had spoiled fruits were either not disinfected in chlorinated water or dripped in chlorinated water in less than 3 minutes. At the last day of storage period, only treatments T10, T16 and T7 maintained the fresh aspects of fruit (0% of spoiled fruits), while treatments T5 and T13 had maintained fruits in the same aspect as from 10 day of storage (10% of spoiled fruits). At 22 days of storage, all treatments that had less than 3 minutes immersion in chlorinated water had realised more than 30 % spoiled fruits.

From the results above, the fruit shelf life was increased in most of the treatments regarding the harvesting stage (red light) since after harvest, ripening continues and tomatoes can become overripe very rapidly, resulting in restricted shelf life; this probably because according to Nasrin et al. (2008), shelf life of tomato can be extended at ambient temperature up to 17 days without excessive deterioration in quality by treating the fruits with chlorine, and packaging in perforated polyethylene bags. It

is also well known that, being a climacteric and a perishable vegetable, tomatoes have a very short life span, usually 2-3 weeks (Dilmacunal et al., 2011) when harvested at mature green stage.

Fruit flavour

Significant differences result among the treatments. A regression analysis for flavour prediction was obtained in order to explain the effect of different variables on the tomato flavour. According to this equation,

Flavour score = 0.436+0.007*[HE] +0.0024*[CL]*[t]-0.0031* [t]*[HE]-
Where, HE refers to REO; CL refers to NaOCl, and t to immersion time

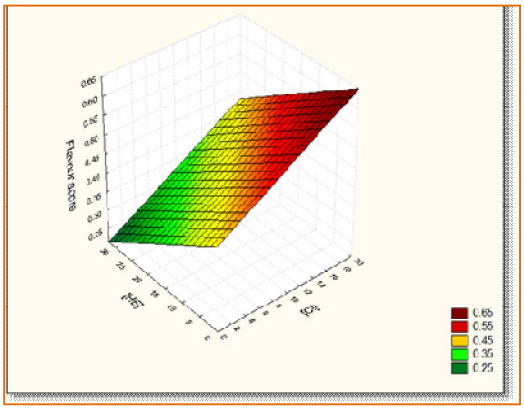

Figure 1: Influence of chlorine and EO at 4.5 min treatment on the flavour of fresh tomatoes

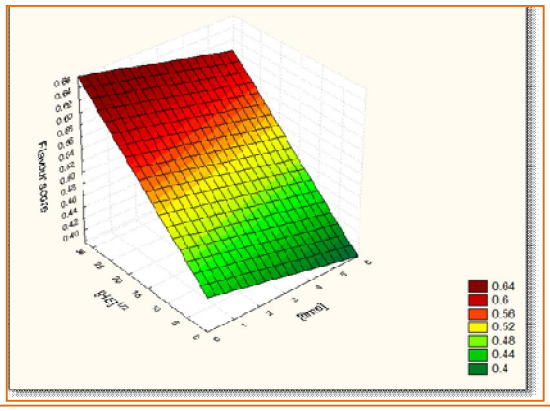

Figure 2: influence of time of treatment and EO when chlorine is used at 200ppm on the flavour of fresh tomatoes

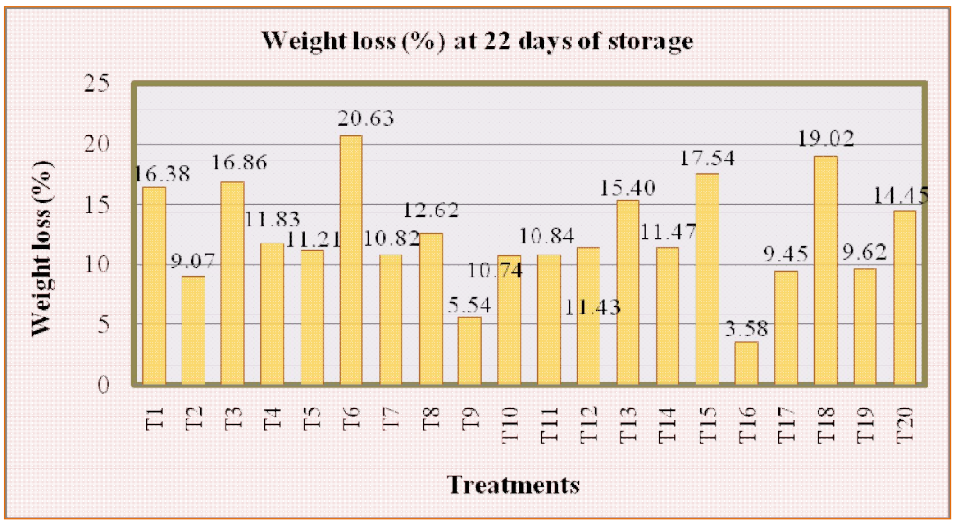

Figure 3. Percentage of fruit Weight loss after 22 days of storage

Results from the above figures indicate that the higher the concentrations of chlorine and REO, the higher the tomato flavour. It should be remembered here that high tomato flavour means flavour associated to freshly harvested product. It is noted that flavour of tomato results mainly from a combination of volatile compounds for aroma and of sugars and acids for taste (Barret et al., 2010; Aoun et al., 2013). Several studies report aroma composition by cultivars, stages of ripeness, different culture conditions, and treatments suggesting that these parameters influence the aroma composition of tomato (Messina et al., 2012). Alteration and ripeness during storage contributes to flavour decay.

Weight loss

Weight change is most related to storage duration; the weight loss of fresh tomatoes is primarily due to transpiration and respiration which can lead to wilting and shrivelling (Znidarcic and Pozrl, 2006; Gharezi et al., 2012). The lowest percentage in weight loss was observed in T16 (3.57%) whereas the highest was in T6 (20.63%) (Fig.3).

There was significant fruit weight loss among the treatments after 22 days of storage at ambient temperature among different treatments (Table 4). Significant differences observed in weight loss of fruits could be due to the interaction effects of treatments. Tables (5, 6 and 7) show differences among treatments in regards to interactions among factors. Results indicate that there was a significant decrease in percentage weight loss where there was an increase in the concentration of rosemary essential oil (REO). This reduction in weight loss is probably due to the effects of the coating as a semi-permeable barrier against O2, CO2, moisture and solute movement, thereby reducing respiration, water loss and oxidation reaction rates (Abd-Alla et al., 2009; Dilmacunal et al., 2011; Gharezi et al., 2012; Tabaestani et al., 2013).

Table 4. ANOVA for percentage in fruit weight loss

Source	Df	SS	MS	F	Pr > F
Treatments	16	346.497	21.656	15.428	0.022
Error	3	4.211	1.404		
Total	19	350.708			

(*α=0.05*)

Table 5. Mean separation of NaOCl and immersion time for the % in weight loss

Factors	Mean	Standard error	Groups				
NaOCl400*min0	26.977	1.616	A				
NaOCl400*min6	17.582	1.616		B			
NaOCl100*min1,5	16.997	1.039		B			
NaOCl200*min0	16.471	1.294		B			
NaOCl300*min1,5	13.230	1.039		B	C		
NaOCl200*min6	12.538	1.294		B	C	D	
NaOCl400*min3	12.502	1.294		B	C	D	
NaOCl0*min3	11.905	1.294		B	C	D	
NaOCl200*min3	11.536	0.604			C	D	
NaOCl0*min0	8.441	1.616			C	D	E
NaOCl100*min4,5	8.436	1.039				D	E
NaOCl300*min4,5	7.174	1.039					E

Table 6. Mean separation of NaOCl and REO for the % in weight loss

Factors	Mean	Standard error	Groups			
NaOCl200*REO0	20.986	1.452	A			
NaOCl400*REO500	19.414	1.378	A			
NaOCl00*REO0	16.925	1.428	A	B		
NaOCl200*REO500	13.908	0.820		B	C	
NaOCl100*REO700	12.658	1.142		B	C	
NaOCl400*REO1000	12.524	0.881		B	C	
NaOCl100*REO200	11.407	1.142		B	C	D
NaOCl300*REO200	10.032	1.142		B	C	D
NaOCl0*REO500	9.847	1.523			C	D
NaOCl300*REO700	9.005	1.142			C	D
NaOCl200*REO1000	7.018	1.317				D

Table 7: Mean separation of immersion time and REO for the % in weight loss

Factors	Mean	Standard error	Groups			
min1,5*REO700	23.143	1.283	A			
min0*REO0	20.374	1.246	A	B		
min1,5*REO200	18.898	1.283	A	B	C	
min4,5*REO200	15.721	1.283	A	B	C	D
min3*REO0	15.059	1.584	A	B	C	D
min0*REO500	13.296	0.996	A	B	C	D
min4,5*REO700	11.701	0.897		B	C	D
min6*REO500	11.701	0.897		B	C	D
min3*REO500	7.981	0.837			C	D
min0*REO1000	6.406	1.517			C	D
min6*REO1000	4.811	1.086				D
min3*REO1000	1.091	1.805				D

CONCLUSION

Physiological changes occurred during storage of fresh tomatoes. At 10 days of storage, all fruits converted to full red independently of the factors, though they were stored at the red light stage. Results have established that the presence of defects is related to the shorter time of immersion in chlorinated water for fruits surface disinfection, that immediately impact on the fruit shelf life. The longer the time of fruits immersion in chlorinated water coupled with high concentration of REO, the better the results. Reduction in fruit weight loss was effective in treatments with REO application. At the end of storage, most fruits softened in relation to the ripening stage and storage time. The study also established that chlorine and REO concentrations had a positive influence on the preservation tomato fresh flavour.

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Nutritional Quality and Shelf Life of Processed Tomato Juice and Paste Using *Rosmarinus officinalis* Essential Oil Combined with Low Heat Treatment in Challenged Conditions with *Bacillus cereus* Spores

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CIS, SLSK, GMN and JJEN designed the study. Author CIS wrote the protocol, managed the analyses of the study and literature searches with author SLSK. Author SLSK performed the statistical analysis with author CIS. Authors CIS, SLSK, GMN and JJEN wrote the first draft of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This experiment was done to investigate the effect of adding *Rosmarinus officinalis* essential oil (REO) in tomato juice and paste during processing in condition of reduced thermal treatment on nutritional qualities and evaluate their postharvest shelf life under *Bacillus cereus* spore contamination.
Place and Duration of Study: Laboratory of Microbiology, University of Yaoundé I, Cameroon, from January 2017 to June 2018.

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Methodology: Total polyphenol content was determined using the Folin Ciocalteu coloric method; the Titatrable acidity and vitamin C content were determined by titration with indicator method. The effect of level of Bacillus cereus contamination, time of thermal treatment and concentration of REO was assessed using a experimental CCD plan. The shelf life was estimated using the storage efficacy of the product on a period of time, and calculated based on the percentage of spoiled products. Data were analysed using XLSLTAT and STATISTICA packages for non-linear regression analyses.

Results: Only 10 min pasteurization at 95°C in the presence of 100ppm of ROE was necessary to totally deactivate 10^4 *Bacillus cereus* spores/g in tomato juice while for tomato paste the same concentration of ROE needed 20 min pasteurization at 95°C to totally deactivate up to 10^8 *Bacillus cereus* spores/g. Total polyphenols, Titratable acidity and Vitamin C content were affected under this combined processing technique. The storage efficacy of tomato products and hence the extended shelf life was as a result of combined effects between cooking time and microbial load percent reduction that depended on the spore load concentration.

Conclusion: Using *Rosmarinus officinalis* essential oil can help reduce the thermal impact during tomato juice and paste processing even in condition of high contamination of spores of *Bacillus cereus*. Moreover in this processing condition, nutritional compounds are not significantly impacted.

Keywords: B. cereus; postharvest quality; rosemary essential oil; shelf life; tomato products.

1. INTRODUCTION

Attention to the concept of postharvest food loss reduction as a significant means to increase food availability was drawn by the World Food Conference held in Rome in 1974 [1] Postharvest losses are much higher for perishable fresh fruits and vegetables than for cereals and other field crops. Once harvested, fruits and vegetables have a limited postharvest life. It has been reported that in developing countries, up to 30% of the food produced cannot be used due to the spoilage by the actions of microorganisms, rodents, or insects according to UNDP reports, food wastes represent 33% of food production. Storage and processing technologies have been utilised for centuries to transform these perishable fruits and vegetables into safe, delicious and stable products. Washing, peeling and blanching steps prior to processing are responsible for some loss of water-soluble nutrients. Depending on how processing is carried out, it may result in changes in colour, texture, flavour and nutritional quality [2]. Food processing techniques, among other goals, reduce the number of microorganisms present or inhibit their growth in food [3,4], although there is a risk that processing steps may also contaminate the final product [5]. Thermal treatment (pasteurisation and sterilisation) is the most common and widely employed method for the inactivation of microorganisms and enzymes in the food industry [6]. Tomato (*Lycopersicon esculentum* Mill.) is a plant fruit of great economic importance in most of the developing countries because of its availability in different daily meals. The demand for tomato processing typically arises from a need to preserve the product for cooking purposes. Tomatoes are processed into many different tomato-based products such as sauce, soup, paste and juice [7]. Tomatoes and tomato-based products provide a wide variety of nutrients and many health-related benefits to the body [8]; these health benefits are related to the ingestion of bioactive components such as essential vitamins, minerals and polyphenolic compounds. Microbial contamination has been always considered to be due to bacteria, moulds or yeasts, which can survive in the processed products. These microorganisms may promote the deterioration of food products by degrading some of their compounds (such as carbohydrates, protein and vitamins), producing undesirable odour and off-flavour, coloration, pH and texture changes [9]. Bacterial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety of these foods [10]. Sporulating forms have a distinct role in food deterioration; this, due to the high thermal resistance of the spores, which in some species are still viable after the high temperature treatments associated with the pasteurization process. *Bacillus* and *Clostridium* are the few genera that can grow in such substrate. *Bacillus cereus* is the most important contaminant of the spore forming microorganisms because its spores are ubiquitous and can therefore survive cooking and dry storage [11,12]. Being a soil resident, *B. cereus* can spread easily into many types of foods such as plants, eggs, meat, fish, milk, and

dairy products, and is known for causing 25% of food-borne intoxications [13]. Recent food-borne microbial outbreaks are driving a search for innovative ways to inhibit microbial growth in foods while maintaining quality, freshness, and safety [14,15]. The use of essential oils is becoming popular to increase the shelf life of food products, since consumers are more conscious about the health problems caused by several synthetic preservatives [16,17,18,19]. Spices and herbal essential oils have been used by the food industry as natural agents for extending the shelf life of foods. Addition of spices in foods not only imparts flavour and pungent stimuli but also provides antimicrobial property [15]. A variety of plant based antimicrobials is used for reducing or eliminating pathogenic bacteria and increasing the overall quality of food products. Among several essential oils that may be useful as antimicrobial agents, rosemary *(Rosmarinus officinalis)* has several applications especially in the food processing and preserving because of its natural antioxidant and antimicrobial properties [10,20,21]. This study aimed to investigate if the use of rosemary essential oil *(Rosmarinus officinalis)* could permit to reduce thermal treatment time and nutrient loss during the production of tomatoes paste and juice, while maintaining food safety in situation of *Bacillus cereus* contamination.

2. MATERIALS AND METHODS

2.1 Sample Preparations

Tomato fruits (*Rio Grande cv*.) were purchased from a local supplier at a commercial stage of ripeness. Fruits were sorted and washed with tap-water to remove dirt and soil, before processing into juice and paste (Fig. 1).

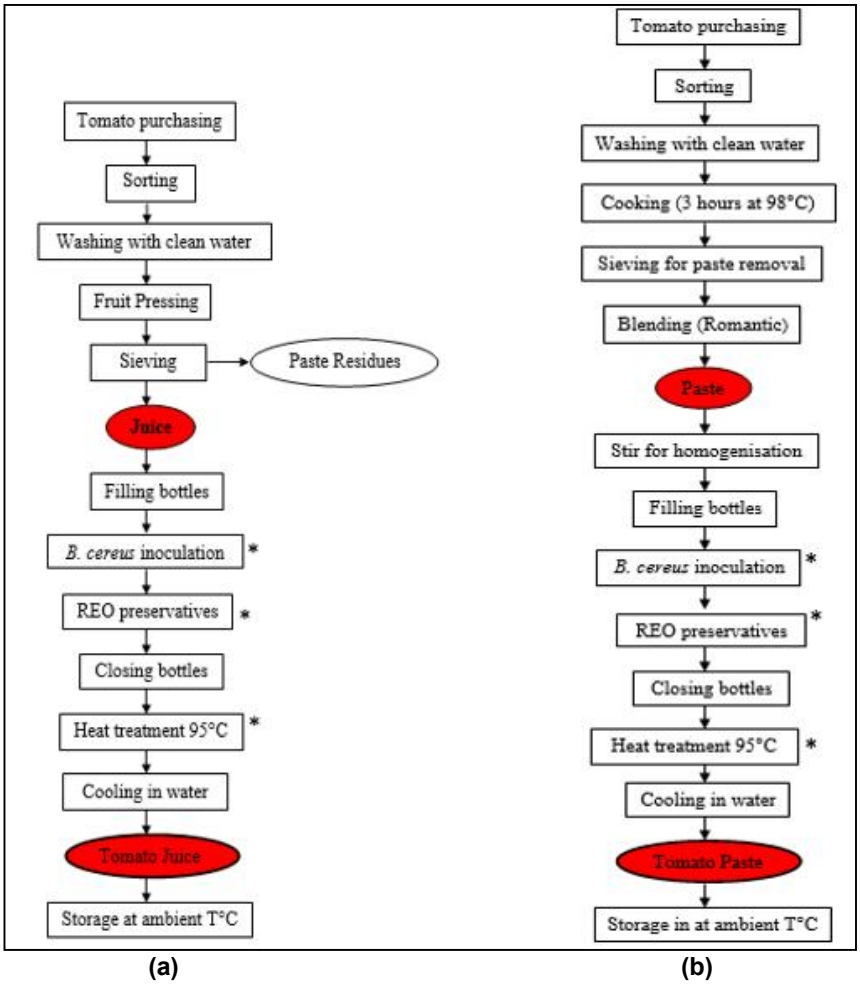

Fig. 1. Flow chart of processing tomato juice (a) and tomato paste (b)
** According to the experimental plan*

2.2 Preparation of *Bacillus cereus* Spores

To induce sporulation of vegetative cells of *B. cereus* (ATCC 11966), the procedures described by Etoa, et al. [22] were used. Briefly, spores stored on nutrient agar slants were cultured in nutrient broth and incubated at 37°C for 24 hours. Later, 10^3 cells/ml of the inoculum were sporulated on nutrient agar supplemented with salts (0.5 g of dissodic phosphate, 0.1 g of calcium chloride, 0.04 g of manganese sulphate), then incubated for 7 days at 37°C. After these days, spores were harvested by flooding the plates with sterile distilled water. Purification of spores was performed by several centrifugations at 4000 g/15 min at 4°C and stored at 4°C for 1 month before use.

2.3 Experimental Design and Treatments

Three treatment factors namely *Bacillus cereus* concentration deliberately inoculated (F_1), Cooking time (F_2) and Rosemary essential oil (REO) concentration (F_3) were considered at 5 levels (-2, -1, 0, 1, 2) each in a central composite experimental design (CCD) reinforced at the edges as indicated in Tables 1-2.

2.4 Enumeration of Residual Spores in Juice and Paste

Residual spores or vegetative cells of *Bacillus cereus* in juice or paste treated according to the reinforced experimental CCD were determined using a three repetition most probable number protocol with dilutions ranging from 1 to 10^{-5} dilutions. After incubation of the diluted samples at 37°C for 24 hours, observations on the number of tubes per repetitions presenting *B. cereus* growth were recorded. The generated code was used to calculate the MPN cells/g or ml using a MPN Excel add-in provided by Jarvis, et al. [23].

2.5 Physicochemical Analyses

2.5.1 Total polyphenols

Total polyphenols content were determined using a modified Folin-Ciocalteu colorimetric method [24,25]. After incubation at room temperature for 90 minutes, the absorbance was read at 760 nm using a UV-VIS Spectrophotometer. Gallic acid was used as the standard, and results were expressed as mg of Gallic acid equivalents (mg GAE) per ml of tomato juice or per mg of tomato paste. The correlation coefficient for the calibration curve was 0.9958.

Table 1. Corresponding values for tomato juice in a Central Composite Design (CCD) plan

Coded values in the CCD				Corresponding real values			
Condition	**F_1**	**F_2**	**F_3**	**Condition**	***B. cereus* (log)**	**Time (min)**	**REO (ppm)**
1	-1	-1	-1	1	2	5	50
2	-1	1	1	2	2	20	150
3	1	-1	1	3	6	5	150
4	1	1	-1	4	6	20	50
5	0	0	0	5	4	10	100
6	-1	-1	1	6	2	5	150
7	-1	1	-1	7	2	20	200
8	1	-1	-1	8	6	5	50
9	1	1	1	9	6	20	150
10	0	0	0	10	4	10	100
11	-2	0	0	11	0.1	10	100
12	2	0	0	12	8	10	100
13	0	-2	0	13	4	0	100
14	0	2	0	14	4	30	100
15	0	0	-2	15	4	10	0
16	0	0	2	16	4	10	200
17	0	0	0	17	4	10	100
18	2	-2	2	18	8	0	200
19	2	2	2	19	8	30	200
20	-2	-2	-2	20	0.1	0	0

F_1: Bacillus cereus spores (log cell/mL); F_2: Cooking time (minutes); F_3: Rosemary essential oil (ppm), v/v

Table 2. Corresponding values for tomato paste in a Central Composite Design (CCD) plan

Coded values in the CCD				Corresponding real values			
Condition	F_1	F_2	F_3	Condition	*B. cereus* (log)	Time (min)	REO (ppm)
1	-1	-1	-1	1	2	10	50
2	-1	1	1	2	2	30	150
3	1	-1	1	3	6	10	150
4	1	1	-1	4	6	30	50
5	0	0	0	5	4	20	100
6	-1	-1	1	6	2	10	150
7	-1	1	-1	7	2	30	200
8	1	-1	-1	8	6	10	50
9	1	1	1	9	6	30	150
10	0	0	0	10	4	20	100
11	-2	0	0	11	0.1	20	100
12	2	0	0	12	8	20	100
13	0	-2	0	13	4	0	100
14	0	2	0	14	4	40	100
15	0	0	-2	15	4	20	0
16	0	0	2	16	4	20	200
17	0	0	0	17	4	20	100
18	2	-2	2	18	8	0	200
19	2	2	2	19	8	40	200
20	-2	-2	-2	20	0.1	0	0

F_1: Bacillus cereus spores (log cell/mL); F_2: Cooking time (minutes); F_3: Rosemary essential oil (ppm), v/v

2.5.2 pH and titratable acidity

The pH values of tomato juice and paste were measured by using electronic pH meter (Hanna) [26]. Titratable acidity (TA) was obtained by titrating 5 ml of tomato extracts with an alkaline solution (0.1 N NaOH) using phenolphthalein indicator up to pH 8.1. The appearance of light pink colour was marked as the end point. Results were expressed as grams of citric acid per 100 g of tomato products [27,28,29].

2.5.3 Vitamin C concentration

Vitamin C in tomato juice and paste was estimated by the indicator method. The vitamin C contents of fruit juices were reported as mg/100 ml [26,30].

2.5.4 Shelf life of tomato juice and paste

In order to analyse the data for shelf life recorded for one year, a storage efficacy of the process was calculated using the following formula developed during this study:

$$\text{Shelf life} = \frac{[\Sigma(\text{number of good samples on Day i X Day i})]}{(\text{Maximum sample repetition X } \Sigma \text{ Day i})}$$

The value ranges from 0 to 1: 0 being a condition where all repetitions got spoiled on the first day of storage, and 1, a process condition that have assured all sample repetitions stable until 366 days (1 year).

3. RESULTS

3.1 Microbial Deactivation

Deliberately inoculated tomato juice and paste were produced as indicated in Fig. 1. After appertisation, the percentage of microbial reduction was assessed before the beginning of storage at ambient temperature. These results are presented in Tables 3-4.

The results (Table 3) were analysed using a non-linear regression in order to assess the relations between factors and the percentage reduction. The percentage reduction of *B. cereus* in juice is given in the below equation with R^2= 0.32 and SE= 1351

% Reduction = 93.5 + 0.1542*[*B. cereus*]*[Time] - 0.0158*[*B. cereus*]*[REO] (Equation 1)

It indicates that interactions between treatments (*Bacillus cereus* concentration and cooking time; *Bacillus cereus* concentration and REO concentration) were responsible for the spore reduction. Maximum reduction (100%) of *B. cereus* was observed in J5, J10, J11, J13, J14, J17 J19 and J20 where there was mostly a concentration of 10^4 *B. cereus*/ml, cooking time

(10 minutes) and 100 ppm of REO. It can be observed that the natural spore content of the product estimated to 0.1 Log ufc/ml (treatment J20) could not be detected in the product before storage.

In Table 4, the combination of a time of 20 minutes and REO of 100 ppm seems the best, since 100% reduction were noted in P10 and P12. The increase in essential oil over 100 ppm while maintaining thermal treatment time to 20 minutes did not provide a better bacterial reduction. In order to assess the relation between factors and the percentage reduction, a non-linear regression was performed and the following equation obtained:

% Reduction = 92.422 + 0.179*[REO]*[*B. cereus*] - 0.428*[*B. cereus*]*[Time] (Equation 2)

[R^2= 0.35 and SE= 62.9]

It can be observed that the combination of *B. cereus* concentration with time and REO concentration affected significantly the percentage reduction of the strain.

3.2 Total Polyphenols

Significant differences among treatments with regards to the concentration of total polyphenols (µg/ml juice and µg/mg paste) were observed (Figs. 2 and 3), and different groups were formed statistically ($p<0.05$). In Fig. 2, three different groups were formed statistically ($p<0.05$), and the higher concentration was recorded in J2 (167.22 µg/ml juice), whereas the lower concentration was observed in J9 (40 µg/ml juice). In Fig. 3, significant differences ($p<0.05$) were observed in most treatments and 2 groups were formed from the mean comparison. The most high concentrations of total polyphenols (µg/mg paste) was recorded in P1 (220 µg/mg paste) and the lower concentration in P5 (85.56 µg/mg paste).

3.3 pH and Titratable Acidity

The ph of tomato juice and tomato paste were 4.6±0.3 and 4.7±0.4 respectively. Results on the concentration of titratable acidity in tomato products (Figs. 4 and 5) were quite constant during the different treatments although a significant difference was observed between the highest and lowest values. In tomato juice (Fig. 4), the highest and lowest values were found respectively in J18 (29.44 g/100 ml juice); and in J11 (16.32 g/100 ml juice) while In tomato paste (Fig. 5), they were respectively found in P1 (35.84 g/100 g paste) and in P12 (28.8 g/100 g paste).

3.4 Vitamin C Concentration

Statistical analyses have indicated significant differences among treatments with regards to the vitamin C concentration level (Figs. 6 and 7). In tomato juice (Fig. 6), four different groups resulted in the mean comparison, though independently of the factors. The high concentration was found in J1 (20.43 mg/ml) and the low concentration in J20 (14.40 mg/ml). The concentration of vitamin C in tomato paste (Fig. 7) has been reduced compared to tomato juice; and significant differences were found among treatments with the highest concentration being in P12 (2.93 mg/mg) and the lowest P20 (0.47 mg/mg).

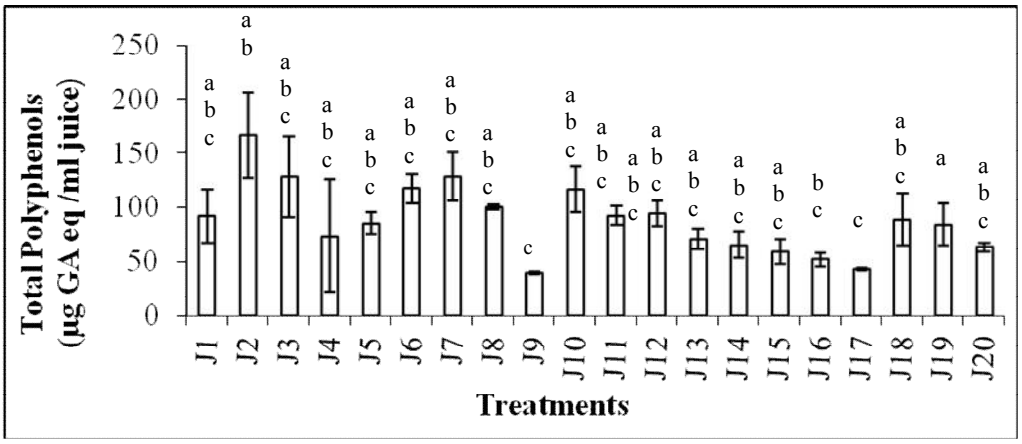

Fig. 2. Levels of total polyphenols in tomato juice as affected by different treatments
J1, J2, J3… J20: Labels for Juice different treatment conditions

Table 3. Microbial load reduction in tomato juice, storage efficacy and shelf life obtained after treatment at 95°C for varied time of appertisation, concentrations in *B. cereus* and REO

Juice Treatments	*B. cereus* (log CFU/g)	Time (min)	REO (ppm)	MPN/ml	% reduction	Juice storage efficacy	Shelf life (months)
J1	2	5	50	38.01	61.99	1.00	12
J2	2	20	150	3.00	97.00	1.00	12
J3	6	5	150	18.00	99.99	1.00	12
J4	6	20	50	9.16	99.99	0.79	9.6
J5	4	10	100	0.00	100.00	1.00	12
J6	2	5	150	3.57	96.43	1.00	12
J7	2	20	200	3.00	97.00	0.79	9.5
J8	6	5	50	21.00	99.99	0.93	11.2
J9	6	20	150	146.63	99.99	0.75	9
J10	4	10	100	0.00	100.00	0.75	9
J11	0.1	10	100	0.00	100.00	0.54	6.5
J12	8	10	100	920.00	99.99	0.75	9
J13	4	0	100	0.00	100.00	0.50	6
J14	4	30	100	0.00	100.00	0.54	6.5
J15	4	10	0	3.00	99.97	0.75	9
J16	4	10	200	3.00	99.97	0.77	9.2
J17	4	10	100	0.00	100.00	0.79	9.5
J18	8	0	200	43653877.62	56.35	0.79	9.5
J19	8	30	200	198.25	100.00	0.79	9.5
J20	0.1	0	0	0.00	100.00	0.54	6.5

J1, J2, J3… J20: Labels for Juice treatments

Table 4. Microbial load reduction in tomato paste, storage efficacy and shelf life obtained after treatment at 95°C for varied time of appertisation, concentrations in *B. cereus* and REO

Paste Treatments	*B. cereus* (log CFU/g)	Time (min)	REO (ppm)	MPN/ml	% reduction	Paste storage efficacy	Shelf life (months)
P1	2	10	50	1.0025	99.95	0.84	10.1
P2	2	30	150	1.0025	99.95	0.79	9.5
P3	6	10	150	1.0025	99.98	0.50	6
P4	6	30	50	92	67.27	0.54	6.5
P5	4	20	100	1.0025	99.97	1.00	12
P6	2	10	150	1.0025	99.95	1.00	12
P7	2	30	200	1.0025	99.95	0.59	7.1
P8	6	10	50	21	77.96	0.59	7.1
P9	6	30	150	9.17704	83.95	0.54	6.5
P10	4	20	100	0.0000	100.00	1.00	12
P11	0.1	20	100	1.0025	98.92	0.75	9
P12	8	20	100	930000	100.00	0.79	9.5
P13	4	0	100	9300	0.79	0.17	2
P14	4	40	100	1.0025	99.97	0.54	6.5
P15	4	20	0	1.0025	99.97	1.00	12
P16	4	20	200	1.0025	99.97	1.00	12
P17	4	20	100	1.0025	99.97	0.84	10.1
P18	8	0	200	$2.4x\ 10^{+08}$	0.10	0.03	0.4
P19	8	40	200	42	79.71	0.66	7.9
P20	0.1	0	0	23	0.00*	0.84	10.1

** affected because an increase in cells was observed; P1, P2, P3… P20: Labels for Paste treatments*

3.5 Shelf Life of Tomato Juice and Paste

The conservation length based on microbial macroscopic observations for both tomato juice (Table 3) and paste (Table 4) was affected by *B. cereus* contamination, REO concentration and time of appertisation. The best shelf life obtained in this experiment was one year.

In tomato juice (Table 3), treatments that had initial spore load of 2 log concentration of *B. cereus* combined with different REO concentrations and appertisation had a prolonged shelf life. However, the interaction between REO and thermal treatments is not very straight forward. In fact, when using 200 ppm of REO, the same level of inactivation was observed without treatment (J18) and after 20 minutes (J4) at 95°C.

In tomato paste (Table 4), treatments with initial spore load of 4 *B. cereus* log concentration and pasteurization time of 20 minutes combined with different REO concentrations had a shelf life of at least 1 year. Moreover, when the inoculated load was 2 log, a treatment time of 10 minutes and REO of 50 ppm could assure a 1 year of shelf life. It can also be observed that tomato paste with 20 minutes appertisation and no REO concentration (P15) demonstrated also a 1 year of storage level. Time of treatment higher than 30 minutes in combination of REO were not in favour of the storage efficacy. The use of REO alone gave lower storage times inversely proportional to the microbial initial load.

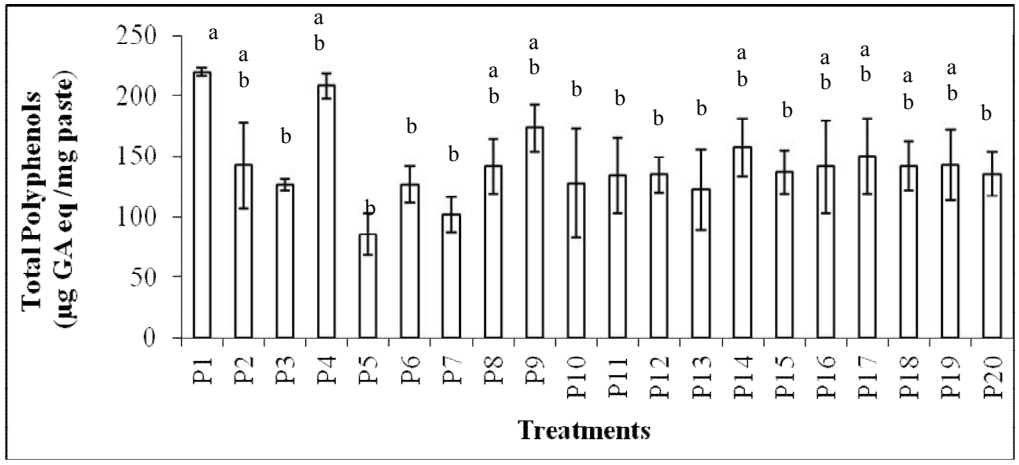

Fig. 3. Levels of total polyphenols in tomato paste as affected by different treatments
P1, P2, P3 … P20: Labels for Paste different treatment conditions

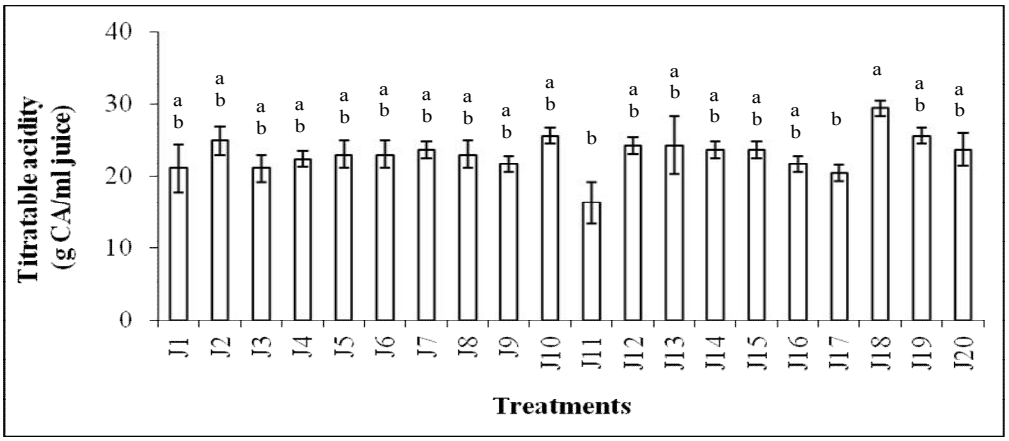

Fig. 4. Levels of Titratable acidity in tomato juice as affected by different treatments
J1, J2, J3… J20: Labels for Juice different treatment conditions

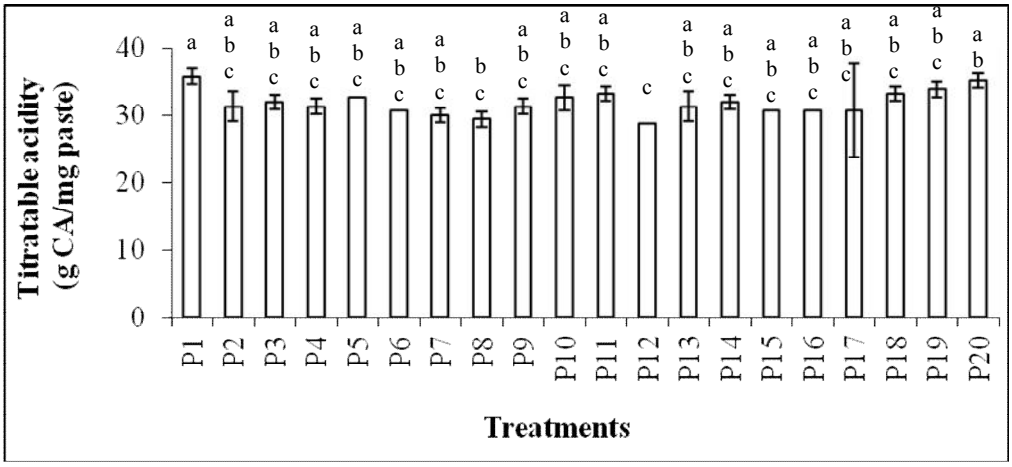

Fig. 5. Levels of Titratable acidity in tomato paste as affected by different treatments
P1, P2, P3 … P20: Labels for Paste different treatment conditions

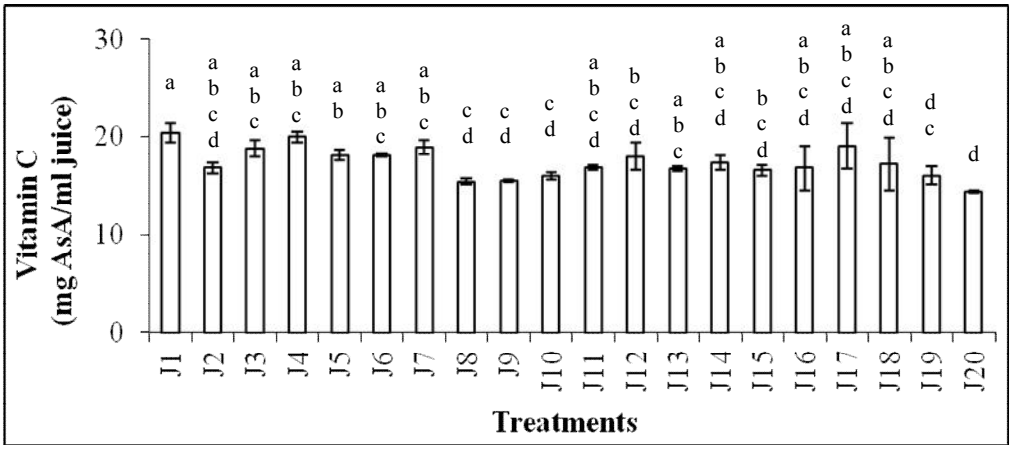

Fig. 6. Levels of Vitamin C in tomato juice as affected by different treatments
J1, J2, J3… J20: Labels for Juice different treatment conditions

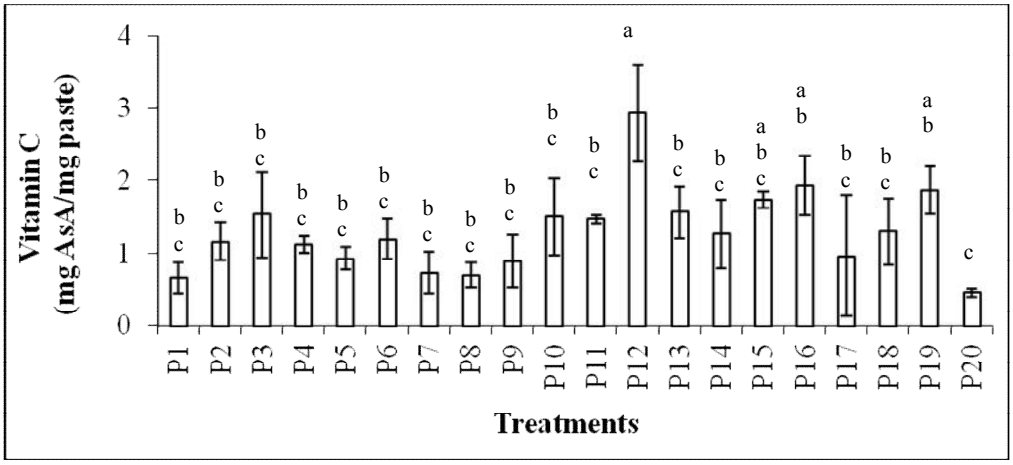

Fig. 7. Levels of Vitamin C in tomato paste as affected by different treatments
P1, P2, P3 … P20: Labels for Paste different treatment conditions

4. DISCUSSION

Before applying any treatment, the tomato juice and paste had in average pH value readings of 4.6 and 4.7 respectively. These products can be considered as being low-acid foods, and so need sufficient thermal treatments. However, when processing tomatoes, the pH should be lower than 4.4 to avoid potential spoilage with thermophilic organisms [31]. Our results indicated that these pH are not suitable for preservation because they are higher than the pH 4.5 that is the limit to prevent easy growth of most pathogens and toxin production. This little discrepancy with the recommended levels was compensated with the use of essential oil during thermal treatments.

The percentage of microbial reduction was assessed before the beginning of storage at ambient temperature. According to the equations (Equations 1 and 2), no direct effect of the independent variables were observed. In both tomato juice and paste, the percentage reduction of spores depended on the combination of Rosemary essential oil (REO) with spore concentration and heating time with spore concentration. However, these equations (Equations 1 and 2) only explained a maximum of 35% of the variability of the data, indicating that other important factors explaining these reduction are still to be identified. B. cereus spores are heat and radiation resistant [32,33]; the heat resistance might vary with different strains [11], pH and water activities of the heating and recovery medium [34], sporulation conditions and the nature of suspending media [32]. Despite the fact that inactivation of bacterial spores requires high temperature and long heating time, food containing > 10^4 *B. cereus*/g is not safe for the consumption, as the real infectious dose vary from about 10^5–10^8 viable cell or spores/g [11]. Our results have indicated that all treatments in both tomato juice and paste inoculated with 4 log CFU/g have registered a certain level of *B. cereus* reduction, except where there was no heating for deactivation (Tables 3-4). This reduction is a combined effect of heat and rosemary essential oil (REO). Previous studies have demonstrated that the efficacy of REO in inhibiting a variety of pathogens depends on many factors including the plant location and seasonal variations, the phenotype stage of the plant, the method of extraction of the essential oil, the procedure used in the antimicrobial assays, the type of organism, the cultivation conditions (incubation time, temperature, oxygen), the culture medium, the concentration of the test substance and the solvents used to dilute the oil, among other factors [35,36,37]. Rosemary extracts at a level of 0.06 -1% inhibit the growth of Gram-positive pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus* [20].

Regarding total polyphenols, results (Fig. 3) indicate an increase in the total polyphenols content of tomato paste compared to tomato juice (Fig. 2), independently of the treatments (*B. Cereus* spores, REO, cooking time). Thermal processing has been shown to release more bound phenolics due to the breakdown of cellular constituents [24,33,38]. This can explain better the increase in total polyphenols content observed in tomato paste after being subjected to processing techniques.

The loss of vitamin C in different tomato products increase with heating time and number of processing steps [39]. During processing, vitamin C is destroyed mainly due to oxidation reactions and the heat applied in the presence of air; and in addition to the effect of oxygen, such high temperature applications themselves cause oxidative stress. Results in Figs. 6 and 7 are consistent with data from the literature, where numerous examples can be found of vitamin C degradation during thermal processing of tomato products [40,41,42,43]. Loss of vitamin C occurs primarily by chemical degradation that involves oxidation of ascorbic acid to dehydroascorbic acid (DHAA), followed by hydrolysis to 2,3-diketogulonic acid and further polymerization to form other nutritionally inactive products [24].

Tomato juice typically has a commercial shelf life of 12 months [30] and concentrated tomato paste is typically stored for 1 year or more [44]. The active components of spices at low concentrations may interact synergistically with other factors to increase preservative effect. The findings of Eissa, et al. [45] have demonstrated that the use of volatile oil extracts (lemon grass, clove and rosemary) has been an effective method of quality improvement and shelf life extension in apple juice, stored at 4°C.

5. CONCLUSION

Using *Rosmarinus officinalis* essential oil can help reduce the thermal impact during tomato juice and paste processing even in condition of high contamination of spores of *Bacillus cereus*. Moreover in this processing condition, nutritional

compounds are not significantly impacted. 12 months shelf life can be achieved after high *Bacillus cereus* spores contamination lower or equal 10^4 *Bacillus cereus* spores/g of the raw material by applying Only 10 min appertisation at 95°C in the presence of 100 ppm of ROE in tomato juice while for tomato paste the same concentration of ROE needs 20 min appertisation at 95°C.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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