Effect of Selected Preservatives on Microbial Growth and Stability of β-carotene During Storage of Orange Fleshed Sweetpotato Puree

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(BSc. Food Science and Technology, University of Nairobi)

A Research Dissertation submitted in partial fulfillment of the requirements for the award of Master of Science degree in Food Safety and Quality in the University of Nairobi

Department of Food Science, Nutrition and Technology

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Declaration

I declare that this research dissertation is my original work and has not been submitted for a degree or any other award in any other university.

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Supervisors’ approval

We confirm that the dissertation has been submitted with our approval as Supervisors:

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I dedicate this work to my parents Mr. Jackson Mwambui and Theresia Musyoka for the unending support, encouragement and love that they showed me during the study period. To my brother James and my sisters Liz, Gilly, Esther, Jane and Dama for the support all through the study period, the encouragement and the prayers that ensured that my stay in campus was not full of struggles.
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<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>CA</td>
<td>Citric acid</td>
</tr>
<tr>
<td>CfU</td>
<td>Colony forming unit</td>
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<tr>
<td>CfU/g</td>
<td>Colony forming units per gram</td>
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<tr>
<td>CIP</td>
<td>International Potato Centre</td>
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<tr>
<td>G</td>
<td>Grams</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>GRAS</td>
<td>Generally Regarded as Safe</td>
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<tr>
<td>IMF</td>
<td>Intermediate Moisture Foods</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilograms</td>
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<tr>
<td>Log cfu/g</td>
<td>Log colony forming units per gram</td>
</tr>
<tr>
<td>MCT</td>
<td>Microbiological Challenge Test</td>
</tr>
<tr>
<td>Mg</td>
<td>Milligrams</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliters</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
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<tr>
<td>OFSP</td>
<td>Orange Fleshed Sweetpotato</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>PS</td>
<td>Potassium sorbate</td>
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<tr>
<td>RAE</td>
<td>Retinol Activity Equivalence</td>
</tr>
<tr>
<td>Rpm</td>
<td>Rotations per minute</td>
</tr>
<tr>
<td>SB</td>
<td>Sodium benzoate</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>VAD</td>
<td>Vitamin A deficiency</td>
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General Abstract

The orange fleshed sweetpotato (OFSP) varieties are being promoted in Kenya due to their nutritional benefits and their importance in enhancing income and food security in the rural areas. In Kenya, OFSP roots are processed into a mashed form or puree that is incorporated into processing of various products both at household and commercial level. OFSP puree is highly perishable thus limiting its wide adoption and all year availability for incorporation into products. The puree is prone to contamination by both pathogenic and non-pathogenic microorganisms during its preparation and therefore limiting its safety and shelf life. There is also limited information on stability of β-carotene in OFSP puree during storage.

The aim of the current study was to contribute towards production of shelf-stable OFSP puree while ensuring the safety of the product with minimal nutritional loss. The current study evaluated the effect of different combinations of sodium benzoate, potassium sorbate and citric acid in controlling the growth of pathogenic microorganisms (Staphylococcus aureus and Escherichia coli) through a challenge test, their effect on growth of yeast and molds and total viable counts and the stability of β-carotene in OFSP puree during storage. OFSP puree was prepared and dosed with preservative combinations: non-supplemented puree (A), 0.05 % sodium benzoate + 0.05 % potassium sorbate + 1 % citric acid (B), 0.1 % sodium benzoate + 0.1 % potassium sorbate + 1 % citric acid (C), 0.2 % sodium benzoate + 0.2 % potassium sorbate + 1 % citric acid (D) and 1 % citric acid (E). All OFSP puree samples (each 100 g) were inoculated with Escherichia coli and Staphylococcus aureus pathogens at inoculum levels of 5.2x10⁹ cfu/100 g puree and 1.53x10⁹ cfu/100 g puree respectively before being evaluated during storage for 10 weeks at prevailing ambient
temperature (15-25 °C) and refrigeration temperature of 4 °C. Total aerobic counts, yeast and molds were also evaluated during the storage period. All the samples were evaluated for β-carotene biweekly for 8-week storage of OFSP puree at ambient and refrigeration temperatures.

Results showed that E. coli and S. aureus population declined significantly (p<0.05) in all puree treatments at the storage conditions except for non-supplemented puree which recorded a 2-log increase in counts at ambient conditions. Total viable counts, yeast and molds were completely inhibited during the storage period except for the puree with citric acid only. Pack distention and alcoholic odors were noted in OFSP puree treated with citric acid only after one week of storage. β-carotene content significantly (p<0.05) declined with storage period at both storage conditions. At the end of the storage period, the highest β-carotene content of 6.66 mg/100 g was noted in OFSP puree with 0.2 % sodium benzoate + 0.2 % potassium sorbate + 1 % citric acid while the lowest of 5.21 mg/100 g was found in puree with 0.1 % sodium benzoate + 0.1 % potassium sorbate + 1 % citric acid at ambient temperature. At refrigeration temperature, the highest content of β-carotene of 6.70 mg/100 g was noted in puree treated with 1 % citric acid while the lowest of 5.37 mg/100 g was noted in puree with 0.05 % sodium benzoate + 0.05 % potassium sorbate + 1 % citric acid. The treatment with 0.05 % sodium benzoate + 0.05 % potassium sorbate + 1 % citric acid was found to be the best combination in controlling growth of pathogenic microorganisms and ensuring extensive use of the puree. This treatment is therefore recommended for use by producers since it is cost effective and the low levels do not alter the sensory characteristics of the puree. Treatment of puree with preservatives ensures minimal β-carotene loss during storage.
Chapter One

1 General Introduction

1.1 Background information

Sweetpotato (Ipomea batatas) is the seventh most important food crop in the world (FAOSTAT, 2014). About 80% of the world's sweetpotatoes are produced in China (FAOSTAT, 2014). Other major producers include; Uganda, Indonesia, Vietnam and Nigeria. Sweetpotato is considered a staple crop in many parts of sub-Saharan Africa (Wheatly & Loechl, 2008) including Kenya where it is mostly grown in the Western and Nyanza parts (Kaguongo et al., 2012). It is one of the many crops that assist farmers in generation of income and subsistence food security (Wheatly & Loechl, 2008). Sweetpotato is increasingly being recognized in the improvement of food security, health and livelihoods of poor families in Kenya. Duvernay et al. (2013) and Oloo et al. (2014) pointed out that sweet potato has some good attributes over other root crops which include; its ability to tolerate drought, a low demand for soil nutrient, the capability to provide reasonable yields, flexibility in planting and harvesting period, low inputs requirements like fertilizer and shorter growing period. In Kenya, sweetpotato is mainly eaten as a staple crop and the roots are mainly steamed, boiled or mashed and roasted while the vines are eaten as vegetables (Odondo et al., 2013).

Sweetpotato roots are in various colors such as the commonly found white fleshted, the anthocyanin rich purple fleshted and the orange fleshted rich in β-carotene, a precursor of vitamin A. The orange fleshted sweetpotato (OFSP) varieties are being promoted in Kenya
due to their high β-carotene content. OFSP consumption has been shown to improve vitamin A intake in vulnerable groups (Low et al., 2007). Vitamin A deficiency (VAD) is a very serious nutritional and health problem affecting mainly children under the age 5, pregnant and lactating women in Kenya (Mitra, 2012). According to Odebode et al. (2008), VAD causes blindness, leads to decline in the capacity of a child to fight other diseases and therefore the importance of appreciating the potential of OFSP in improving vitamin A intake. OFSP are affordable sources of vitamin A to the urban and rural poor families (Tumwegamire et al., 2004) since they cannot afford the expensive vitamin A rich foods which include milk, eggs, butter and fish oils.

Utilization of OFSP is being promoted in order to improve the nutrition and health of the households in Kenya in addition to generating income for households through small scale businesses (Abidin et al., 2001). OFSP roots are processed into flour or puree that is incorporated in the processing of various products such as porridge, chapatti, mandazi, cakes and biscuits (Adeyuno, 2016). OFSP puree is preferred to flour because the former is more nutritious and economical to produce compared to the latter (Low et al., 2015). Currently in Kenya, OFSP puree is incorporated in making of chapatti and mandazi for children at the household level (Stathers et al., 2013) and commercially as a partial wheat substitute in bakery products such as bread, doughnuts and cakes. However, the major bottleneck in producing OFSP puree is posed by the high perishability of the puree and its high potential of contamination during production. Therefore, there is a need to produce shelf-stable OFSP puree that can ensure continuous supply to the bakeries and continuous income gain to the farmers.
Shelf-stable OFSP puree can be achieved through controlling growth of pathogenic microorganisms while ensuring minimal nutritional loss. One of the approaches of producing shelf storable puree is by using preservatives. Natural preservatives have been used in extending the shelf life of various products. For instance, nisin has been used in extending the shelf life of strawberry puree by reducing the growth of aerobic bacteria, yeast and molds and *Escherichia coli* (Jin *et al.*, 2010). Chemical preservatives such sodium benzoate and potassium sorbate can also be used in extending the shelf life and minimizing nutrient loss in food products. For instance, Ceylan *et al.* (2004) recorded a decline in *E. coli* counts in apple juice treated with sodium benzoate and potassium sorbate while Sarkar *et al.* (2015) showed the effect of sodium benzoate on β-carotene retention in tomato puree during storage.

However, the effect of different combinations of potassium sorbate, sodium benzoate and citric acid in retarding or inhibiting the growth of both spoilage and food pathogens in stored OFSP puree is not known. Additionally, information on the stability of β-carotene in OFSP puree treated with different preservatives during storage is limited. Therefore, the current study was aimed at determining the effects of different combinations of sodium benzoate, potassium sorbate and citric acid in controlling the growth of *Escherichia coli* and *Staphylococcus aureus* and evaluating the stability of β-carotene in OFSP puree treated with preservatives during storage.

### 1.2 Statement of the problem

Orange fleshed sweetpotato (OFSP) puree is a relatively new product in Kenya that is produced by one processor in Homa Bay County. OFSP puree is utilized in Kenya as an
ingredient in bakery products like bread, buns and cakes. OFSP puree is highly perishable and prone to post-heating microbial contamination if good manufacturing practices are not adhered to during handling (Pérez-Díaz et al., 2008). For instance, *S. aureus* and *E.coli* can contaminate the puree due to improper handling and use of non-potable water during cleaning of equipment surfaces. These microorganisms grow in the puree during storage thus posing food safety hazards to the consumer (Raybaudi-Massilia et al., 2009) as well as spoilage leading to economic losses. The current processor in Kenya uses fresh or frozen puree. However, it is expensive to freeze puree and transport while frozen and this limits its production. There is, therefore, an urgent need to produce shelf-stable puree to ensure continuous supply to bakeries for incorporation into processing of bakery products. Preservatives have been known to inhibit the growth of microorganisms in various products. However, the effect of combinations of potassium sorbate, sodium benzoate and citric acid on the growth of *S. aureus*, *E. coli*, total viable counts and yeast and molds and stability of β-carotene in OFSP puree during storage is not known.

1.3 Justification

Processing of orange fleshed sweetpotato (OFSP) roots in Kenya initially involved the production of flour for incorporation into bakery products. There is, however, the production of OFSP puree (boiled and mashed) currently which is used as a partial substitute for wheat flour in bakery products. OFSP puree is highly perishable compared to flour and it is prone to contamination by both pathogenic and non-pathogenic microorganisms during post-heating processing. However, according to research, OFSP equivalent bakery products made using puree are more viable compared to those made using OFSP flour (Sindi et al., 2015a) given its high conversion rate of 4-5 kg of fresh root
required to produce 1 kg of OFSP flour compared to 1.2-1.3 kg of fresh roots required to produce 1 kg of OFSP puree (Bocher *et al*., 2017; Low *et al*., 2015). There is more β-carotene loss during drying in flour processing compared to that lost during preparation of puree (Low *et al*., 2015). OFSP puree products have been found to be more acceptable to consumers compared to those made from flour (Sindi *et al*., 2015a). To address the problems of food safety related to the growth of pathogens, *E. coli* and *S. aureus* in OFSP puree and economic losses as a result of food spoilage by pathogenic microorganisms in the puree, it is therefore important to explore the use of combinations of locally available and affordable preservatives, sodium benzoate, potassium sorbate and citric acid in preserving OFSP puree. This will ensure extensive use of the puree since the consumers (bakeries) will be able to prolong the storage of the puree in their warehouse before use at minimal cost and therefore continuous supply of bakery products with sufficient β-carotene.

### 1.4 Objectives

#### 1.4.1 Overall objective

The main objective of the current study was to evaluate the effect of combinations of sodium benzoate, potassium sorbate and citric acid on growth of selected microorganisms and stability of β-carotene in orange fleshed sweetpotato (OFSP) puree during storage.

#### 1.4.2 Specific objectives

1. To determine the effect of different combinations of selected preservatives on microbial growth in OFSP puree during storage at ambient (15-25 °C) and refrigeration (4 °C) conditions.
2. To determine the stability of β-carotene in OFSP puree treated with combinations of selected preservatives during storage at ambient (15-25 °C) and refrigeration (4 °C) conditions.

1.5 Hypotheses

1. Different combinations of potassium sorbate, sodium benzoate and citric acid have no effect on the growth of microorganisms in OFSP puree during storage.

2. There is no significant retention of β-carotene in OFSP puree treated with different combinations of preservatives during storage.
Chapter Two

2 Literature Review and Analysis

2.1 Production of Sweetpotato in Kenya

Sweetpotato (*Ipomea batatas*) is an important food security crop grown in Kenya mostly by women for household consumption and as a source of income (Mukhopadhyay *et al.*, 2011). Sweetpotato has some good attributes which makes it easy to produce such as the ability to thrive under adverse weather and soil conditions, flexibility in planting schedules and low input requirements (Abong’ *et al.*, 2016). As a food security crop, sweetpotato can be harvested piecemeal as required and therefore being a source of food and income to households. Sweetpotato is also an excellent source of vitamin A especially the orange fleshed varieties (Kaguongo *et al.*, 2012).

Sweetpotato production in Kenya is mostly done in Nyanza and Western provinces, with a small proportion in the coastal and central provinces (Kaguongo *et al.*, 2012). According to the Kenya National Bureau of Statistics (KNBS, 2013) approximately 48% of the population in these provinces live below the poverty line and a great proportion do not have access to adequate quality and quantity food. Sweetpotato is therefore important in contributing to food security, increased income and reduction of nutritional deficit in these areas (Kaguongo *et al.*, 2012).

In Kenya, both local and improved varieties of sweetpotato are grown. Sweetpotato varieties differ in the color of the tuber skin (white, reddish purple and brown yellow), color of the tuber flesh (white, yellow, purple and orange), shape of the leaves and tuber, resistance to diseases and time of maturity among others (Fetuga *et al.*, 2013). The orange
fleshed varieties are high in β-carotene, pro-vitamin A carotenoid which is important in Western, Coastal, Nyanza and Eastern parts of the country where vitamin A deficiency (VAD) is mostly prevalent (Low et al., 2007). Some of the improved varieties of sweetpotato grown in Kenya are SPK 013 (Kalamb nyerere), SPK 004 (Nyathi odiewo), Kemb 20 (Nyamisambi), Kemb 23 (Nya migori), Kemb 10 (united), KSP 20 (Kuny kibuonjo), KSP 11, Mugande (Amina), Ex-Diani, Mafuta, Japanese pumpkin and CIP selection 420009 (Kwach et al., 2010).

The predominant varieties of sweetpotato in Kenya are white and yellow that contains small amounts of β-carotene (Kapinga et al., 2011). New and improved high-yielding sweetpotato varieties have been introduced in Kenya; the orange fleshed sweetpotato (OFSP) has high β-carotene content and enough dry matter for consumers’ satisfaction (Harvest Plus, 2012). OFSP promotion in Kenya as a food-based strategy was done mainly because of three reasons: first, they are high in β-carotene, white fleshed roots that are majorly grown is low in β-carotene yet VAD is very common in the western parts of Kenya (KNBS, 2010). Secondly, OFSP is an affordable all year round source of Vitamin A. Fruits being cheap sources of vitamin A, they are only available for a period of four months (Oiye et al., 2009). On the other hand, other sources of vitamin A such as cod liver oil are relatively expensive for the rural poor households and some are not available in the local stores (Hagenimana et al., 2001).

Lastly, sweetpotato is majorly farmed by women who are again the main actors in household’s food security. Women plant sweetpotatoes in very small plots and after harvesting they keep some for household consumption and sell small amounts to for
income generation (Hagenimana et al., 2001). Therefore, in order to increase vitamin A intake in Kenya through utilization of foods, women controlling both production and consumption make it easier (Laurie & Van Heerden, 2012). The developed orange fleshed sweetpotato varieties that are rich in β-carotene are Kenspot 3, Kenspot 4, Kenspot 5, Vitaa and Kabode (Kwach et al., 2010). OFSP is gaining importance as the most affordable source of antioxidants having several attributes such anti-cancer, anti-oxidation and it is important in combating VAD in the communities of Kenya which is a major public health concern (Mitra, 2012).

2.2 Utilization of sweetpotato in Kenya

Utilization of sweetpotato in Kenya was initially limited to roasting, chewing raw or boiling (Odondo et al., 2013). However, this has been changing and currently sweetpotato are processed into a variety of products (Odondo et al., 2013). Sweetpotato utilization and processing is capable of enhancing the productivity of the crop further improving food security, income generation and nutritional security in both rural and urban households (Aurélie, 2010). Sweetpotato can be processed and used in a variety of ways in both households and in the markets. Sweetpotato is utilized as human food, livestock feed and in the industrial processing of alcohol and starch. In addition, the leaves can be utilized by both humans and animals as food as they provide vitamins, minerals and proteins (Yanggen & Nagujia, 2006).

Sweetpotato is processed into dry chips that are utilized by humans at household level. The dry chips can be made into flour which is then used in the enrichment of various products such as weaning food, or can be combined with wheat flour to make other products such as
biscuits, bread, *chapatti*, *mandazi*, cakes, *ugali*, crankies and porridge among others (Adeyonu, 2016). The leaves are utilized as vegetables while fresh or preserved while dry. A study by Abubakar *et al.* (2010) revealed the nutritional quality of sweetpotato leaves that contained high amounts of crude fiber and proteins important in addressing colon and deficiency diseases. This study revealed further that both the leaves and the tubers contained micronutrients that are important for a healthy body.

Sweetpotato offers various processing opportunities in Kenya at the household level. They are processed into juice for human consumption and chips used as an animal feed. Sweetpotato juices are produced at household level and are of different types notably 100% sweetpotato or mixed with either mangoes, oranges or passion (Westby *et al.*., 2003). Sweetpotatoes are also made into various liquid and semi-solid products such as beverages, soup, baby foods and snacks among others. Both sweetpotato roots and leaves can be used as feed since they provide a variety of nutrients such as energy and proteins and are used fresh, fermented into silage or used while dry (Abubakar *et al.*, 2010).

OFSP roots are processed into flour or puree (boiled and mashed) that is incorporated in the processing of various products. At household level, OFSP puree is incorporated in the making of fried products (*mandazi*, *chapatti*) doughnuts, crankies and fried chips that have been accepted by both urban and rural consumers (Tumwegamire *et al.*, 2004). Commercially, OFSP puree is used as a partial substitute for wheat flour in the making of bread. Studies show that OFSP bread has been widely accepted by consumers; it’s a good source of vitamin A (Jenkins *et al.*, 2015) and has proven to be profitable for the bakers. This is a food based strategy aimed at improving vitamin A intake mostly in urban areas.
2.3 Microbiological Spoilage of Sweetpotato Puree

Quality losses in puree may result from microbial, chemical, physical or enzymatic changes. Microbiological losses in puree are important because they constitute a hazard to the consumers due to the presence of pathogenic microorganisms or microbial toxins and because of economic losses that result from microbial spoilage (Raybaudi-Massilia et al., 2009). The microbiology of vegetables differs depending on nutrient availability, water activity and the pH of the medium among others (Kalia & Gupta, 2006). The causal agents of microbial spoilage in sweetpotato puree can be bacteria as well as yeast and molds. The former are considered the main spoiling agents due to the high pH of sweetpotato puree.

OFSP puree falls in the category of intermediate moisture foods (IMF). IMFs are foods whose water activity falls in the range of 0.6-0.9 (Loveday et al., 2010). They are semi-moist with some of the water being bound (therefore unavailable for microbial growth) by the addition of organic acids or humectants and therefore preventing microbial growth. Reducing water activity below certain levels prevents the growth of pathogenic microorganisms in the product (Abbas et al., 2009). However, there is still presence of some microorganisms that can pose spoilage and health concerns to the consumers when their conditions of growth are favorable. *Staphylococcus aureus* is the major microorganism of concern in IMF such as OFSP puree. *S. aureus* has the ability to grow at water activity above 0.95 when the pH of the product is favorable (pH 4-9) (Aydin et al., 2011). Thus, formulation of OFSP puree requires additional measures to inhibit *S. aureus* during storage. This can be achieved by the use of chemical preservatives such sodium benzoate, potassium sorbate and citric acid which have been known to inhibit the growth of microorganisms in food (Abdulmumeen et al., 2012).
OFSP puree is categorized as a low acid food (pH>4.6). Low acid foods are known to support the growth of various pathogenic microorganisms such as *Clostridium botulinum* (Park *et al*., 2014). However, addition of an acidulant such as citric acid to OFSP puree reduces the pH of the puree to below 4.6 at which *Clostridium botulinum* spores do not germinate or produce neurotoxin (Breidt *et al*., 2010). At this pH, the survival of acid resistant bacterial pathogens such as *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica* that may be present in the puree are of concern (Silvaa & Gibbs, 2010). However, a temperature of 65°C for a few seconds is sufficient to destroy these microorganisms hence they are destroyed during steaming of roots into puree. OFSP puree is prone to post heating contamination with *E. coli* and therefore it becomes an issue of concern in the puree. Other spoilage organisms such as yeast and molds are still of concern in the puree (Breidt *et al*., 2014).

### 2.4 Microbiological contamination of sweetpotato puree

Sweetpotato puree is susceptible to microbial contamination and inappropriate food handling practices by food handlers is one of the potential sources of contamination (Perez-Diaz *et al*., 2008). Microbial contamination of puree also arises from raw materials and contact with processing equipment. Many types of microorganisms can be found in the raw materials including Gram-positive bacteria, Gram-negative bacteria, and fungi (yeast and molds) (Mandrell *et al*., 2006). The high water activity of the raw materials, nutrient availability and pH of higher than 4.6 makes the raw material susceptible to contamination by most microorganisms (Barth *et al*., 2009). However, these microorganisms are destroyed during steaming of the roots to puree at high temperatures of 100°C.
Although many microorganisms are destroyed during processing of OFSP puree by steaming, there is always a potential of post-heating contamination by pathogenic and non-pathogenic microorganisms (Perez-Diaz et al., 2008). This is attributable to excessive handling if good manufacturing practices are not adhered to during puree processing leading to contamination of OFSP puree with *Staphylococcus aureus*. Handlers can be a source of contamination of the puree through uniform, gloves and unclean hands, when sneezing or coughing while handling the OFSP puree (Aluko et al., 2014). Therefore, *S. aureus* is a good choice for a Microbiological Challenge Testing (MTC) as a model for post-processing handling of puree.

Contamination can also arise from use of unclean equipment. When water is used as an ingredient in the food, contamination occurs if the water supply is not potable (Sperber and Doyle 2009). Contamination of OFSP puree with *E. coli* can occur after post-heat process treatment when unclean water is used for cleaning equipment and surfaces that come into contact with the puree (Sharma, 2013). These are the real behaviors that occur in small-scale production setting. It has been pointed out by Alemu (2014) that *E. coli* can survive on surfaces and persist for as long as months. MCT using *E. coli* was therefore selected as a model for processing surfaces.

### 2.5 Preservation of OFSP puree

One of the major hurdles that can be used in stabilization of IMF such as puree use of chemical preservatives and storage at different temperatures. Many food preservation methods such as freezing, chilling, acidification, fermentation, modified atmosphere packaging, use of antimicrobials and water activity reduction have been used to control the
growth of microorganisms in food products (Davidson, 2011). The use of preservatives in controlling microbial growth while ensuring the safety and quality of food products have increased due to demand of healthy and safe foods (Soliva-Fortuny & Martín-Belloso, 2003).

Preservatives are food additives and their application in foods is governed by both national and international regulations. Preservatives are able to delay or destroy microorganisms in a food matrix (Davidson & Zivanovic, 2003). Many preservatives are found in nature such as citric acid (in lemons), sorbic acid (in rowanberries), benzoic acid (in cranberries) or tartaric acid (in grapes) (Raybaudi-Massilia et al., 2009). Most of the food preservatives are biostatic and are not biocidal and this limits their action in a food thus the foods’ shelf-life is dependent on the storage conditions. On the other hand, the use of combination of preservatives is more effective compared to the use of one preservative because some of the microorganisms are not destroyed by the legally approved doses (Beuchat, 2001). Combination of two or more preservatives leads to a synergistic or antagonistic effects. The same effects are expected when preservatives are combined with other preservation methods such ultra violet light and heat among others (Raybaudi-Massilia et al., 2009).

The efficiency of preservatives in inhibiting microbial growth depends on the concentration, the species of microorganism, type and genus of the microorganism. It is also dependent on environmental factors such as water activity, temperature, acidity of the food and the initial microbial load (Davidson, 2011). The environmental factors are themselves preservation methods and can also be used in combination forming the basis of
the hurdle concept which involves the use of more than one treatment in order to enhance microbial safety (Raybaudi-Massilia et al., 2009).

2.5.1 Sodium benzoate (E 211)

Benzoic acid is a phenyl formic acid found naturally in plums, prunes, cranberries, cinnamon and other berries. Sodium benzoate is produced through the process of neutralizing benzoic acid using sodium hydroxide. Sodium benzoate is generally regarded as safe (GRAS) for normal use and the maximum recommended usage is 0.1 % (Adeshina & Onaolapo, 2012; Code of Federal Regulations, 1977/1988). Sodium benzoate is used in food preservation rather than benzoic acid because it is more soluble compared to benzoic acid (Chipley, 2005). It is mostly suitable for use as a preservative in foods with pH below 4.5 or the pH can be brought to that level by the use of an acidulant (Wibbertmann et al., 2005) and its activity is against yeast, molds and bacteria.

2.5.1.1 Mode of action

The action of sodium benzoate against microorganisms is greater in yeasts than in bacteria and molds but its dependent on pH, the microorganisms present and water activity (El-Ziney, 2009). Benzoates have bacteriostatic effect but not bacteriocidal and they act against microorganisms by inhibiting the cellular uptake of substrates (Mani-López et al., 2012). The antimicrobial activity of sodium benzoate is due to the undissociated form of the compound which reduces the internal pH of microorganisms (Chipley, 2005). In undissociated form, they cause leakage of protons into the cytoplasm of the cells and energy from the cells is directed out in an effort to maintain an optimum internal pH. The disruption of the membrane activity affects amino acids transport (Jay, 2000). Benzoates
are also known to inhibit specific enzymes within cells such as α-ketoglutarate and succinate dehydrogenases (Chipley, 2005).

The antimicrobial activity increases with a decrease in the pH of the food and the maximum antimicrobial activity is encountered at pH values of 2.5-4.0 (El-Ziney, 2009). At very low pH values, sodium benzoate is known to impart a slight tang in taste due to the undissociated benzoic acid while at concentrations above 0.1%, benzoates have a negative impact on the flavor of food. However, this can be overcome by using sodium benzoate in combination with other preservatives like potassium sorbate to lower its concentration below its taste threshold and to increase its bacteriostatic effect (Chipley, 2005). Several microorganisms have been found to utilize sodium benzoate under both aerobic and anaerobic conditions leading to its degradation. These include; fungi such as *Rhodotorula glutinis* (Hazan *et al.*, 2004), moulds such as *Penicillium frequentans* and bacteria such as *Alcaligenes denitrificans* (Hazan *et al.*, 2004) and several strains of denitrifying pseudomonas.

### 2.5.1.2 Clearance from the body

Sodium benzoate is rapidly absorbed after oral uptake from the gastrointestinal tract (GIT) and it is metabolized in the liver by conjugation with glycine resulting into the formation of hippuric acid which is rapidly excreted through the urine (Wibbertmann *et al.*, 2005).

### 2.5.2 Potassium sorbate (E 202)

Sorbic acid is a straight chain unsaturated monocarboxylic fatty acid obtained from rowanberry. Potassium sorbate is generally regarded as safe (GRAS) for normal use with a maximum recommended level of use is 0.1 % (Code of Federal Regulations, 1977/1988).
Levels above 0.1 % may pose undesirable off-flavors in food products and its high solubility in water makes it very important and useful in food preservation (Hussain et al., 2011).

2.5.2.1 Mechanism of action

Sorbates are known to have activity against Gram-positive and Gram-negative, aerobic and anaerobic pathogenic and spoilage bacteria. Some of the pathogenic bacteria inhibited by sorbates are *Bacillus spp.*, *Clostridium botulinum, S. aureus, E. coli* and *V. parahemolyticus* (El-Ziney, 2009). Sorbates are bacteriostatic and not generally bacteriocidal against microorganisms and are effective against microorganism in foods with low pH because its antimicrobial activity is due to the undissociated form of the compound (Mani-López et al., 2012). The antimicrobial activity increases as the pH of the medium decreases and it is more effective in foods with low pH values (< 6.5) (Hussain et al., 2011). Potassium sorbate is lipophilic, can move across the bilipid layer of the microbial cytoplasm and interacts with the microbial membrane leading to the interference of the membrane-associated cellular functions that are important for the microbial growth (Hussain et al., 2011).

Inhibition of cell metabolism by sorbates can be attributed to alteration of the functions, integrity and morphology of the cells' membrane, inhibition of nutrient uptake, enzyme activity and cell transport (Beales, 2004). Inhibition of uptake of nutrients may be caused by ATP depletion, inhibition of enzymes involved in transport, proton motive force neutralization and electron transport system inhibition (Aouadhi et al., 2015). Sorbates have also been shown to interfere with proteinase, or inhibit respiration through competitive action with acetate in acetyl CoA formation (El-Ziney, 2009). Sorbates are able to prevent the action of various enzyme systems and this leads to the destruction of various
processes that take place in cell metabolism, cell growth, cell replication and transport functions (Beales, 2004). Sorbates also inhibit amino acid and glucose uptake by the cell thus interfering with its electron transport system (Aouadhi et al., 2015).

Potassium sorbate undergoes decomposition and oxidation in the presence of moisture and various acids. However, citric acid has no effect on potassium sorbate degradation and it can help in stabilizing potassium sorbate solutions (Gliemmo et al., 2009). Several bacteria are known to be resistant to potassium sorbate in foods. For instance, certain species of staphylococcus and pseudomonas are known to be more resistant to potassium sorbate compared to other bacteria like Clostridium parfringens (Beales, 2004). S. aureus is less sensitive to potassium sorbate compared to other bacteria and it is usually inactivated by potassium sorbate at levels of 0.1 % with the pH of the medium at 5.0 (Davidson & Harrison, 2002). Certain yeasts such as the osmotolerant yeast develop resistance to inhibition by sorbates and certain mold strains are also resistant to sorbates with some being able to metabolize sorbates with the production of unsaturated fatty acids (Davidson & Harrison, 2002).

### 2.5.2.2 Clearance from the body

Potassium sorbate is almost absorbed completely after intake, well distributed in the body and is oxidized to carbon (IV) oxide (CO₂) and water and therefore 80 to 86 % is excreted as CO₂ through the lungs (El-Ziney, 2009) while Less than 10 % of the excreted radioactivity was discovered in urine as urea and as sorbic and muconic acid in minor concentrations (El-Ziney, 2009).
2.5.3 Citric acid (E 330)

Citric acid is a tricarboxylic acid produced naturally by citrus fruits and it is GRAS (Code of Federal Regulations, 1977/1988). Citric acid is used in the food industry as a preservative because pH has a significant effect on microbial growth and survival. Usually, bacteria grow in pH close to neutrality (pH 6.5-7.5) but are able to tolerate a pH range of 4-9. On the other hand, yeasts are more tolerant to low pH than bacteria while molds can grow in a wide range of pH conditions (Raybaudi-Massilia et al., 2009). Therefore, microbial growth can be limited through reducing the pH (increasing the acidity) of a food by the use of an acidulant such as citric acid (Ricke, 2003).

2.5.3.1 Mechanism of action

The action of citric acid is dependent on concentration, temperature, pH and the target organism and due to the metabolic complexity of the microbial cell; citric acid is likely to affect several systems in the target microorganism (Chipley, 2005). The mechanisms of action of citric acid include: pH reduction of the growth medium due to increased proton concentration (Raybaudi-Massilia et al., 2009). Citric acid gives away protons in solution leading to the reduction in pH of solution. It is also known to disrupt the protein motive force which provides energy for nutrient absorption thereby hindering the uptake of nutrients by the microorganisms (Chipley, 2005). Citric acid also alters the cell wall of Gram-positive bacteria, causing death and is also able to chelate metal ions that are present in the cell wall of microorganisms, thereby damaging them (Helander & Mattila-Sandholm, 2000).
2.5.3.2 Clearance from the body

Excess citric acid in the body undergoes metabolism after which it is eliminated from the body (Ricke, 2003).

2.6 Microbiological Challenge Test

Microbiological Challenge Test (MCT) is a tool performed on food products to determine the growth pattern of microorganisms. MCT involves intentional introduction of the selected microorganism into the food product and incubating the food product under controlled conditions (NACMCF, 2010). The main aim of conducting a MCT on a food product is to evaluate the potential of microbial growth in that food which is the difference between the initial and final concentration of inoculated pathogens (Feroz et al., 2013).

MCT is important in assessing whether the selected pathogen is able to grow in the food product if the properties of the food such as water activity and pH are unable to control the pathogen (NACMCF, 2010). Microorganisms to be used in conducting MCT are selected based on the knowledge of the food formulation, history of the food (association with known outbreaks), intrinsic and extrinsic parameters of the food, the packaging involved and the storage temperature of the food (NACMCF, 2010). MCT has been carried out in various food products. For instance MCT carried out in potato puree at different temperatures to determine the antimicrobial activity of nisin and carvacrol against Bacillus cereus and Bacillus circulans (Rajkovic et al., 2005). One of the most important considerations that should be considered before carrying out a MCT is selection of the appropriate pathogens for the study.
2.7 Stability of β-carotene in OFSP puree during storage

In plant foods like sweetpotato, carotenoids exist as pro-vitamin A and β-carotene is the carotenoid with the highest pro-vitamin A activity of 100 % (Tanumihardjo, 2002). Processing of sweetpotatoes leads to the disruption of the food matrix rendering the carotenoids vulnerable to oxidative degradation (Kósambo, 2004). Carotenoids structures are lipophilic and unsaturated and are unstable (Gayathri et al., 2004). Degradation of carotenoids in OFSP puree during storage can result from either natural or chemical causes. The natural causes are heat, light or oxygen while chemical degradation of β-carotene occurs through oxidation and isomerization (Colle et al., 2013). In nature, carotenoids are found as trans-carotenoids. Carotenoids light exposure leads to their degradation and one of the mechanisms of light degradation is through photo oxidation which leads to production of carotenoid radical cations (Konovalova et al., 2001) and light can also excite chlorophyll leading to formation of singlet oxygen. Exposure of carotenoids to acids is known to produce ion-pairs which form carotenoid carbocations or radicals upon dissociation (Pénicaud et al., 2010) and metals such as iron and iodide are known to interact with carotenoids directly forming degradation products (He & Kispert, 2001). Thermal treatment of carotenoids in presence of oxygen leads to the formation of volatile and non-volatile compounds (Colle et al., 2010a). Isomerization occurs in the presence of heat and light exposure in which Trans carotenoids are isomerized into cis carotenoids leading to less β-carotene since cis-isomers have less pro-vitamin A activity compared to trans isomers (Deming et al., 2002). Oxidation (enzymatic and non-enzymatic) is known to be the most cause of degradation of β-carotene during storage and it takes place through free radical reactions. β-carotene can
react directly with singlet oxygen which is the excited state of molecular oxygen in air that initiates free radical reactions with food compounds (Dhuique-Mayer et al., 2007) leading to the damage of food components such as fatty acids and other unsaturated plant components (Boon et al., 2013). Unsaturated fatty acids in sweetpotato are susceptible to oxidation leading to the formation of peroxides (Bou et al., 2011). As an antioxidant, β-carotene inhibits peroxidation process through the trapping of hydroperoxides that lead to radical chain reaction propagation. During this reaction, β-carotene is oxidized into epoxides (Bechoff et al., 2010) thus reducing β-carotene content of sweetpotato or its products such as puree.

Lipid peroxides usually lead to degradation of highly unsaturated β-carotene molecules. Pénicaud et al. (2010) pointed out that sometimes the antioxidant reaction by β-carotene can reverse and therefore results into complete degradation of β-carotene by a free radical chain reaction. Tissue disruption of sweetpotato during puree making frees enzymes that oxidize and isomerize carotenoids (Rodriguez-Amaya & Kimura, 2004). Antioxidants in sweetpotato are known to protect the tissues by quenching singlet oxygen and scavenging free radicals that can be involved in lethal processes such lipid peroxidation (Pénicaud et al., 2010).

### 2.8 Knowledge gaps

No studies have been reported on the effect of combinations of potassium sorbate, sodium benzoate and citric acid on food-borne pathogens that could potentially contaminate orange fleshted sweetpotato (OFSP) puree. The stability of β-carotene in OFSP puree dosed with combinations of potassium sorbate, sodium benzoate and citric acid during storage is yet to be established.
Various studies show that preservatives such as sodium benzoate and potassium sorbate have been used in extending the shelf life of various products. Ceylan et al. (2004) studied the effect of sodium benzoate and potassium sorbate on E. coli counts in apple juice during storage. The effect of citric acid, sodium benzoate and potassium sorbate on the growth of L. monocytogenes in sweetpotato puree have been studied (Pérez-Díaz et al., 2008). However, no studies have reported the effects of combinations of potassium sorbate, sodium benzoate and citric acid on the growth and survival potential of E. coli and S. aureus which are known to contaminate OFSP puree.

Studies on the stability of β-carotene during processing and subsequent storage have been reported in products treated with preservatives. For instance, work done by Sarkar et al. (2015) showed the effects of sodium benzoate on the retention of β-carotene in tomato puree during storage. However, information on the stability of β-carotene in OFSP puree treated with combinations of potassium sorbate, sodium benzoate and citric acid during storage is not available.
Chapter Three

3 Effect of Selected Chemical Preservatives and Acidification on Microbial Growth During Storage of Orange Fleshed Sweetpotato (OFSP) Puree

3.1 Abstract

Orange Fleshed Sweetpotato (OFSP) puree is a versatile ingredient in food processing. However, it is highly perishable limiting its use in resource constrained environments. OFSP puree is susceptible to contamination by both pathogenic and non-pathogenic microorganisms during preparation. It is therefore important to develop a safe and shelf-stable OFSP puree. The current study was carried out as a challenge test to determine the effects of different combinations of chemical preservatives and acidification on microbial growth in stored OFSP puree. OFSP puree was prepared, treated with preservative combinations as follows: Non-supplemented puree (A), 0.05 % potassium sorbate + 0.05 % sodium benzoate + 1 % citric acid (B), 0.1 % potassium sorbate + 0.1 % sodium benzoate + 1 % citric acid (C), 0.2 % potassium sorbate + 0.2 % sodium benzoate + 1 % citric acid (D) and 1 % citric acid (E). Puree samples (100 g) were inoculated with Escherichia coli and Staphylococcus aureus pathogens at inoculum levels of 5.2 x 10^9 cfu/100 g and 1.5 x 10^9 cfu/100 g respectively. The samples were then evaluated during storage for 10 weeks at prevailing ambient temperature (15-25 °C) and refrigeration temperature of 4 °C. E. coli, S. aureus, total aerobic counts, yeasts and molds were evaluated during the storage period. Results showed that E. coli and S. aureus counts significantly (p<0.05) declined by 4 log cycles in all puree treatments under the storage conditions except for the control and the puree with 1 % citric acid only. Total Viable count, Yeasts and molds were completely inhibited during the storage period except for the puree with 1 % citric acid only.
Combinations of potassium sorbate, sodium benzoate and citric acid were found to control the growth of pathogenic microorganisms and ensure extensive use of the puree.

3.2 Introduction

Sweetpotato (*Ipomoea batatas*) is widely used in Kenya as an on-farm supplementary food crop in a subsistence economy (Wheatley & Loechl, 2008). In addition to being a food security crop, it helps farmers obtain cash income. In Kenya, sweetpotato roots occur in various colors ranging from white, yellow, purple and the orange that is rich in β-carotene, an important pro-vitamin A carotenoid (Fetuga *et al.*, 2013). Biofortified orange fleshed sweetpotato (OFSP) has been promoted in Kenya as an effective and sustainable source of vitamin A (Saltzman *et al.*, 2013) that can be used to mitigate vitamin A deficiency (VAD), a major public health concern in the Western and Nyanza parts of Kenya (WHO, 2009).

Sweetpotato roots are utilized in Kenya to make various products such as flour which is used as an ingredient in making of weaning food, cakes and biscuits among others and also in the making of livestock feed. The leaves are utilized as vegetables or used to make fodder for livestock (Yanggen & Nagujia, 2006). OFSP roots are processed in Kenya into puree (boiled and mashed) that is used at household level to make food products such as fried doughnuts (*mandazi*), *chapatti* and porridges for children. At commercial level OFSP puree is being used as a partial substitute for wheat flour in bakery products. There are several economic advantages of using OFSP puree compared to OFSP flour. According to Low *et al.* (2015), 1.25kg of OFSP fresh roots makes 1kg of puree while 4-5 kg of OFSP fresh roots is needed to make 1 kg of flour.
The major challenge in the use of OFSP puree is that it is difficult to store requiring refrigeration. An advanced technology of processing sweetpotato puree in the USA is done through sterilization and aseptic packaging using a continuous flow microwave system (Coronel et al., 2005) giving a shelf-life of up to 36 months. However, this system is expensive in low income countries such as Kenya where OFSP puree is processed at small-scale level. Therefore, there is an urgent need for an affordable way of producing ambient stable OFSP puree which does not require a significant cold chain to ensure a continuous supply for bakeries throughout the year. Natural preservatives which give the much desired “clean label” have been shown to be effective against microbial growth in food products (Jin et al., 2010; Theivendran et al., 2006). However, natural preservatives such as nisin and natamycin are not cost-effective for small-scale processors in Kenya.

Chemical preservatives such as sodium benzoate (E211) and potassium sorbate (E202) are used commonly worldwide to retard or stop the growth of pathogenic microorganisms in food. Potassium sorbate and sodium benzoate are permitted in food products in levels of 0.1 % (Gören et al., 2015). These two compounds have been used before in inhibiting microbial growth in food products. For instance, Jin et al. (2010) recorded the antimicrobial effect of sodium benzoate and potassium sorbate against Escherichia coli in strawberry puree. Citric acid (E330) also increases the acidity of the food product thus increasing the effectiveness of sodium benzoate and potassium sorbate since a greater proportion of the acids is in undissociated form (Mani-Lopez et al., 2012). Therefore, developing a shelf-stable OFSP puree using sodium benzoate, potassium sorbate and citric acid may be effective as well as cost effective.
Contamination of OFSP puree with *S. aureus* could take place during post process handling of the puree by operatives (Chawla & Chander, 2004). This pathogen is commonly found on human skin and can be transferred to foods if good hygienic practices are not followed. OFSP puree could also be contaminated with *E. coli* through the use of non-potable water for cleaning surfaces and equipment (Reddi *et al.*, 2015). *E. coli* has been known to survive and persist on equipment surfaces for months (Wilks *et al.*, 2006). These are real scenarios during OFSP puree processing at small-scale level in rural settings.

Therefore, the current study was designed to examine survival and growth potential of *S. aureus* and *E. coli* in preserved and stored OFSP puree. Conducting the challenge test in OFSP puree will help in establishing the ability of potassium sorbate, sodium benzoate and citric acid to inhibit the growth of *S. aureus*, *E. coli*, total aerobic microorganisms, yeast and molds in OFSP puree held under ambient (15-25 °C) and refrigeration (4 °C) conditions and therefore facilitate more extensive use of the puree. Results from this study will determine the feasibility of developing shelf-stable OFSP puree which can be stored without refrigeration. Shelf-stable OFSP puree will help reduce post-harvest losses for OFSP farmers and economic losses due to spoilage of puree during storage by consumers.
3.3 Design and Methodology

The current study employed an experimental study design by preparing orange fleshed sweet potato (OFSP) puree and carrying out microbial and analytical evaluation. The experimental study design included two independent variables: use of different combinations of preservatives and two different storage temperatures. The dependent variables were *E. coli* counts, *S. aureus* counts, Total aerobic counts, yeast and mold counts.

3.4 Preparation of OFSP puree

About 25 kg of root samples were randomly collected from a pool of five batches from farmers in Homabay-Kenya. Kabode variety which is the most adopted and widely grown OFSP varieties was used for the current study. The processed puree was packaged in 5 kilogram polyethylene bags of 300 micron and vacuum sealed before being transported overnight to the Department of Food Science, Nutrition and Technology (University of Nairobi) laboratory for evaluation. Figure 3.1 shows a flow diagram of processing sweetpotato puree. Sweetpotato roots were sorted to remove the diseased and badly damaged roots before being washed to remove soil and loose dirt. The roots were then peeled manually using knives before being trimmed to remove the fibrous ends, surface blemishes and the diseased ends of the roots. The washed roots were cut into approximately 0.5-0.75 cm slices in thickness and steamed at 100 °C for 30 minutes in a steaming pot and then cooled for 30 minutes at ambient temperature. Cooked and cooled roots were then comminuted into puree using a pureeing machine (OMAS Food Machinery-AEE1T0- Euro ingredients limited, Italy), packaged in 5 kilogram polyethylene bags and vacuum sealed using vacuum packaging machine (MINIPACK-TORRE S.p.A MVS 45X, ANNO-2015-Euro ingredients, Italy). This activity was carried out in the current puree
processing plant in Kenya and with the help of workers in the plant in order to ensure that the study utilized similar product usually produced in the set up.

Figure 3.1: Preparation of OFSP puree

3.5 Bacteria load in OFSP puree

Prior to challenging OFSP puree with specific bacteria and before treatment with preservatives, OFSP puree was assessed microbiologically for _E. coli_ and _S. aureus_ pathogens, then microwaved and assessed again. The initial enumeration of _E. coli_ and _S. aureus_ in the puree was carried out as described in previous studies (Baluka et al., 2015;
Güngör E., & Gökoğlu, 2010). Puree sample (25 g) was homogenized with 0.85 % NaCl and serial dilutions prepared up to $10^{-6}$. A volume of 0.1 mL from each dilution was spread in triplicate onto Brilliance *E. coli*/*Coliform* agar (Oxoid, Hampshire, England) and incubated at 37 °C for 24 hours for enumeration of *E. coli*. Similarly, 0.1 mL of each dilution was spread in triplicate onto Baird parker agar (Oxoid, Hampshire, England) and incubated at 37 °C for 48 hours for the enumeration of *S. aureus*. Enumeration was done for plates with 30-300 colonies. All microbial counts were expressed as mean base-10 logarithms of colony forming units per gram (log cfu/g). Data points were expressed as mean from the triplicate experiments and samples. The results indicated high levels of *E. coli* and *S. aureus* in puree before treatment with preservatives. However, after the puree sample was microwaved, the counts declined to non-detectable levels as shown in Table 3.1. This formed the basis for the level of inoculation of *E. coli* and *S. aureus* into the puree.

**Table 3.1:** Counts of bacteria in OFSP puree (log cfu/g)

<table>
<thead>
<tr>
<th></th>
<th>Raw OFSP puree (log cfu/g)</th>
<th>OFSP puree after microwaving (Log cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>6.9 ± 0.04</td>
<td>nd*</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>5.7 ± 0.05</td>
<td>nd*</td>
</tr>
</tbody>
</table>

*Each value is mean ± standard deviation for triplicate experiments. nd*=Not detected*
3.6 Microbiological challenge test

3.7 Preparation of bacterial inoculum

*Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538 pellets were obtained from the American Type Culture Collection (Microbiologics, MN 56303-USA). The pellets were activated by suspending a single pellet of each microorganism in phosphate buffer (0.1 M) and incubating at 38 °C for 30 minutes. From the buffer, 1mL was transferred to nutrient broth and incubated at 35 °C for 24 hours to allow for growth of the bacteria. The levels of inoculum obtained after plating were $5.2 \times 10^9$ cfu/mL for *E. coli* and $1.5 \times 10^9$ cfu/mL of *S. aureus*. The inoculum was then stored at -80 °C to avoid changes that may affect growth (Corry et al., 2010).

3.8 Treatment of OFSP puree samples with combination of chemical preservatives

OFSP puree was first sterilized for 3 minutes in a microwave. OFSP puree samples (100 g) were then dosed with combinations of selected chemical preservatives as shown in Table 3.1.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Puree without preservatives</td>
</tr>
<tr>
<td>B</td>
<td>Puree with 0.05 % sodium benzoate + 0.05 % potassium sorbate + 1 % citric acid</td>
</tr>
<tr>
<td>C</td>
<td>Puree with 0.1 % sodium benzoate + 0.1 % potassium sorbate + 1 % citric acid</td>
</tr>
<tr>
<td>D</td>
<td>Puree with 0.2 % sodium benzoate + 0.2 % potassium sorbate + 1 % citric acid</td>
</tr>
<tr>
<td>E</td>
<td>Puree with 1 % citric acid</td>
</tr>
</tbody>
</table>
3.9 **Inoculation strategy and growth assessment**

Puree samples (100 g) treated with preservative combinations and that without preservatives were inoculated with 1000 µL of bacterial suspension containing $5.2 \times 10^9$ cfu of *E. coli* and $1.5 \times 10^9$ cfu of *S. aureus* resulting in a load of $5.2 \times 10^7$ cfu (7.8 log cfu) of *E. coli/*g of puree and $1.5 \times 10^7$ cfu (7.2 log cfu) of *S. aureus/*g of puree and vacuum sealed. Some inoculated samples were incubated at ambient (15-25 °C) and others at refrigeration temperatures (4 °C) with enumeration of bacterial load at weekly intervals. Serial dilutions of all samples were prepared up to $10^{-6}$ and each dilution plated in triplicate for the different tested microorganisms. The experiments were performed independently three times.

3.10 **Determination of total viable count (TVC) and yeast and moulds in OFSP puree during storage**

OFSP puree was prepared as shown in Figure 3.1 with the addition of selected chemical preservatives (as in Table 3.1 section 3.8) before packaging in 100 g polyethylene bags. Some of the puree was packaged without preservatives and analyzed for TVC and yeast and molds. The puree samples (100 g) were then stored at ambient temperature of (15-25 °C) and refrigeration temperature of (4 °C) with TVC and yeast and mold evaluation weekly for a period of 10 weeks puree storage. A sample of puree (25 g) was placed into 225 mL of sterile saline solution (0.85 % NaCl), vortexted for 1 minute to homogenize and serially diluted to a dilution of $10^{-7}$. TVC was determined by transferring 1 mL of each sample dilution to sterile Petri dishes in triplicates to which approximately 20 mL of Plate Count Agar (PCA, LAB, UK) were added. The plates were swirled and allowed to solidify before
being incubated at 30 °C for 72 hours (Pérez-Díaz et al., 2008). Yeasts and moulds were
determined by spread plating 0.1 mL of each sample dilution in triplicate onto Dichloran-
Rose Bengal Chloramphenicol (DRBC) agar (Oxoid, Hampshire). The plates were incubated
at 25 °C for 5 days (Landl et al., 2010). Enumeration was done for plates with 30-300
colonies. All microbial counts were expressed as mean base-10 logarithms of colony
forming units per gram (log cfu/g). Data points were expressed as mean from the triplicate
experiments and results expressed as logarithm of colony forming units per gram (log
cfu/g). The experiment was performed three times independently.

3.11 Determination of pH in OFSP puree during storage

One gram of OFSP puree sample was homogenized in 1mL of distilled water in a test tube. The
pH values of the samples were measured using pH meter (model HI 98107, USA) by immersing
the electrode directly into the sample in the test tube. Before the measurements, pH meter was
calibrated using pH 4.0 and 7.0 buffers.

3.12 Statistical analysis

Experiments were carried out in triplicates and quality control measures were taken into
account. Data were analyzed by analysis of variance (ANOVA) using SPSS software (Version
20.0 SPSS Inc). Tukey test was used to determine the significant difference of mean values.
The significance level was expressed at 5 % level. Microsoft Excel was used to draw line
graphs on the growth of microorganisms.
3.13 Results

3.13.1 Changes in pH in OFSP puree during storage

The initial pH of OFSP puree was 5.23 before treatment with preservatives. Addition of different combinations of sodium benzoate and potassium sorbate and citric acid led to a decline in pH of the puree kept at ambient (15-25 °C) and refrigeration (4 °C) conditions as shown in Table 3.2.

Immediately after treatment of puree with preservatives, the highest pH values of 5.19 and 5.18 were obtained in the control sample at ambient and refrigeration temperatures respectively, while least values of 4.60 and 4.61 were recorded in the sample with 1 % citric acid at ambient and refrigeration temperatures respectively. At the end of the storage period, highest pH values of 4.99 and 4.63 were recorded in the control sample, while least values of 3.94 and 3.98 were obtained in samples with 1 % citric acid at ambient and refrigeration temperatures respectively. Significant differences (p<0.05) were observed in different treatments in a specific storage time.
Table 3.2: Changes in pH in OFSP puree during storage at ambient (15-25 °C) and refrigeration temperature (4 °C)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Storage Temperature</th>
<th>0 Days</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 5</th>
<th>Week 7</th>
<th>Week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Ambient</td>
<td>5.18±0.02f</td>
<td>5.12±0.02f</td>
<td>5.10±0.01d</td>
<td>5.07±0.02g</td>
<td>5.02±0.02e</td>
<td>4.99±0.04g</td>
<td>4.99±0.03g</td>
</tr>
<tr>
<td></td>
<td>Refrigeration</td>
<td>5.19±0.01f</td>
<td>5.18±0.01f</td>
<td>5.17±0.02d</td>
<td>5.15±0.01h</td>
<td>5.12±0.02f</td>
<td>5.13±0.04h</td>
<td>4.63±0.06f</td>
</tr>
<tr>
<td>B</td>
<td>Ambient</td>
<td>4.70±0.01b</td>
<td>4.60±0.01c</td>
<td>4.64±0.03bc</td>
<td>4.57±0.02e</td>
<td>4.23±0.02b</td>
<td>4.19±0.01c</td>
<td>4.15±0.03b</td>
</tr>
<tr>
<td></td>
<td>Refrigeration</td>
<td>4.82±0.02c</td>
<td>4.76±0.02d</td>
<td>4.64±0.01bc</td>
<td>4.40±0.01c</td>
<td>4.39±0.01c</td>
<td>4.24±0.01cd</td>
<td>4.22±0.01bc</td>
</tr>
<tr>
<td>C</td>
<td>Ambient</td>
<td>4.74±0.02b</td>
<td>4.55±0.02b</td>
<td>4.47±0.01bc</td>
<td>4.41±0.01c</td>
<td>4.37±0.02c</td>
<td>4.30±0.01de</td>
<td>4.28±0.01cd</td>
</tr>
<tr>
<td></td>
<td>Refrigeration</td>
<td>4.87±0.01cd</td>
<td>4.78±0.01de</td>
<td>4.62±0.02bc</td>
<td>4.48±0.01d</td>
<td>4.36±0.01c</td>
<td>4.28±0.01d</td>
<td>4.29±0.03de</td>
</tr>
<tr>
<td>D</td>
<td>Ambient</td>
<td>4.90±0.01de</td>
<td>4.82±0.03e</td>
<td>4.67±0.06bc</td>
<td>4.64±0.04f</td>
<td>4.56±0.02d</td>
<td>4.40±0.01f</td>
<td>4.39±0.01e</td>
</tr>
<tr>
<td></td>
<td>Refrigeration</td>
<td>4.95±0.03e</td>
<td>4.81±0.01de</td>
<td>4.76±0.01c</td>
<td>4.53±0.01e</td>
<td>4.40±0.01c</td>
<td>4.37±0.01ef</td>
<td>4.36±0.02de</td>
</tr>
<tr>
<td>E</td>
<td>Ambient</td>
<td>4.60±0.05a</td>
<td>4.45±0.03a</td>
<td>4.37±0.02a</td>
<td>4.28±0.01a</td>
<td>4.06±0.06a</td>
<td>3.95±0.04a</td>
<td>3.94±0.04a</td>
</tr>
<tr>
<td></td>
<td>Refrigeration</td>
<td>4.61±0.03a</td>
<td>4.54±0.03b</td>
<td>4.38±0.24a</td>
<td>4.36±0.01b</td>
<td>4.26±0.01b</td>
<td>4.10±0.06b</td>
<td>3.98±0.07a</td>
</tr>
</tbody>
</table>

Each value is mean ± standard deviation for triplicate samples. Values in a column with different superscript letters are significantly different (p<0.05). A=Puree without preservatives, B=puree with 0.05% sodium benzoate + 0.05% potassium sorbate + 1% citric acid, C=puree with 0.1% sodium benzoate + 0.1% potassium sorbate + 1% citric acid, D=puree with 0.2% sodium benzoate + 0.2% potassium sorbate + 1% citric acid, E=puree with 1% citric acid.
3.13.2 Growth and survival of *Escherichia coli* in stored OFSP puree

Figure 3.2 shows the growth of *E. coli* in OFSP puree treated with a combination of selected chemical preservatives and that without preservatives and stored at ambient (15-25 °C) and refrigeration temperatures (4 °C) for 10 weeks.
Figure 3.2: Growth of *E. coli* in OFSP puree with preservatives during storage at ambient temperatures (15-25 °C) and refrigeration temperature (4 °C). **PS**=Potassium sorbate, **SB**=sodium benzoate, **CA**=citric acid

In non-supplemented puree, *E. coli* counts increased significantly after inoculation by 2 logs with subsequent increase in storage from 7 log cfu/g to 9 log cfu/g. All combinations of potassium sorbate, sodium benzoate and 1 % citric acid led to a significant (p<0.05) 4 log
reduction in *E. coli* counts in puree kept at ambient temperature from 7 log cfu/g to 3 log cfu/g while 1 % citric acid gave a 3 log reduction from 7 log cfu/g to 4 log cfu/g.

Similarly, preservative treatment with all combinations of potassium sorbate, sodium benzoate and citric acid led to a significant (p<0.05) reduction in the *E. coli* population by 4 log cycles (from 7 log cfu/g to 3 log cfu/g) in OFSP puree stored at refrigeration temperature. 1 % citric acid also resulted in a 4 log reduction in the numbers of *E. coli*. *E. coli* counts in non-supplemented puree increased significantly by 2 logs immediately after inoculation with subsequent increase in counts before declining after 3 weeks of puree storage from 7 log cfu/g to 6 log cfu/g. There was no significant difference (p>0.05) in *E. coli* populations in OFSP puree with different combinations of sodium benzoate, potassium sorbate and citric acid in the two storage conditions.

### 3.13.3 Growth and survival of *Staphylococcus aureus* in stored OFSP puree

Figure 3.3 shows the growth of *S. aureus* in OFSP puree treated with a combination of selected chemical preservatives and that without preservatives and stored at ambient (15-25 °C) and refrigeration temperatures (4 °C) for a period of 10 weeks.
Figure 3.3: Growth of *S. aureus* in OFSP puree with preservatives during storage at ambient temperatures (15-25 °C) and refrigeration temperature (4 °C). PS=Potassium sorbate, SB=sodium benzoate, CA=citric acid
Combined use of sodium benzoate and potassium sorbate at different concentrations with 1 % citric acid led to a 4-log reduction in *S. aureus* counts in OFSP puree kept at ambient and refrigeration temperatures from 7 log cfu/g to 3 log cfu/g. *S. aureus* counts in non-supplemented puree kept at ambient temperature recorded a 2-log increase immediately after inoculation with subsequent increase in counts during storage from 7 log cfu/g to 9 log cfu/g while that at refrigeration recorded 2-log increase after inoculation with subsequent decline in counts during storage from 7 log cfu/g to 6 log cfu/g.

The treatment with 1 % citric acid recorded a reduction in *S. aureus* population by 1 log cycle at ambient temperature from 7 log cfu/g to 6 log cfu/g and by 2 log cycles at refrigeration temperature from 7 log cfu/g to 5 log cfu/g. Treatment of OFSP puree with potassium sorbate and sodium benzoate at different concentrations together with 1 % citric acid had a slightly greater effect on *S. aureus* growth compared to 1 % citric acid when used alone.

### 3.13.4 Growth of aerobic microorganisms in stored OFSP puree

Figure 3.4 shows the growth of aerobic microorganisms in OFSP puree with and without preservatives and stored at ambient (15-25 °C) and refrigeration temperatures (4 °C) for 10 weeks.
Figure 3.4: Total aerobic microorganisms in OFSP puree with preservatives during storage at ambient temperatures (15-25 °C) and refrigeration temperature (4 °C). PS=Potassium sorbate, SB=sodium benzoate, CA=citric acid

OFSP puree was found to contain high levels of bacteria (9.0 log cfu/g) immediately after preparation. The counts declined significantly (p<0.05) from 9 log cfu/g to non-detectable levels at the end of the storage period in OFSP puree treated with different combinations of potassium sorbate and sodium benzoate together with 1 % citric acid both at ambient and
refrigeration conditions. However, in puree with 1 % citric acid at ambient temperature, aerobic microorganisms declined from 9 log cfu/g to 3 log cfu/g at week 10 of puree storage.

3.13.5 Growth of yeasts and moulds in OFSP puree during storage

Figure 3.5 shows the growth of yeast and molds in OFSP puree with and without preservatives and stored at ambient (15-25 °C) and refrigeration temperatures (4 °C) for 10 weeks.
Figure 3.5: Microbial counts of yeast and molds in OFSP puree with and without preservatives during storage at ambient temperatures (15-25 °C) and refrigeration temperature (4 °C). PS= Potassium sorbate, SB=sodium benzoate, CA=citric acid

OFSP puree contained a total of 7.92 log cfu/g yeasts and molds immediately after preparation. The counts declined significantly (p<0.05) with storage from 7.92 log cfu/g to non-detectable levels at week 7 of puree storage in all the treatments kept at ambient temperature except for the puree with 1 % citric acid in which yeast and mold counts declined from 7.92 log cfu/g to 5 log cfu/g at week 10 of puree storage. For the puree kept...
at refrigeration temperature, yeast and mold counts declined significantly (p<0.05) from 7.92 log cfu/g to non-detectable levels at week 3 of puree storage.

Pack distention and alcoholic odors were noted in OFSP puree treated with citric acid only after one week of storage.

3.14 Discussion

3.14.1 Effect of pH on microbial growth in orange fleshed sweetpotato (OFSP) puree

Preservative effectiveness is dependent on among other factors, the pH of the product (Stanojevic et al., 2009) and pH is also one of the factors that determine the growth and survival of microorganisms during processing and storage (Beales, 2004). The interest of food processors is to determine the pH of a food product and maintain that pH at a certain level in order to control microbial growth thus preventing product spoilage (Ricke, 2003). The reduction in pH of puree treated with preservatives was mainly because of addition of citric acid. pH is important to the antimicrobial effect of potassium sorbate and sodium benzoate because their effect is due to the undissociated form of their molecule which is dependent on pH (Lotte Dock et al., 2000). Preservatives such as sodium benzoate and potassium sorbate have also been shown to have an effect on the pH of a food product and therefore the decrease in pH of puree during storage would also be attributed to the presence of preservatives (Beales, 2004).

3.14.2 Growth and survival of Escherichia coli and Staphylococcus aureus in OFSP puree

Information on the survival potential of pathogenic microorganisms in orange fleshed sweetpotato (OFSP) puree is limited and such knowledge would be of significance in OFSP
puree storage period by the consumer further contributing to food safety and quality. If post processing contamination of OFSP puree by *E. coli* and *S. aureus* would occur, the data presented indicate that these pathogens would grow extensively in the puree under ambient temperature assuming a 10-week storage period. The increase in the counts of *E. coli* and *S. aureus* in non-supplemented puree (control) would be attributed to nutrient availability and favorable environment for their growth (Olaimat & Holley, 2012) which include the pH and water activity.

The decline in *E. coli* and *S. aureus* populations in stored OFSP puree with preservatives can be attributed to various factors. Sodium benzoate and potassium sorbate activity is largely dependent on the pH of a food product. The optimum inhibitory activity of these preservatives takes place at low pH which favors the undissociated form of the molecule that freely moves across the plasma membrane into the cytoplasm (López-Malo et al., 2007). The low pH of the puree was achieved through the addition of citric acid in combination with the preservatives. Due to the neutral pH of the cytoplasm, the acid dissociates into anions and protons. These molecules are not able to diffuse back across the cell membrane hence accumulate in the cytoplasm. Acidification of the cytoplasm and the energy depletion leads to physiological malfunction finally inhibiting microbial growth (Ghaly et al., 2010; Hazan et al., 2004). In addition to the preservatives, the storage conditions and vacuum packaging of the puree also contributed towards inactivation of *E. coli* and *S. aureus* in the puree. The results agreed with those reported by Chikthimmah et al. (2003), who demonstrated that the use of chemical preservatives was critical for a significant reduction in *E. coli* counts in apple cider stored under ambient and refrigeration conditions.
The antimicrobial activity of the citric acid would be due to reduction of pH of OFSP puree below the optimal range of pH values for *E. coli* and *S. aureus* growth which is 6-7 or due to the disruption of the pathogens’ membrane permeability thus preventing entry of essential nutrients for its growth. Other researchers have demonstrated the effectiveness of citric acid in inhibiting the growth of *S. aureus*. For instance, Seo *et al.* (2013) found that 2 % citric acid was the most effective in reducing counts of *S. aureus* in chicken meat in 5 days. Work done by Abu-ghazaleh (2013) showed that 0.03 % citric acid significantly inhibited *S. aureus* growth in growth medium after 24 hours of incubation. Similarly, the use of 2 % citric acid alone on chicken meat led to the decline in *E. coli* counts by 4 log cycles in 12 hours (Seo *et al.*, 2013).

There were no significant differences (p>0.05) in *E. coli* and *S. aureus* populations in OFSP puree with different combinations of sodium benzoate, potassium sorbate and citric acid in the two storage conditions. This suggests that even the lowest concentration of preservatives used in combinations was effective in inhibiting the growth of *E. coli* and *S. aureus* in OFSP puree during the storage period. There was a slightly better inhibition of *E. coli* and *S. aureus* in treatments with different combinations of potassium sorbate, sodium benzoate and citric acid as compared to citric acid alone at ambient and refrigeration temperatures. This suggests that combination of a number of hurdles (preservative factors) gives higher or multiple inhibitory effects against microorganisms compared to a single hurdle. According to Lotte Dock *et al.* (2000), the effect of combined treatments with preservatives in apple cider was significantly greater than that of a single preservative used alone. For instance, antimicrobial activity against *E. coli* was enhanced through the combined use of 0.1 % potassium sorbate and 0.1 % sodium benzoate at 8 °C with survival.
time being reduced by 50 % compared with the one with 0.1 % sodium benzoate alone (Lotte Dock et al., 2000).

Temperature is also known to be one of the significant factors affecting microbial growth in food products (Islam et al., 2002). The enzyme activity of microorganisms is optimum at a certain temperature range beyond which the enzyme undergoes denaturation thus microbial growth inhibited. As expected, growth of *S. aureus* and *E. coli* was more rapid at 25 °C compared to 4 °C. This is because at low temperatures, the fluidity of the cytoplasmic membrane of microorganisms is reduced thus interfering with transport mechanisms (Valík et al., 2008). Therefore, microbial growth rate increases with increasing temperature until the maximum temperature for growth is reached (Fujikawa et al., 2004). *E. coli* is able to grow at a temperature range of 4-45 °C with an optimum of 37 °C but can survive refrigeration and freezing temperatures. A study carried out on the effect of temperature on the growth of *E. coli* revealed that it can grow and survive well on a range of temperatures but can grow well at 37 °C compared to other temperatures (Nguyen, 2006). *S. aureus* on the other hand grows on a temperature range of 7-48 °C with an optimum of 37 °C (Lindqvist et al., 2002).

### 3.14.3 Growth of aerobic microorganisms, yeast and molds in OFSP puree

The high levels of aerobic microorganisms in OFSP puree before treatment with preservatives would be attributed to poor handling during preparation. The reduction in levels of aerobic microorganisms in OFSP puree samples treated with different combinations of potassium sorbate, sodium benzoate and citric acid indicates the benefits of the combination of antimicrobial chemicals having multiple effects against bacterial...
growth in OFSP puree. Vacuum packaging of the puree eliminates oxygen which is essential for the growth of aerobic microorganisms thus eliminating them even in the non-supplemented puree. Other researchers showed the effect of preservatives on the growth of aerobic microorganisms. For instance, Ogiehor & Ikenebomeh, (2005) recorded a decline in aerobic microorganisms in garri product treated with 0.2 % sodium benzoate stored for 6 months at 30 °C. Results obtained by Udobi et al. (2011) showed that 0.1 % sodium benzoate along with refrigeration was able to inhibit aerobic microorganism multiplication of up to 13 days of storage while at room temperature the inhibition lasted for only 4 days.

The complete inhibition of yeast and molds in OFSP puree with and without preservatives would be attributed to the vacuum packaging of the puree that eliminates oxygen and therefore prevents the growth of molds and oxidative yeasts since they do not grow in the absence of oxygen (Sperber & Doyle, 2009). However, yeast and molds counts were still detected in puree with 1 % citric acid and kept at ambient temperature even at week 10 of puree storage, the packages were found to have expanded after 1 week of storage and the puree was characterized by alcoholic odours. This could be attributed to the growth of fermentative yeasts and/or lactic acid bacteria in the puree metabolizing simple sugars into ethanol and carbon dioxide. According to Rawat (2015), yeasts can grow at very low pH values. Other researchers have demonstrated the effect of preservatives on fungal growth in food products. For instance, Omojowo et al. (2009) reported that 3-5 % potassium sorbate led to a decline in levels of yeast and molds in smoked fish stored for 8 weeks. A study by Guynot et al. (2005) demonstrated that potassium sorbate at concentrations of 0.15-0.30 % were effective in preventing fungal growth.
3.15 Conclusion

In the present study, it was found that sodium benzoate, potassium sorbate and citric acid at different concentrations are able to retard the growth of *E. coli* and *S. aureus* pathogens in OFSP puree during storage. Citric acid when used alone was also found to be effective in retarding the growth of these pathogens. These preservative combinations were found to be effective in stopping the growth of aerobic microorganisms, yeast and moulds in stored OFSP puree. However, citric acid on its own did not eliminate aerobic microorganisms and yeast and moulds in puree kept under ambient temperature.

3.16 Recommendations

The treatments with combinations of sodium benzoate, potassium sorbate and citric acid were found to be the best combination in controlling growth of pathogenic microorganisms and ensuring extensive use of the puree compared to the use of citric acid alone. These treatments are therefore recommended for use by producers since they are cost effective and the low levels do not alter the sensory characteristics of the puree.
Chapter Four

4 Stability of β-carotene in Orange Fleshed Sweetpotato (OFSP) Puree Treated with Selected Chemical Preservatives During Storage

4.1 Abstract

Orange fleshed sweetpotato (OFSP) puree is used as an ingredient in bakery products to increase Vitamin A consumption due to its high β-carotene content. There is limited information regarding the stability of β-carotene in OFSP puree treated with chemical preservatives during storage. β-carotene retention during processing of OFSP roots into puree and the storage of puree for incorporation into products is important in defining the importance of Vitamin A consumption of OFSP puree formulated products. The current study was carried out to determine the stability of β-carotene in OFSP puree with combinations of sodium benzoate, potassium sorbate and citric acid during storage. Puree was prepared, dosed with preservative combinations and kept at ambient (15-25 ºC) and refrigeration (4 ºC) temperatures for eight weeks with baseline and biweekly β-carotene analysis. The treatments were: non-supplemented puree (A), 0.05 % potassium sorbate + 0.05 % sodium benzoate + 1 % citric acid (B), 0.1 % potassium sorbate + 0.1 % sodium benzoate +1 % citric acid (C), 0.2 % potassium sorbate + 0.2 % sodium benzoate + 1 % citric acid (D), 1 % citric acid (E). Results indicated that β-carotene content decreased significantly (p<0.05) with storage period at all conditions. At the end of the storage period, the highest β-carotene content of 6.66 mg/100 g was found in OFSP puree with 0.2 % sodium benzoate + 0.2 % potassium sorbate + 1 % citric acid while the lowest of 5.21 mg/100 g was found in puree with 0.1 % sodium benzoate + 0.1 % potassium sorbate + 1
\% citric acid at ambient temperature. At refrigeration temperature, the highest content of β-carotene of 6.70 mg/100 g was found in puree treated with 1 \% citric acid while the lowest of 5.37 mg/100 g was found in puree with 0.05 \% sodium benzoate + 0.05 \% potassium sorbate + 1 \% citric acid. Low temperatures of puree storage together with combinations of preservatives ensure minimal β-carotene loss.

4.2 Introduction

Sweetpotato (Ipomea batatas) is a staple crop in most parts of Africa. They are very nutritious with minerals, vitamins, anthocyanins, tocopherols and β-carotene (Donado-pestana et al., 2012). Sweetpotato roots are in various fleshed colors depending on the carotenoid content with the orange fleshed having β-carotene as the major carotenoid (Vimala et al., 2011). The orange fleshed varieties have been introduced to communities in sub-Saharan Africa (SSA) in order to alleviate Vitamin A Deficiency (VAD). This is because these varieties are rich in β-carotene, easy to grow and are relatively cheaper sources of vitamin A for most poor families in SSA (Anderson et al., 2007). The International Potato Center (CIP) tested some varieties of orange fleshed sweetpotato (OFSP) that yielded up to 8000 µg/100 g β-carotene of the roots on fresh weight basis (Tumwegamire et al., 2004).

Sweetpotato is an important crop in various parts of Kenya for household consumption and as a source of income from small businesses. The main types grown are the white and the yellow fleshed (Kaguongo et al., 2012). The orange fleshed sweetpotato (OFSP) varieties have been introduced in these communities to help in alleviating vitamin A deficiency (VAD) because they have high β-carotene content which is a pro-vitamin A carotenoid (Low et al., 2007). Sweetpotatoes are utilized in various ways in Kenya. OFSP varieties are
processed into puree (boiled and mashed) that is incorporated in the making of bakery products such as bread and buns. Provitamin-A carotenoids are unsaturated and unstable and are therefore easily degraded by UV light, heat and oxygen and are influenced by water activity (Boon et al., 2013).

With the increasing awareness of the health benefits of vitamin A, the stability of β-carotene during processing and the subsequent storage has drawn much attention lately. Data from literature (Zanoni et al., 2003), states that β-carotene generally remains stable during processing practices with the exception of extreme conditions like long heating times or very high temperatures. Carotenoids have double bonds in their carbon chain which make them susceptible to reactions such as isomerization (cis-trans) and oxidation during processing and storage of foods (Rao & Rao, 2007). Several factors have been named that affect the stability of β-carotene in products during storage which includes presence of metals, oxygen, heat treatment, exposure to light among others (Provesi et al., 2011). Hence, the stability of β-carotene varies greatly in foods during storage and therefore it is very important to understand the β-carotene losses that occur in OFSP puree during storage inorder to achieve products that are of adequate nutritional quality.

Knowledge on the mechanisms of β-carotene degradation in OFSP puree during storage is important in order to be able to come up with technologies that will limit or decrease the loss for a good nutritional quality product. Some of the approaches that lead to reduced β-carotene loss include the addition of ascorbic acid to foods such as vegetables purees since its interaction with carotenoids leads to decreased oxidation (Gliemmo et al., 2009). Another approach is the choice of packaging material whereby the nature of the packaging
material affects the content of β-carotene since certain characteristics such as light, presence of metals and oxygen are some of the factors affecting degradation of β-carotene (Hymavathi & Khader, 2005).

Many studies have been carried out correlating to effects of processing and storage conditions on stability of β-carotene (Vásquez-Caicedo et al., 2007b). For instance, Sarkar et al. (2015) studied the effects of sodium benzoate and storage conditions on retention of β-carotene in tomato puree. However, there is no information on the stability of β-carotene in OFSP puree with preservatives during storage. In order to limit the loss of β-carotene in OFSP puree during storage, preparation of puree with selected combinations of chemical preservatives followed by vacuum packaging is important. There is therefore a need to determine β-carotene stability in OFSP puree treated with combinations of preservatives during storage. The objective of the current study was to evaluate the stability of β-carotene in OFSP puree treated with different combinations of sodium benzoate, potassium sorbate and citric acid during storage at ambient temperature (15-25 °C) and refrigeration temperature (4 °C).
4.3 Design and Methodology

The current study employed an experimental study design by preparing orange fleshed sweet potato (OFSP) puree and carrying out analytical evaluation. The experimental study design included two independent variables: use of different combinations of preservatives and two different storage temperatures. The dependent variable was β-carotene content.

4.4 Preparation of OFSP puree

OFSP puree was prepared as shown in Figure 3.1 (Section 3.4) and dosed with combinations of chemical preservatives as shown in Table 3.1 (Section 3.8) before being packaged and vacuum sealed in 100 g polyethylene bags. The samples were then stored at ambient temperature (15-25 °C) and refrigeration temperature (4 °C) and analyzed for β-carotene content biweekly for a period of eight weeks.

4.5 Chemicals and standards

The solvents used in the analysis were all HPLC grade. These were: Methanol (Sigma-Aldrich, USA), Tetrahydrofuran (Sigma-Aldrich, USA), Hexane (Sigma-Aldrich, USA), Ethanol (Sigma-Aldrich, USA) and tert-butyl methyl ether (Sigma-Aldrich, USA). Analytical standards of β-carotene (Sigma-Aldrich, USA) were used to calibrate and quantify the carotenoids.

4.6 Sample extraction

Samples were analyzed in triplicate as described in the method by Pillay et al., (2011) but with slight modifications. Extraction was performed by incubating 1 g of sample with 5 mL of methanol in water bath (SW23GB, JULABO) at 70 °C for 1 hour. The mixture was vortexed for 1 minute and centrifuged (Eppendorf, Centrifuge 5810, Germany) for 10
minutes at 3000 rpm. Methanol layer was transferred into a 25 mL volumetric flask and the sample extracted using 10 mL Tetrahydrofuran (THF) with vortexing and Centrifugation steps. Extraction was repeated three more times using 5 mL of THF at each time with the layers being transferred into the volumetric flask. Methanol was added to make final volume to 25 mL. To 3 mL of the extract was added 3 mL of methanol, 5 mL of hexane and 5 mL of HPLC water in a 25 mL glass tube. The mixture was vortexed for 1 minute and centrifuged at 3000 rpm for 3 minutes. The upper phase was transferred into a 15 mL glass tube and dried completely under nitrogen gas using N-Evap machine (Organomation, Model OA-8125, USA and a water bath set at 40 °C. Dried tubes were reconstituted with 1 ml of ethanol. The tubes were vortexed and sonicated (JIRCAS, 101489, JAPAN) for 30 seconds before transferring 1 mL to HPLC vials.

4.7 Analysis of carotenoids

Carotenoid analysis was carried out by the use of Waters 2695 HPLC separation module consisting of 2996 PDA detector. The carotenoids were separated on a C$_30$ carotenoid column (3µm, 150X4.6 mm, YMC Wilmington, NC) utilizing a reverse phase gradient HPLC method. Two mobile phases were employed consisting of mixtures of methanol, tert-butyl methyl ether and water (Mobile phase A: 85:12:3, v/v/v, with 1.5 % ammonium acetate in the water) and (Mobile Phase B: 8:90:2, v/v/v, with 1 % ammonium acetate in the water). Gradient Elution Programme was set with (i) 0-1 min with 100 % A, (ii) 1-10 min with linear gradient to 90 % A, (iii) 10-22min with a linear gradient to 45 % A, (iv) 22-33min with a linear gradient to 5 % A, (v) 33-37 min held at 5 % A, (vi) 37-39 min with a linear gradient to 100 % A, (Vii) 39-40 min held at 100 % A. The injection volume was 50 µl and the carotenoids were monitored at a wavelength of 450 nm. All Trans and cis β-carotene in
the samples were identified by comparing their peak retention times and absorption spectra with that of known β-carotene standards. The concentration of β-carotene standards was calculated using its absorption coefficient (A1 %) which is 2,620 for all-trans β-carotene in ethanol at 450 nm (Rodriguez-Amaya & Kimura, 2004).

4.8 Statistical analysis

The experiments were carried out in triplicate and quality control measures were taken into account. The results were expressed in terms of mean. Results were analyzed using SPSS (Version 20.0 SPSS Inc.) where one-way analysis of variance (ANOVA) was performed. The significant differences between means were evaluated by Tukey test with p<0.05 used as the level of significance. Microsoft excel was used for presenting data in line graphs.
4.9 Results

Figure 4.1 shows the content of β-carotene in OFSP puree samples with preservatives and kept at ambient conditions (15-25 °C). The content of β-carotene in OFSP puree before treatment with preservatives was 9.88 mg/100 g. At the end of the storage period, the highest β-carotene content of 6.66 mg/100 g was found in OFSP puree with 0.2 % sodium benzoate + 0.2 % potassium sorbate + 1 % citric acid while the lowest of 5.21 mg/100 g was found in puree with 0.1 % sodium benzoate + 0.1 % potassium sorbate + 1 % citric acid.
Figure 4.1: Levels of β-carotene in OFSP puree during storage at ambient temperatures (15-25 °C). The bars represent standard error of means (n=3).

Figure 4.2 shows the content of β-carotene in OFSP puree samples with preservatives and kept at refrigeration temperature (4 °C). At the end of the storage period, the highest content of β-carotene of 6.70 mg/100 g was found in puree treated with 1 % citric acid while the lowest of 5.37 mg/100 g was found in puree with 0.05 % sodium benzoate + 0.05 % potassium sorbate + 1 % citric acid.
Significant differences (p<0.05) in β-carotene content at the end of the storage period were observed only between samples preserved with 0.1 % potassium sorbate + 0.1 % sodium benzoate + 1 % citric acid and those with 0.2 % potassium sorbate + 0.2 % sodium benzoate + 1 % citric acid at ambient temperature. At refrigeration temperature, significant differences were observed between samples with 0.05 % potassium sorbate + 0.05 % sodium benzoate + 1 % citric acid and those with 1 % citric acid only.
4.10 Discussion

All the puree samples dosed with different combinations of sodium benzoate, potassium sorbate and citric acid and kept at ambient and refrigeration temperatures recorded a significant (p<0.05) decline in β-carotene content throughout the storage period. Even though, β-carotene was not completely degraded during the storage period. In spite of the significant β-carotene reductions, OFSP puree had good amount of β-carotene left which could be a rich source of β-carotene. The lowest β-carotene content of 5.21 mg/100g at the end of the storage period corresponds to Retinol Activity Equivalence (RAE) of 434.17 using the conversion factor of 12 µg β-carotene to 1 µg retinol which was based on the bioefficacy of β-carotene in mixed diet in developed countries (Trumbo et al., 2001). This is more than enough for children under the age of 10 and half requirement for adults (FAO/WHO, 2002).

The stability of β-carotene is affected by oxidation and isomerization reactions during both processing and storage. Factors such as light, presence of metals, oxygen, temperature, the type of packaging, enzymes and pro-oxidants can lead to β-carotene degradation during processing and storage (Truong, & Avula, 2010). These factors lead to degradation of β-carotene eventually resulting to loss of nutritional quality of OFSP puree. Some of the factors that would have contributed to significant β-carotene content in OFSP puree during the storage period include the packaging of puree in polythene bag with vacuum sealing. Vacuum packaging removes oxygen from the package and therefore little oxygen was present thus protecting the puree against oxygen and light leading to minimal loss of β-carotene through oxidation.
Another factor is the storage of puree under low temperatures (ambient and refrigeration temperatures). Low temperatures are known to slow down chemical reactions such as enzymatic reactions and therefore assist in maintaining the quality of the puree. According to studies by Tang & Chen (2000) and Cinar (2004), a simple fridge (4°C) was able to preserve β-carotene content of sweetpotato. There may also be possible presence of trace antioxidants in the puree thereby deactivating free radicals that may lead to oxidation and loss of β-carotene. Other researchers have studied β-carotene degradation during storage of different food products. For instance, Sarkar et al. (2015) recorded a 48.8 % decrease in β-carotene content in tomato pulp with 0.05 % and 0.1 % sodium benzoate during storage. Work done by Lin & Chen (2005) showed loss of β-carotene in tomato juice during storage.

4.11 Conclusion

This study showed that levels of β-carotene in OFSP puree are susceptible to loss during storage. Even though, the storage of puree at ambient and refrigeration conditions together with the combination of preservatives achieved significant stability of β-carotene during storage of OFSP puree and therefore nutritional quality of the puree.

4.12 Recommendations

The study was only carried out for 8 weeks and therefore more research is recommended for prolonged storage of puree to determine the stability of β-carotene during storage.
Chapter Five

5 General Conclusions and Recommendations

5.1 Conclusions
The study sought to investigate the effect of different combinations of preservatives on microbial growth and stability of β-carotene in OFSP puree at different storage conditions and time. Different combinations of sodium benzoate, potassium sorbate and citric acid improved microbial keeping quality and inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* in OFSP puree as indicated by the microbial challenge test. Use of citric acid alone was less effective in controlling the growth of these pathogens. There was a significant loss of β-carotene in OFSP puree during storage but the loss was not significant between the two storage conditions.

5.2 Recommendations
Adoption of the use of combinations sodium benzoate, potassium sorbate and citric acid preservatives would produce a shelf-stable orange fleshed sweetpotato puree. The challenge study was limited to only 10 weeks and therefore there is no information on the tentative time the pathogens were not recovered from the product. Further studies should therefore investigate the effect of the combinations of these preservatives on microbial growth in orange fleshed sweetpotato puree when stored for more than 10 weeks.

The study focused on only two pathogens. Further studies focusing on the effect of the preservatives on the growth of other pathogens that can be introduced to puree during
preparation should be studied. The stability of β-carotene in orange fleshted sweetpotato puree during storage for more than 8 weeks should be investigated.
References


