ORANGE FLESHED SWEET POTATO AS A POTENTIAL COLOURANT IN BREAD

BY
EGIDE NSENGIMANA
2015/HD02/2927X

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN FOOD SCIENCE AND TECHNOLOGY

IN
THE DEPARTMENT OF FOOD TECHNOLOGY AND NUTRITION
COLLEGE OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES
MAKERERE UNIVERSITY

JUNE 2018
DECLARATION

I, Egide Nsengimana, declare that this thesis is my original piece of work and has not been published and or submitted to any University or institution for the award of a degree.

Signed........................................ Date........................................

Egide Nsengimana

This thesis has been submitted with approval of the following supervisors:

Signed........................................

Assoc. Prof. Yusuf Byaruhanga

Department of Food Technology and Nutrition

Date........................................

Makerere University

Signed........................................

Dr. Ivan Muzira Mukisa

Senior Lecturer and Graduate Co-ordinator

Department of Food Technology and Nutrition

Date........................................

Makerere University
DEDICATION

This work is dedicated to:

My father and my mother who made the foundation of my education and patiently allowed me to achieve this level of education;

My brothers and my sisters for their joy and understanding during the moments I missed them;

Aline Niyonsaba for her love and patience.
ACKNOWLEDGMENT

First and foremost, I thank the Agricultural Sciences Faculty Council at the University of Burundi, particularly the former Dean Prof. King Freedom, for the transparency and impartiality used in identifying me as suitable candidate to the Master’s degree.

I am extremely grateful to the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) for its financial support both for my studies and research leading to the award of Master of Science in Food Science and Technology.

Most sincere thanks to Assoc. Prof. Yusuf Byaruhanga and Dr. Ivan Muzira Mukisa, my supervisors for the perfect collaboration, scientific guidance and encouragement during the entire research. I will always be grateful for the skills and for the gentleness I learnt from them.

I am also grateful to the technical assistance from all the technicians in the different laboratories and sections under the Food Technology and Nutrition department at Makerere University, especially in the pilot plant, bakery section, research laboratories and sensory evaluation laboratory. In the same way, I thank all the participants in the sensory evaluation tests.

Finally, I would like to thank my M.Sc. colleagues, especially those I joined with in the first year of my Food Science and Technology program, for the compassion, kindness and understanding they granted me as a foreign student from a French-speaking country.
# TABLE OF CONTENTS

DECLARATION ........................................................................... Error! Bookmark not defined.

DEDICATION .................................................................................. ii

ACKNOWLEDGMENT .................................................................. iii

LIST OF TABLES ........................................................................ viii

LIST OF FIGURES ........................................................................ i

LIST OF ABREVIATIONS ................................................................. x

ABSTRACT ..................................................................................... xi

CHAPTER ONE: INTRODUCTION .................................................... 1

1.1 Background .............................................................................. 1

1.2 Problem statement .............................................................. 2

1.3 Research objectives .............................................................. 3

1.3.1 Overall objective ........................................................... 3

1.3.2 Specific objectives .......................................................... 3

1.4 Hypotheses ............................................................................. 3

1.5 Justification of the study ...................................................... 4

CHAPTER TWO: LITERATURE REVIEW ......................................... 5

2.1 Bread ..................................................................................... 5

2.2 Ingredients used in bread making ...................................... 5

2.2.1 Wheat flour ................................................................. 5

2.2.1.1 Proteins ............................................................... 6

2.2.1.2 Starch .................................................................. 7

2.2.1.3 Enzymes ............................................................ 8

2. 2.2 Yeast .............................................................................. 10

2.2.3 Water .............................................................................. 10
### 2.2.4 Sugars


### 2.2.5 Lipids


### 2.2.6 Salt


### 2.2.7 Colourants


### 2.2.8 Other ingredients


### 2.3 Methods and steps in bread making


### 2.3.1 Sponge dough method


### 2.3.2 Straight dough method


### 2.4 Significance of the main steps in bread making


### 2.4.1 Mixing


### 2.4.2 Proofing


### 2.4.3 Baking


### 2.5 Bread perception


### 2.6 Quality properties of bread


### 2.6.1 Loaf volume


### 2.6.2 Crumb texture of bread


### 2.6.3 Colour of bread


### 2.7 Staling of bread


### 2.8 Orange fleshed sweet potato in Sub-Saharan Africa (SSA)


### 2.8.1 Development process of Orange fleshed sweet potato in Sub-Saharan countries


### 2.8.2 OFSP adoption in Uganda


### 2.8.3 Performances of OFSP varieties released in Uganda


### 2.8.4 Production and consumption of OFSP in Uganda


### 2.9 Composition of OFSP


### 2.9.1 Carbohydrates
2.9.2 Proteins ........................................................................................................................................30
2.9.3 Vitamins ......................................................................................................................................30
2.9.4 Phytochemicals ..........................................................................................................................31
2.10 Utilization of OFSP .....................................................................................................................33

CHAPTER THREE: MATERIALS AND METHODS .............................................................................34

3.1 Materials .........................................................................................................................................34
3.2 Methodology ....................................................................................................................................34
3.2.2 Preparation of OFSP wet mash and dry flour ..........................................................................34
3.2.2.1 Preparation of OFSP wet mash .........................................................................................34
3.2.2.2 Preparation of OFSP flour .................................................................................................36
3.3 Bread making ..................................................................................................................................37
3.3.1 Dough preparation .....................................................................................................................37
3.3.2 Proofing and baking ....................................................................................................................38
3.4 Determinations and measurements of loaf quality properties ......................................................38
3.4.1 Loaf volume and loaf specific volume ......................................................................................38
3.4.2 Loaf textural properties ............................................................................................................39
3.4.3 Moisture content ........................................................................................................................39
3.4.4 Determination of beta-carotene content ....................................................................................40
3.4.5 Colour measurement ..................................................................................................................40
3.4.6 Staling measurement ..................................................................................................................40
3.4.7 Sensory evaluation .....................................................................................................................41
3.4.7.1 Triangle test ........................................................................................................................41
3.4.7.2 Preference test .......................................................................................................................41
3.4.7.3 Consumer acceptability test ..................................................................................................42
3.5 Data analysis ....................................................................................................................................42
CHAPTER FOUR: RESULTS AND DISCUSSION ................................................................. 43

4.1 Effect of the form of OFSP on loaf specific volume and crumb texture ................. 43

4.2 Effect of different amounts of OFSP flour on loaf volume, weight, crumb texture, colour, beta-carotene content and staling ............................................................. 45

4.2.1 Effect of different amounts of OFSP flour on loaf volume, specific volume (SV) and weight ................................................................. 45

4.2.2 Effect of different amounts of OFSP flour on crumb texture .......................... 46

4.2.3 Effect of different amounts of OFSP flour on loaf colour and beta-carotene content .... 47

4.2.4 Effect of OFSP flour on sensory properties and consumer acceptability of the bread .... 50

4.2.4.1 Triangle test ................................................................................................. 50

4.2.4.2 Preference test ......................................................................................... 50

4.2.4.3 Acceptability test .................................................................................... 51

4.2.5 Staling ........................................................................................................ 53

4.2.6 Effect of amount of OFSP on beta-carotene content and colour retention ........ 55

4.3 Effect of bread improver on properties of bread made from OFSP-wheat composite flours 58

4.3.1 Bread volume and specific volume ................................................................ 58

4.3.2 Crumb texture .............................................................................................. 58

4.3.3 Colour and Beta-carotene ............................................................................ 59

4.3.4 Effect of bread improver on composite bread staling ..................................... 61

REFERENCES ................................................................................................................. 66

Appendix 1 ......................................................................................................................... 91

Appendix 2 ......................................................................................................................... 92

Appendix 3 ......................................................................................................................... 93

Appendix 4 ......................................................................................................................... 94
LIST OF TABLES

Table 1: Bread properties improved by enzymes used as flour additives ................................................................. 9
Table 2: Examples of commonly used additive foodstuffs and their major corresponding pigments .................................................................................................................................................................................. 13
Table 3: Examples of dough conditioner ingredients used in bread making .............................................................. 15
Table 4: Yield and beta carotene content of some OFSP varieties in Uganda .......................................................... 28
Table 5: Nutritional composition of OFSP .................................................................................................................. 32
Table 6: Formulations used for making OFSP-wheat composite bread (g/100g of flour) ............................................ 37
Table 7: Loaf properties of bread made from wheat-wet mashed OFSP and wheat-OFSP flour composites ............................................................................................................................................................................... 44
Table 8: Loaf properties of bread made from Wheat-OFSP composites flours of varying composition ......................................................................................................................................................................................... 46
Table 9: Crumb texture properties of bread made from Wheat-OFSP composites flours of varying composition ......................................................................................................................................................................................... 47
Table 10: Colour and beta-carotene content of bread made from Wheat-OFSP composites flours of varying composition ......................................................................................................................................................................................... 48
Table 11: Triangle test results for bread made from Wheat-OFSP composites flours of varying composition ......................................................................................................................................................................................... 50
Table 12: Preference test results for bread made from Wheat-OFSP composites flours of varying composition ......................................................................................................................................................................................... 51
Table 13: Consumer acceptability score for bread made from Wheat-OFSP composites flours of varying composition ......................................................................................................................................................................................... 52
Table 14: Volume and specific volume of bread made from Wheat-OFSP composites flours of varying composition with and without improver ........................................................................................................... 58
Table 15: Crumb texture of the bread made with or without improver at different levels of OFSP ......................................................................................................................................................................................... 59
Table 16: Beta-carotene content and colour of the bread made with or without improver at different levels of OFSP ......................................................................................................................................................................................... 60
LIST OF FIGURES

Figure 1: Sponge and dough bread process ................................................................. 17
Figure 2: Straight dough bulk fermentation bread process .............................................. 18
Figure 3: Production of OFSP wet mash ....................................................................... 35
Figure 4: Production of OFSP flour .............................................................................. 36
Figure 5: Crust colour (A) and crumb colour (B) of bread made using OFSP-wheat composite flour versus bread coloured with synthetic colourant (C) ................................................. 49
Figure 6: Crumb hardness (A), crumb resilience (B) and moisture content (C) of bread made from Wheat-OFSP composite flours of varying composition ........................................ 54
Figure 7: Loaves attacked by molds at day 8 ................................................................... 55
Figure 8: Beta-carotene (A), red colour (B) and yellow colour (C) of bread made from Wheat-OFSP composites flours of varying composition ......................................................... 57
Figure 9: Crumb hardness (A), crumb resilience (B) and moisture content (C) of bread at different levels of OFSP with and without improver at day 1, 3 and 6 ........................................... 62
Figure 10: Beta-carotene content (A), red colour (B) and yellow colour (C) of bread at different levels of OFSP with and without improver at day 1, 3 and 6 .................................................. 64
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AACC</td>
<td>American Association of Cereal Chemists</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AOAC</td>
<td>Association of Official Analytical Chemists</td>
</tr>
<tr>
<td>AOCS</td>
<td>American Oil Chemists’ Society</td>
</tr>
<tr>
<td>BBI</td>
<td>Best bread improver</td>
</tr>
<tr>
<td>CIP</td>
<td>International Potato Center</td>
</tr>
<tr>
<td>ha</td>
<td>Hectare</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>N</td>
<td>Newton</td>
</tr>
<tr>
<td>OFSP</td>
<td>Orange fleshe sweet potato</td>
</tr>
<tr>
<td>RAE</td>
<td>Retinol activity equivalent</td>
</tr>
<tr>
<td>REU</td>
<td>Reaching End Users</td>
</tr>
<tr>
<td>SD</td>
<td>Straight dough</td>
</tr>
<tr>
<td>SPD</td>
<td>Sponge and dough</td>
</tr>
<tr>
<td>SSA</td>
<td>Sub-Saharan Africa</td>
</tr>
<tr>
<td>SPVD</td>
<td>Sweet potato virus disease</td>
</tr>
<tr>
<td>SV</td>
<td>Specific volume</td>
</tr>
<tr>
<td>t</td>
<td>Tone</td>
</tr>
<tr>
<td>VAD</td>
<td>Vitamin A deficiency</td>
</tr>
</tbody>
</table>
ABSTRACT

Orange fleshed sweet potato (OFSP) is rich in energy and pro-vitamin A carotenoids. Although OFSP has been used to produce composite flour for baked goods, there is lack of information about the use of OFSP as a natural bread colourant. This study aimed at evaluating the applicability of OFSP as a natural colourant in making yellow bread. The effect of using a wet mash of OFSP on bread texture and specific volume (SV) was evaluated against using OFSP flour. Loaves were prepared from 300 g dough containing wheat and 0, 10, 20, and 30% NASPOT 9 O and with and without inclusion of improver. Bread made using 100% wheat flour served as a control while yellow bread made using artificial colourant was used as a reference. The loaves were stored for a week under ambient temperature. Bread load properties namely weight, volume crumb texture, colour, and amount of beta-carotene were determined for the fresh bread and bread stored for 1, 3 and 6 days. Sensory difference and consumer acceptability tests for the breads were done at day 1.

The results showed that wet mash of OFSP had a negative effect on bread loaf volume and textural properties. When OFSP flour was used, loaf volume and specific volume decreased (p<0.05) while loaf weight increased with increasing of amount of OFSP flour in the bread. Use of bread improver had no effect on the treatments except for the control where the loaf specific volume was higher when the improver was used than in the absence of improver. The addition of OFSP flour (10–30%) resulted in detectable sensory differences but all products were generally acceptable. Increasing the quantity of OFSP increased (p<0.05) the beta-carotene content and affected colour of bread. Bread containing OFSP had lower yellow colour score (2.21–4.45) than artificially coloured bread (6.4). Crumb hardness and resilience increased (p<0.05) with increasing of amount of OFSP, storage time and in the absence of improver.

Although it increased the pro-vitamin A carotenoids, OFSP in wet paste or dry flour form was not a good colourant in making yellow bread. Further studies are recommended on how to better use OFSP as a yellow colourant in bread.

Key words: Orange fleshed sweet potato, Wheat flour, Improver, Pro-vitamin A, Yellow brea
CHAPTER ONE: INTRODUCTION

1.1 Background

Bread is among the most popular foods all over the world (Karimi et al., 2012). It is an important food in both developing and developed countries where it is accepted by the entire population including the rich and poor, rural and urban.

Bread is a good source of carbohydrates, proteins, fats, minerals and vitamins necessary for human health (Al-Mussali et al., 2009). Wheat flour is the major ingredient used in bread making because of the ability of its gluten proteins to form a viscoelastic network when mixed with water (Zaidel et al., 2008).

The technology of bread making has changed over the years and diverse substances and additives are currently used to make bread with additional value. The quality of bread is appreciated based on many aspects such as nutritive value, taste, flavor, shelf life and attractiveness for consumers (Krala et al., 2014). The quality of the raw material used in bread making greatly affects the quality of the final product (Dabija et al., 2011). Nowadays, researchers are interested in the use of composite flours for food products manufacturing in general and bread-making in particular. Due to increasing price of wheat, composite flours help in reducing the cost of bread making. The positive effects of using composite flour can be expressed in the functional and physicochemical properties of the final product (Hasmadi et al., 2014).

Sensory quality is considered as a key factor for food acceptability because consumers seek food with certain sensory characteristics (Costell et al. 2010). Colour is one of the properties that have a major effect on consumer acceptability. It is an important factor in food choice and can be used to identify the suitability of a food source or improve the ability in food detection (Cole et al., 2015). In the food industry, colour has become more important in terms of how food is displayed. It is used as quality control parameter and is added to foods to stimulate appetite (Miranda et al., 2012). According to Mortensen (2006), colourants are added to food to replace colour lost during processing, to enhance colour already present and to minimize batch-to batch variations or to colour otherwise uncoloured food.
In Uganda, sweet yellow bread loaves are made using artificial yellow food colourants and the yellow colour imparted is an important characteristic in acceptability of bread (Hagenimana, 1996). However, natural colourants can also be used to impart or improve the colour of food products. For instance, yellow-orange carotenoids naturally present in some plants are one group of the natural pigments that can be used as food colourants in the production of yellow bread (Shen et al. 2014).

Due to its high carotenoids content, Orange fleshed sweet potato (OFSP) has been successfully incorporated in flat unleavened bread making for purposes of enriching bread with vitamin A (Zegeye et al., 2015). According to Nzamwita et al. (2017), the use of OFSP in baked products such as bread can add natural colour and help change the perception of people towards sweet potato. Thus, OFSP can potentially be used to enhance yellow colour of bread whilst improving its pro-vitamin A content.

1.2 Problem statement

Colourants are non-nutritive food additives used in bakery products, and other foods and seasonings to impart desirable colours (Pasias et al., 2015). In fact, consumer demand toward multicoloured baked products is increasing (Tarek, 2015). However, when abused, artificial colourants can negatively impact on human being health. For instance, tartrazine and chocolate brown cause liver and kidney damage when used in amounts beyond the recommended levels (Hassan, 2010). Sunset yellow, one of the colourants used in coloured bakery goods is known to cause severe hypersensitivity reactions especially in children (Culver et al., 2012). In Uganda, colourants are abused by use in quantities beyond the recommended, for the acceptable ones; but is also not uncommon to find unscrupulous bakers using none food grade colourants in bread. This information has changed the perception of consumers about artificial colourants in that these colourants are perceived as unhealthy regardless of the current empirical evidence that proves otherwise. Conversely, the consumers perceive natural colourants as safer and healthier than artificial colourants. Contemporary customers express a pivotal interest in natural pigments for colouring foods and their perception, opinions and desires doubtlessly impact on food industries (Martins et al., 2016).
It is therefore, imperative that alternative natural colourants are explored to address the needs of the health conscious customers. Therefore, the aim of this study was to assess NASPOT 9, a variety rich in the yellow pro-vitamin A carotenoids (Mwanga et al., 2009; HarvestPlus, 2012) as a potential colourant in in the production of yellow leavened pan bread. Nevertheless, it is anticipated that use of OFSP in the leavened bread will affect loaf quality characteristics. However, the form and extent of such effects and how they can be mitigated are not fully established. Thus this work will investigate the effect of varying amounts and form of OFSP on loaf volume, colour, texture, staling and pro-vitamin A content wheat-OFSP composite bread.

1.3 Research objectives

1.3.1 Overall objective

The overall objective of this study was to evaluate the applicability of OFSP in colouring leavened bread

1.3.2 Specific objectives

1. To determine the effect of the form (mash or flour) and amount of OFSP on bread loaf quality properties.

2. To determine the effect of varying amount of OFSP on loaf crumb colour and beta-carotene content

3. To determine the effect of bread improver on quality properties of OFSP-wheat flour composite bread.

1.4 Hypotheses

1. The form and amount of OFSP do not affect the quality properties of the bread.

2. Varying amount of OFSP does not affect colour and beta-carotene content of the bread.

3. Bread improver does not affect the quality properties of OFSP-wheat flour composite bread
1.5 Justification of the study

Consumers have concerns over the safety of artificial colourants while many natural colourants provide health benefits (Culver, 2012). Therefore, the use of OFSP as a natural colourant in yellow bread may help increase bread acceptability while substituting untrusted colourants. It could also ensure protection of consumers from the use of abused quantities of allowed artificial colourants. Moreover, it has been found that expectations and perceived taste, flavor, liking and decision making by the consumer are affected by food colour and appearance (Wei et al., 2012). OFSP as a yellowish natural colourant will make bread more appealing. On the other hand, demand for Orange fleshed sweet potato will be enhanced if profitable processed food products are developed using OFSP as a major ingredient (Namutebi et al., 2015). It is worth noting that the use of OFSP as a natural yellow colourant can be extended to several other baked foods once it shows success in bread applications. The incorporation of OFSP in baked products such as bread can contribute towards reducing post-harvest losses due to OFSP perishability by transforming OFSP tubers into flour. This application will also help to improve the nutrient and health status of communities by alleviating vitamin A deficiency and related diseases. In fact, OFSP is the cheapest crop with high amount of β-carotene available to poor households (Tumwegamire et al., 2004; Mkumbira et al., 2015). Furthermore, since wheat production has failed to keep up with growing demand of consumers in Sub-Saharan Africa (Mason et al., 2012), the substitution of a certain amount of wheat flour by OFSP flour in bread making will help save wheat flour for bread making or for other applications. Using a wet mash of OFSP would help cut costs involved in drying. Fortunately, OFSP has already demonstrated its ability to be easily incorporated into many processed products.
CHAPTER TWO: LITERATURE REVIEW

2.1 Bread
Bread is one of the most substantially consumed food products (Alp, 2011). It is a basic dietary item dating back to the Neolithic era with the first bread reportedly made around 10,000 years before Christ (Mondal & Datta, 2008). Bread offers a positive effect on human health due to its nutritional components, such as dietary fibers, minerals and vitamins (Gellynck et al., 2009). It is a staple food product made from wheat in artisan bakeries and large commercial bakeries as well as in-store supermarket bakeries (Swami et al., 2015). The nutritional composition of bread varies with wheat composition which in turn varies between wheat varieties. In Uganda, bread is widely consumed in Kampala and other cities scattered around Kampala where there are people with high income (Florkowski et al., 2012; Hagenimana, 1996). The fact that wheat, the main ingredient in bread production, is only grown in Western Uganda at the elevation above 1,500 meters (Florkowski et al., 2012), this has impact on bread production and consumption. Uganda is obligated to import wheat flour because the internal production has failed to keep up with growing demand due to some factors such as increasing urbanization and growing populations.

2.2 Ingredients used in bread making
The major ingredients used bread making include: wheat flour, water, sugar, fat, salt and yeast. Water and flour are the most significant ingredients in a bread recipe as they affect texture and crumb the most (Mondal & Datta, 2008). According to Swami et al. (2015), the essentials of any bread dough are flour, water, and yeast. However, presence of only these basic ingredients is not sufficient to produce bread of high quality. Thus, many bakers have tried to improve the performance of bread by adding artificial sweeteners, colourants, flavorings, preservatives and improvers (Chin et al., 2010).

2.2.1 Wheat flour
Wheat is the principal cereal used for bread making (Różyło & Laskowski, 2011). According to Swami et al. (2015), the composition of wheat is responsible for the nutritional properties of bread which properties are dependent on climate, soil and genetic variations.
Triticum aestivum is the commonest variety of wheat used for baking applications (Chase, 2007). According to Malik (2009), wheat flour consists mainly of starch (70–75%), water (14%) and proteins (10–12%). Apart from those, wheat flour also contains many other types of substances including: enzymes, non-starch polysaccharides and lipids. These components of wheat flour are important in terms of their impact on the process-ability of the raw material and in terms of the quality of the final product (Goesaert et al., 2005). Cereals, pseudocereals or legumes can fully or partially replace wheat flour in bread-making but these produce bread of relatively inferior quality. Composite flours can produce the optimal rheological profile of bread dough (Hadjadev et al., 2011). However, at least 70% of wheat flour is required in the bread formulation for the dough to rise (Olaoye et al., 2006).

2.2.1.1 Proteins
The ability of wheat flour to be used in making different products relies on its proteins which range between 8 and 20% (Žilić et al., 2011, Swami et al., 2015, Šramková et al., 2009 & Kumar et al., 2011). The amount of proteins in wheat depends on the type of wheat (Chase, 2007). Proteins particularly gluten and starch granules are the main constituents of wheat flour and many wheat flour products (Flint & Moss, 1970). Gluten proteins represent 80–85% of total wheat protein and constitute the main storage proteins of wheat (Van Der Borght et al., 2005). Gluten is the mixture of gliadin and glutenin, the two basic types of protein in wheat flour. It gives dough its ability to form thin sheets that will stretch and hold gas (Alp, 2011).

Gliadins and glutenins cover about 75% of the total protein content (Šramková et al., 2009). According to Žilić et al., (2011), the gliadins are polymorphic mixture of proteins constituting 30–40% of total flour proteins while glutenins are made up of single polypeptides linked through intermolecular disulfide bonds that account for about 45% of the total proteins in the grain endosperm. These two types of proteins have different properties. The proteins are usually categorized according to their solubility. Gliadins are soluble in 70% ethyl alcohol whereas glutenins are soluble in dilute acid or sodium hydroxide solutions (Šramková et al., 2009). Moreover, glutenin forms a rubbery material that is more elastic and tenacious while gliadin forms a viscous liquid which is sticky and inelastic (Alp, 2011). Both glutenin and gliadin exert the most influence on the strength and elastic properties of dough (Sissons, 2008).
They form a strong, cohesive and viscoelastic network that allows the wheat flour dough to retain yeast fermentation gases and to produce a light, aerated baked product (Van Der Borght *et al.*, 2005). They form disulfide bonds as they are mixed and stretched, which can trap the gas released due to the action of yeast (Chase, 2007).

The viscosity and plasticity are promoted by gliadin whereas the elasticity and the strength are attributed to the action of glutenin (Goesaert *et al.*, 2005). The unique gluten-forming properties of proteins contribute to the excellent baking quality of wheat flour for bread making (Pomeranz, 2012). Besides those main proteins, wheat grain contains albumins and globulins. These proteins comprise of 15–20% of total wheat flour proteins (Malik, 2009). Albumins are smaller than globulins and are soluble in water while globulins are only soluble in dilute NaCl solutions (Šramková *et al.*, 2009). Both globulins and albumins are not involved in bread making performance (Hoseney *et al.*, 1971). However, albumins and globulins, which are non-gluten proteins are nutritionally well reputed due to their very good amino acid balance and they are also involved in metabolic activities (Ţilić *et al.*, 2011).

### 2.2.1.2 Starch

Wheat flour contains 78.1% carbohydrate (Kumar *et al.*, 2011). Starch accounts for 65–75% of the grain dry weight (Kuktaite, 2004) and is therefore a major constituent of wheat flour. This polysaccharide is produced by most green plants as an energy store. It is the most common carbohydrate in human diets and is contained in large amounts in staple foods such as potatoes, wheat, maize, rice, and cassava. According to Regina *et al*. (2015), starch comprises of two polymers: amylose (20–30%) and amylopectin (70–75%). Amylose is linear polymer consisting of α-1, 4 glycosidic linkages of glucose while amylopectin consists of linear α-1, 4 bonds with the inclusion of glucose branches held by α-1, 6 bonds (Chase, 2007). The important property of starch in relation to its functionality is its ability to absorb water, resulting in gelatinization and loss of granular organization (Blazek & Copeland, 2008). Starch absorbs up to about 46% water during dough preparation (Goesaert *et al.*, 2005). At the baking stage, the starch granules gelatinize and swell due to the combination of heat, moisture and time during baking (Goesaert *et al.*, 2005). The gelatinization of hydrated starch during baking helps enforce the structure of the baked product (Chase, 2007). Starch is tightly connected to gluten during bread making and this influences the crumb texture of the loaf (Pomeranz, 2012).
Starch also determines bread aging because of the rapid retrogradation of amylose (Goesaert et al., 2005). The changes in texture and moistness of the bread crumb are primarily caused by starch reactions. Indeed, in the presence of limited amounts of water, the branched starch fraction undergoes a weak association which finally causes collapse of branched molecules thus leading to staling of bread (Pomeranz, 2012).

2.2.1.3 Enzymes
Wheat grain contains a large number of enzymes whose levels vary due to genetic and climatic conditions during growing and harvesting (Ral et al., 2016). Wheat flour contains several technologically important enzymes such as amylases, proteases, lipoxygenase, polyphenol oxidase and peroxidase which become active and give functional attributes to the flour when water is added (Rani et al., 2001). For example, the proteases are known to have the ability of softening flour proteins and therefore, can cause great changes in the structure and properties of the dough. Beta-amylase and alpha-amylase play an important role in bread production. Beta-amylase converts dextrins and a portion of soluble starch to maltose which is essential for active yeast fermentation. Alpha-amylase hydrolyzes starch into sugars, which are further, fermented by the yeast leading to increased bread volume by production of carbon dioxide. Certain amylases also have the ability to delay bread staling (Miguel et al., 2013).

Some other enzymes such as peroxidases or glucose oxidase are used to improve bread making performance of dough (Dunnewind et al., 2002). The gluten-cross linking enzymes contribute to the functional properties of dough. Moreover, polysaccharide-degrading enzymes allow fresh quality enhancement and staling prevention of bakery products (Caballero et al., 2007). In combination with proteins, the enzymes can improve the texture, structure, specific volume and colour of bread depending on their nature and concentrations (Moore et al., 2006). The combination of various enzymes between themselves are reported to have a positive effect on volume, colour, taste, aroma, crust and crumb texture, crumb softness, freshness and shelf life of the baked product. Apart from the enzymes contained in wheat flour, there are other enzymes added in dough conditioners to replace chemical ingredients and to perform other functions such as enhancing the properties of the bread as indicated in Table 1.
Table 1: Bread properties improved by enzymes used as flour additives

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Amount of Enzyme added (U/kg of flour)</th>
<th>Bread attribute improved</th>
<th>Reference*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gluten network</td>
<td>Volume</td>
<td>Colour &amp; Flavor</td>
</tr>
<tr>
<td>Amylase</td>
<td>30 – 200 mg/kg</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Protease</td>
<td>0.02 g/kg</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Xylanase</td>
<td>500 – 1000 U/kg</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Oxidase</td>
<td>0.01– 0.05 g/kg</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Lipase</td>
<td>0.004 g/kg</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Reference*: Reference for the amount of enzyme added

X: Property improved by the corresponding enzyme (Whitehurst & Van Oort, 2009)
2.2.2 Yeast

Yeast are eukaryotic, single-celled microorganisms classified as members of the fungus kingdom. They belong to ascomycetes that are good source of vitamin B and protein (Ali et al., 2012). Baker’s yeast is mainly marketed as either compressed cakes or as a dry powder. Bread can be prepared by using yeast in concentrations of 2.5–2.8% (Swami et al., 2015). Yeast is used to leaven bread and works by converting sugar into carbon dioxide, which causes the dough to rise (Swami et al., 2015). The yeast used in baking is a commercial preparation consisting of dried cells of one or more strains of Saccharomyces cerevisiae (Ali et al., 2012). The advantages of Saccharomyces cerevisiae include their ability to grow actively at lower pH values, ease and low cost of harvesting and storage (Abulhassan, 2015). A secondary contribution of baker’s yeast is to increase bread flavoring and aroma (Ali et al., 2012). This is due to the chemical compounds produced during the fermentation and baking that contribute to the flavour of the bread (Abulhassan, 2015). The primary compounds reported to contribute to the flavor of bread are 2-acetyl-1-pyrroline, (E)-2-nonenal, 3-methylbutanal, 2,3-butanedione, methional, and (Z)-2-nonenal, whereas for bread crumb, (E)-2-nonenal, (E,Z)-2,6-nonadienal, (E,E)-2,4-decadienal, 2,3-butanedione, methional, 1-octen-3-ol, and (E,E)-2,4-nonadienal (Cho & Peterson, 2010).

The production of flavor compounds in is a result of complex chemical and enzymatic reactions which lead to physical and chemical changes contributing to the flavor of the finished product (Johnson & El-Dash, 1969). The penetration and regulation of the movement of these compounds which are mainly the fusel alcohols, the fatty acids and their esters into yeast cells depend on the lipophilic nature of yeast cells (Suomalainen & Lehtonen, 1979). The yeast used and the fermentation conditions have been reported in influencing the formation of aroma compounds such sulphur compounds and phenols.

2.2.3 Water

The quantity of water used affects yeast activity and other ingredients involved in bread making. According to Gil et al. (1997), dough water content ranges between 0.67–0.85 g/g of flour based on dry matter. Water facilitates the dispersion of yeast cells and is necessary for the dissolution of salt and sugars (Alp, 2011).
In combination with flour, water helps in gelatinization of starch (Primo-Martin et al., 2007). It activates flour enzymes and leads to the development of new bonds between the macromolecules in the flour, and changes the rheological properties of dough (Alp, 2011). The moisture content and the physicochemical properties of the flour are reported to be the main determinants of the amount of added water.

2.2.4 Sugars

Chemically, there are simple sugars such as glucose, fructose and galactose and complex sugars which include sucrose, maltose and lactose. In chemistry, sugar refers to mono-, di-, and the lower oligosaccharides that give sweet taste (Clemens et al., 2016). Monosaccharides contain a single polyhydroxy aldehyde or ketone unit and the disaccharides consist of two monosaccharide units linked together by a covalent bond while oligosaccharides contain from 3–10 monosaccharide units. In baking, sugars are used for many reasons. They are usually used by yeasts as source of energy during the first stages of fermentation (Alp, 2011). This helps to increase the volume of the bread because of the carbon dioxide released when yeasts break down the sugars. Glucose, fructose, sucrose and maltose are mostly used because they can be easily metabolized by the yeast cells (Giannou et al., 2003). They are also used as sweeteners to enhance flavor, aroma and taste and contribute to the colour of the final product.

However, concentration of glucose, fructose and sucrose above 5% negatively affects fermentation by creating inhibitory effect against yeast due to the high osmotic pressure imparted by the high concentrations of these sweeteners (Abulhassan, 2015). Sugars influence the colour of baked products, depending on some conditions such as temperature through chemical reactions including Maillard reactions and caramelization which cause browning during baking (Estelle & Lannes, 2008). Caramelization results from the melting of sugars above 120°C which leads to brown colour and new flavors (Kroh, 1994). The brown colour from sugar caramelization is due to the formation of dark compounds called melanoidins (Bastos et al., 2012). The Maillard reaction takes place between the carbonyl group of the sugar and the amino groups of the amino acids in the protein. Beside these two reactions where sugars are involved, studies have revealed that in bread making specifically at mixing level, sugars compete with gluten for available water.
This behavior of the sugar of avoiding over-development of gluten improves the texture of the final product by reducing its rigidness and increasing its tenderness (Meiske et al., 1960).

2.2.5 Lipids
Lipids originate from where they are indigenously present or are added as shortening and/or surfactants and are used as fats or oils (Pareyt et al., 2011). Fats are used in the form of solid shortening, butter or margarine; or the liquid form of oil which contribute tenderness, moistness and to the smooth mouth feel of the baked product (Lauterbach & Albrecht, 1994). The role of fats at the initial stages of baking is to retain gas bubbles in dough and prevent their coalescence (Renzyaeva, 2013). During baking, fats participate in the formation of the leavening structure and texture of the goods (Renzyaeva, 2013). According to Rogers (2004), fat or oil eases the expansion of the dough, improves dough handling, tenderizes the crust and improves shelf life by slowing or masking the staling process. They prevent the formation of tougher product by impairing free development of gluten (Lauterbach & Albrecht, 1994). Bread can contain up to 5% added lipid with a mean of 3~4% (Stauffer, 1996).

2.2.6 Salt
Salt is another important ingredient used in bread making. In baking, salt acts as a stabilizing agent of yeast fermentation rate, strengthens the dough, enhances the flavor of the final product and increases dough mixing time (Miller & Hoseney, 2008). Salt is used in bread making for processing reasons, sensory reasons, and preservation reasons.

It acts as a seasoning and colour enhancer by reducing the action of sugar within the dough and retards bacteria growth (Luchian & Canja, 2010). The important function of salt in bread making is its stabilizing effects on fermentation and offers the baker a tool to control carbon dioxide production (Miller & Hoseney, 2008). The amount of salt to be used depends on the properties needed. However, 2% salt has shown high dough expansion rate (Toyosaki & Sakane, 2013). The amount of yeast has to be increased to enhance fermentation in case the amount of salt is increased for the purpose of improved flavor (Abulhassan, 2005).
2.2.7 Colourants

Colour is an important attribute of food quality and affects consumer acceptance (Shen et al., 2014). Food colour often dominates over other sources of information regarding flavor and greatly influences marketing strategy (Chaitanya, 2014). Most manufacturers use a variety of colours in their bakery products (Saleem et al., 2013). Food colourants are mainly classified into natural and synthetic colourants. Natural food colourants are made from renewable sources mostly plants, animals and microorganisms while synthetic food colourants are man-made (Aberoumand, 2011). The commonly used additive foodstuffs are shown in Table 2.

Table 2: Examples of commonly used additive foodstuffs and their major corresponding pigments

<table>
<thead>
<tr>
<th>Colouring foodstuff</th>
<th>Main colouring pigment</th>
<th>Colour shades</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot</td>
<td>Beta-carotene</td>
<td>Yellow/Orange</td>
</tr>
<tr>
<td>Blackcurrant, black carrot, radish, red cabbage, elderberries, grape</td>
<td>Anthocyanin</td>
<td>Pink/red/purple</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>Carotenoids</td>
<td>Yellow</td>
</tr>
<tr>
<td>Spinach, nettle</td>
<td>Chlorophyll</td>
<td>Orange</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>Riboflavin</td>
<td>Green</td>
</tr>
<tr>
<td>Tomato</td>
<td>Lycopene</td>
<td>Red</td>
</tr>
<tr>
<td>Turmeric</td>
<td>Curcumin</td>
<td>Red/Orange</td>
</tr>
<tr>
<td>Caramelized sugar syrup</td>
<td>Similar properties to caramel</td>
<td>Yellow/Brown</td>
</tr>
</tbody>
</table>

Source: Chapman (2011)

These sources of natural colourants are not adequate for making high quality bread because of their high moisture content, low starch and fiber content. Most of them also have less β-carotene than OFSP and are costly. *Curcuma longa* (Turmeric) is the most commonly used natural colourant from plants globally (Chaitanya, 2014). Another great challenge with naturally derived colourants is their relatively low stability especially when they are exposed to particular conditions such as light or during processing (Wrolstad et al., 2012).
The use of natural colourant might also result in the development of unwanted colours and flavors by interacting with other ingredients due to their chemical structure. For instance, structural variation in anthocyanins comes from various patterns of substitution between rings with OH and OCH3 groups, presence of different sugar substituents, and the possibility of acylation of sugar substituents with cinnamic and aliphatic acids (Wrolstad et al., 2012). The carotenoids can differ from the number of double bonds, conjugation with carbonyl groups or ring structures (Wrolstad et al., 2012). All these structural variations can impact on both colour and stability of the end product.

The most commonly used artificial colourants are Allura Red, Ponceau, Tartrazine, Sunset yellow, Quinoline yellow and Carmoisine (Chapman, 2011). Sunset yellow is the most commonly used colourant in the food industry (Martins et al., 2016). However, most artificial colourants are reported to be a public health problem (Arnold et al., 2012). Several food additives that were used over decades are therefore currently not allowed, due to evidence of their side effects including toxicity and high frequency of health disturbance incidents (Martins et al., 2016). Apart from the food colourant regulations, artificial colourants are being replaced by natural colourants due to the adverse effects of the former on consumers (Wrolstad & Culver, 2012).

### 2.2.8 Other ingredients

Apart from the main ingredients discussed above, several other ingredients can be added to the dough formulation, usually in small amounts in order to improve baking performance and prolong shelf-life (Alp, 2011). The use of bread improvers and other bakery ingredients ensures reliable dough preparation for the production of breads and fine bakery wares of high quality. Preservatives extend the shelf life of baked goods by inhibiting microbial growth. They are commonly used in breads because economic losses from bread spoilage caused by bacteria or by molds are substantial (Ali et al., 2012). Some of the ingredients added to the dough are indicated in Table 3.
Table 3: Examples of dough conditioner ingredients used in bread making

<table>
<thead>
<tr>
<th>Dough ingredient</th>
<th>Function</th>
<th>Level</th>
<th>Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium sulfate</td>
<td>Yeast nutrient</td>
<td>0.04%</td>
<td>Nitrogen source</td>
</tr>
<tr>
<td>Ammonium phosphate</td>
<td>Yeast nutrient</td>
<td>0.04%</td>
<td>Nitrogen and phosphorous source</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>pH regulator</td>
<td>0.1– 0.5%</td>
<td>Raises pH</td>
</tr>
<tr>
<td>Calcium sulfate</td>
<td>pH regulator</td>
<td>0.1– 0.6%</td>
<td>Raises pH</td>
</tr>
<tr>
<td>Potassium bromate</td>
<td>Oxidizing agent</td>
<td>10 –75 ppm</td>
<td>Slow oxidizer</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>Oxidizing agent</td>
<td>10 –100 ppm</td>
<td>Intermediate oxidizer</td>
</tr>
<tr>
<td>Calcium peroxide</td>
<td>Oxidizing agent</td>
<td>10 –75 ppm</td>
<td>Dries dough surface</td>
</tr>
<tr>
<td>Potassium iodate</td>
<td>Oxidizing agent</td>
<td>10 – 45 ppm</td>
<td>Fast oxidizer</td>
</tr>
<tr>
<td>SSL (Sodium stearoyl</td>
<td>Emulsifier</td>
<td>0.25 – 0.5%</td>
<td>Strengthens and softens</td>
</tr>
<tr>
<td>lactylate)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSL (Calcium stearoyl</td>
<td>Emulsifier</td>
<td>0.25 – 0.5%</td>
<td>Strengthens and softens</td>
</tr>
<tr>
<td>lactylate)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Lallemand Inc. (1996)

2.3 Methods and steps in bread making

All bread making processes rely mainly on three key steps: mixing, proofing or fermenting and baking. However, these steps are accompanied by other sub-steps and requirements depending upon the method being used. There are generally two methods that can be used for bread making: sponge and dough (SPD) method and straight dough (SD) method.
2.3.1 Sponge dough method

The sponge dough method is a two-step bread making process. The first step consists of making a sponge using part of the total quantity of the formulation while the second step consists of adding the rest of the ingredients to produce the final dough. Depending on the desired properties of the final product, parts of flour, water, yeast, fat, salt and sugar and even some other ingredients such as milk or improver are thoroughly mixed in proportions which depend on the individual taste and location (Cauvain, 2015). According to Khatkar (2005), generally two-thirds of the flour-, water and yeast are used as indicated in Figure 1. The mixture is covered and left to ferment under defined conditions of temperature (24–30°C) and humidity (70–75 %) depending on the amount of yeast used but mainly on the required flavors (Cauvain, 2015). The final dough is prepared by mixing the remainder of the flour, sugar and water, fats, salt and yeast with the knocked sponge. The importance of sponge consists of modifying the flavor and contributing to the development of the final dough through the modification of its rheological properties (Cauvain, 2015). The aroma of the dough produced by the SPD method is superior to that produced by the SD method. Nevertheless, it has been observed that the SPD method requires a lot of time for the two mixing and fermentation steps, more equipment, more energy and more labor.
2.3.2 Straight dough method

Straight dough is the most traditional, natural and used method for the bread making. Contrary to the sponge dough method, the straight dough method consists of only one step combining all the ingredients together at the same time (Figure 2) and mixing them until the gluten is properly formed (Sahli, 2015). The dough is left to ferment in a warm, moist environment leading to increase in volume. The resting time in bulk of the formed dough depends on the quality of the flour, yeast level, dough temperature and the bread variety in production (Cauvain, 2015).
For bulk fermentation in excess of one hour, a knock-back or punching down is recommended after about three quarters of the bulk fermentation time has passed. This operation leads to the equilibration of dough temperatures throughout its bulk and the incorporation of more air into the dough which improves yeast activity (Cauvain, 2015). The knocked dough is subjected to mixing during a few minutes in order to release the air and level the temperature throughout the dough ready for further rising.

Weigh all ingredients

Develop dough by mixing & ferment (2 ½ hour)

Punching and ferment for 55 min

Dividing and intermediate proofing (25 min)

Molding and putting in bread tins & final proofing (55 min)

Baking

*Figure 2: Straight dough bulk fermentation bread process (Khatkar, 2005)*
2.4 Significance of the main steps in bread making

2.4.1 Mixing

The mixing of precisely measured ingredients is important because numerous functional and crucial dough characteristics, such as consistency, gluten development, and dough temperature are determined during mixing. The first step of mixing consists of blending and homogenizing the ingredients with formation of a wet and sticky dough. Mixing promotes hydration and interactions between mixing components. The aim of the mixing process is to prepare a homogeneous dough from all ingredients involved in the mixture and to bring about the physical development of gluten into a uniform structure (Abulhassan, 2015). Optimum hydration of ingredients enables the development of a gluten-network whose coherence, consistency and mobility reflect the quality of loaf. The dough at optimum stage is smooth and elastic. The mixing time varies with many factors such as the type of mixer, speed of mixing and type of the flour. For instance, it has been found that the gluten water content of the flour increases with overmixing (Kuktaite et al., 2007). Moreover, it has been found that gliadin and its subgroups decrease the mixing time of flour. Depending on the technique used, mixing can impact the rheology of flour and the sensory properties of whole wheat bread (Kihlberg, 2004).

2.4.2 Proofing

Before baking, the dough has to be proofed for maturation and ripening due to yeast action which leads to carbon dioxide production. Proofing refers to the dough resting in bread tins after molding has been accomplished. It allows for fermentation, which is important for obtaining gaseous dough and thus a voluminous bread (Lilleberg, 2012). The time, temperature and humidity during proofing vary for different processes (Siffring & Bruinsma, 1993). Generally, proofing is carried at 30–35°C and at 85% relative humidity, conditions under which yeast can perform well. Inversely, the quantity of yeast influences the proofing time and temperature. Indeed, Siffring & Bruinsma (1993) found that the level of yeast has to be increased when the temperature is decreased. In addition, increased amount of yeast requires a time relatively short because the proofing rate becomes high. According to Liu et al., (2012), proofing time, temperature and relative humidity of 35 min, 35°C and 80%, respectively produce bread with optimal quality.
Temperature, humidity and time also influence the physical, textural and nutritional properties of the finished product. By affecting yeast activity, these parameters have impact on gas production hence the volume of the bread being produced. For instance, the temperature within the dough which is related to that surrounding it was found to affect the volume of the bread (Siffring & Bruinsma, 1993). The proofing conditions affect the flavor, the physical, textural and nutritional properties of the finished product. According to Alp (2011), crumb structure and volume of bread are determined by gas cells stabilization and gas retention. Indeed, the gas produced during fermentation leavens the dough into foam. This foam is transformed into sponge structure during baking which determines the structure of the bread crumb. It has been observed that high humid condition is required to minimize weight loss during proving while lower humidity leads to development dough with dry crust.

2.4.3 Baking

Baking is the last step and has to occur in a proper manner and under controlled conditions because it is the most important step in the bread making operation as it changes the unpalatable dough into expedient bread (Abulhassan, 2015). Baking can be defined as the process which transforms dough into a food with unique sensory features by application of heat inside an oven (Swami et al., 2015). A series of physical, chemical and biological changes such as evaporation of water, formation of porous structure, volume expansion, protein denaturation, starch gelatinization and crust formation take place during bread baking (Mondal & Datta, 2008). Starch granules gelatinize and swell due to the combination of heat, moisture and time during baking, (Goesaert et al., 2005). The time and temperature conditions of the baking process significantly influence the quality and shelf life of baked product and vary from dough to another. Baking temperature should be 239.5–345.62°C whereas optimum baking time has to be 20–21 min (Swami et al., 2015).

2.5 Bread perception

Appearance, taste, flavor, texture and nutritional aspect are the most important quality parameters of foods including bread. They can be summarized in sensory, physical and nutritional properties. Sensory tests of bread depend on its crust colour, crumb appearance, texture, flavor which give prediction to overall acceptance (Alam et al., 2013).
Sensory evaluation is defined as a scientific method used to evoke measure, analyze and interpret the responses to products as perceived through sensory receptors of consumers (Pohjanheimo, 2010). In leavened bread, texture is important for quality perception and bread crumb is described based on textural properties such as softness and strength (Kihlberg, 2004).

Descriptive test is used to understand product characteristics in a controlled environment and quantitative descriptive analysis is the most commonly used method. It is performed by a small number of panelists (8–15) who provide intensity ratings for a set of selected attributes (Valentin et al., 2012). Discriminatory test and hedonic test are mostly used in sensory evaluation. Discriminatory tests are done to determine whether samples are perceptibly different or identical and are extremely useful for evaluation of a new or an improved version of an already existing product. The hedonic test is very useful for measuring food acceptability and commonly uses a 9-point Hedonic scale ranging from ‘extremely dislike’ to ‘extremely like’. The hedonic test can be used for determining the consumer acceptability of bread (Kihlberg, 2004). In the hedonic test, samples may be tested monadically or in combination depending on the statistical design. Hedonic scale ratings are converted to numerical scores and the difference in degree of liking between samples is determined by statistical analysis.

In addition to acceptability test, preference test can be carried out to allow consumers to express a choice between two samples or to assign an order to the samples according to his or her preference in case of more than two samples. The results from a paired-preference test are analyzed using a 2-tailed binomial test while the significant differences of the samples analyzed using the multiple-sample ranking preference test are tested using the Friedman statistical Tables at different levels of significance (Watts et al., 1989 & Carpenter et al., 2000). The number of assessors required to carry out a particular sensory analysis test depend on many factors including the test procedure, the purpose of the test and the nature (trained or untrained) of the assessors (Carpenter et al., 2000). For all sensory assessment methods, humans are the measuring instrument (Singh-Ackbarali & Maharaj, 2014).
2.6 Quality properties of bread

Loaf volume and crumb texture are considered as the most important parameters in bread quality assessment (Magdić et al., 2006 and Różyło & Laskowski, 2011). However, according to Hadnađev-Dapčević et al. (2013), colour and freshness of bread are also among the important quality features determining consumer choice. Specific loaf volume is the main parameter related to protein content and quality in evaluating bread quality (Jirsa et al., 2007).

2.6.1 Loaf volume

Loaf volume and specific volume are among the parameters used in assessing bread physical properties. The specific volume of the loaf is equal to the volume of the loaf divided by its weight. The size of the bread is first of all related to the size of the dough. Loaves of varying sizes can be made to get different consumer needs especially in terms of affordability (Umelo et al., 2014). The method used in making bread is one of the factors that affect loaf volume and thus specific volume. Amr & Ajo (2005) reported that the specific volume of the bread produced by SPD method is significantly higher than that produced by the SD method. The protein content of the flour of the formulation also affects the loaf volume of bread hence the specific volume (Różyło & Laskowski, 2011). This is due to the fact that, be it during proofing or baking, the dough containing more proteins expands at higher rate as compared to that containing less protein (He & Hoseney, 1992). Dough improvers are usually used to enhance all the bread properties including loaf volume. The improvement of loaf volume is attributed to the positive effect of the improver on the flour which allows formation of dough with medium strength (Horvat et al., 2007). However, the level at which the loaf volume increases depends on the type of improver used (Umelo et al., 2014). Other substances such enzymes can be used to improve the volume of the loaf bread. In fact, xylanase was found to increase the specific volume of the loaf bread when incorporated at 8 g/100 kg of flour formulation (Jaekel et al., 2012).

2.6.2 Crumb texture of bread

The crumb texture of bread is described by its textural properties and attributes such softness and strength and constitutes an important parameter for perception in leavened bread (Kihlberg, 2004). Unlike dough quality which gives information about the baking suitability of combined ingredients, the analysis of texture informs about the quality of the final product (Korczyk-Szabó & Lacko-Bartošová, 2013).
According to Karimi et al. (2012), the mechanical texture features are some of the most important quality aspects that affect the sensory perception and the shelf life of bread. The crumb texture is analyzed by determining the crumb firmness. This involves application of compression tests using a penetrating probe to determine the rheological characteristics of the product including the elastic and plastic parameters with respect to time (Kaszab et al., 2002).

Besides the effect of mixing, proofing and baking conditions on crumb texture as mentioned earlier, other factors especially related to the formulation and bread making method affect the texture. A study carried out by Magdić et al. (2006) has shown that crumb texture properties are strongly correlated to the gluten index which is a typical property of different cultivars. In fact, high protein quality and quantity make the flour very strong leading to stronger dough (Salehifar et al., 2010). Apart from the effect of the flour which is the main raw material in bread making, it has been found that other ingredients have effect on bread texture. For example, emulsifiers such as sodium stearoyl lactylate, diacetyl tartaric acid ester of monoglyceride and mono-and diglycerides of fatty acids are reported to affect the bread texture. Karimi et al. (2012) found that bread hardness decreases up to five times by increasing the concentration of these emulsifiers. The dough produced by SPD method was found to be softer than that produced by the SD method which was attributed to the fact that the dough was mixed twice and fermented for a longer time as compared with that produced by the SD method (Amr & Ajo, 2005). This softness of the dough due to the type of method used in making bread may affect the crumb texture of the bread.

2.6.3 Colour of bread
Colour is one of the most important physical and sensory quality properties of bread. Bread colour assessment involves determining or describing both crumb colour and crust colour. Many factors affect colour in food production. According to Bastos et al. (2012), bread colour is the primary characteristic of the Maillard reactions which involve reducing sugars and amino acids. Caramelization is also reported among the factors responsible for formation of colour in baked products. The final crumb and crust properties differ depending on their heat-moisture dynamic (Vanin et al., 2009). Apart from the effect of some phenomena such as mixing, proofing time and baking conditions, colour is mainly influenced by the formulation used in bread making.
The colour of the flour from which the bread is made is the most important while the apparent bread colour depends on crumb grain (Pomeranz, 1960). Natural and artificial colourants are used to enhance bread appeal hence stimulate consumers to enjoy its flavour and texture. However, due to their many bioactive functions, carotenoids content and antioxidativity, natural colourants could be more easily accepted by the consumers than the artificial colourants which have been reported to correlate with the hyperactivity in children (Kaimainen, 2014).

Other ingredients such emulsifiers were found to affect the colour. Karimi et al. (2012) found that the lightness of crumb and crust of bread increased by adding emulsifier. Since many years back, Lovibond glasses have been used in grading colour of foods (McNicholas, 1935). They are easy to use with main objection of high cost to obtain them (Broeg & Walton, 1952). Colourimeter is widely used for colour evaluation of food (Pathare et al., 2013). However, it has been found that this method is not representative in heterogeneous food items such as bread crumb and crust since it measures L*(lightness),a*(+a= redness, -a= greenness) and b*(+b = yellowness, -b = blueness), coordinates only over a very few square centimeters. Novel techniques such as computerized image analysis by capturing food images using a digital colour camera or using a scanner allow rapid analysis of colour (Angioloni & Collar, 2009).

2.7 Staling of bread

Staling refers to the undesirable changes other than microbial spoilage that take place between the time bread is baked and consumed (Angioloni & Collar, 2009). Staling is detected by evaluating the organoleptic properties such as texture, taste and aroma. In fact, the crumb generally becomes harder, dry and crumblly during storage and the crust becomes soft and leathery (Pateras, 2007). Starch retrogradation is the main factor affecting the staling of bread crumb (Alp, 2011). During storage, bread gradually loses its freshness and stales (Goesaert et al., 2005). The ingredients, enzymes and emulsifiers involved in bread making, processing and packaging may also affect also bread the process of bread staling. Moisture content changes and starch retrogradation can be taken into account in staling evaluation of bread (Fadda et al., 2014). According to Goesaert et al. (2005), bread staling is often evaluated by measuring crumb firmness which is also influenced by loaf volume and crumb structure.
Other bread aspects such as crust softening and flavor changes can inform about staling. Shelf-life of baked products may be extended by supplementing either flour or dough with natural or synthetic additives including colourants in bread making. These might interact with cereal proteins during dough mixing thereby strengthening their structure, increasing elasticity of crumb and extending bread freshness when a suitable quantity is applied regardless the nature of the additive (Kwaśniewska-Karolak et al., 2014).

2.8 Orange fleshed sweet potato in Sub-Saharan Africa (SSA)

Orange fleshed sweet potato (OFSP) is a conventionally bio-fortified crop with β-carotene contents of 30–100 ppm, compared with the 2 ppm in local varieties (HarvestPlus, 2012). OFSP was introduced in SSA to address vitamin A deficiency. OFSP varieties are recognized to represent the least expensive and year-round source of vitamin A available to poor families (Tumwegamire et al., 2004).

2.8.1 Development process of Orange fleshed sweet potato in Sub-Saharan countries

OFSP clones were introduced during the 1990s by the International Potato Center (CIP) through collaboration with National Agricultural Research Institute (NARI) partners (Mwanga & Ssemakula, 2011). The first clones spread in SSA countries from CIP were not adapted to the growing conditions and were not suitable for local consumption. The Uganda National Sweet Potato Program at the National Crops Resources Research Institute (NaCRRI) under the National Agricultural Research Organization (NARO) in collaboration with the Regional Network for the Improvement of Potato and Sweet Potato in East and Central Africa (PRAPACE) Network was responsible for improving sweet potato for sweet potato virus disease (SPVD) resistance (Mwanga et al., 2012). Breeding populations were generated between 2002 and 2009 and 20–40% of the seeds were sent to collaborating countries including: Burundi, Ethiopia, Rwanda, Kenya, Tanzania, Madagascar, Ghana, Nigeria, Malawi, Mozambique, Zambia, South Africa and Burkina Faso (Mwanga & Ssemakula, 2011). Kakamega, Ejumula, NASPOT 9 O and NASPOT 10 O varieties have been introduced into several African countries (Mwanga et al., 2012).
2.8.2 OFSP adoption in Uganda

Uganda is the second largest producer of sweet potato throughout of the world after China (Mwanga and Ssemakula, 2011). OFSP cultivars have been promoted by government, nongovernment organizations and farmer groups. OFSP promotion started in Luwero, Central Uganda, in 1999 as a joint multi-sectorial effort of different partners coordinated by Micronutrient Operational Strategies and Technology (Mwanga and Ssemakula, 2011). In Uganda, Kakamega, Ejumula, NASPOT 9 O and NASPOT 10 O varieties have been deployed under the Dissemination of New Agricultural Technologies in Africa (DONATA) project and the Reaching End Users (REU) project of the HarvestPlus Program in Uganda (HarvestPlus, 2012). In 2006, the level of OFSP adoption was substantially higher at 22% in the intervention communities (Yanggen & Nagujja, 2006).

The REU project successfully promoted OFSP in Uganda by leading the adoption to a 57–64 % in the country in 2009 with 26–39% in Eastern and 73–76% in Central Uganda (HarvestPlus, 2012). The spread rate of OFSP was well appreciated since 14,406 farmers were growing the OFSP varieties Vita and Kabode by 2009 in districts of Bukeeda, Kamuli and Mukono, five seasons after the farmers received the varieties (Mwanga and Ssemakula, 2011). NASPOT 12 O and NASPOT 13 O are grown by local farmers in Isingiro, Buyende, Rakai, Oyam, Bushenyi,Kabale,Kamwenge,Wakiso, Mukono,Mpigi, Soroti, Gulu, Lira, Kisoro, Mbarara, Masaka, Kibaale, Kole, and Kamuli where HarvestPlus and collaborating partners promote dissemination of OFSP cultivars to alleviate vitamin A deficiency (Mwanga et al., 2016).

2.8.3 Performances of OFSP varieties released in Uganda

Many sweet potato cultivars such as Kakamega, Ejumula, NASPOT 7, NASPOT 8, NASPOT 9 O, NASPOT 10 O and Dimbuka-Bukulula have been released in Uganda. Kakamega and Ejumula are mostly accepted due to their good yield and appealing fresh colour (Tumwegamire et al., 2007). NASPOT 9 O and NASPOT 10 O are the improved varieties resistant to SPVD and these were released by the Uganda National Sweet Potato Program (UNSP) in 2007 after Kakamega and Ejumula were released in 2004 (Mwanga & Ssemakula, 2011). NASPOT 9 O and NASPOT 10 O are high yielding (Table 4).
The newer varieties of NASPOT 12 O and NASPOT 13 O have moderate β-carotene content and moderate field resistance to SPVD (Mwanga et al., 2016). NASPOT 9 O variety is the best among others in β-carotene according to the performances revealed by Mwanga et al. (2009) and HarvestPlus (2012). Therefore, NASPOT 9 O variety was selected for this study mainly because of its high β-carotene content that would be assumed to also result into a high colour intensity. Table 4 shows the characteristics of some OFSP varieties grown in Uganda.

2.8.4 Production and consumption of OFSP in Uganda

The OFSP varieties developed in Uganda have root yields above 10 t/ha (Mwanga and Ssemakula, 2011). According to Ssebukiba et al. (2006), OFSP cultivars give lower yield than local white cultivars. However, HarvestPlus (2012) reported that the yields of OFSP compared favorably with the yields of white sweet potato in almost all areas of Uganda. About 64% of farmers had already at least some area in production in 2006 (Yanggen & Naguji, 2006). Table 4 shows the yields of some varieties of OFSP in Uganda. OFSP consumption is important in the countries like Uganda especially because it is ideal in addressing hunger, poverty, macro and micro undernutrition due to their ability to generate superior levels of food per unit area per unit time during relatively short rainy periods (Mwanga and Ssemakula, 2011). In Uganda, there is increased consumption of OFSP which can be attributed to the alleviation of vitamin A, one of the commonest problems in the country (Ssebukiba et al., 2006). As the non-OFSP, OFSP varieties have other advantages such as flexible planting and harvesting times, tolerate high temperatures and low-fertility soils, are drought-tolerant, are easy to propagate and maintain, yield well in adverse conditions and form ground cover rapidly to alleviate soil erosion (Mwanga and Ssemakula, 2011). These qualities could help understand the production and consumption of OFSP.
Table 4: Yield and beta carotene content of some OFSP varieties in Uganda

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mean of root yields (t/ha)</th>
<th>Beta-carotene content (μg/100 g fresh weight basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPK 004</td>
<td>16.5</td>
<td>3760</td>
</tr>
<tr>
<td>Ejumula</td>
<td>14.7</td>
<td>7760–14370</td>
</tr>
<tr>
<td>Dimbuka-Bukulula</td>
<td>17.3</td>
<td>20</td>
</tr>
<tr>
<td>NASPOT 8</td>
<td>20</td>
<td>2878–4000</td>
</tr>
<tr>
<td>NASPOT 9 O</td>
<td>16.5</td>
<td>11030</td>
</tr>
<tr>
<td>NASPOT 10 O</td>
<td>16.0</td>
<td>11030</td>
</tr>
<tr>
<td>NASPOT 12 O</td>
<td>25.6</td>
<td>7230</td>
</tr>
<tr>
<td>NASPOT 13 O</td>
<td>17.2</td>
<td>11030</td>
</tr>
</tbody>
</table>

Source: HarvestPlus (2012); Mwanga et al. (2009), Tumwegamire et al. (2014)

2.9 Composition of OFSP

Orange fleshed sweet potato varieties have very large genetic diversity in terms of proximate composition (Krochmal-Marczak et al., 2014 & Alam et al., 2016). OFSP have been reported to contain carbohydrates, proteins, vitamins, anti-oxidants, dietary fiber and minerals (Table 5). Sweet potatoes are also recognized as a very good source of energy and phytochemicals which are important in human and animal feeding (Mohanraj & Sivasankar, 2014).
2.9.1 Carbohydrates
Carbohydrates are the fundamental components in sweet potatoes with regard to their industrial use and eating quality (Islam, 2014). Dry OFSP roots contain 72–90% carbohydrates (Haile et al., 2015; Nicanuru et al., 2015) while fresh roots contain 18–26.8% carbohydrates (Nicanuru et al., 2015). The primary carbohydrate constituent associated with the texture of sweet potato storage roots is starch (Kitahara et al., 2017). Starch constitutes 60–70% of the total carbohydrates, the rest part being constituted by lesser amount of pectins, hemicelluloses and cellulose (Islam, 2014). The starch content varies with variety of sweet potato. For example, Ejumula contains 68.4% while Esapat contains 73.9% starch (Nabubuya et al., 2012). Orange fleshed varieties contain lower amounts of starch than cream, white and yellow-fleshed varieties. High starch containing varieties generate flours with high pasting viscosities while the low starch varieties contain high amylase activities, high sugar content and low pasting viscosities (Nabubuya et al., 2012). Amylase activity is also influenced by the maturity of cultivars (Adu-Kwarteng et al., 2014). Although starch is known as source of energy, amylase regulates the blood sugar levels and is recommended as a healthy food substance, even for patients with diabetes, especially type 2 diabetes (Mohanraj & Sivasankar, 2014; Van Chuyen & Eun, 2013).

Sweet potato starch is composed of 60–70% amylopectin and 30–40% amylose (Van Chuyen & Eun, 2013). Orange fleshed sweet potato varieties have higher amylose than other varieties (Nabubuya et al., 2012). In ten sweet potato varieties investigated by Nabubuya et al. (2012), sucrose was found to be the major sugar with values ranging from 5.79% (Dimbuka) to 14.42% in New kawogo followed by glucose, maltose and fructose ranging from 0.15–1.37%, 0.28–0.44% and 0.21–1.10% respectively. Maturity of cultivars affects total soluble sugar content (Adu-Kwarteng et al., 2014). The sweetness in sweet potato is due to the presence of endogenous sugars such as sucrose, glucose, and fructose present at harvest and additional sugar such as maltose formed through starch hydrolysis by amylase enzymes during cooking (Adu-Kwarteng et al., 2014). Because of its sweetness and textural properties, starch is used in many areas with different purposes such as manufacture of starch syrup, glucose and isomerized glucose syrup, lactic acid beverages, bread and other confectionaries, as well as distilled spirits such shochu in Japan (Odebode et al., 2008).
2.9.2 Proteins

Plant tubers including sweet potato contain proteins which are, however, lower in amounts when compared to seeds (Shewry, 2003). The tuberous roots of sweet potato contain large quantities of sporamins A and B, two proteins which accounts for more than 80% of the total proteins (Maeshima et al., 1985). Sporamin accounts for about 60%–80% of total soluble protein in sweet potato tubers (Yeh et al., 1997). The protein content in sweet potato varies with varieties. According to Islam (2014), sweet potato contains 0.46–2.93% of proteins. In OFSP varieties, protein content ranges between 1.9–9.29% depending on the state, the cultivar and treatment and drying methods used (Alam et al., 2016, Haile et al., 2015, Nicanuru et al., 2015). According to Nicanuru (2016), the amount of protein increases in dried samples due to the fact that during drying, food loses a significant amount of moisture resulting in the concentration of the nutrients in the remaining mass. Sweet potato proteins act as a nutrient component for energy supply and as exhibitor of some other important biological functions (Van Chuyen & Eun, 2013). Other proteins in sweet potato have been shown to possess antidiabetic and antiproliferative properties (Maloney et al., 2012).

2.9.3 Vitamins

The tubers are constituted by many essential vitamins such as pantothenic acid, pyridoxine, and thiamin, as well as niacin and riboflavin (Mohanraj & Sivasankar, 2014). These vitamins are required by the body as external sources in order to be replenished and as cofactors for various enzymes during metabolism (Mohanraj & Sivasankar, 2014). OFSP varieties are rich in carotenoids and β-carotene, a precursor of vitamin A (Haile et al., 2015 & Alam et al., 2016). They contain 24.2 – 73.9 mg/100 g of β-carotene on dry basis (Nicanuru et al., 2015). OFSP, owing to their high β-carotene content, contribute towards alleviating vitamin A deficiency (Haile et al., 2015). Vitamin A helps to maintain the integrity of mucus membranes and skin and contributes for visual acuity (Mohanraj & Sivasankar, 2014). Carotenoids are organic pigments found in plants usually C₄₀ tetraterpenoids and built from eight C₅ isoprenoid units (Bechoff et al., 2010). They are the principal pigments responsible for red, orange, yellow and green colour of vegetables and fruits (Mbwaga et al., 2007).
According to Bechoff et al. (2010), most of the carotenoids in sweet potato are found in trans-carotenoids nature. Both α and β-carotene are pro-vitamin A carotenoid (Mbwaga et al., 2007). OFSP varieties contain trans and cis-β carotene, the former being dominant (Islam, 2015). Light and enzymatic or non-enzymatic oxidations are some of the factors that affect carotenoids. Light leads to the isomerization of the carotenoids into 9-cis; 13-cis and 15-cis carotenoids which have about a half of trans-carotene pro-vitamin A activity (Bechoff et al., 2010). Carotenoids are extremely lipophilic compounds, non-polar and non-soluble in water (Mbwaga et al., 2007). Carotenoids have also antioxidant capabilities and reduce or inhibit mutagenesis in cells (Mohanraj & Sivasankar, 2014). Beta-carotene content in sweet potato is influenced by many factors including genotype, age, farming area and farming conditions (Van Chuyen & Eun, 2013; Mbwaga et al., 2007). At handling stage, it has been observed that cooking methods and storage time cause minimal losses (0–20%) in β-carotene (Burri, 2011).

2.9.4 Phytochemicals

Beside the major phytochemicals present in the leaves such triterpenes or steroids, alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, and phenolic acids, sweet potato have specific phytochemicals such as quercetin and chlorogenic acid that prevent against cancer (Mohanraj & Sivasankar, 2014). These compounds are reported to have antioxidant activity that enables them intercept free radicals (Mohanraj & Sivasankar, 2014). In addition to β-carotene, OFSP contains other antioxidants such phenolic acids, anthocyanins and tocopherol (Mamo et al., 2014). The levels of phenolic acids, anthocyanins and flavonoids are relatively higher in purple-fleshed sweet potatoes than in OFSP varieties (Park et al., 2016). Flavonoids and phenolic compounds are rich in antioxidants and help in fighting lung and cavity cancers while anthocyanins have important antioxidant and anti-inflammatory properties (Mohanraj & Sivasankar, 2014). Tannins and phytate are known as anti-nutritional factors. These anti-nutritional factors vary with cultivar and processing conditions. Indeed, Haile et al. (2015) reported 74–108 mg/100 g and 51–98 mg/100 g respectively for tannins and phytate content of one variety of OFSP depending on the treatment and method used. Sun-dried, OFSP flour contained 92.49–108.46% tannins and 80.61–98.39% phytate (Haile et al., 2015). Blanching and fluidized bed drying has been shown to reduce the tannins and phytate (Haile et al., 2015).
These anti-nutrients and oxalate which is another anti-nutrient are found in tubers and leaves of the sweet potato (Nicanuru, 2016). They hinder the digestion and availability of nutrients in the body unless their levels are reduced through processing or boiling (Nicanuru, 2016).

### Table 5: Nutritional composition of OFSP

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Amount per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw roots</td>
</tr>
<tr>
<td>Vitamin A (µg)</td>
<td>300–1300</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>0.32–0.88</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>0.18–0.57</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>0.08</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.06</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>0.56</td>
</tr>
<tr>
<td>Vitamin B6 (mg)</td>
<td>0.21</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>0.26</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>22.7</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>1.6</td>
</tr>
<tr>
<td>Fiber (mg)</td>
<td>3</td>
</tr>
<tr>
<td>Phytate</td>
<td>10</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>18–26.8</td>
</tr>
<tr>
<td>Starch (g)</td>
<td>11.7–17.42</td>
</tr>
<tr>
<td>Total fats (g)</td>
<td>0.17–0.63</td>
</tr>
</tbody>
</table>

Source: Low et al. (2009); Nicanuru et al. (2015), Alam et al. (2016)

NA: Not available
2.10 Utilization of OFSP

OFSP is being promoted in the developing world as a source of β-carotene that has been shown to be beneficial towards alleviating vitamin A deficiency (Laurie & Heerden, 2012). It improves nutritional security of households since it can be grown at lower cost and the vines can be fed fresh or as silage to livestock (Mkumbira et al., 2015). OFSP can be processed into diverse products such as pancakes, mandazis, chapatti, doughnuts, fried chips and crankies, all of which have been reported to be acceptable in urban and rural areas (Tumwegamire et al., 2004). In Uganda, a range of OFSP based new products such as composite flour, porridge, weaning foods, doughnuts and animal feed have been developed (Yanggen & Naguijja, 2006). The production of juice based on OFSP and use of OFSP leaves as nutritious vegetables in many countries has also been reported (Mkumbira et al., 2015). In Uganda, an OFSP based drink was developed for use in alleviating VAD (Muhammad et al., 2014). In Tanzania, OFSP based snacks have been developed for school children (Honi et al., 2017). OFSP has also been incorporated in bread making to develop nutritious and enriched vitamin A bread (Kidane et al., 2013 & Bibiana et al., 2014). Bread based on OFSP has been developed as a source of vitamin A for lactating mothers (Zegeye et al., 2015). In Ethiopia, complementary porridges using OFSP have been formulated as baby and weaning foods (Tumwegamire et al., 2004; Jemberu et al., 2016). Beyond the utilization of OFSP at home level and in baked products, OFSP enriched yoghurt has been developed in Kenya (Njeri, 2014). Furthermore, complementary foods based on OFSP which meet the stipulated energy and nutrient densities as specified in the Codex Standard with advantage of having lesser phytate compared to cereal based complementary foods have been developed in Ghana (Amagloh & Coad, 2014).
CHAPTER THREE: MATERIALS AND METHODS

3.1 Materials
Mature wholesome OFSP tubers of NASPOT 9 O variety, without any sign of deterioration were obtained from a farmer in Mukono district, Uganda. NASPOT 9 O variety was selected for use in this study because of its high β-carotene content and deep orange colour (Mwanga et al., 2009; HarvestPlus, 2012). The maturity of the OFSP tubers was considered looking at the size and characteristics of the tubers by comparing them with others since the grower had other varieties in harvest. Knowing that the maturity period of NASPOT 9 O is 4 months, the grower was asked about the age of the tubers at harvest to ensure the maturity of them. Baker’s wheat flour, yeast, fat, sugar, improver, salt and calcium propionate as preservative were purchased from established baking ingredients suppliers in Kampala.

3.2 Methodology
This research focused on the properties of bread incorporating different ratios of NASPOT 9 O flour (10–30%) and wheat flour (70–90%) for the assessment of NASPOT 9 O applicability in producing yellow bread. The study was limited to evaluation of the following bread loaf properties: loaf volume, loaf specific volume, crumb texture, colour, carotene content, staling and sensory acceptability. Applicability of a wet mash of NASPOT 9 O was initially compared against dry NASPOT 9 O flour. The effect of using improver on the loaf properties of bread made from OFSP-wheat flour composites was assessed.

3.2.2 Preparation of OFSP wet mash and dry flour

3.2.2.1 Preparation of OFSP wet mash
The OFSP tubers were washed and peeled. They were mashed using a blender (Robot coupe R23, A510R23, Henri Biaugaud, Bordeaux- France) for four minutes at maximum speed. The wet mash was placed in a sachet and stored in a cold room at 2°C before being used in bread making. The procedure for preparing wet OFSP mash is summarised in Figure 3.
Figure 3: Production of OFSP wet mash
3.2.2 Preparation of OFSP flour

The procedure for preparing OFSP flour is summarized in Figure 4. OFSP tubers were washed, peeled and grated. The grated OFSP was spread out on trays and oven dried at 60°C for 24 hours according to the method of Vimala et al. (2011). The dried OFSP was milled using a hammer mill, sieved through a 250 μm mesh following the method of Bibiana et al. (2014) and stored in air tight containers in a cold room at 2°C prior to use.

Figure 4: Production of OFSP flour
3.3 Bread making

3.3.1 Dough preparation

OFSP-whole wheat composite flours were prepared by adding 10%, 20% and 30% OFSP flour or wet mash and straight dough method was used. Reference bread (Tuskys sweet yellow bread, Tusker Mattresses Ltd, Makerere branch, Kampala, Uganda) and bread made with 100% wheat flour (control) were used for comparison. A summary of the experimental runs is shown in Table 6.

<table>
<thead>
<tr>
<th>Runs</th>
<th>Wheat</th>
<th>OFSP</th>
<th>Water</th>
<th>Sugar</th>
<th>Fat</th>
<th>Yeast</th>
<th>Salt</th>
<th>CaPro*</th>
<th>Improver*</th>
<th>Egg yolk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using OFSP flour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>90</td>
<td>10</td>
<td>65</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>0.3</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>20</td>
<td>65</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>0.3</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>30</td>
<td>65</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>0.3</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>0</td>
<td>65</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>0.3</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Reference</td>
<td>100</td>
<td>0</td>
<td>70</td>
<td>8</td>
<td>3</td>
<td>0.4</td>
<td>0.8</td>
<td>0.4</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Using OFSP wet mash</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>10</td>
<td>65</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>0.3</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>20</td>
<td>65</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>0.3</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>30</td>
<td>65</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>0.3</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>0</td>
<td>65</td>
<td>2</td>
<td>2</td>
<td>2.5</td>
<td>2.5</td>
<td>0.3</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Reference</td>
<td>100</td>
<td>0</td>
<td>70</td>
<td>8</td>
<td>3</td>
<td>0.4</td>
<td>0.8</td>
<td>0.4</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

(g/100g of flour*): Recipe based on Islam et al. (2012)

CaPro* = Calcium propionate

Improver*: Loaves were prepared in two sets with one set having improver added and the other without improver in order to study the effect of improver on the quality of OFSP-wheat flour composite bread. This was done in triplicates: for each set, the experiment was carried out three independent times so as to ensure unbiased results.
The dough was first kneaded using hands for 10 minutes and finally using a dough mixer (ABSFBM-80T, Spiral Mixer, Australia) for 5 minutes at medium speed. Molded dough (about 300 g) was placed in oiled bread baking pans for proofing and baking.

3.3.2 Proofing and baking

Proofing was carried out at 35°C and 85% relative humidity for one hour in accordance with the method of Amal (2015) and Nzamwita et al. (2017). Baking was carried out at 180°C for 45 minutes. The loaves were allowed to cool for one hour. Samples were stored at room temperature (20–25°C) and analyzed at days 1, 3 and 6 for different parameters, except for weight whose measurements were taken one hour after baking.

3.4 Determinations and measurements of loaf quality properties

3.4.1 Loaf volume and loaf specific volume

Loaf volume was determined using the rapeseed replacement method described by AACC (2000) with one modification: rapeseed was replaced with millet grain. A saucepan with a flat bottom and with known volume was filled with millet grains and leveled on top using a ruler. The millet seeds were then withdrawn from the pan and the bread to be measured was placed in the saucepan. The millet grains were then gently poured into the pan until the top of the bread was covered and the saucepan was leveled again. The volume of the remaining millet grains displaced by the bread was measured using a measuring cylinder and was considered as the volume of the bread expressed in milliliters. The specific volume was obtained by dividing the loaf volume by its corresponding loaf weight as described by Bibiana et al. (2014) using equation (1).

\[
\text{Specific volume} = \frac{\text{Loaf volume (ml)}}{\text{Loaf weight (g)}} \quad (1)
\]

The volume (ml) and specific volume (ml/g) were measured 24 hours after baking. The weight (grams) of cooled loaves (one hour after baking) was measured using a weighing scale (Digital scale, SF-400, Mode function: g/oz, capacity: 7000g x 1g/280 oz x 0.1oz).
3.4.2 Loaf textural properties

Loaf texture analysis involved measurement of crumb hardness and crumb resilience. Four slices (about 3 cm of thickness per slice) were taken from each of 2 loaves and used for analysis. Crumb firmness (N) and crumb resilience (N.mm) were measured using a Texture Analyzer (TA-XTi2 Stable Microsystems, Surrey, United Kingdom). The test was done in compression mode using a 36mm diameter aluminum probe. Three crumb compression tests were performed for each slice making 12 readings per loaf and 24 readings per treatment (2 loaves per treatment per day) by applying the aluminum plate probe of 36mm at three different points of the bread crumb. A total of 72 readings were taken per treatment in three replicates. The peak force of compression was reported as firmness (AACC, 2000). The test modes were pre-test speed with a compression of 1.00 mm/sec, test speed of 1.70 mm/sec, post-test speed of 10 mm/sec and a trigger force of 0.049 N. Each slice was allowed to be compressed up to 40% in accordance with Kaszab et al. (2002) who found that a compression between 30 and 50% is suitable for the analysis of the elastic properties of bread.

3.4.3 Moisture content

Moisture content was determined using the conventional oven method (AOAC, 2000). Aluminum weighing dishes were numbered, dried in a hotbox oven at 100°C for one hour and cooled for 10 minutes. The dish to be used was first weighed ($w_1$) using an electronic balance. The weight of both sample and dish was measured and recorded ($w_2$) and the weight of the wet sample (before drying) was calculated as $w_2 - w_1$. Using a pair of tongs, the aluminum weighing dish containing the sample was placed in the hotbox oven with fan size 2 (Gallenkamp/SG93/08/850, Earth ground, United Kingdom) set at 100°C.

After sixteen hours, the samples were removed from the oven and placed in the desiccator to cool for 10 minutes and reweighed ($w_3$). The weight of the sample after drying was determined as $w_3 - w_1$. Analyses were performed in triplicate. Percentage moisture content of the sample was calculated using equation (2).
% MC = \frac{w_2 - w_3}{w_2 - w_1} \times 100 \quad (2)

3.4.4 Determination of beta-carotene content

Beta-carotene content of wheat flour, fresh and dry OFSP, fresh bread (within 24 hours) and stored bread (at days 1, 3 and 6) was determined using the HarvestPlus Method (Rodriguez-Amaya & Kimura, 2004).

The total carotenoid content was calculated using equation (3).

\text{Total carotenoids content (μg)} = \frac{A \times \text{volume (ml)} \times 10^4}{2592 \times \text{Sample weight}^* \ (g)} \quad (3)

Where A= Absorbance, volume (ml) = Total volume of extract (50 ml), 2592 = Absorption coefficient of β-carotene in petroleum ether, Sample weight*: on dry basis.

3.4.5 Colour measurement

Colour was measured using a Lovibond® colour scale (Tintometer model E, Tintometer Ltd, England) according to AOCS Cc 13e-92 (AOCS, 1992). The colour of the sample was expressed in Lovibond® units and 3 colour readings were made for a given sample. The recording of colour values relied on divisions of the three colour basis where orange was the combination of red and yellow, green the combination of yellow and blue, violet the combination of blue and red and white the combination of red, yellow and blue.

3.4.6 Staling measurement

Staling was evaluated by determining the crumb hardness, crumb resilience and moisture content of the bread samples at day 1, 3 and 6. At day 1, loaves were considered as fresh because most of the changes in the bread crumb occur during 24 hours after baking (Kaszab et al., 2002). Crumb hardness, crumb resilience and moisture content were determined using the methods previously mentioned in the section of loaf textural properties. Crumb hardness was generated by the Texture Analyzer at the same time with crumb resilience.
The results of crumb hardness and crumb resilience were expressed as average and standard deviation from 24 readings per day, per replicate and per treatment), meaning 72 readings per treatment for 3 independent replicates and for each day (1, 3, 6) either by using improver or not. Analysis of variance was used to determine the effect of OFSP level and storage time on crumb hardness and crumb resilience.

3.4.7 Sensory evaluation

Sensory evaluation of the bread was carried out in three stages. The samples were initially differentiated by using a triangle test and then subjected to preference and acceptability tests. Panelists for all three tests were students from Makerere University in the departments under the School of Food Technology, Nutrition and Bio-Engineering. Only panelists who showed interest, willingness and availability were selected.

3.4.7.1 Triangle test

Three bread samples made from composite flours containing OFSP at levels of 10, 20 and 30% and coded with 3-digit random numbers were presented to the panelists for identification using the triangle test (Appendix 1). The bread samples were presented in the following order: set 1 (10%, 10%, 20%), set 2 (10%, 10%, 30%) and set 3 (20%, 20%, 30%). Twenty-four untrained assessors were used as recommended by Carpenter et al. (2000). The number of assessors required in triangle test to give correct judgments at 5% significance level (13 correct responses for a panel size = 24), as established by Carpenter et al. (2000) was used to conclude on the odd sample.

3.4.7.2 Preference test

The preference test was done to allow people to express a choice between the bread samples prepared from flours containing 0, 10, 20 and 30% OFSP. Fifty untrained panelists were used (Carpenter et al., 2000). All the samples were simultaneously presented to the panelists and the multi-sample ranking test for preference was used. The panelist’s preferences were scored with 1 for the most preferred, 2 for the next, 3 for the third and 4 for the least preferred. The panelists were asked to rank the samples in order of their preferences (Appendix 2). The ranks assigned to each sample were totaled. The samples were then tested for significant difference by comparing the differences between all the pairs of the samples to the tabulated critical values at p= 0.05 as described by Watts et al. (1989).
3.4.7.3 Consumer acceptability test

In order to find out the degree of liking and disliking of each sample over another, the three top ranked samples were subjected to consumer acceptability test. Sixty panelists were used with respect to the required number reported by Mammasse & Schlich (2010). The panelists were asked to rate the acceptability of taste, texture and mouth feel, flavor, aroma, appearance, colour and overall acceptability. For each sensory attribute, the acceptability was ranked using the 9-point Hedonic scale (9 = Like extremely, 8 = Like very much, 7 = like moderately, 6 = Like slightly, 5 = Neither like nor dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = dislike very much, 1= dislike extremely as described by Peryam (1998) (Appendix 3). The numerical scores were tabulated and analyzed by analysis of variance (ANOVA) to determine whether significant differences in mean degree of liking scores existed among the samples.

3.5 Data analysis

The analysis of the effect of the form of OFSP on loaf specific volume and crumb texture and the effect of improver on bread properties were analyzed using two-tailed test. The analysis of the effect of OFSP flour at different levels on bread properties was carried out using one-way ANOVA. Duncan’s multiple range tests was used to separate the means. The statistical analyses were performed using GenStat package (Fourteenth Edition, Copyright 2011, VSN International Ltd, United Kingdom). Confidence limits of 95% were used.
CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Effect of the form of OFSP on loaf specific volume and crumb texture

The form of OFSP (wet mash versus dry flour) affected (p<0.05) crumb hardness and crumb resilience at levels of 20% and 30% OFSP (Table 7). Using OFSP flour led to an increase in the textural properties. According to He & Hoseney (1990), high moisture content results in low firmness of bread. The fact that crumb hardness and crumb resilience were higher for bread made using OFSP flour than for that made using wet OFSP shows that the latter had higher moisture content than the former. Moreover, Hera et al. (2014) reported high resilience in bread made of fine flour, which is OFSP flour against wet mashed OFSP in this study. The high moisture content for wet OFSP could be attributed to the moisture content of the fresh OFSP tubers (67.62% moisture as opposed to 5.17% in the dry flours) and the moisture content of the wet mash added in formulation to balance the dry matter content of OFSP. About 3% of wet OFSP was added to the initial amount of each level of wet OFSP in order to achieve the dry matter content of the OFSP flour of the corresponding level. The water content of the dough containing wet OFSP in the formulation was about 50% based on the quantity of wet OFSP used and the moisture content of fresh OFSP tubers. During mixing, about 25 ml were added. The final water content of the bread made from wet OFSP was estimated to be 75 ml against 65 ml of that made from dry OFSP. In addition, although weights were not different (p>0.05), the higher values observed for bread made using wet OFSP can be attributed to the high moisture content contained in the samples (Iwe et al., 2017). High softness negatively impact on textural properties with risk of high staling rate (Salehifar et al., 2010). This and the difficulty to balance water in the formulation for wet OFSP were taken into consideration in opting for OFSP flour as a suitable way in making bread with inclusion of OFSP. Moreover, although the specific volumes were not (p>0.05) different, high values were observed for bread made using dry OFSP.

Furthermore, although neither bread made from wet OFSP nor that made from OFSP flour had yellow colour intensity comparable to that of artificially coloured bread, the bread made using OFSP flour was more appealing than that made using wet OFSP which had big particles at the surface due to the inability of the blender to mash OFSP into fine particles comparable to flour. The high resilience found for the bread made using OFSP flour shows its higher ability to resist deformation as compared to that made from wet OFSP.
Table 7: Loaf properties of bread made from wheat-wet mashed OFSP and wheat-OFSP flour composites

<table>
<thead>
<tr>
<th>OFSP (%)</th>
<th>Crumb hardness (N)</th>
<th>Crumb resilience (N.mm)</th>
<th>Specific volume (ml/g)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wet</td>
<td>Dry</td>
<td>Wet</td>
<td>Dry</td>
</tr>
<tr>
<td>10</td>
<td>9.4±0.2</td>
<td>8.7±0.3</td>
<td>55.4±4.8</td>
<td>58.3±7.1</td>
</tr>
<tr>
<td>20</td>
<td>10.1±0.9</td>
<td>11.9±0.9</td>
<td>58.7±5.8</td>
<td>67.5±6.0</td>
</tr>
<tr>
<td>30</td>
<td>16.2±4.0</td>
<td>21.8±4.0</td>
<td>147±29.0</td>
<td>150.9±13.2</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations (n= 3).

*Means in the same row for a given loaf property with different superscripts letters are different (p < 0.05).
4.2 Effect of different amounts of OFSP flour on loaf volume, weight, crumb texture, colour, beta-carotene content and staling

4.2.1 Effect of different amounts of OFSP flour on loaf volume, specific volume (SV) and weight

Increasing the amount of OFSP flour generally led to an increase (p<0.05) in loaf weight and decrease (p<0.05) in loaf volume and SV (Table 8). The reduction in volume and SV with increase in amount of OFSP in the formulation might be due to the decrease in gluten network in dough that prevents it from rising during proofing (Bibiana et al., 2014). The decrease in volume due to the decrease in gluten network was also reported by (Kiin-Kabari, 2015). That effect of amount of OFSP on volume and SV is in accordance with the findings of Adeleke and Odedeji (2010) who found lower swelling power in sweet potato flour than in wheat flour. Weight increased as OFSP level increased except that no significant difference was found between the weight of the bread at 20% OFSP and that of the bread at 30% OFSP (Table 9). This observation was also made by Oluwalana et al. (2012). This can be attributed to high fiber content of OFSP (72 – 90% carbohydrates for dry OFSP roots, Haile et al., 2015; Nicanuru et al., 2015) compared to wheat flour (78.1% carbohydrate, Kumar et al., 2011). Indeed, high fiber content led to high water absorption which increased the weight through the increase of moisture content as OFSP level was increased (Table 8). Additionally, since moisture content was 11.6% and 5.17% for wheat flour and OFSP flour respectively, the latter absorbed more water than the former, which was accompanied by increase in weight. Furthermore, because of the high water absorption and lower protein network due to the increase of the amount of OFSP, the amount of moisture and carbon dioxide that diffused out of the loaf was also low, which increased the weight as OFSP increased in amount. Lastly, as the weight was taken just after cooling, its increase might have also been influenced by less water which evaporated from the samples at the time of weighing (Nzamwita et al., 2017). The fact that the weight varied between 260 and 267 g is related to the size of the dough which was sliced in small pieces of 300 g before baking. This is in agreement with Wu et al. (2009) who reported that loaf weight is affected by the quantity of dough baked.
Table 8: Loaf properties of bread made from Wheat-OFSP composites flours of varying composition

<table>
<thead>
<tr>
<th>OFSP (%)</th>
<th>Volume (ml)</th>
<th>Weight (g)</th>
<th>Specific volume (ml/g)</th>
<th>Moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1115 a ±6.3</td>
<td>260.4 a ±0.6</td>
<td>4.3 a ±0.0</td>
<td>35.9 a ±1.6</td>
</tr>
<tr>
<td>10</td>
<td>1120 a ±10.7</td>
<td>263.6 b ±1.6</td>
<td>4.25 a ±0.0</td>
<td>36.2 ab ±1.0</td>
</tr>
<tr>
<td>20</td>
<td>991 b ±36.2</td>
<td>266.3 c ±1.1</td>
<td>3.7 b ±0.1</td>
<td>36.5 ab ±1.3</td>
</tr>
<tr>
<td>30</td>
<td>806 c ±12.1</td>
<td>267.5 c ±1.3</td>
<td>3.0 c ±0.0</td>
<td>37.7 b ±1.3</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations (n=3).
*Means in the same column with different superscripts letters are different (p<0.05).

4.2.2 Effect of different amounts of OFSP flour on crumb texture

Increasing the amount of OFSP in the composite flours led to an increase (p<0.05) in crumb hardness and crumb resilience (Table 9). At 30% OFSP, crumb hardness was closer to the average of 15.6 N reported by Korczyk-Szabó & Lacko-Bartošová (2013) from different varieties of wheat. The high crumb hardness observed for 30% OFSP (higher than for other levels) shows that hardness increases as OFSP amount is increased. Changes in crumb hardness could be attributed to changes in maltose content of the composite flours used. Wu et al. (2009) reported that there is a negative correlation between maltose content and hardness. This shows that OFSP flour used contained less maltose compared to wheat flour in agreement with the findings of Nabubuya et al. (2012) reporting that NASPOT 9 O, the OFSP variety used in this study, contains 0.28% maltose on basis of dry matter while Codină et al. (2013) reported 0.57 mg of maltose per gram of wheat flour. The increase of the amount of OFSP in the formulation might have therefore led to a low maltose content in the mixture which would have led to the high hardness. This increase in hardness with the increase of OFSP level could explain the increase in resilience as OFSP increases in amount which shows the resistance to deformation of the bread with the increase of the amount of OFSP.
Table 9: Crumb texture properties of bread made from Wheat-OFSP composites flours of varying composition

<table>
<thead>
<tr>
<th>OFSP (%</th>
<th>Crumb hardness (N)</th>
<th>Crumb resilience (N.mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.6(^a) ±0.9</td>
<td>66.3(^a) ±8.5</td>
</tr>
<tr>
<td>10</td>
<td>9.4(^a) ±1.1</td>
<td>65.0(^a) ±7.5</td>
</tr>
<tr>
<td>20</td>
<td>10.6(^a) ±0.4</td>
<td>74.4(^a) ±2.3</td>
</tr>
<tr>
<td>30</td>
<td>14.4(^b) ±2.0</td>
<td>103.4(^b) ±11.1</td>
</tr>
</tbody>
</table>

Values are means ± standards deviations (n=3).
*Means in the same column with different superscripts letters are different (p<0.05).

4.2.3 Effect of different amounts of OFSP flour on loaf colour and beta-carotene content

Increasing the amount of OFSP led to an increase (p<0.05) in amount of beta-carotene (Figure 10). The increase of beta-carotene content with OFSP level was due to the replacement of wheat flour by OFSP flour. This shows that OFSP can be used to improve beta-carotene content of bread. This is similar to the findings of Laelago et al. (2015) who found that beta-carotene content increased when more OFSP was added to wheat flour in the composite cookies.

Beta-carotene content can therefore be boosted by inclusion of OFSP flour (Kidane et al., 2013). While beta-carotene content of bread varied from 4.14 to 10.46 µg RAE /100g at 10–30% OFSP, that of the artificially coloured bread was 0.46 µg RAE /100g (Table 10).

With regard to colour, only red and yellow colours were detected in the bread crumb. Increasing the amount of OFSP flour in the composite flour led to an increase (p<0.05) in the intensity of red and yellow colours (Table 10). This increase of colour with the increase of amount of OFSP is also illustrated in Figure 5 B and can be explained by the increase of carotenoids contained in OFSP. This observation of significant effect of carotenoid source levels on bread colour was also made by See et al. (2007) in bread supplemented with pumpkin flour. However, the colour intensity of OFSP-wheat composite bread was significantly lower than that of artificially coloured bread (Table 10 and Figure 5 C).
The low colour intensity for OFSP-wheat composite bread could be due to the lower concentration of beta-carotene. Therefore higher amount of OFSP might be required but care should be taken in increasing amount of OFSP in the formulation as this could negatively impact on other bread characteristics. In conclusion, while the colour intensity imparted by OFSP (10–30%) was significantly lower than that of the commercial coloured bread, the advantage of including OFSP at these levels with regard to boosting carotene content should not be ignored.

Table 10: Colour and beta-carotene content of bread made from Wheat-OFS composites flours of varying composition

<table>
<thead>
<tr>
<th>OFSP (%)</th>
<th>Beta-carotene content (µg RAE /100g)</th>
<th>Colour readings (Tintometer Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Red</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yellow</td>
</tr>
<tr>
<td>0</td>
<td>0.86(^b) ±0.5</td>
<td>0.5(^a) ±0.1</td>
</tr>
<tr>
<td>10</td>
<td>4.14(^c) ±2.5</td>
<td>0.9(^b) ±0.0</td>
</tr>
<tr>
<td>20</td>
<td>7.08(^d) ±3.4</td>
<td>1.2(^c) ±0.1</td>
</tr>
<tr>
<td>30</td>
<td>10.46(^c) ±1.4</td>
<td>1.5(^d) ±0.0</td>
</tr>
<tr>
<td>Reference bread</td>
<td>0.46(^a) ±0.4</td>
<td>1(^b) ±0.0</td>
</tr>
</tbody>
</table>

Values are means ± standards deviations (n=3).

*Means in the same column with different superscripts letters are different (p<0.05).
Figure 5: Crust colour (A) and crumb colour (B) of bread made using OFSP-wheat composite flour versus bread coloured with synthetic colourant (C)
4.2.4 Effect of OFSP flour on sensory properties and consumer acceptability of the bread

4.2.4.1 Triangle test

Panelists were able to detect differences between all three sets of bread (set 1: 10%, 10% and 20%, set 2: 10%, 10% and 30%, set 3: 20%, 20% and 30%) presented to them (Table 11) indicating that varying amounts of OFSP in the composite flour leads to detectable sensorial differences. Based on comments from the panelists, bread made with composite flour containing 30% OFSP had a “stronger brown colour” compared to that made with 10% OFSP which was regarded as being “pale”. These significant effects of the amounts of OFSP on colour with dark brown in colour at high level of OFSP have also been reported by Laelago et al. (2015). It is therefore important to note that increasing the amounts of OFSP in the composite flour contributes to detectable brown colour development which might positively or negatively impact on acceptability.

Table 11: Triangle test results for bread made from Wheat-OFSP composites flours of varying composition

<table>
<thead>
<tr>
<th>Triangle presentation of samples</th>
<th>(10%, 10%, 20%)</th>
<th>(10%, 10%, 30%)</th>
<th>(20%, 20%, 30%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panelists (out of 24) with correct answers</td>
<td>14</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Difference at 5%</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Yes: samples compared are significantly different at 5%.

4.2.4.2 Preference test

Only bread made using the OFSP-wheat composite flour with 20% OFSP flour was more preferred than the control (0% OFSP). The rest of the bread samples were equally preferred at 5% significance level. This may be attributed to the competitiveness between different attributes of the samples such as colour, texture, aroma, taste, appearance and mouth feel with regard to the panelist’s expectations since differences could be detected between the samples in triangle test. The fact that 20% OFSP showed more preference than the control implies that bread made using that formulation could be more acceptable by the consumers.
Table 12: Preference test results for bread made from Wheat-OFSP composites flours of varying composition

<table>
<thead>
<tr>
<th>Difference between rank total pairs</th>
<th>DP1</th>
<th>DP2</th>
<th>DP3</th>
<th>DP4</th>
<th>DP5</th>
<th>DP6</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP1=100% wheat – 10% OFSP, DP2= 100% wheat – 20% OFSP, DP3= 100% wheat–30% OFSP, DP4= 10% OFSP–20% OFSP, DP5= 30% OFSP–10% OFSP, DP6= 30% OFSP – 20% OFSP.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comparison* 25&lt;34 34=34 4&lt;34 9&lt;34 21&lt;34 30&lt;34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance No Yes No No No No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison*: Rank total pair difference compared to 34 (34 = tabulated critical value at p = 0.05, for 50 panelists and 4 samples according to the statistic Table of Friedman described by Watts et al., 1989). A rank total for a given sample was the total of the ranked values given to that sample by all the 50 panelists.

No: No significant difference in preference between the ratios compared

Yes: There is significant difference in preference between the ratios compared

4.2.4.3 Acceptability test

Increasing the levels of OFSP (10 – 30%) in the composite flour did not affect (p>0.05) acceptability scores for appearance, aroma, colour, mouth feel and taste but lowered (p<0.05) the acceptability of texture (Table 13). The loaves made using the three formulations were generally liked (with acceptability scores ranging from 6 to about 7 or like slightly to like moderately). Although there were discernible differences (as shown by the triangle test) and preference for the 20% (preference testing) the consumer acceptability scores were not affected (p>0.05). This means that composite flours containing up to 30% can be used to produce bread that is acceptable to consumers. There is thus potential for saving on costs of flour whilst producing a more nutritious and naturally coloured loaf by replacing up to 30% of wheat flour in bread formulations by OFSP flour.
Table 13: Consumer acceptability score for bread made from Wheat-OFSP composites flours of varying composition

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Acceptability scores for different formulations</th>
<th>90% wheat: 10% OFSP</th>
<th>80% wheat: 20% OFSP</th>
<th>70% Wheat: 30% OFSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td></td>
<td>6.8a ±1.4</td>
<td>6.8a ±1.3</td>
<td>6.5a ±1.8</td>
</tr>
<tr>
<td>Aroma</td>
<td></td>
<td>6.5a ±1.4</td>
<td>6.5a ±1.5</td>
<td>6.2a ±1.5</td>
</tr>
<tr>
<td>Colour</td>
<td></td>
<td>6.9a ±1.3</td>
<td>6.9a ±1.2</td>
<td>6.4a ±1.8</td>
</tr>
<tr>
<td>Mouth feel</td>
<td></td>
<td>6.5a ±1.4</td>
<td>6.3a ±1.4</td>
<td>6.5a ±1.5</td>
</tr>
<tr>
<td>Taste</td>
<td></td>
<td>6.2a ±1.5</td>
<td>5.8a ±1.5</td>
<td>6.1a ±1.6</td>
</tr>
<tr>
<td>Texture</td>
<td></td>
<td>6.9a ±1.3</td>
<td>6.9b ±1.2</td>
<td>6.3b ±1.6</td>
</tr>
<tr>
<td>O.A*</td>
<td></td>
<td>6.8a ±1.1</td>
<td>6.4a ±1.3</td>
<td>6.5a ±1.5</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations (n = 60). *Means across the rows with different superscripts letters are different (p<0.05).

O.A*: Overall acceptability
4.2.5 Staling

Both loaf moisture content and crumb texture were affected (p<0.05) by storage time. Moisture content decreased with storage time but increased with increase of amount of OFSP in the formulation (Figure 6 C). Similar observations were reported by Adeleke and Odedeji (2010) and Kidane et al. (2013). The reduction of moisture content with storage time might be due to the loss of water which may evaporate from crumb to the crust (Lallemand, 1996). Crumb hardness and crumb resilience increased (p<0.05) with duration of storage (Figures 6 A and 6 B). This is in agreement with the results of Dvořáková et al. (2012) who found that all texture parameters deteriorated with storage time. The differences in crumb hardness and crumb resilience for the different OFSP levels might be associated with the differences in water binding capacities of the blended flours related to water loss and water redistribution during storage which affect the starch retrogradation. This is in close agreement with the findings of He & Hoseney (1990) which showed that when the moisture content decreases, it accelerates the formation of cross-links between starch and protein which leads to bread firmness. The highest hardness for 20% and 30% OFSP shows a higher staling rate of OFSP-wheat composite bread compared to the control. This is due to the lower protein content of OFSP-wheat composite flour as compared to that of the whole wheat flour (Salehifar et al., 2010).
Figure 6: Crumb hardness (A), crumb resilience (B) and moisture content (C) of bread made from Wheat-OFSP composite flours of varying composition.
Measurements were not taken on day eight since the samples had visible mold growth (Figure 7). The attack by molds showed that the bread on the eighth day was not safe to be consumed and therefore there was no reason of taking measurements of such bread.

![Loaves attacked by molds at day 8](image)

**Figure 7: Loaves attacked by molds at day 8**

### 4.2.6 Effect of amount of OFSP on beta-carotene content and colour retention

Beta-carotene content was not affected (p>0.05) by the storage time (Figure 8 A). On the contrary, colour was affected (p<0.05) by storage time. According to Bechoff *et al.* (2010), the stability of beta-carotene in OFSP is mainly related to environmental storage conditions such as temperature, oxygen and humidity. The fact that beta-carotene was not significantly different after six days of storage means that beta-carotene in the composite breads is stable in a period of time not less than one week at ambient conditions. This refers to the study that showed that serious carotenoid losses in OFSP dried chips stored at ambient in polyethylene bags were observed after four months (Bechoff *et al.*, 2010).

Generally, colour decreased with the increase of storage time (Figures 8 B and 8 C). According to Nzamwita *et al.* (2017), redness and yellowness of breads made with inclusion of OFSP are strongly and positively correlated to the amount of carotene retained after baking. For the current study, the fact that storage time affected only colour retention shows that, although the beta-carotene content was not significantly affected by time of storage, the amount of beta-carotene was not as much as for the fresh bread due to exposure of the samples to changes in humidity, light, temperature and oxygen.
The yellowness was more affected by storage time than the redness. This is in accordance with the results of Nzamwita et al. (2017) which showed that the loss of colour over time was higher for the dominant colour which is yellow in the current study. According to Bechoff et al. (2010), high percentage losses of OFSP total carotenoids are correlated with high carotenoid content. This indicates that OFSP had higher amount of yellow than red colouring carotenoids. In combination, β-carotene retention can help to predict both red and yellow colour during storage. It can also be concluded that the attractive aspect of bread due to yellow colour on consumers is more profitable for fresh breads than for stored breads.
Figure 8: Beta-carotene (A), red colour (B) and yellow colour (C) of bread made from Wheat-OFSP composites flours of varying composition
4.3 Effect of bread improver on properties of bread made from OFSP-wheat composite flours

4.3.1 Bread volume and specific volume

Addition of improver only affected (p < 0.05) loaf volume and specific volume (SV) at 100% wheat (Table 14). This increase of volume and SV for the control due to the use of dough improver is in accordance with the findings of Horvat et al. (2007), Veluppillai et al. (2010) and Umelo et al. (2014). The fact that the improver did not show any significant effect on volume and SV of the bread made with inclusion of OFSP may suggest that the amounts used (0.5%) were suboptimal and that these amounts are only sufficient for improving the volume of bread made from 100% wheat flour and not composite flours.

Table 14: Volume and specific volume of bread made from Wheat-OFSP composites flours of varying composition with and without improver

<table>
<thead>
<tr>
<th>OFSP (%)</th>
<th>Volume (ml)</th>
<th>Specific volume (ml/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without improver</td>
<td>With improver</td>
</tr>
<tr>
<td>0</td>
<td>1109 ±18.4</td>
<td>1120 ±50.7</td>
</tr>
<tr>
<td>10</td>
<td>1111 ±7.4</td>
<td>1129 ±26.5</td>
</tr>
<tr>
<td>20</td>
<td>958 ±27.7</td>
<td>955 ±53.9</td>
</tr>
<tr>
<td>30</td>
<td>798 ±21.7</td>
<td>813 ±57.3</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations (n = 3). *Means in the same row for a given bread quality property with different superscripts letters are different (p<0.05).

4.3.2 Crumb texture

Crumb texture was affected (p<0.05) by improver except for 20% OFSP. Crumb hardness and crumb resilience were higher in absence of improver than in its presence (Table 15). The effect of the improver on the crumb texture might have occurred during the stage of dough preparation and kneading leading to the creation of smooth crumb cells of the bread. The improver used seems to have acted as hydrocolloids in reducing crumb hardness through their ability to retain water (Maleki & Milani, 2013).
Table 15: Crumb texture of the bread made with or without improver at different levels of OFSP

<table>
<thead>
<tr>
<th>OFSP (%)</th>
<th>Crumb hardness (N)</th>
<th>Crumb resilience (N.mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With improver</td>
<td>Without improver</td>
</tr>
<tr>
<td>0</td>
<td>8.7b ±0.2</td>
<td>10.45a ±0.4</td>
</tr>
<tr>
<td>10</td>
<td>8.4b ±0.3</td>
<td>10.4a ±0.0</td>
</tr>
<tr>
<td>20</td>
<td>10.4a ±0.2</td>
<td>10.8a ±0.5</td>
</tr>
<tr>
<td>30</td>
<td>12.6b±0.3</td>
<td>16.2a ±0.4</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations (n = 3). *Means in the same row for a given bread quality property with different superscripts letters are different (p<0.05).

4.3.3 Colour and Beta-carotene

Beta-carotene content and colour intensity were affected (p<0.05) by improver. They were more in bread made with improver than in bread made without improver (Table 16). The higher amount of beta-carotene in bread made with improver than in those made without improver indicates that the improver led to a form of carotenoids enhanced expression hence enhanced colour expression. This might be associated to the findings of Horvat et al. (2007) according to which, improver enhances flour properties hence its content. The fact that colour was not significantly affected by improver in the control bread shows that the amount of carotene in that sample was too low compared to that of the bread at 10%, 20% and 30% such that it could not be boosted by improver. This could also be the reason why red colour was not affected by improver because all the samples (10–30% OFSP) had more yellow colour than red. The significance of this is that the dominant colour is more sensitive to changes in formulation ingredients and improver is recommendable in increasing the yellowness of bread.
**Table 16: Beta-carotene content and colour of the bread made with or without improver at different levels of OFSP**

<table>
<thead>
<tr>
<th>OFSP (%)</th>
<th>Beta-carotene content (µg RAE/100g)</th>
<th>Colour readings (Tintometer Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With improver</td>
<td>Without improver</td>
</tr>
<tr>
<td>0</td>
<td>0.86&lt;sup&gt;a&lt;/sup&gt; ±0.5</td>
<td>0.87&lt;sup&gt;a&lt;/sup&gt; ±0.6</td>
</tr>
<tr>
<td>10</td>
<td>4.34&lt;sup&gt;a&lt;/sup&gt; ±1.7</td>
<td>3.93&lt;sup&gt;b&lt;/sup&gt; ±0.6</td>
</tr>
<tr>
<td>20</td>
<td>7.38&lt;sup&gt;a&lt;/sup&gt; ±1.1</td>
<td>6.77&lt;sup&gt;b&lt;/sup&gt; ±0.4</td>
</tr>
<tr>
<td>30</td>
<td>10.74&lt;sup&gt;a&lt;/sup&gt; ±0.1</td>
<td>10.38&lt;sup&gt;b&lt;/sup&gt; ±1.4</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations (n = 3). *Means in the same row for a given bread property with different superscripts letters are different (p<0.05).
4.3.4 Effect of bread improver on composite bread staling

Moisture content, crumb hardness and crumb resilience were affected (p<0.05) by improver during storage. Moisture content generally decreased in presence of improver (Figure 9 C). These results match with the results of Veluppillai et al. (2010) who found that the moisture content decreased when the amount of bread improver was increased. This shows the ability of the improver to enhance dough properties which might have led to less required water. The crumb hardness was higher for bread made without improver than those made with improver except for 20% OFSP at day 1. The resilience was higher for the bread made without improver than those made with improver except for 10 and 20% at day 3 and 6 (Figures 9 A and 9 B). The improver used might have acted as an anti-staling agent by preventing starch-gluten interactions hence slowing the rate of starch retrogradation by slowing the rate of moisture migration and by its water retention capacity.
Figure 9: Crumb hardness (A), crumb resilience (B) and moisture content (C) of bread at different levels of OFSP with and without improver at day 1, 3 and 6.
4.3.5 Carotene and colour retention

Beta-carotene content and colour were affected (p<0.05) by improver during storage. Beta-carotene content and yellow colour expression was higher in OFSP-wheat composite breads made using improver than in the bread samples made without improver (Figures 10 A and 10 C). This is in accordance with findings of Wassermann (2000) who reported that bread staling can be retarded by the inclusion of improver. The high yellow colour intensity observed in the presence of improver is related to the higher expression in beta-carotene in the samples made with improver than in those made without improver. Actually, the improver did not increase the beta-carotene content and colour. It acted as a beta-carotene and colour expression enhancer. The improver might have led to a form of carotenoids capable of boosting beta-carotene expression hence colour expression. The use of improver can thus help predict both red and yellow colour. Including improver in the formulation when making bread has a positive implication on carotene content and yellow colour retention.
Figure 10: Beta-carotene content (A), red colour (B) and yellow colour (C) of bread at different levels of OFSP with and without improver at day 1, 3 and 6
CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1. Use of OFSP in a wet mash form negatively impacted on loaf volume and crumb texture of the bread. Its use therefore, might require adjusting the amount of formulation water to ensure that products of desired quality are attained.

2. Although use of OFSP increased the pro-vitamin A carotenoids, OFSP in wet paste or dry flour form did not act as a good colourant in making yellow bread. Further studies are recommended on how to better use OFSP (rich in yellow beta-carotene pigment) as a colourant in making yellow bread.

3. Use of bread improver can boost yellow colour expression and also reduce staling rate of bread. However, the amount of improver used needs to be optimized so as to realize significant increase in loaf volume and further delay staling.

5.2 Recommendations

Use of OFSP is recommended for use in boosting the pro-vitamin A carotenoid content of bread for addressing vitamin A deficiency.

Further research is recommended

- To establish how best to use OFSP in imparting yellow colour to leavened pan bread
- Use of external gluten to mitigate the negative effects of wheat substitution with OFSP
REFERENCES

Aberoumand, A. (2011). A review article on edible pigments properties and sources as natural
bio-colourants in foodstuff and food industry. World Journal of Dairy & Food Sciences, 6(1),
71-78.
Abulhassan, A. M. H. (2015). Effect of Baker’s Yeast Storage On its Viability, Activity and
Bread Making (Doctoral dissertation, University of Khartoum).
Adeleke, R. O., & Odedeji, J. O. (2010). Functional properties of wheat and sweet potato flour
Adu-Kwarteng, E., Sakyi-Dawson, E. O., Ayernor, G. S., Truong, V. D., Shih, F. F., & Daigle,
K. (2014). Variability of sugars in staple-type sweet potato (Ipomoea batatas) cultivars: the
the nutritional and sensory quality of functional breads prepared from whole wheat and soybean
Alam, M. K., Rana, Z. H., & Islam, S. N. (2016). Comparison of the Proximate Composition,
Total Carotenoids and Total Polyphenol Content of Nine Orange-Fleshed Sweet Potato Varieties
role in fermentation during bread making process-A. Pakistan Journal of Food Sciences, 22(3),
171-179.
temperature and time, and fiber content. Chalmers University of Technology (Sweden).


Nicanuru, C. (2016). Effect of pretreatments and drying on nutrient content of orange fleshed sweet potato tubers and cowpea leaves used in Maswa District, Tanzania (Doctoral dissertation, Jomo Kenyatta University of Agriculture and Technology).


Pomeranz, Y. (1960). Determination of bread crumb colour as related to the colour of flour used to bake the bread. *Cereal Chemistry*, 37(6), 765-773.


Siffring, K., & Bruinsma, B. L. (1993). Effects of proof temperature on the quality of pan bread. *Journal of Cereal Chemistry (USA).*


Singh-Ackbarali, D., & Maharaj, R. (2014). Sensory evaluation as a tool in determining acceptability of innovative products developed by undergraduate students in Food Science and Technology at the University of Trinidad and Tobago. *Journal of Curriculum and Teaching, 3*(1), 10-27.


Appendix 1: Triangle test

Sex……… Date………………

Instructions
You have been given three samples among which two are identical and one is different. Taste the samples and mention the code number of the odd sample.

<table>
<thead>
<tr>
<th>Sample Group</th>
<th>The two similar samples</th>
<th>Odd sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>256, 347, 423</td>
<td></td>
<td></td>
</tr>
<tr>
<td>687, 875, 724</td>
<td></td>
<td></td>
</tr>
<tr>
<td>569, 624, 479</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 2: Preference test

Sex........... Date..............

Instructions
Please taste the samples you have been given. Using their respective codes, assign the most preferred sample a rank value of 1, the next preferred sample a rank value of 2, the third a rank value of 3 and the least preferred sample a rank value of 4.

<table>
<thead>
<tr>
<th>Code</th>
<th>Rank assigned</th>
</tr>
</thead>
<tbody>
<tr>
<td>556</td>
<td></td>
</tr>
<tr>
<td>634</td>
<td></td>
</tr>
<tr>
<td>492</td>
<td></td>
</tr>
<tr>
<td>378</td>
<td></td>
</tr>
</tbody>
</table>

Any other comments (please note the sample code)

..................................................................................................................................................

Thank you for your participation
Appendix 3: Acceptability test

Sex……… Date……………..

Instructions
You have been provided with three coded samples of bread. Please assess these samples for overall acceptability and the acceptability of different sensory attributes as shown in the Table using the scale given below. Write down the Figure that corresponds to your response in the Table below. Please rinse your mouth with water provided before and after tasting each sample. Feel free to give any comments about these samples.

Scale

| Dislike extremely | 1 |
| Dislike very much | 2 |
| Dislike moderately | 3 |
| Dislike slightly | 4 |
| Neither like nor dislike | 5 |
| Like slightly | 6 |
| Like moderately | 7 |
| Like very much | 8 |
| Like extremely | 9 |

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Appearance</th>
<th>Colour</th>
<th>Texture</th>
<th>Aroma</th>
<th>Mouth feel</th>
<th>Taste</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>435</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>508</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>357</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>626</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Any other comments (please note the sample code)

...........................................................................................................................................................................

Thank you for your participation
Appendix 4: Preference scoring

Preference scoring totals

<table>
<thead>
<tr>
<th>Panelist</th>
<th>0% NASPOT 9 O</th>
<th>10% NASPOT 9 O</th>
<th>20% NASPOT 9 O</th>
<th>30% NASPOT 9 O</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>17</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>22</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>23</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>24</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>25</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>26</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>27</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>28</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>29</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>31</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>32</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>33</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>34</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>