QUALITY AND STORAGE STABILITY OF PROVITAMIN A BIOFORTIFIED AMAHEWU, A NON-ALCOHOLIC CEREAL BEVERAGE

by

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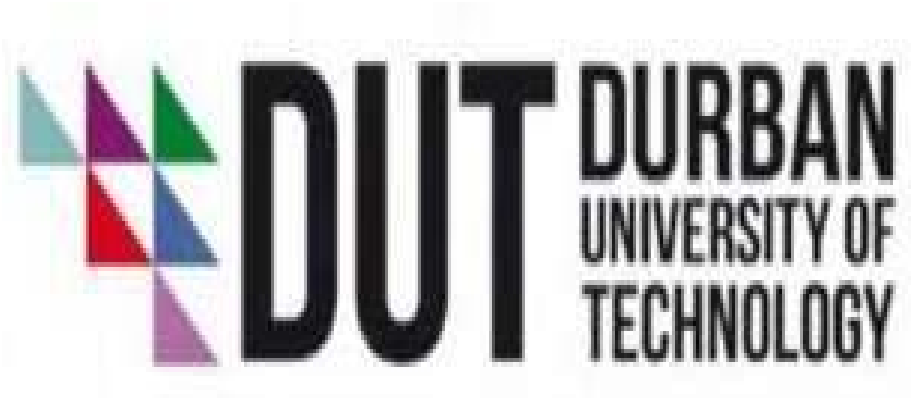

PREFACE

The work described in this thesis was carried out in the Department of Biotechnology and Food Technology, Durban University of Technology, under the supervision of Dr Eric Oscar Amonsou, Dr Muthulisi Siwela and Dr Oluwatosin Ijabadeniyi.

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DECLARATION

I, Temitope Deborah Awobusuyi, declare that:

1. The research reported in this thesis, except where otherwise stated, is my original research.

2. This thesis or any part of it has not been submitted for any degree or examination at any other university.

Signed:

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Finally, I thank God, for the strength and the blessings that have made this work possible.

DEDICATION

This thesis is dedicated to God Almighty.

He alone sustained me.

To my parents, Mr and Mrs J.O AWOBUSUYI,

thank you for the precious gift of education.

And my siblings OLUWASEUN AND OLUWASEGUN AWOBUSUYI,

thank you for your support and prayers.

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ABSTRACT

Vitamin A deficiency (VAD) is a major health problem in sub-Saharan Africa where maize is a staple food. Amahewu, a fermented non-alcoholic,maize-based beverage is a popular drink in southern Africa.The aim of this study is to produce a provitamin A enriched and acceptable amahewu, using provitamin A biofortified maize which can be used to alleviate VAD.

The optimal processing parameters for the production of amahewu using provitamin A-biofortified maize were determined. Amahewu samples were prepared with reference to a traditional method by boiling a mixture of maize meal and water (rato:1:7) at 90^{O}C, with occasional stirring, for 15 minutes. The resulting porridge was left to cool to approximately 40^{O}C, before inoculation and fermentation at 37^{o}C. Processing parameters investigated were inoculum types (wheat bran (WB), maize malt (MM) and *Lactobacillus* mixed starter culture) and inoculum concentration (0.5,1 and 2% (w/w)) and varieties of provitamin A maize (PVAH 62 and PVAH 19). Wheat flour (at 2%) was used as reference inoculum to conform to the traditional practice. White maize amahewu samples processed in the same way as those of provitamin A-biofortified maize were used as references.

Provitamin A amahewu samples were produced using the optimized processing parameters and then analysed for nutrient composition, including carotenoids, protein, ash, amino acids, mineral profile and *invitro* protein digestibility. The consumer acceptability of amahewu samples was evaluated using regular consumers of amahewu (n= 54), who rated the acceptability of the samples on a 9-point hedonic scale (1:disliked extremely, 9:liked extremely). The storage stability of the provitamin A biofortified amahewu samples was assessed by subjecting the samples to different storage conditions: 4^{O}C, 25^{O}C and 37^{O}C. The microbiological quality of the stored samples was monitored by taking samples every day for a period of five days to analyse for the presence of aerobic and anaerobic bacterial spore formers, *E.coli* and moulds.

The provitamin A maize variety did not influence pH and Total titratable acidity (TTA) of amahewu samples during fermentation. As expected, there was a substantial drop in pH with fermentation time. After 24 hours, all the samples of amahewu, including those made with white maize, prepared using malted maize and wheat bran inocula reached a pH of 3.3-3.8 and TTA of 0.3-0.6, which were within acceptable range for amahewu. The addition of a starter culture substantially reduced fermentation time, from 24 to six hours. The inoculum of WB and MM, respectively, at a concentration of 0.5%, with or without starter culture (5%), were found to be suitable for the production of amahewu using provitamin A biofortified maize.

The total provitamin A content of amahewu samples, produced using optimised parameters (i.e one variety of provitamin A biofortified maize, 0.5% MM, WB with or without starter culture), ranged from 3.3-3.8 μg/g (DW). The percentage retention of total provitamin A ranged from 79%- 90% (DW). The lowest percentage retention was observed in products fermented with the addition of starter culture. The gross energy of the amahewu samples was approx. 20 MJ/kg. There was a slight increase in the lysine content of amahewu after fermentation. The protein digestibility (approx. 91%) of amahewu samples was slightly higher than that of raw provitamin A maize (86%). Amahewu processed using starter cultures had a slightly higher iron content than those processed without a starter culture.

Consumer acceptability data showed that amahewu samples made with provitamin A biofortified maize were slightly more acceptable (average rating for overall acceptability was 7.0 ± 1.2), compared to those made with white maize (average rating for overall acceptability was 6.4 ± 0.8). Principal component analysis (PCA) of Amahewu sensory data showed that 71% of variation was due to maize types and 18% of variation may be due to the inoculum used during fermentation. The use of a starter culture improves the taste and aroma acceptability of amahewu. Segmentation of consumers based on overall linking for amahewu revealed three clusters, named A, B and C. Cluster A consisted of most consumers (43%), who liked amahewu moderately. About 60% of these consumers were females. Cluster B consisted of most of the consumers (31%) who were undecided about their liking for the product. Approximately 52% of the consumers in this cluster were female.

Cluster C consisted of consumers (26%) who liked amahewu very much. Sixty-four percent (64%) of these consumers were female. It appeared that gender may have some influence on overall liking for amahewu, as cluster B, consisting of undecided consumers, had more male consumers compared to clusters A and C. Age did not seem to be significantly associated with the liking of amahewu.

Provitamin A biofortified amahewu samples stored under refrigerated conditions ($4^{O}C$) had better microbiological quality compared to those stored at $25^{O}C$ and $37^{O}C$. Refrigeration effectively maintains the microbiological quality of amahewu for about three of days.

Provitamin A biofortified maize can be used to produce β-carotene enriched amahewu that is acceptable to consumers following the processing method that is traditionally employed for white amahewu at both domestic and commercial level. Provitamin A biofortified amahewu has the potential to make a significant contribution towards alleviating VAD among rural communities, who are the most vulnerable to VAD.

CHAPTER 1

INTRODUCTION

1.1 IMPORTANCE OF THE STUDY

Maize is the most important cereal crop in sub-Saharan Africa. It is a major staple, and constitutes an important source of energy for many people in many countries of Africa. Maize is traditionally consumed in several food forms, including breads, porridges, steamed and roasted products, beverages and snacks (Ortiz-Monasterio *et al.,* 2007). In southern Africa, amahewu is a very popular beverage produced by the lactic acid fermentation of maize. It is used as a refreshing drink by children and adults. Amahewu is used as a weaning food for infants, especially in rural poor communities (Chelule *et al.,* 2010). However, the quality of the maize and processed products is nutritionally poor, because maize is deficient in essential amino acids such as lysine and tryptophan (WHO, 2010). Also, the most commonly used white maize has a nutritionally insignificant amount of provitamin A, the precursor of vitamin A. Therefore the high consumption of the white maize, especially by rural communities whose diet has little diversity, is of public health concern, as it predisposes the communities to malnutrition, including vitamin A deficiency (VAD).

VAD is a major public health problem in developing countries, including South Africa (Pillay, 2011a). Population groups that are vulnerable to VAD include children under the age of five; children with infection and children from poor socio-economic backgrounds. Non-breastfed infants, pregnant and lactating women are also affected (Ahmed and Darnton-Hill, 2004). Globally, approximately 250 million preschool children have VAD and 250 000 to 500 000 of these children become blind every year as a result of VAD (WHO, 2010). Low dietary intake of vitamin A causes VAD (WHO 2009). VAD results in loss of appetite, poor growth in children and an impaired tissue function and immune response (Gibson, 2005). Signs of VAD include xerophthalmia, anaemia and reduced immune function, which can increase the severity of infections (WHO, 2009). Xerophthalmia is the leading cause of childhood blindness (WHO, 2009).

Many attempts are being made to improve the nutritional quality of maize, since it represents a major staple in many developing countries,. These efforts include genetic manipulation and fortification during processing. Biofortification is a genetic manipulation technique that seeks to improve the micronutrient content of staple foods consumed by people from poor socioeconomic backgrounds (Meenakshi *et al.,* 2010). Biofortification targets poor people living in remote rural areas who are not reached by commercial fortification and supplementation programmes (Li *et al.,* 2010, Nestel *et al.,* 2006).

Provitamin A biofortified maize has been developed through plant breeding as a part of strategic intervention to alleviate or eliminate VAD in vulnerable communities. The consumer acceptance of provitamin A-biofortified maize has been investigated for some popular foods such as thin porridge and samp consumed in South Africa (Pillay, 2011a, 2011b). According to Pillay *et al*. (2011b), provitamin A biofortified maize has potential as a complementary strategy to address VAD among pre-school and younger school children, because they preferred biofortified maize food products to corresponding white maize products. However, adults were found to prefer white maize products over the provitamin A products. Pillay (2011a) suggested that the acceptance of yellow maize was dependent on the product type being evaluated. Therefore more foods still need to be evaluated to establish the food types in which the biofortified maize is acceptable across all the demographic groups of the targeted consumers. The knowledge of the nutritional quality and consumer acceptability of amahewu made with provitamin A biofortified maize will be important in the utilisation of provitamin A maize to address VAD.

1.2 AIM

To produce a provitamin A enriched and acceptable amahewu using provitamin A biofortified maize which can contribute to the alleviation of VAD.

1.3 HYPOTHESES

1) Amahewu produced using lactic acid culture, wheat bran or malt will have a reduced fermentation time compared to the traditional spontaneous

fermentation. This is because the active culture initiates lactic acid production. Wheat bran and malt acts as a source of enzymes, which produces a small amount of maltose, which is utilised in the fermentation (Mugocha 2001).

2) Amahewu produced using provitamin A maize will have a better nutrient composition (e.g. β-carotene, protein and amino acid content), compared to white maize. Fermentation was found to increase the essential amino acids such as lysine, tryptophan and methionine (Olukoya, 2012). The total protein content of fermented products has also been found to increase after fermentation (Mugocha, 2001).
3) Provitamin A biofortified amahewu will have better functional properties such as high protein digestibility, compared to white maize. Fermentation improves digestibility by partial hydrolysis of storage proteins and carbohydrates by endogenous and microbial enzymes (Onyango *et al.*, 2009). β-carotene acts as an antioxidant due to genetic factors (Pillay *et al*., 2011b).
4) The sensory properties in terms of colour, taste and aroma of provitamin A-biofortified amahewu will be improved and the product will be acceptable to consumers. This is because the fermentation enhances the organoleptic properties of foods such as the aroma, flavour and texture (Osungbaro 2009, Oyewole 2012a), thereby making it more acceptable to consumers.
5) Provitamin A biofortified amahewu stored under refrigerated conditions will keep its quality longer than that stored at room temperature. The lowering of the pH through lactic acid production inhibits pathogenic organisms which can cause food spoilage. By doing this, the shelf-life of fermented foods is prolonged (Abdel, 2009; Olukoya *et al*., 2012).

1.4 OBJECTIVES

1) To determine the optimal processing condition for the production of provitamin A biofortified amahewu
2) To determine the nutrient composition and *invitro* protein digestibility of provitamin A biofortified amahewu
3) To determine the consumer acceptability of provitamin A biofortified amahewu
4) To determine the microbial quality of processed provitamin A biofortified amahewu

CHAPTER 2

LITERATURE REVIEW

Maize (*Zea mays)*, also known as corn, is one of the leading cereal grains in the world (Nuss and Tanumihardjo, 2010). Worldwide consumption of maize is more than 116 million tons, with Africa consuming 30% and sub-Saharan Africa (SSA) 21%. Lesotho has the largest consumption *per capita* (174 kg per year). Eastern and southern Africa uses 85% of maize production as food and Africa as a whole uses 95%. Ninety per cent of white maize consumption is in Africa and Central America (International Institute of Tropical Agriculture (IITA, 2009). Maize is processed into a wide variety of traditional and modern food products. Food products made from maize include breads, porridges, steamed and roasted products, beverages and snacks.

In many countries of SSA, one of the common products made from white maize is amahewu. Amahewu is a very popular sour gruel produced by the lactic fermentation of a cereal grain. Amahewu is usually made with maize. Amahewu contributes significantly to the daily calorie intake of a large segment of the southern African population. Also, amahewu has been commercialised on a large scale and the low pH and high acidity of this product contributes to its bacteriostatic and bactericidal properties (Nyanzi *et al.,* 2010). Amahewu serves as a refreshing energy drink for adults and children. Amahewu is also known as 'amahewu' (Zulu) and 'mahewu' (original African spelling) (Mugocha, 2001). Since white maize is deficient in vitamin A, amahewu processed from white maize without vitamin A fortification is devoid of vitamin A.

2.1 VITAMIN A DEFICIENCY (VAD)

VAD affects 190 million pre-school children in the World Health Organization (WHO) regions of Africa and South East Asia. This section will focus on VAD trends in South Africa.

2.1.1 Trends of vitamin A deficiency in South africa

In South Africa, in particular, 63.6 % of children aged between one and nine years were found to be vitamin A deficient in 2005 by the National Food Consumption Survey (NVASPGSA, 2012).

In South Africa in 1994, a national survey done by the South African Vitamin A Consultancy Group (SAVACG) for the Department of Health showed that one out of three children under the age of six years in the country had poor vitamin A status. The provinces most seriously affected by VAD were the Northern Province, KwaZulu-Natal, Mpumalanga, North West Province and the Eastern Cape. Children living in rural areas and in low socio-economic environments were found to be more severely affected than those living in urban areas and in better socio-economic environments. The Department of Health launched a national vitamin A supplementation (VAS) programme in 2001 following the 1994 SAVACG survey, which showed that VAD was a public health problem in South Africa. The 2005 National Food Consumption Survey (NFCS) indicated that other micronutrient deficiencies among women and children still persist and nutritional status may be deteriorating. Very recently, the HSRC (2014) reported that 11 in 25 (44%) of South African children under the age of five suffered from VAD. Previous findings identified VAD to be a significant public health issue for young children in the country and that key intervention strategies were needed to alleviate this nutritional disorder. South Africa, like many other countries, has adopted multiple strategic approaches to prevent VAD, namely food fortification, vitamin A supplementation and dietary diversification.

2.1.2 Strategies employed to address VAD

Many strategies have been set to increase the production, availability and access to foods rich in micronutrients and to increase the consumption of foods rich in micronutrients and the bioavailability of micronutrients from the diet. One of these strategies is through the biofortification of staple foods.

2.1.2.1 Biofortification

Biofortification is a public health intervention that seeks to improve the micronutrient content of staple foods consumed by the majority of poor people (Meenakshi *et al.,* 2010). Biofortification involves breeding staple crops for increased vitamin and mineral content, using the best traditional breeding practices and modern biotechnology (De Groote and Kimenju, 2008). According to Li *et al.* (2010), biofortification programme target the poor, vulnerable groups living in remote rural areas. One of the most important advantages of biofortification is that it is cost-effective (Nestel *et al.,* 2006). Research currently focuses on iron, zinc and provitamin A, which are three micronutrients that have been identified as limiting by the World Health Organization (Ortiz-Monasterio *et al.,* 2007). The food fortification approach, to fortify food with essential nutrients, is a highly effective strategy to address micronutrient deficiency in developing countries (UNICEF).

2.2 PROVITAMIN A BIOFORTIFICATION OF MAIZE

Maize is one of the food vehicles used for fortification, as it was found to be one of the most commonly consumed food items. Biofortification takes advantage of the fact that staple crops are a predominant part of the diets of poor populations who have VAD or are at risk of VAD. There is also a large number of staple foods consumed by all family members in poor households at risk of VAD (Bouis, 2003). Biofortification can deliver naturally fortified foods to people who may not have access to commercially fortified foods that are more readily available in urban areas (Nestel *et al*., 2006). Biofortification and commercial fortification can therefore be regarded as complementary strategies to address micronutrient malnutrition (Bouis, 2003). Another advantage of biofortification is that there is less risk of vitamin A toxicity from biofortification, compared to excessive consumption of fortified foods and massive doses of vitamin A supplements, as the conversion of carotenoids into vitamin A in the body is controlled and regulated (Penniston and Tanumihardjo 2006).

A (Yellow)

B (Orange)

(Source: CIMMYT)

Figure 1: Provitamin A biofortified maize types

The maize kernel contains two fat-soluble vitamins: provitamin A or carotenoids, and vitamin E. Carotenoids are found mainly in yellow maize, in amounts that may be genetically controlled, while white maize has little or no carotenoid content. Most of the carotenoids are found in the hard endosperm of the kernel and only small amounts in the germ. The β-carotene content of yellow maize is an important source of vitamin A (Wurtzel *et al.,* 2012).

2.2.1 Conversion of beta carotene to retinol

There are 600 known carotenoids, approximately 50 have vitamin A activity, but food composition data are available for only three of these carotenoids (α-carotene, β-carotene and β-cryptoxanthin) (Tura *et al.,* 2010); α-carotene, β-carotene and β-cryptoxanthin are precursors of vitamin A that can be converted into retinol by the body (Strobel *et al.,* 2007). Natural β-carotene contains a mixture of different isomers (cis and trans) of the β-carotene molecule. The trans-isomer is the most common form in human tissue, comprising up to 60 percent of the total β-carotene content. Although many cis-isomeric forms of each carotenoid exist, the all-trans isomers are the most common and stable (Institute of Medicine, 2001).

The all-trans isomers have the greatest vitamin A activity and are the main forms of retinoid and carotenoids found naturally in foods (Fig.4); β-carotene exhibits the

greatest vitamin A activity of all the carotenoids. Two molecules of vitamin A can be produced from each molecule of dietary β-carotene (Sherry, 2010).

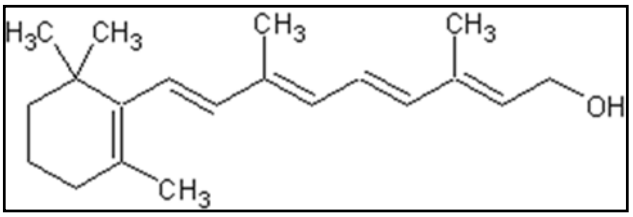

Figure 2: Structure of vitamin A
(Institute of Medicine, 2001)

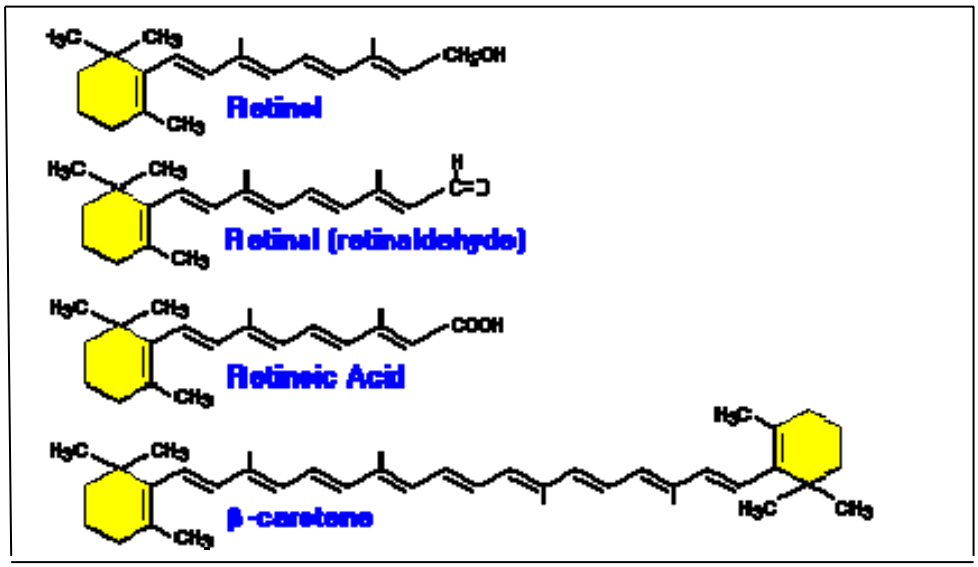

Figure 3: Conversion of β-carotene to retinol

(Institute of Medicine, 2001)

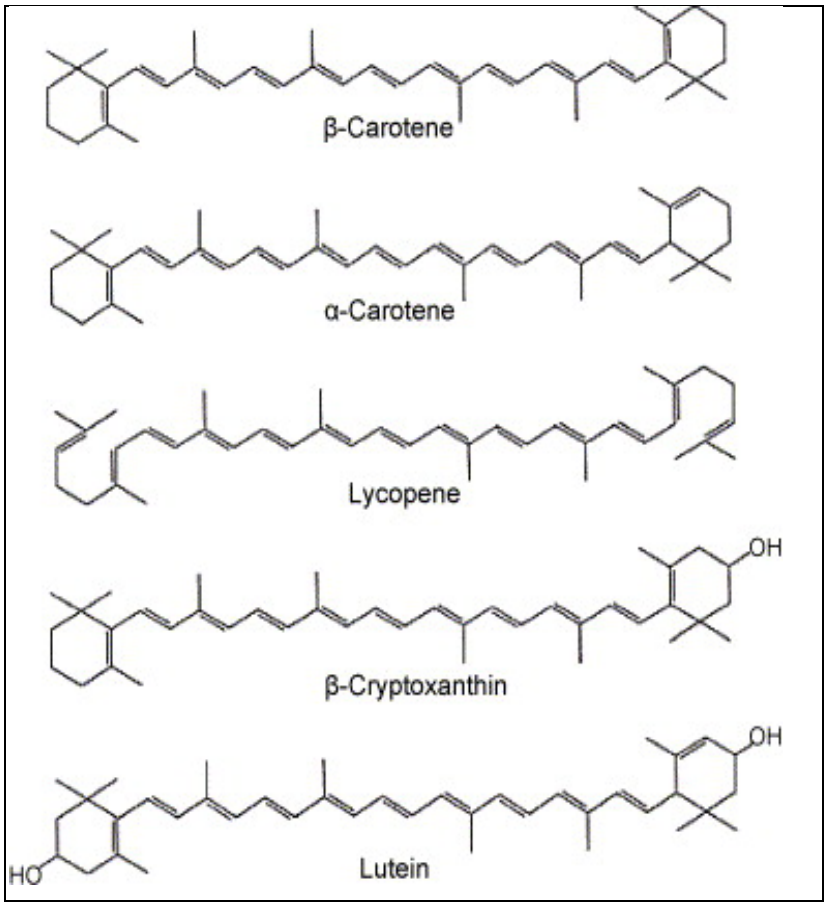

Figure 4: Major carotenoids
(Institute of Medicine, 2001)

2.3 MAIZE KERNEL STRUCTURE AND NUTRIENT COMPOSITION

Maize is a cross-pollinating species, with the female (silk) and male (tassel) flowers located separately on the plant. Maize kernels develop in the ear and each ear may hold between 300 and 1000 single kernels, weighing between 19 and 40 g per 100 kernels. Figure 5 shows the maize kernel, which is made up of the following major anatomical structures: the pericarp (hull or bran) (6% of kernel weight); the germ or embryo (11% of kernel weight); and the endosperm (83% of kernel weight) (Johnson 2000; FAO 1992).

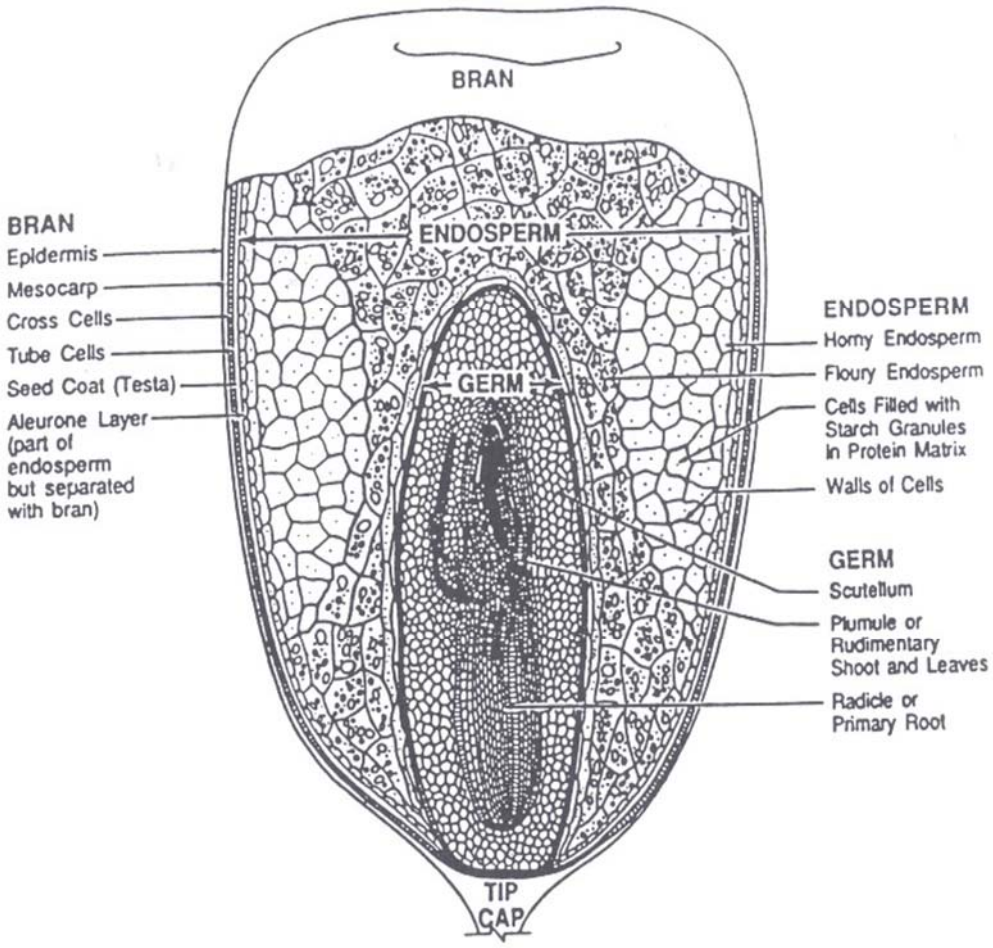

Figure 5: Structure of maize kernel (Johnson 2000)

The chemical composition of maize is known to vary due to genetic make-up, environmental factors and agronomic practices. Although maize is an important source of energy it contains limited amounts of some macronutrients and micronutrients, which makes it inadequate for consumers that depend on maize as a major food source (Nuss and Tanumihardjo, 2010). Starch is the major chemical component of the maize kernel and is concentrated in the endosperm. Starch consists of amylose, a linear glucose polymer, and amylopectin, a branched glucose polymer. Other carbohydrates present include simple sugars such as glucose, sucrose and fructose, that form 1-3% of the kernel. Protein is the next largest chemical component of the kernel and is mostly found in the endosperm of the kernel (Fig. 1) (Machida *et al.,* 2010).

Table 1: Chemical composition of normal dent maize

Component	Normal Dent
Starch (%)	71.3
Protein, N X 6.25 (%)	8.7
Fat (%)	4.1
Fibre (%)	3.0
Sugars (%)	11.4a
Ash (%)	1.5
Amylose (g/100 g starch)	24b
Amylopectin (g/100 g starch)	76b
Lysine (g/100 g protein)	2.7c

[a]Keener *et al.,* 1985, [b]Johnson 2000, [c]McCann 2000

2.3.1 Maize-based food products

Maize is consumed in several food forms. Some of the traditional foods made from maize are breads, porridges, steamed and roasted products, beverages and snacks (Ortiz-Monasterio *et al.*, 2007). Fresh or fermented maize porridges are widely consumed, depending on the country. Ground maize is prepared into porridge in eastern and southern Africa, while maize flour is prepared into porridge in west Africa (IITA, 2013). Examples of fermented maize-based food products are described below.

2.3.1.1 Amahewu

Amahewu is a pure lactic fermented maize gruel that is well-known and appreciated throughout SSA (Fig.6). Traditionally, amahewu is made by adding one part of maize meal into nine parts of water. It is left to boil, with occasional stirring, for 10-

15 minutes and cooled to about 40^{O}C. A small amount of flour is added as a source of inoculum and allowed to ferment for one to three days in a warm place (Chelule *et al.* 2009).

Amahewu is produced on an industrial scale through two fermentation processes. One process is through a mesophilic mixed strain fermentation and another, a thermophilic fermentation. An example of the commercial product is shown in Figure 6. The standardised product contains 8-10% solids and has a pH of about 3.5 and titratable acidity is 0.4-0.5% (Madoroba, 2009). The main fermenting organisms are *L. acidophilus. L. bulgaricus, L. delbrueckii* and *Streptococcus lactis. Lactobacillus* starter culture is normally added to initiate lactic acid fermentation. Amylolytic enzymes are introduced by means of sorghum, wheat flour and malt, these materials also function as a source of innoculum. Studies conducted on lactic acid fermentation for the improvement of amahewu quality showed an increase in protein and amino acid yield, as a result of natural lactic acid fermentation (Chelule *et al.,* 2010). In addition, fermentation can reduce the amount of mycotoxins in contaminated food (Chelule *et al.,* 2010).

Figure 6: Amahewu
(Source: from Wikipedia)

2.3.1.2 Kanun-zaki

Kanun-zaki is a non-alcoholic, fermented, cereal-based beverage consumed in northern Nigeria (Fig.7). Kanun-zaki can be prepared from pearl millet, sorghum or maize. This product is popularly served as a breakfast dish. It was reported that this beverage is nutritionally, medically and economically important in the regions where it is widely consumed (Nyanzi and Jooste, 2012).

Figure 7: Kanun-Zaki
(Source: from wikipedia)

2.3.1.3 Ogi

Ogi is a traditional African acid-fermented cereal gruel prepared from maize, although sorghum and millet flours are also used (Fig.8). During fermentation, *Lb.plantarum* is the predominant micro-organism, although bacteria such as *Corynebacterium* spp. hydrolyse the corn-starch, following which yeast genera such as *Saccharomyces* and *Candida* contribute to the flavour. Ogi has a sour flavour and a characteristic aroma (Nyanzi and Jooste, 2012).

Figure 8: Ogi
(Source: from Wikipedia)

2.3.2 Nutritional quality of maize-based food products

Fermentation has been found to remove or reduce the anti-nutritional factors, such as phytic acid, tannins and polyphenols, present in some cereals (Oyewole, 1997b). This results in better bioavailability of nutrients, such as iron, zinc and calcium (Holzapfel 2002, Blandino *et al*., 2003). According to Holzapfel (2002), fermentation decreases the activity of the proteinase inhibitors in cereals, resulting in an increase in the availability of essential amino acids such as lysine, leucine, isoleucine and methionine. The protein quality and nutritive value of products such as kenkey, iru and ugba (Iwuoha and Eke, 1996) and ogi (Teniola and Odunfa, 2001) was improved during fermentation, due to either microbial synthesis or loss of non-protein material. Fermentation in many instances results in increased vitamin content in the final product. *Lactobacilli* involved in fermentation may require vitamins for growth but several of them are capable of synthesizing B-vitamins in excess of what they need. Cereal-based products such as ogi, mageu and kenkey have been reported to have improved B-vitamin content (Campbell-Platt, 1994; Iwuoha and Eke, 1996).

2.3.3 *Invitro* protein digestibility of fermented foods

Lactic acid bacteria are known to release various enzymes and vitamins into the intestinal lumen. This exerts synergistic effects on digestion, alleviating symptoms of intestinal mal-absorption, produces lactic acid, lowers the pH of the intestinal content and helps to inhibit the development of invasive pathogens such as *Salmonella* spp. or strains of *E. coli* (Parvez *et al.*, 2006).

Onyango *et al.* (2009) reported an increase in protein digestibility. This was attributed to the partial degradation of complex storage proteins by endogenous and microbial proteolytic enzymes into soluble products.

Pranoto *et al.* (2013) reported that fermentation improved the nutritional value of the sorghum, because it leads to increased *invitro* protein digestibility.

2.3.4 Other health-promoting properties of fermented foods

2.3.4.1 Anti-fungal effect

Lactic acid bacteria (LAB) have been found to show antifungal activity. Fungal diseases are difficult to treat. Different strains of LAB have been screened out to identify their potential anti-fungal activity (Mugocha, 2001). Among various strains of LAB, *Lactobacillus fermentum* has been shown to possess a strong anti-fungal property, especially against *Candida albicans* and *Candida glabrata.* As LAB possesses anti-mycotic property they can be used as probiotics against various lethal fungal diseases. Fungal infection caused by *Candida glabrata* and *Candida albicans* are common. LAB used as probiotics may address these issues in a better way (Masood *et al.,* 2011)

2.3.4.2 Anti-carcinogenic effect

Research conducted by Purhit *et al.* (2009) confirmed the effectiveness of lactic acid bacteria in colon cancer. Kim *et al.* (2006) found that LAB such as *Lactobacillus rhamnosus* ATCC 9595 was useful in preventing colon cancer in human beings. They conducted experiments on two cell lines of cancer; PANC-I (pancreas) and HI-29 (colon). They found that lactic acid bacteria successfully decreased the cancer growth. Some types of LAB were investigated and the strains with anti-carcinogenic property included *Lactobacillus helveticus, Bifidobacterium*, *Lactobacillus acidophilus*, or a mixture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. *Lactobacillus helveticus* was observed to be the most effective one in inhibiting the uncontrolled growth of colonic cells.

2.3.4.3 Anti-ulcer effect

Lactococcus rhamnosus is not only is used as an adjunct in anti-ulcerative therapy, but also reduced ethanol-induced mucosal lesions. Pre-treatment with *Lactococcus rhamnosus* also significantly increases the basal mucosal prostaglandin level and attenuates the suppressive actions of ethanol on mucus-secreting layer and trans-mucosal resistance and reduces cellular apoptosis in the gastric mucosa (Myllyluoma

et al., 2007). It can be said that *Lactococcus rhamnosus* is, in many ways, an anti-ulcerative. (Lam *et al*., 2007).

2.3.4.4 Probiotics

Probiotics are live microbial food supplements that improve the human intestinal microbial balance. Among the potential benefits that have been claimed are the prevention and treatment of infantile diarrhoea, travellers' diarrhoea and antibiotic-induced diarrhoea, colon cancer, constipation, hypercholesterolemia, lactose intolerance, vaginitis and intestinal infections (Nyanzi *et al.,* 2010).

Fermented foods are associated with 'good bacteria', referred to as probiotics. Probiotics are beneficial bacteria in that they favourably alter the intestinal microflora balance, inhibit the growth of harmful bacterial, promote good digestion, boost immune function and increase resistance to infection (Christine *et al.,* 2010). Most commonly used genera as probiotics are *Lactobacillus* and *Bifidobacterium,* but other LAB such as *Lactococci, Streptococci, Enterococci*, as well as *Propionibacteria*, *Bacilli* (e.g. *Bacillus subtilis*) and yeasts (e.g. *Saccharomyces boulardii*) are used (Kullisaar *et al.,* 2012). Probiotics play a key role in enhancing resistance to colonisation by exogenous, potentially pathogenic organisms. They do this by producing compounds such as lactic acid, hydrogen peroxide and acetic acid that increase acidity of the intestine and inhibit the reproduction of many harmful bacteria (Christine *et al.,* 2010).

Probiotics also produce bacteriocin, which act as natural antibiotics that kill undesirable/pathogenic micro-organisms. They are also known to out-compete the pathogenic micro-organisms, preventing their survival in the gastro-intestinal tract (GIT) (Christine *et al.,* 2010).

2.4 EFFECT OF FERMENTATION ON THE NUTRITIONAL QUALITY OF PROVITAMIN A CAROTENOIDS IN PROCESSED FOODS

Maize is processed, milled, prepared and consumed in different ways (Nuss and Tanumihardjo, 2010). In South Africa, the common products of processed maize include samp, mealie meal and fermented and non-fermented gruels and beverages.

These processes may result in a loss of certain nutrients, including provitamin A carotenoids (Johnson, 2000).

2.4.1 Retention of provitamin A carotenoids

Previous research has indicated the possible loss of provitamin A carotenoids during fermentation (Muntean, 2007).

Heat treatment could contribute to the decrease in provitamin A content. It has been reported that, at high temperatures, the long-chain polyunsaturated carbons undergo isomerisation from the trans to the cis form, leading to loss of the vitamin A activity (Tannenbaum, 1976).

Van Jaarsveld *et al.* (2006) determined the retention of β-carotene in boiled, mashed orange-fleshed sweet potatoes under home-cooking and institutional-cooking conditions. Although retention of trans-β-carotene in orange-fleshed sweet potatoes varied with cooking conditions, overall, the trans-β-carotene content of boiled, mashed sweet potatoes was still substantial, with retention ranging from 83% to 92%.

Li *et al.* (2007) investigated the retention of the major provitamin A carotenoids, α-carotene, β-carotene and β-cryptoxanthin, in high β-carotene content maize during the traditional processing of a fermented African porridge. The major provitamin A carotenoid found in the maize was all-trans β-carotene, with the two prominent cis-isomers of β-carotene being 9-cis and 13-cis. The cumulative losses of β-carotene in the final, cooked products were 24.5% and 24.8% for the fermented and unfermented porridges, respectively. This suggests that traditional fermentation does not adversely affect the retention of provitamin A carotenoids in high β-carotene content maize porridges. Higher losses during the cooking of the unfermented porridge were observed for all carotenoids (Li *et al.*, 2007).

Pillay *et al.* (2011) indicated that the highest retention of provitamin A carotenoids was observed in cooked phutu and cooked samp, whilst the lowest retention of provitamin A carotenoids was observed in cooked thin porridge.

Muzhingi *et al.* (2008) investigated the effect of cooking on the carotenoid content of raw, uncooked yellow maize flour. The cooking of sadza (dumpling), porridge and mangai (snack) resulted in an increase in the carotenoid levels, while muffin preparation resulted in a decrease in carotenoid levels. The aforegoing review indicates that provitamin A losses may occur when maize is processed using different methods. The findings suggest that the extent of the loss of provitamin A carotenoids is influenced by the method of food processing.

2.5 SENSORY EVALUATION OF FOOD PRODUCTS

Sensory evaluation and sensory quality of food are sub-disciplines of sensory science, which is defined as a scientific discipline used to evoke, measure, analyse and interpret reactions for those characteristics of food and materials as they are perceived by the senses (IDF Standard 99A, 2012). Sensory quality, however, refers to "the degree of excellence of fitness for eating in those contributory attributes which are perceived via the senses of sight, smell, taste, touch and hearing" (Williams and Atkin, quoted by Schőnfeldt, 1999). The primary role of sensory analysis in commerce is to provide comprehensible, valid and reliable information for research and development (R&D) production and marketing, in order for management to make sound business decisions about perceived sensory properties of products (Meilgaard *et al.,* 1999).

2.5.1 Descriptive versus consumer panels

The descriptive analysis technique is a method of sensory evaluation that identifies, describes and quantifies the sensory attributes of a product (Gillette, 1984). Descriptive analysis of products involves the use of a trained panel of five to 10 judges (for typical products on the grocery shelf) and five to 100 judges (for products of mass production, such as beers and soft drinks) (Meilgaard *et al.,* 1999).

2.5.2 Consumer testing

Consumer testing is used to determine whether customers like the food product or prefer one product to the other (O'Mahony, 1988). This is established by using the data from a panel of consumers (O'Mahony, 1988). According to Stone *et al*. (1974),

large-scale consumer testing may yield information consistent with what small Quantitative descriptive analysis (QDA) panels may have already yielded, but it does not disqualify the need for consumer testing. This is because consumer testing provides additional information about the possible outcome of subsequent consumer testing and expanded understanding of not only company products, but also competitor products.

2.6 CONSUMER ACCEPTANCE OF PROVITAMIN A BIOFORTIFIED MAIZE

In Africa, white maize is produced and sold by farmers and is preferred by consumers. African consumers prefer white maize as their staple crop, compared to yellow maize, which is preferred for livestock feeding (McCann, 2005). Increasing the provitamin A content of maize through breeding changes its colour to yellow/orange and can also change other characteristics such as flavour and aroma (Stevens and Winter-Nelson, 2008). In Zimbabwe, the main source of supply of yellow maize is through imported food aid, which has two negative associations for consumers. Firstly, it is considered a "poor man's grain" and inferior to white maize. Secondly, yellow maize undergoes chemical changes, resulting in unacceptable organoleptic properties, if poorly handled during importation (Muzhingi *et al.,* 2008). Biofortification of maize with provitamin A carotenoids changes the grain colour from white to yellow, as well as the aroma and flavour of the maize. In particular, the yellow colour of provitamin A-biofortified maize may pose a challenge with regard to consumer acceptance (De Groote *et al.,* 2008). In eastern and southern Africa there is a cultural preference for white maize (Muzhingi *et al.,* 2011). Several studies have been done to assess the consumer acceptability of provitamin A biofortified maize. Pillay *et al.* (2011) reported the acceptance of commonly consumed maize products (phutu, thin porridge and samp), prepared with provitamin A biofortified maize. They reported that primary school children preferred the yellow maize, while secondary school children and adults preferred the white maize.

A study conducted by Muzhingi *et al.* (2008) on consumer acceptability of yellow maize in urban and rural Zimbabwe found that more than 94% of households were

willing to consume yellow maize if they knew it was more nutritious than white maize. However, only 2% of households had some knowledge about the nutritional qualities of yellow maize. Although more than 50% of respondents liked the taste of the yellow maize, almost a third disliked the smell. The overall preference for white maize was based on its visual appeal.

White maize is also the most common staple crop produced and consumed in Mozambique (Stevens and Winter-Nelson, 2008). Previous studies in Mozambique indicated that white maize was preferred over yellow maize; however, poor consumers were more willing to purchase yellow maize if it was offered at a discounted price (Low *et al.,* 2007). The market survey conducted by Stevens and Winter-Nelson (2008) found that many participants had a favourable response to the orange maize. The appearance of the orange maize was rated lower by men than by women. Although participants preferred the appearance of the white maize over the yellow maize, participants preferred the aroma of the orange maize over the white maize, which may increase the chances of acceptance. Results from Mozambique suggests that provitamin A biofortified maize may be a self-targeting nutritional intervention, as those who are most vulnerable to VAD were the most likely to accept the orange maize (Stevens and Winter-Nelson, 2008).

Studies conducted on urban consumers have confirmed that white maize is preferred by Kenyans, but there is a preference for yellow maize in some parts of Kenya (De Groote *et al.,* 2010, De Groote and Kimenju, 2008). Consumers with a higher education seem to prefer white maize. Ethnic background plays a role in the preference (De Groote and Kimenju, 2008). De Groote *et al.* (2010) concluded that Kenyans were more interested in commercially fortified maize and would buy yellow maize only at a discount of 11%. Poor acceptance of yellow maize in Kenya seems to come from prejudice and negative associations, such as food aid and animal feed, rather than from sensory characteristics such as taste.

Although the consumer acceptability studies in Mozambique showed a favourable response to yellow maize, studies in Zimbabwe and Kenya have shown a definite preference for white maize over yellow maize (De Groote and Kimenju, 2008, Muzhingi *et al.,* 2008, Stevens and Winter-Nelson, 2008). The feasibility of using

provitamin A biofortified maize to alleviate VAD is dependent on consumer acceptance of the provitamin A biofortified maize. The yellow/orange provitamin A-biofortified maize needs to be widely accepted by consumers who are vulnerable to VAD and are traditionally consumers of white maize. The present study will provide useful data on the consumer acceptability of provitamin A biofortified amahewu among children and adults of poor socio-economic status, who are highly likely to be at risk of VAD.

2.7 SAFETY OF FERMENTED FOOD PRODUCTS

Fermented foods are those which have been subjected to the action of micro-organisms or enzymes, so that the desirable biochemical changes cause significant modification to the food. Fermented foods constitute a major portion of human diets all over the world and provide 20-40% of the total food supply (Abdel *et al*., 2009). Fermented foods, unlike non-fermented foods, have a longer shelf-life, making fermentation a key factor in the preservation of such foods (Nyanzi and Jooste, 2012). The commonest organisms responsible for fermentation of foods are acid-forming bacteria such as lactic acid bacteria (LAB) as, for example, *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Streptococcus, Aerococcus* and *Pediococcus* (Chelule *et al.,* 2010, Agarry *et al.,* 2010).

Most pathogenic micro-organisms found in food cannot survive the low pH of fermented foods. The fermentation of food has been found to reduce the risk of pathogenic micro-organisms growing in the food (Abdel *et al.,* 2009). However, there have been reported cases of persistent pathogens in fermented foods (Colak and Hampikyan, 2007; Ijabadeniyi, 2007; Dineen *et al.,* 1998). Fermentation leads to the significant lowering of anti-nutrients of cereal products (Oyewole and Isah, 2012a). Athoughly fermention improved, to some extent, the nutritional quality and safety of maize-based food products, some including those fermented, are still deficient in micronutrients such as vitamin A, which calls for the need for fortification.

2.7.1 Effects of fermentation on the storage stability of fermented foods

Fermented foods have a longer shelf-life. Fermentation is a key factor in the preservation of foods (Nyanzi and Jooste, 2012). The commonest organisms

responsible for fermentation of foods are acid-forming bacteria such as the LAB genera, *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Streptococcus, Aerococcus* and *Pediococcus* (Chelule *et al.,* 2010, Agarry *et al.,* 2010).

Chako *et al*. (2010) studied the effect of storage conditions on the microbial quality of fermented foods and reported that the shelf life of fermented food products lengthened considerably over the unfermented food products. Shelf life stability of ogi, a fermented corn meal, was studied by Ohenhen *et al.* (2007) who found that fermentation increases the shelf life of the product. Dike and Sanni (2010) studied the influence of starter culture and shelf life of agidi and reported that the use of a starter culture improved the shelf life of the maize product. Lactic acid fermentation of food has been found to reduce the risk of having pathogenic micro-organisms growing in the food (Abdel *et al*., 2009). In addition to this, fermentation irreversibly degrades mycotoxins, without adversely affecting the nutritional value of the food (Ari *et al*., 2012) and without leaving any toxic residues.

RESEARCH

CHAPTER 3

PROCESS OPTIMISATION OF PROVITAMIN A-BIOFORTIFIED AMAHEWU

ABSTRACT

Amahewu, a fermented non-alcoholic maize-based beverage, is a popular drink in southern Africa. Traditionally, amahewu is processed using white maize, which is deficient in Vitamin A. In this study, the suitable processing conditions for the production of amahewu using provitamin A biofortified maize was determined. Two varieties of provitamin A maize (PVAH-62 and PVAH-19) were used. Other processing variables investigated were inoculum types and concentrations. Inoculums used were malted provitamin A biofortified maize (MM), wheat bran, (WB), *Lactobacillus* mixed starter cultures (at 5%), with either MM or WB. These were added at concentrations of 0.5%, 1% and 2%. Wheat flour (at 2%) was used as reference inoculum to conform to the traditional practice. Titratable acidity and pH were monitored at six-hour intervals. Total soluble solids (TSS) of amahewu were determined. One-way analysis of variance was conducted on the results and the means compared, using Fisher's Least Significant Difference Test at $p<0.05$. The variety of provitamin A maize did not influence pH and TTA during fermentation. As expected, there was a substantial drop in pH with fermentation time. After 24 hours, all amahewu products reached a pH of 3.3-3.8 and TTA of 0.3-0.6, which were within an acceptable range for amahewu. The inoculum types did not substantially affect the pH changes after 24 hours of fermentation. However, 0.5% inoculum concentration appeared to be as effective as 2% and the pH and TTA values compared favourably with those obtained with traditional amahewu produced using 2% wheat flour as inoculum. The addition of starter culture substantially reduced fermentation time by from 24 to six hours with pH 3.3 and TTA 0.6. Provitamin A biofortified maize can be used to produce enriched provitamin A biofortified amahewu. The wheat bran and malted inoculum concentration of 0.5%,

with or without starter culture, could be recommended for the production of amahewu using provitamin A biofortified maize.

3.1 INTRODUCTION

Maize (*Zea mays*) is the most important staple crop in sub-Saharan Africa. In South Africa, white maize is commonly consumed in the form of thick and thin porridge and amahewu, a non-alcoholic fermented beverage. Amahewu is consumed by various groups of people, including children, youths and adults. It is used as complementary food for infants by weaning mothers (Simango, 1997). White maize, known to be deficient in vitamin A, is normally used for processing and, consequently, the consumption of amahewu may contribute to vitamin A deficiency among the low income population group.

Traditionally, amahewu is made by adding white maize meal into water (ratio 1: 9) and the mixture is boiled, with occasional stirring, for 10-15 minutes, until cooked. The resulting porridge is cooled to about 40^{O}C and then allowed to ferment in a warm place for one to three days (Chelule *et al.,* 2010). Wheat flour (approximately 2% of the grist w/w) is used as a source of inoculum during fermentation. The standardised amahewu product contains 8-10% solids and has a pH of 3.4-3.9 and titratable acidity is 0.4-0.5%. The main fermenting organisms identified in traditional amahewu processing are *L. acidophilus. L. bulgaricus, L. delbrueckii* and *Streptococcus lactis* (Mugocha, 2001). The use of provitamin A biofortified maize in the production of amahewu may assist to combat VAD among vulnerable rural people. However, processing parameters (conditions and formulation) for provitamin A-biofortified maize amahewu are not known and there is a need to optimise the processing of the innovative product, so that it is of acceptable quality.

Previous research reported the use of malt and wheat bran as sources of inoculum to aid the fermentation process of porridges (Firibu *et al.* (2012), Kure *et al.* (2013) and Onesmo (2011). According to Onesmo (2011), fermentation with added malt considerably lowered the pH and increased the total acidity of gruels. The addition of wheat bran increased the fibre content. In the present study, optimal processing

conditions for the production of amahewu using provitamin A biofortified maize were determined.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Design

Two varieties of provitamin A biofortified maize, PVAH-62 and PVAH-19, were chosen. White maize was used as a reference. Malted provitamin A biofortified maize (MM) ,wheat bran (WB) and *Lactobacillus* starter culture were added to the at concentrations of 0.5, 1 and 2% (w/w). Analyses were carried out in duplicates. The maize varieties were grown in the same location and under the same conditions.

3.2.2 Preparation of provitamin A biofortified maize amahewu

Provitamin A-biofortified maize amahewu was processed by adding one part of maize meal to seven parts of water (w/v) and boiled at 90^{O}C, with occasional stirring, for 15 minutes. The resulting porridge was left to cool to approximately 40^{O}C. Inocula: malted provitamin A biofortified maize (MM) and wheat bran (WB) were added to the porridges, at concentrations of 0.5, 1 and 2% (w/w) and the porridges allowed to ferment at 37^{O}C.

Amahewu samples made with white maize, which were prepared in the same manner as the amahewu samples made with provitamin A maize, were used as references. The literature indicated that approximately 2% wheat flour is added during the traditional processing of amahewu. A traditional amahewu sample was thus prepared, using 2% wheat flour, for comparison (Chelule *et al.* 2010).

Use of starter culture: after identifying the appropriate concentration for malted maize and wheat bran, amahewu was prepared, but with a mixed *Lactobacillus* starter culture at 5% concentration (w/w), with either 0.5% malted maize or 0.5% (w/w) wheat bran.

3.2.3 pH and titratable acidity

The pH and titratable acidity (TTA) of the amahewu samples were monitored at six-hour intervals. pH was measured by calibrating the probe and meter according to manufacturer's instructions, buffers 3, 7 and 10 was used for calibration as

recommended. Probe was rinsed with water before placing itin the sample. TTA was measured by adding 10 ml of amahewu into a beaker. 5 drops of phenolphthalein was added as an indicator and titrated against 0.1 NaOH and expressed as percent lactic acid (Amerine *et al.,* 1967).

3.3 RESULTS AND DISCUSSIONS

The variety of maize did not have any major influence on the progression fermentation, as amahewu samples from the two varieties showed similar trends of pH and TTA. The pH and TTA patterns of provitamin A biofortified maize amahewu samples were similar to those of white maize and the traditional amahewu during the 24-hour fermentation period. However, a variation in pH was observed with the inoculum types.

As expected, the pH of the samples of amahewu decreased with the progression of fermentation (Fig. 9-16). An opposite effect was measured for TTA. After 24 hours of fermentation, all the samples of provitamin A-biofortified amahewu prepared with either maize malt or wheat bran as inoculums reached pH 3.3-3.7 and TTA 0.1- 0.6% lactic acid equivalents. The observed pH and TTA acidity were within the acceptable range for amahewu (Mugocha, 2000).

Amahewu prepared using malted maize as inoculum recorded the lowest pH, pH 3.3, compared to wheat bran, pH 3.5, and wheat flour, pH 3.6. The high fermenting ability of malted maize may be attributed to a high concentration of amylolytic enzyme in malt (Mugocha, 2000). Similar observations and findings have been reported by Wedad *et al.* (2008) and Gernah *et al.* (2011).

With starter culture addition (at 5%), the minimum concentration of the inoculum required to achieve the acceptable pH range for amahewu was found to be 0.5%. Therefore, in the next experiments of amahewu processing optimisation, *Lactobacillus* starter cultures were added at 5% concentration, with either 0.5% (w/w) of maize malt or wheat bran, separately, as inoculum source. The addition of starter culture substantially reduced the fermentation time, from 24 hours to six hours, with final pH of 3.5 and TTA of 0.6%. The remarkable drop in pH within six hours during the fermentation of maize food using starter cultures observed in this

study is in agreement with the results of Onyango (2009). Mugula *et al.* (2003a) reported that the use of starter culture significantly ($p \leq 0.05$) decreased pH during the fermentation of togwa, a non-alcoholic traditional beverage of East Africa. The changes in pH, TTA and fermentation time of amahewu were similar for both varieties of provitamin A biofortified maize and white maize (Fig 9-16).

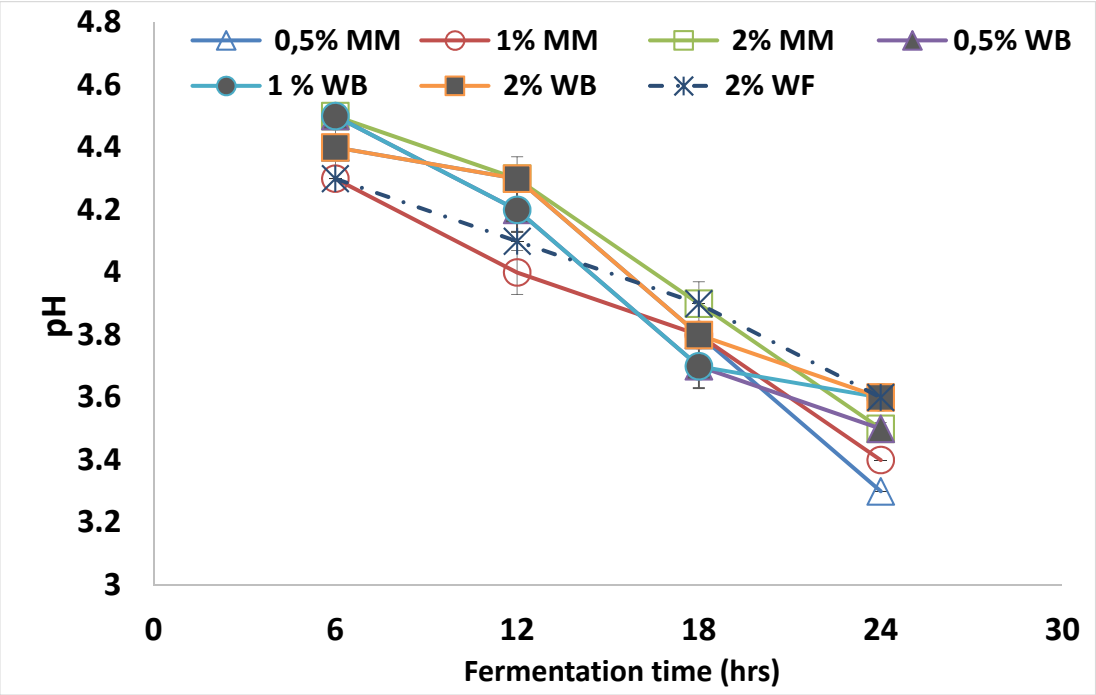

Figure 9: Effect of inoculum types, concentration and fermentation time on the pH of provitamin A biofortified amahewu (variety PVAH-62). MM: malted provitamin A biofortified maize, WB: wheat bran, WF: wheat flour (CV=9.2%; $p<0.05$).

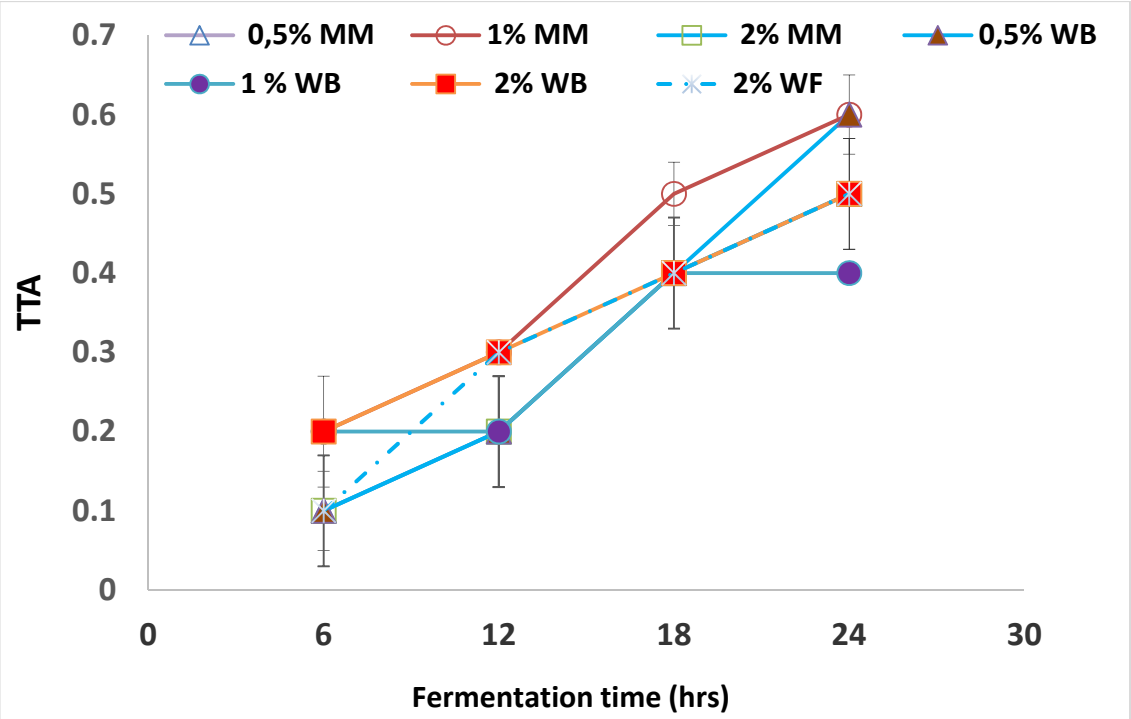

Figure 10: Effect of inoculum types, concentration and fermentation time on the total titratable acidity of provitamin A biofortified amahewu (variety PVAH-62). MM: malted provitamin A biofortified maize, WB: wheat bran, WF: wheat flour (CV=41%; $p<0.05$).

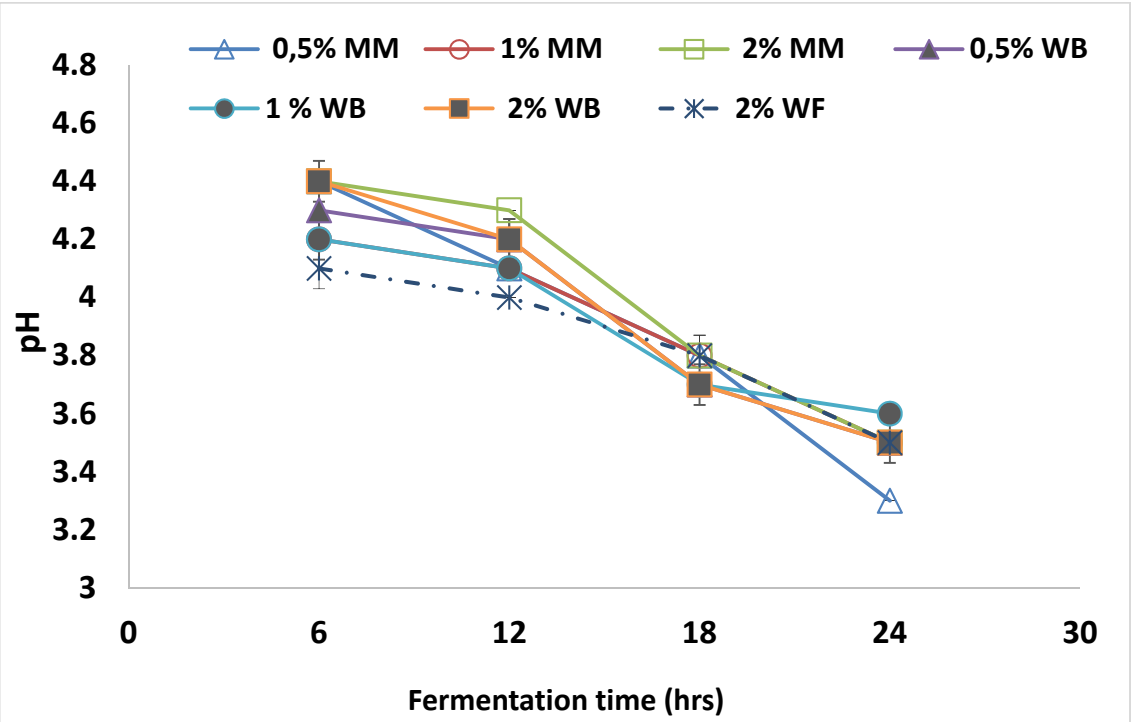

Figure 11: Effect of inoculum types, concentration and fermentation time on the pH of provitamin A biofortified amahewu (variety PVAH-19). MM: malted provitamin A biofortified maize, WB: wheat bran, WF: wheat flour (CV=9.9%; $p<0.05$).

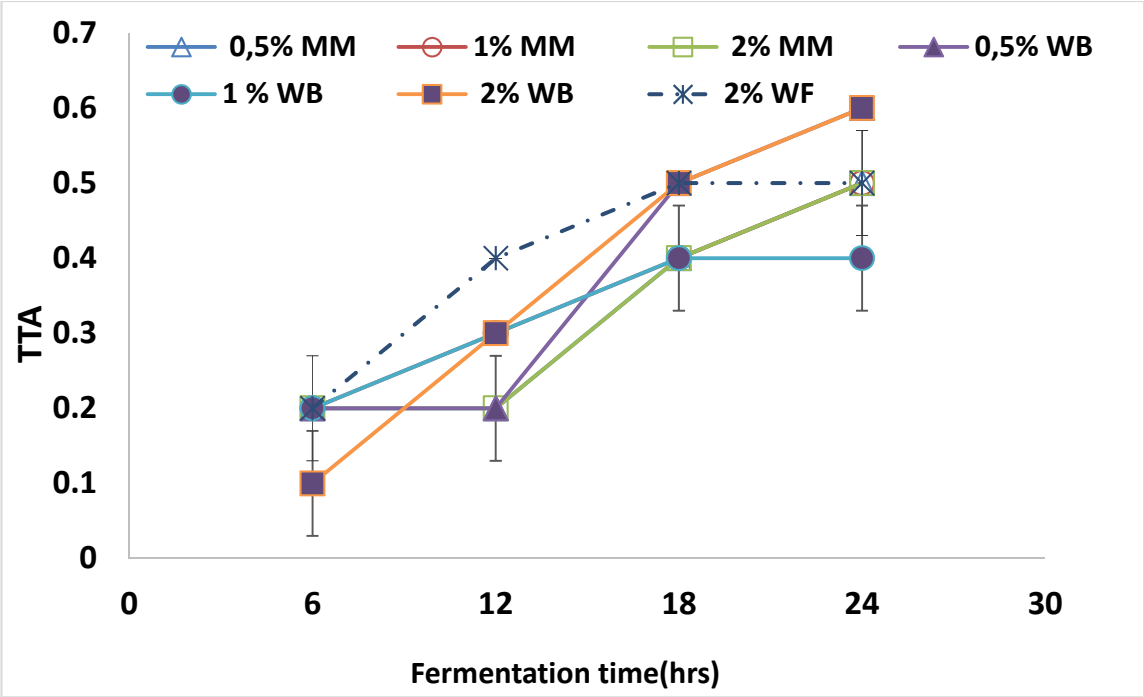

Figure 12: Effect of inoculum types, concentration and fermentation time on the total titratable acidity of provitamin A biofortified amahewu (variety PVAH-19).MM: malted provitamin A biofortified maize, WB: wheat bran, WF: wheat flour (CV=4.2%; $p<0.05$).

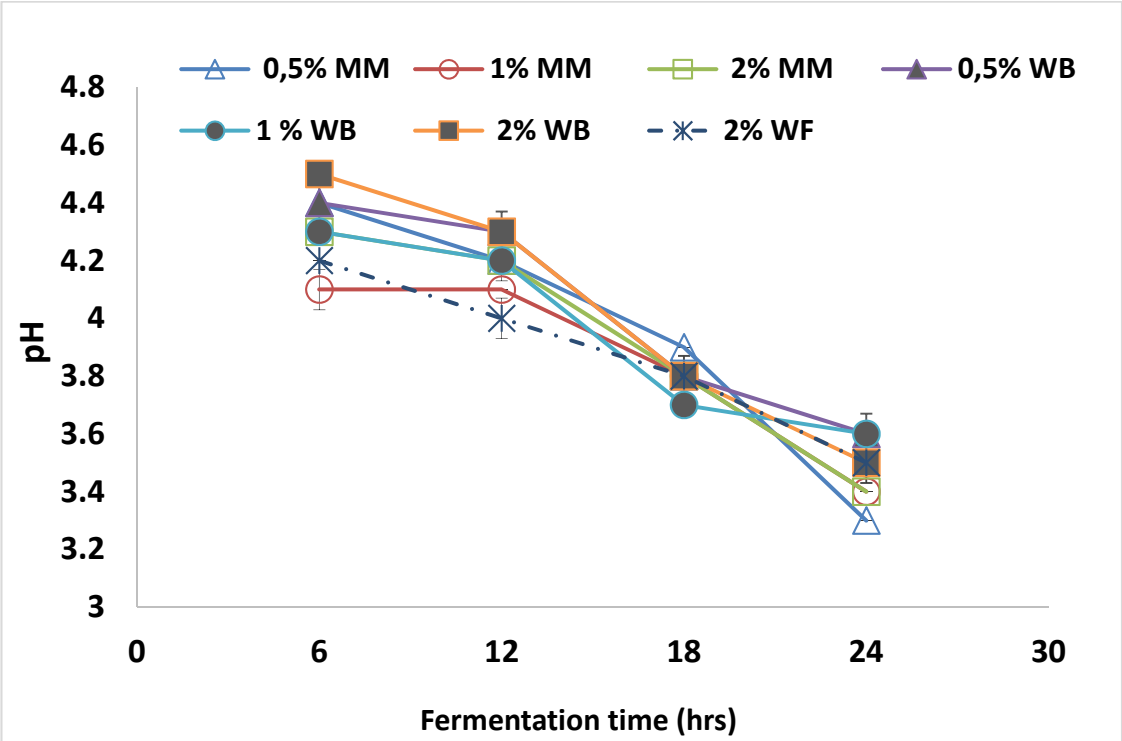

Figure 13: Effect of inoculum types, concentration and fermentation time on the total pH of white maize amahewu (control).MM: malted provitamin A biofortified maize, WB: wheat bran, WF: wheat flour (CV=9.8%; $p<0.05$).

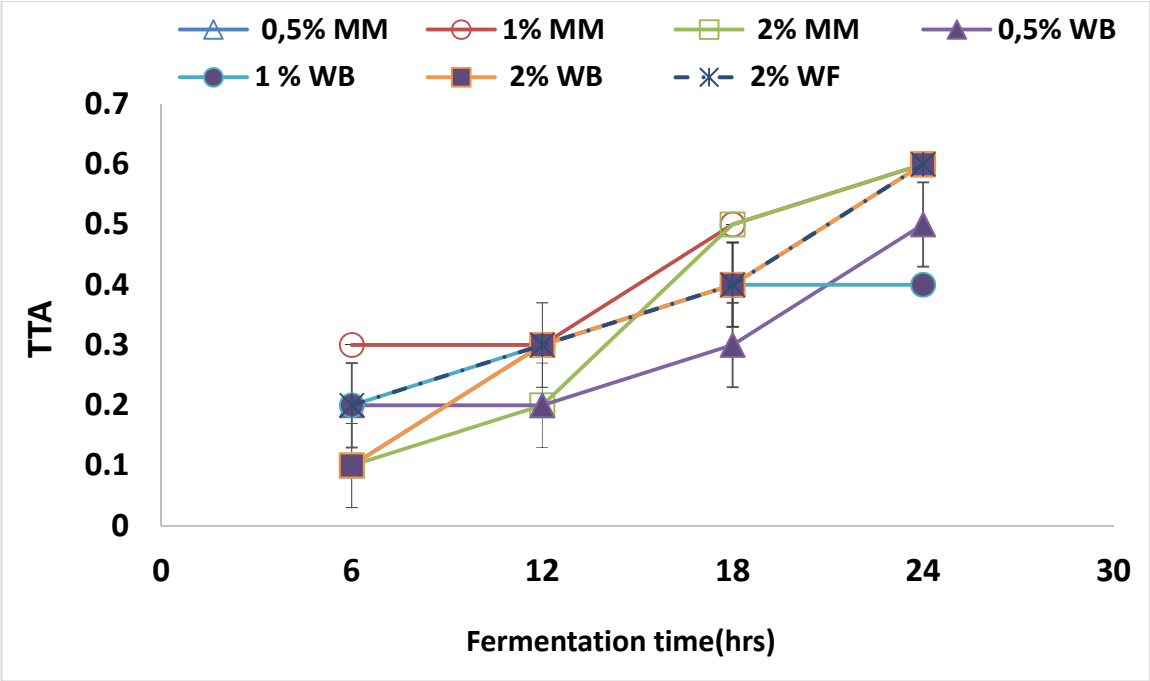

Figure 14: Effect of inoculum types, concentration and fermentation time on the total titratable acidity of white maize amahewu (control).MM: malted provitamin A biofortified maize, WB: wheat bran, WF: wheat flour (CV=43%; $p<0.05$).

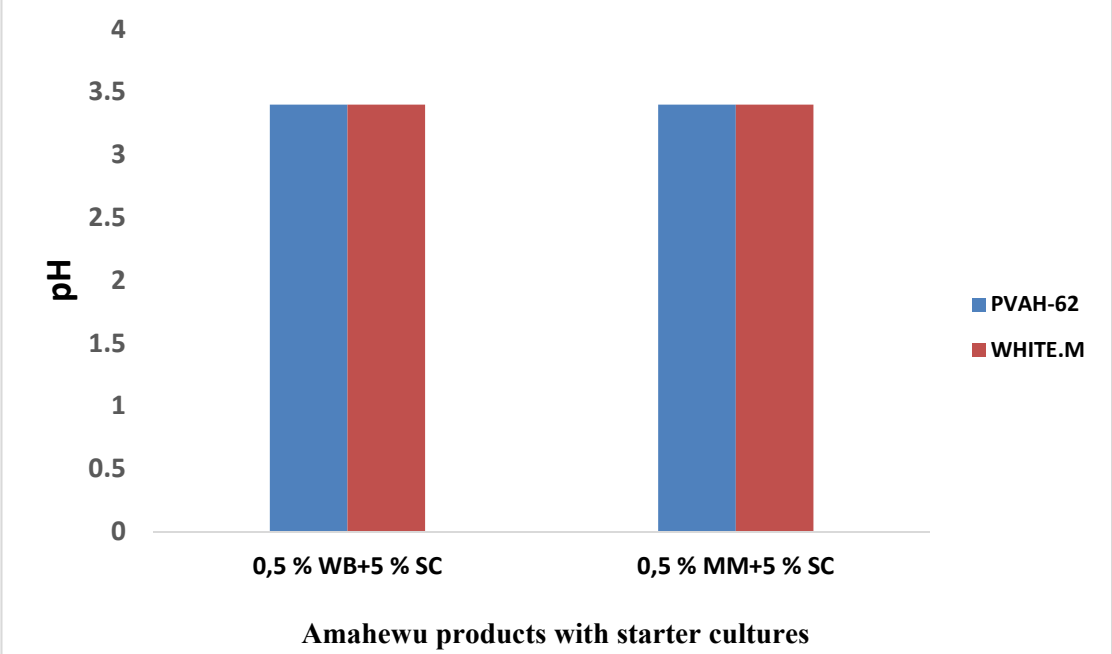

Figure 15: Effect of inoculum types, concentration, fermentation time and the use of starter cultures on the pH of provitamin A biofortified amahewu 0.5 % WB +5% SC: wheat bran + starter culture, 0.5 % MM+ 5% SC: malted provitamin A biofortified maize + starter culture (CV=11%; $p<0.05$).

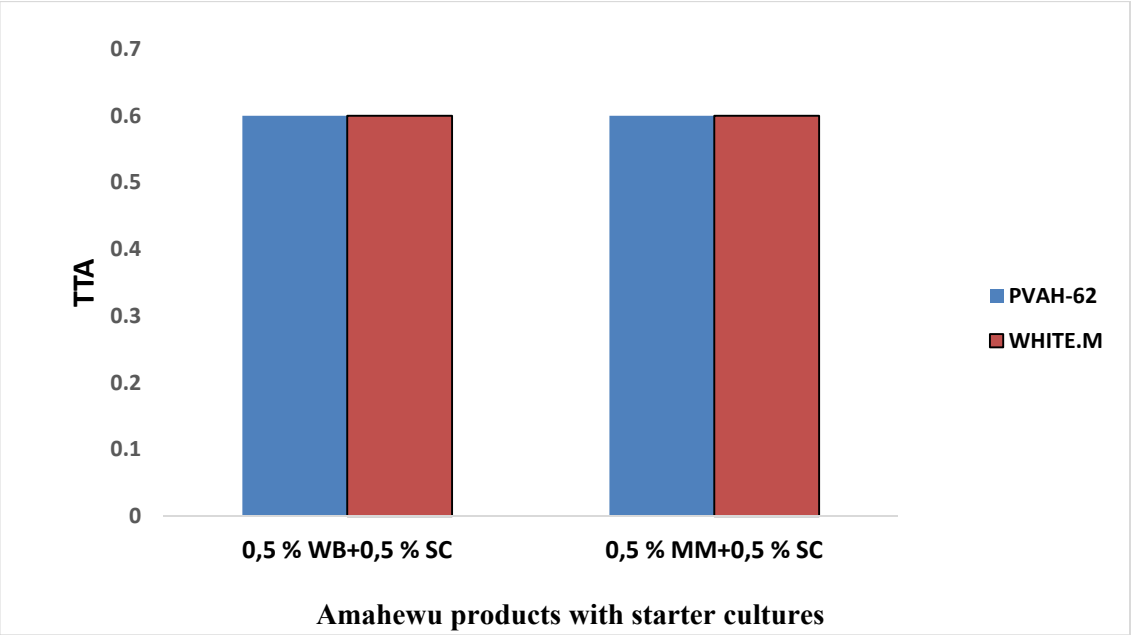

Figure 16: Effect of inoculum types, concentration, fermentation time and the use of starter cultures on total titratable acidity of provitamin A biofortified amahewu 0.5 % WB +5% SC: wheat bran + starter culture, 0.5 % MM+ 5% SC: malted provitamin A biofortified maize + starter culture (CV=25%; $p<0.05$).

3.4 CONCLUSIONS

Wheat bran and malted maize were effective at 0.5, 1 and 2 %.However, wheat bran changes the colour of amahewu at 1 and 2% concentrations which could pose a challenge on the consumer acceptance. Malted maize which is uncooked could also have an effect on the taste of amahewu at these concentrations. Hence, 0.5% concentration of both wheat bran and malted maize can be recommended as a source of inoculum for traditional processing of amahewu using provitamin A- biofortified maize. The addition of starter culture (5%) substantially reduces the fermentation time and this may be appropriate for commercial production of amahewu. The use of starter culture for amahewu production may constitute one major step towards improved safety and quality of traditional fermentation. The two varieties of maize can be used to produce amahewu similar to that produced with white maize. This findings shows that wheat bran and malted provitamin A-biofortified maize can be used to produce commercialized amahewu in the food and beverage industry.

CHAPTER 4

NUTRITIONAL QUALITY OF PROVITAMIN A-BIOFORTIFIED AMAHEWU

ABSTRACT

Amahewu, a lactic acid fermented non-alcoholic maize based beverage is widely consumed in southern Africa., especially by low-income rural communities who are generally vulnerable to malnutrition, including vitamin A deficiency (VAD). Amahewu could be used to deliver provitamin A in biofortified maize to VAD-vulnerable communities. The nutritional quality of amahewu produced using provitamin A biofortified maize was determined. One variety of provitamin A biofortified maize (PVAH-62) was used, Wheat bran, malted maize and *lactobacillus* mixed starter cultures were used as inoculums. Provitamin A biofortified amahewu was processed by fermenting maize porridge using malted provitamin A biofortified maize, wheat bran and a *Lactobacillus* starter culture with either malted maize or wheat bran. One-way analysis of variance was performed on the results and the means compared, using Fisher's Least Significant Difference (LSD) test at $p<0.05$. The total provitamin A content in amahewu products ranged from 3.3-3.8 μg/g (DW). The percentage retention of total provitamin A ranged from 79%-90% μg/g (DW). The lowest percentage retention was observed in products fermented with the addition of the starter culture. The gross energy of amahewu products was about 20 MJ/kg. There was a slight increase in the lysine content of amahewu after fermentation. The protein digestibility of amahewu (approx. 91%) was slightly higher than that of unprocessed provitamin A maize (86%). Amahewu processed using starter cultures had higher iron content than those processed with the addition of malt. These results indicate that provitamin A biofortified amahewu has the potential to make a significant contribution to the alleviation of VAD.

4.1 INTRODUCTION

Provitamin A biofortification of maize through conventional plant breeding is seen as an alternative strategy to alleviate VAD (Ortiz-Monasterio *et al.,* 2007). Provitamin A deep yellow or orange varieties of maize may contain up to 15 µg/g DW of provitamin A (Nuss and Tanumihardjo, 2010), which is substantially high, compared to traditionally cultivated yellow maize, 0.25 and 2.5 µg/g DW. Breeding for provitamin A biofortified maize has become a more sustainable approach as it is cost affective and poses less risk of toxicity. Provitamin A biofortified maize can thus be used in the development of food products. However, evaluation of the quality of the processed foods is essential, especially in assessing the suitability of the processing methods with respect to, among several product quality considerations, their impact on the nutritional quality of the products.

The nutritional quality of maize-based foods, including those of provitamin A, as affected by processing methods, have previously been investigated (Pillay, 2011). For instance, fermentation has been found to result in a lower proportion of dry matter in maize foods and the concentrations of minerals and protein increased when measured on a dry weight basis (Adams, 1990). According to Holzapfel (2002), fermentation decreases the activity of the proteinase inhibitors in cereals, resulting in an increase in the availability of essential amino acids such as lysine, leucine, isoleucine and methionine. Previous studies have indicated possible loss of provitamin A carotenoids during fermentation (Muntean, 2007). Heating has also been found to cause degradation and isomerisation of carotenoids (Robert *et al*., 2005). Amahewu is a very popular beverage in southern Africa. Traditionally, it is processed by fermenting cooked maize porridge. The resulting gruel contains about 10% solids (Chelule, 2010). Wheat flour is the most common source of the fermentation inoculum for home-based processing of amahewu, when compared with industrial processing, where starter cultures are employed. The effects of processing provitamin A biofortified maize, with or without starter cultures, on the nutritional quality of amahewu seems not to have been investigated and is the aim of the current investigation.

4.2 MATERIALS AND METHODS

4.2.1 Chemicals and standards

All solvents used in the carotenoid analysis were HPLC grade. The following solvents were used: methanol (CH_4O), acetonitrile ($C2H_3N$), dichloromethane (CH_2CL_2), ammonium acetate ($C_2H_7NO_2$) and triethylamine ($C_6H_{15}NO_3$). Analytical standards of β-carotene, β-cryptoxanthin, zeaxanthin, lutein and α-carotene (Sigma-Aldrich, St. Louis, MO, USA) were used to calibrate and quantify the carotenoids.

4.2.2 Experimental design

Provitamin A biofortified maize variety PVAH-62 was used. Wheat bran, malted maize and *lactobacillus* mixed starter cultures were used as inoculums at 0.5 % and 5 % concentrations respectively. The maize grains used in this study was obtained from the University of KwaZulu-Natal. South Africa. Analyses were carried out in duplicates.

4.2.3 Preparation of amahewu

Amahewu samples were prepared according to a traditional method, which was described by persons from a community living in rural KwaZulu-Natal. The method involved adding one part of maize meal to seven parts of water and then boiling at 90^{O}C, with occasional stirring, for 15 minutes. The resulting porridge was left to cool to approximately 40^{O}C (Chelule *et al.* 2010). Inocula: malted provitamin A biofortified maize (MM) and wheat bran (WB) were added at 0.5% concentration to porridges and these were allowed to ferment at 37^{O}C. The provitamin A biofortified maize meal was used to prepare the test amahewu samples, whilst the white maize meal was used to prepare the reference amahewu samples.

4.2.4 Total soluble solids

Total soluble solids were determined using a refractometer. The pH and TTA of amahewu which was 3.5 after processing, was measured as described in chapter 3. Total soluble solids (TTS) ranged from 1.9-4.0.

4.3 CHEMICAL ANALYSES

4.3.2 Carotenoids by HPLC

Prior to HPLC analysis, carotenoids were extracted from freeze-dried amahewu samples, using the procedure described by Kurilich and Juvik (1999). Freeze-dried samples of 0.5 g were weighed. Absolute ethanol (6 mL) containing 0.1% butylated hydroxytoluene (BHT) was added to each sample before placing in an 85°C water bath for five minutes. After removal from the water bath, 120 µL of 80% potassium hydroxide (KOH) was added, samples were vortexed for 20 seconds and returned to the water bath for a 10-minute saponification. All samples were vortexed once more during saponification. Upon removal they were immediately placed in an ice bath, where 3 mL cold deionized distilled H2O were added. Each sample then received 3 mL of petroleum ether and diethyl ether (ratio 2:1 v/v) and was vortexed and centrifuged for five minutes at 1400 x g. The upper layer was pipetted into a separate test tube and the pellet was re-extracted twice more. The supernatant of the organic layers was combined in a 10 mL test tube and dried under gas nitrogen steam. The residue was kept at -20^{O}C and reconstituted in 500µl of mobile phase before HPLC analysis. Carotenoid extracts were cleaned up using 0.4mm filter paper before loading onto the HPLC.

Carotenoids were quantified in the sample extracts using a Shimadzu HPLC equipped with a C18 column (Dimension 215mm; Particle size 5µm) in a HPLC and a Photo Diode Array (PDA) detector. The mobile phase consisted of Acetonitrile: Dichloromethane: Methanol: Triethylamine and Ammonium acetate (80:5:15:1:150 mM).The flow rate was set at 1ml/min, injection volume set at 20 µl and absorbance measured at 450 nm. Quantification was based on the peak areas, against a calibration curve obtained using carotene standards (Sigma).

4.3.3 Provitamin A content

Total provitamin A content expressed as β-carotene, which was calculated by using the formula: total provitamin A content = β-carotene + (β-cryptoxanthin+α-carotene)/2. This is because the vitamin A activity of each of β-cryptoxanthin and α-

carotene is half (50%) of that of β-carotene. The percentage retention of provitamin A in the provitamin A biofortified maize amahewu was calculated using the formula:

$$\%\ \text{provitamin A retention} = \frac{\text{Provitamin A content per g sample (dry basis)}}{\text{Provitamin A content per g maize flour (dry basis)}} \times 100$$

4.3.4 Proximate analysis

Provitamin A biofortified amahewu samples were analysed. Analyses were carried out in duplicates.

4.3.4.1 Moisture content

The moisture content of the samples was measured according to the Association of Official Analytical Chemists International (AOAC) Official Method 934.01 (AOAC 2002), in which the samples of known weight were dried in a forced air oven set at 95ºC for 72 hours. The moisture content of the food products was determined by weight difference after freeze drying in a freeze drier.

4.3.4.2 Fat

The fat content of the samples was determined according to the Soxhlet procedure, using a Büchi 810 Soxhlet Fat Extractor (Büchi, Flawil, Switzerland), according to the AOAC Official Method 920.39C (AOAC 2002). A 250ml round bottom flask was weighed, cooled in a dessicator and the mass will be recorded. 3 grams of sample was weighed and the mass was recorded. The sample was transferred to an extraction thimble, and the thimble was placed in an extraction chamber. 100ml of petroleum ether was added to the flask and the flask was connected to the reflux chamber. The condenser was connected to the chamber and the tap was opened to allow for the water to steadily flow through the condenser. The heating mantle was turned on to a medium temperature setting. The fume cupboard extractor fan was turned on. The sample was extracted by refluxing the solvent for at least five minutes; the solvent is topped by via the condenser chamber as soon as the solvent level drops to below the reflux chamber level. The heating mantle will be switched off just before all solvent evaporates from the round bottom flask. The round bottom flask was not be allowed to run dry as the fat extract this will allow the fat extract to

start to burn and this will affect the final extract mass. The flask containing the extract was cooled in a dessicator for 3 to 4 hours and the mass recorded (David Pearson, 1976).

$$\text{Calculate \% fat} = \frac{\text{weight of residue (g)} * 100}{\text{weight of sample (g)}}$$

Where,

Weight of residue = original sample mass – mass of fat extract

4.3.4.3 Ash

Ash was determined by combusting the samples in a furnace set at 550 ºC for four hours, following the AOAC Official Method 923.03 (AOAC 2005).

4.3.4.4 Protein

In clean a dry digestion tubes, 3 grams of samples was weighed. 4 grams of catalyst was added to the mixture and 25 ml concentrated H_2SO_4. The digestion tubes was connected to NaOH trap for absorbing the noxious fumes. The vacuum was used to draw the fumes into the NaOH trap. With a cotton plug the unused opening will be closed, heating commenced and maintained such that the sample will always boiling. The digestion will be allowed to proceed for approximately 1-2 hrs. Digestion is complete when the solution turns light clear green or looks greenish. The Buchi 321 distillation unit was switched on and allowed to preheat. Distillation vessel was inserted with digested sample. The holder was pressed downwards and released when tube is in place. The sample was diluted in water in approximate ratio of 1:3. The water switch was pressed until desired quantity has filled in. 250ml Erlenmeyer receiving flasks was prepared by adding 25ml 2% boric acid and six drops screened methyl red indicator. The Erlenmeyer receiving flask was placed under the long tubes. 32% sodium hydroxide solution was added to sample by pressing on NaOH switch. (Minimum volume of 32% sodium hydroxide required is 100ml of until the solution turns dark brown in colour). The distillation time was set; to $2\frac{1}{3}$ -3 minutes, and the distillation will proceed. The residue aspirations switch was set in ON

position so that at the end of distillation, the distillation switch residues was aspirated off into the sink. The distillate was titrated with standard 0.1N sulphuric acid. The end point is reached when the light blue solution should turn colorless to grey. The protein content is determined by using the equation:

$$\text{Calc \% N} = \frac{\text{titration in ml} - \text{blank in ml} * 1.4 * 0.1}{\text{mass of sample}}$$

% Protein = Factor (for product) x N

4.3.4.5 Gross energy

The gross energy (GE) content of the milled samples and excreta was determined by adiabatic bomb calorimetry, according to the apparatus User's Manual (Gallenkamp, Autobomb, London, UK).

4.3.4.6 Total carbohydrate

Total carbohydrate was obtained by difference. The carbohydrate content was estimated according to the (AOAC, 1995) method as seen below:

Carbohydrate% = (moisture% + fat% + protein% + ash% - 100%).

4.3.5 Individual minerals

Mineral content was determined according to the AOAC method no. 6.1.2 (AOAC 1984), using the Inductively Coupled Plasma (ICP) Spectroscopy. Ground samples of each amahewu sample were acid-digested by addition of 1 mL of 55% (v/v) HNO_3.

4.3.6 Amino acids

The amino acid profile of the samples was analysed by the Waters API Quattro Micro Method, which consists of a column C18, 1.7um, 2.1x 100mm and a binary solvent manager. Samples (400 mg) were subjected to Waters AccQ Tag Ultra Derivatization kit; 10µl of the undiluted sample were added to the Waters AccQ Tag kit constituents and placed in a heating block at a temperature of 55^{O}C for 10 minutes. Injection volume was 1µl.

4.3.7 *Invitro* protein digestibility

According to the method of Bruce Hamaker (1987), a sample 0.2 g was weighed; 35 mL of 0.1 M phosphate buffer; pH 2 containing 1.5 mg pepsin/mL was added. Pepsin-sample mixture was incubated at 37^{O}C for two hours, with continuous shaking. Digestion was stopped by adding 2 mL of 2 M NaOH. The suspension was centrifuged at 4800 rpm at 4^{O}C for 20 minutes. and the supernatant was discarded. The residue was washed with 15 ml of 0.1 M phosphate buffer, pH 7, and centrifuged.

Again the supernatant was discarded and the residue washed on Whatman's No 3 filter paper. The filter paper containing the undigested protein residue was folded and placed in a digestion tube and dried for two hours at 80^{O}C. The dried sample was analysed for protein, using the micro kjeldahl method.

4.3.8 Statistical analyses

Duplicate samples were analysed. Each analysis was repeated at least twice. Mean and standard deviations were calculated and one-way analysis of variance (ANOVA) was done. Mean separation was by Fisher Least Significance Difference ($p < 0.05$).

4.4 RESULTS AND DISCUSSIONS

4.4.2 Carotenoids

Lutein, zeaxanthin, β-cryptoxanthin, β-carotene and α-carotene, which were the major carotenoids in maize, were present in all amahewu samples, with β-cryptoxanthin being the most abundant (Table 2). Similar results were reported by Lozano-Alejo *et al.* (2007). The β-carotene content (approx. 1.8 μg/g) was very similar across amahewu samples, including the raw maize. β-cryptoxanthin content was slightly high compared to other carotenoids, while lutein was the lowest. Except β-carotene, other carotenoids decreased in the amahewu sample prepared using starter cultures, compared to those with wheat bran and malt. The reduction observed may be attributed to increased metabolic activity of the fermenting micro-organisms.

The total provitamin A contents of amahewu samples was estimated from β-carotene, β-cryptoxanthin and α-carotene. The total provitamin A content in provitamin A-

biofortified maize amahewu samples ranged from 3.3-3.8 µg/g β carotene equivalents (DW) (Table 3). The provitamin A content is much higher than the values (0.25-2.5 µg/g DW) reported traditionally for yellow maize (Nuss and Tanumihardjo 2010; Kurilich and Juvick 1999). Overall, provitamin A was substantially retained by 79 %- 90 % β-carotene equivalents (DW) after fermentation in all the amahewu samples. However, the lowest percentage retention was observed when the starter culture was further added as inoculum. Except for β-carotene, β-cryptoxanthin and α-carotene appeared to have experience degradation during fermentation, thus explaining the reduction in provitamin A retention. The low retention of carotenoid in amahewu samples prepared by adding starter culture may be attributed to the metabolic activities of these fermenting micro-organisms, which may have promoted the degradation of the carotenoids, for example through oxidation.

Provitamin A biofortified amahewu samples were cooked before fermentation. Heat treatment could also have contributed to the reduction in carotenoid content of the amahewu samples. It has been reported that, at high temperatures, the long chain polyunsaturated carotenoids undergo isomerisation from the trans to the cis form, leading to the loss of carotenoids (Tannenbaum, 1976).

Table 2: Carotenoid content of amahewu (µg/g)

Samples	α-carotene	β-carotene	zeaxanthin	lutein	β-cryptoxanthin
Raw	2.6^{b}±0.01	1.9^{d}±0.01	1.8^{e} ±0.01	1.7^{f} ±0.01	2.9^{a} ±0.01
WB	1.8^{e} ±0.01	1.8^{e}±0.01	1.8^{e} ±0.01	1.2^{j} ±0.00	2.1^{c} ±0.01
M	2.1^{c} ±0.01	1.7^{f} ±0.01	1.6^{g} ±0.01	1.5^{h}±0.00	2.0^{c} ±0.01
MM+SC	1.5^{h} ±0.00	1.8^{e} ±0.01	1.6^{g} ±0.01	1.3^{j}±0.00	1.6^{g} ±0.01
WB+SC	1.6^{g} ±0.01	1.7^{f} ±0.01	1.4^{i} ±0.00	1.7^{f} ±0.01	1.9^{c} ±0.01

Mean (n=2) is reported, (dry weight basis)[1]**.**WB: wheat bran, MM: malted provitamin A biofortified maize, WB + SC: wheat bran + starter culture, MM + SC: malted provitamin A biofortified maize + starter culture. Mean with different superscript letters in column are significantly different ($p<0.05$), (dry weight basis)

Table 3: Provitamin A retention in amahewu (µg/g)

Samples	Provitamin A content (µg/g)*	Provitamin A retention (%)
WB	$3.8^{a} \pm 0.07$	$90^{a} \pm 0.70$
M	$3.7^{a} \pm 0.07$	$88^{a} \pm 0.70$
MM+SC	$3.3^{b} \pm 0.07$	$79^{a} \pm 0.70$
WB+SC	$3.5^{a} \pm 0.07$	$83^{b} \pm 0.70$

Mean with different superscript letters in column are significantly different ($p<0.05$), (dry weight basis) *Total provitamin A-content expressed β-carotene equivalents.

4.4.3 Proximate composition of amahewu

Protein (approx. 11.5 g) and carbohydrate (approx. 82 g) were the major nutrients in the amahewu sample (Table 4). However, there seemed to be a slight increase in protein in the amahewu samples that were prepared with starter culture, compared to those with wheat bran or malted maize only. This apparent increase in protein content after fermentation may be due to a decrease of carbon ratio in the total mass, resulting in redistribution of nutrient percentages (Onyango, 2004). Micro-organisms utilise carbohydrates as an energy source and produce carbon dioxide as a by-product. This causes the nitrogen in the fermented products to be concentrated and the proportion of protein in the total mass increases. The gross energy contents (approx. 20 kg) were very similar for all amahewu samples. Ash and fat were present in relatively low quantities in all amahewu samples similar to the provitamin maize.

Table 4: Proximate composition of amahewu (g/100g)[1]

Amahewu samples	Moisture	Ash	Fat	Protein	Carbohydrate	Gross energy (MJ/kg)
RAW	$5.0^{e} \pm 0.00$	$3.3^{e} \pm 0.01$	$1.1^{a} \pm 0.02$	$11.6^{b} \pm 0.02$	$79^{a} \pm 0.02$	$19.2^{f} \pm 0.0$
WB	$3.1^{b} \pm 0.13$	$1.8^{b} \pm 0.03$	$2.1^{c} \pm 0.07$	$10.1^{a} \pm 0.03$	$83^{d} \pm 0.21$	$19.2^{e} \pm 0.0$
M	$3.5^{c} \pm 0.02$	$1.6^{a} \pm 0.04$	$3.8^{d} \pm 0.07$	$10.2^{a} \pm 0.02$	$81^{c} \pm 0.02$	$19.2^{e} \pm 0.1$
MM+SC	$4.5^{d} \pm 0.03$	$3.0^{c} \pm 0.08$	$2.0^{c} \pm 0.02$	$11.5^{b} \pm 0.02$	$79^{a} \pm 0.00$	$19.0^{e} \pm 0.0$
WB+SC	$2.6^{a} \pm 0.06$	$3.0^{c} \pm 0.75$	$1.5^{b} \pm 0.10$	$12.5^{c} \pm 0.04$	$80^{b} \pm 0.08$	$19.7^{d} \pm 0.0$

[1]Mean (n=4) is reported. Mean with different superscript letters in column are significantly different ($p>0.05$). Where WB=wheat bran, MM=malted maize, WB+SC= wheat bran + starter culture, MM+SC+ malted maize + starter culture

4.4.4 Mineral composition of amahewu

The mineral profile of amahewu samples did not vary much after fermentation (Table 5). Micronutrients such as iron and zinc contents of amahewu samples were similar to those of provitamin A biofortified raw maize, suggesting that fermentation did not substantially influence the mineral profile. However, there was a slight improvement in the iron content of amahewu fermented with the addition of starter cultures. This apparent increase in iron content of amahewu after fermentation may be attributed to the destruction of anti-nutrient factors such as phytate which bind to iron, thus making it readily assayable (Valérie *et al*., 2011).

The iron and zinc contents in the provitamin A biofortified amahewu reported in this study are higher than the values reported by Šimić *et al.* (2009), Oikeh *et al.* (2004), Oikeh *et al.* (2003a) and Oikeh *et al.* (2003b) in normal white maize.

Table 5: Mineral composition of amahewu (mg/100g)

Minerals	RAW	M	WB	MM+SC	WB+SC
Ca	0.06^{d} ±0.00	0.02^{a} ±0.00	0.03^{a} ±0.00	0.05^{b} ±0.00	0.05^{b} ±0.00
Mg	0.11^{a} ±0.00	0.13^{b} ±0.00	0.16^{c} ±0.00	0.13^{b} ±0.00	0.15^{c} ±0.00
K	0.41^{d} ±0.00	0.26^{a} ±0.00	0.32^{b} ±0.00	0.36^{c} ±0.00	0.41^{d} ±0.00
Na	0.62^{c} ±0.00	0.04^{a} ±0.00	0.04^{a} ±0.00	0.60^{c} ±0.00	0.54^{b} ±0.00
P	0.37^{c} ±0.00	0.29^{a} ±0.00	0.34^{b} ±0.00	0.37^{c} ±0.00	0.40^{d} ±0.00
Zn	2.5^{b} ±0.00	2.1^{a} ±0.00	2.9^{d} ±0.00	2.6^{b} ±0.00	2.8^{c} ±0.00
Cu	0.5^{b} ±0.00	0.4^{a} ±0.00	0.4^{a} ±0.00	0.4^{a} ±0.00	0.4^{a} ±0.00
Mn	1.4^{c} ±0.00	0.6^{a} ±0.00	1.0^{b} ±0.00	1.0^{b} ±0.00	1.4^{c} ±0.00
Fe	3.4^{d} ±0.00	3.1^{a} ±0.00	3.5^{b} ±0.00	3.7^{c} ±0.00	3.5^{b} ±0.00

Mean with different superscript letters in column are significantly different ($p<0.05$). Where, WB=wheat bran, MM=malted maize, WB+SC=wheat bran + starter culture, MM+SC= malted maize + starter culture.

4.4.5 Amino acid composition of amahewu

The amino acid profiles of amahewu samples were very much similar to that provitamin A biofortified maize. However, the contents of some individual amino acids increased slightly after fermentation. The concentration of essential amino acids such as lysine, tryptophan and methionine increased in all amahewu samples (Table 6). This is in agreement with the work of Chelule *et al.* (2010). Transamination may have occurred and this could have led to the observed increase in these amino acids (Mugocha, 2000). As compared to FAO/WHO standard the concentrations of all the essential amino acids in all the provitamin A biofortified amahewu samples were generally higher than the pattern of amino acid requirements for adults.

Table 6: Amino acid composition of provitamin A biofortified amahewu (g/100 g protein)

						FAO/WHO recommended pattern	
Essential AA	**RAW**	**M**	**WB**	**MM+SC**	**WB+SC**	**Pre-school Children (2-5 years)**	**Adult**
Histidine	1.4	2.1	2.2	2.0	1.6	1.9	1.6
Threonine	1.8	2.6	2.7	2.6	2.1	3.4	0.9
Lysine	1.4	1.9	2.2	2.2	1.8	5.8	1.6
Tyrosine	1.8	3.0	3.1	2.9	2.2		
Methionine	0.3	0.5	0.5	0.9	0.3	2.5	1.7
Valine	2.4	3.5	3.6	3.4	2.8	3.5	1.3
Isoleucine	1.6	2.6	2.4	2.6	2.0	2.8	1.3
Leucine	6.6	11	9.9	9.3	7.5	6.6	1.9
Cysteine	0.3	0.5	0.5	0.4	0.3		
Phenylalanine	2.5	3.7	3.7	3.5	2.7	6.3	1.9
Non- essential AA							
Serine	2.5	3.7	3.8	3.5	2.8		
Arginine	2.1	2.8	3.3	2.9	2.4		
Glycine	1.9	2.7	2.9	2.8	2.3		
Asparagine	2.5	3.7	3.8	3.7	3.0		
Glutamine	8.2	13	13.1	12	9.4		
Alanine	3.6	5.7	5.5	5.4	4.3		
Proline	4.9	7.3	7.1	6.4	5.3		

Where, WB=wheat bran, MM=malted maize, WB+SC=wheat bran + starter culture, MM+SC= malted maize + starter culture. FAO/WHO (1989) recommended pattern (pre-school children aged 2-5 years; adults)

4.4.6 *Invitro* protein digestibility of amahewu

The protein digestibility of amahewu was slightly higher (approx. 90%), compared to unprocessed provitamin A maize (Figure 17). Similar improvement in protein digestibility, following the fermentation of maize gruel, has previously been reported (Mohiedeen *et al*., 2010; Mardia *et al.,* 2002; Monawar 1983; Hasseltine, 1983). This could be attributed to the degradation of tannins, polyphenols and phytic acid by microbial enzymes (Mardia *et al.,* 2002). During fermentation, micro-organisms produce proteolytic enzymes which may be responsible for the increased protein digestibility (Hasseltine, 1983). Increased IVPD could also be attributed to the partial degradation of storage proteins into more simple and soluble products (Mohiedeen *et al.* (2010). Monawar (1983) found that the reduction in pH during fermentation also enhances the activity of native proteolytic enzymes and consequently promotes the breakdown of proteins to smaller polypeptides which are easily digested by enzymes.

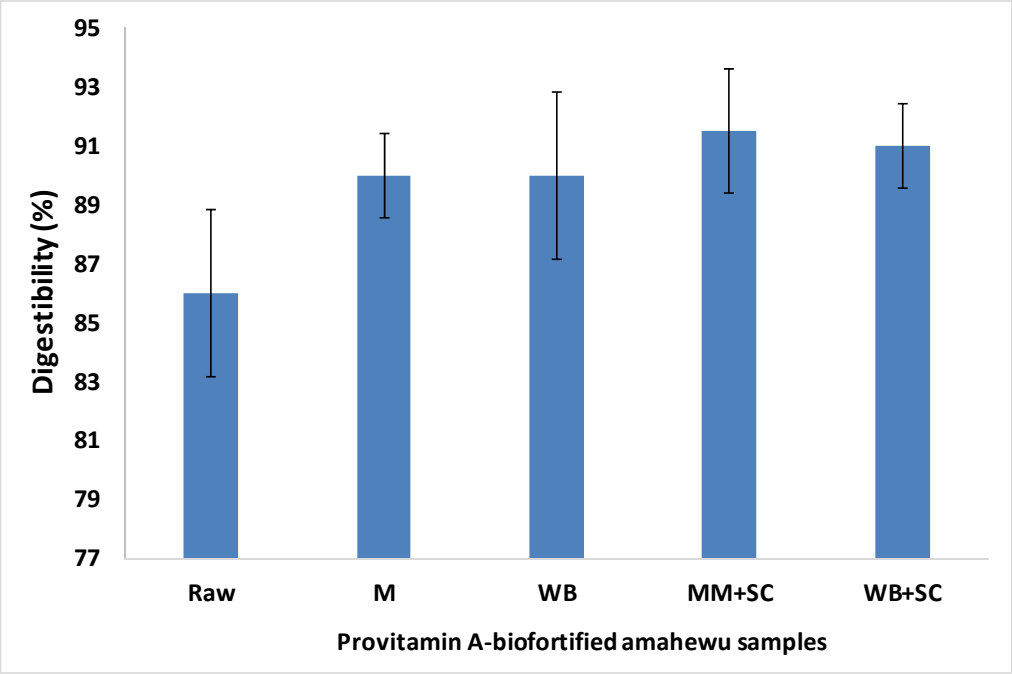

Figure 17: *Invitro* protein digestibility of provitamin A biofortified amahewu. Where, WB=wheat bran, MM=malted maize, WB+SC=wheat bran + starter culture, MM+SC= malted maize+ starter culture ($p<0.05$).

4.5 CONCLUSIONS

Amahewu produced using provitamin A biofortified maize has better nutritional composition, in terms of provitamin A content and protein digestibility. The amino acid content increased after fermentation. Therefore provitamin A biofortified amahewu has the potential to make a significant contribution towards alleviating VAD in rural communities, who are the most vulnerable to VAD.

CHAPTER 5

CONSUMER ACCEPTABILITY OF YELLOW, PROVITAMIN A-BIOFORTIFIED AMAHEWU

ABSTRACT

Provitamin A-biofortified maize, has been developed through plant breeding to alleviate Vitamin A Deficiency (VAD), a major public health problem in sub-Saharan Africa. In this study, the consumer acceptability of amahewu processed using provitamin A-biofortified maize was determined. The provitamin A biofortified maize variety PVAH62 was used. Either malted provitamin A biofortified maize and wheat bran or L*actobacillus* mixed starter cultures, combined with either malted maize or wheat bran, were used as sources of inoculum during fermentation. White maize amahewu made in the same way as that of provitamin A was used as a reference. Eight amahewu samples were produced. One-way analysis of variance was conducted on the results and the means compared using Fisher's Least Significant Difference Test at $p<0.05$. The acceptability of the samples was assessed using regular amahewu consumers (n = 54), who rated sensory attribute acceptability on a 9-point hedonic scale (1= dislike extremely, to 9 = liked extremely). Overall, amahewu samples made with provitamin A-biofortified maize were slightly more acceptable (7.0 ± 1.2) compared to those made with white maize (6.4 ± 0.8).The likeness for colour and mouth feel of provitamin A amahewu (6.0 ± 1.3 and 6.2 ± 1.1, respectively) were very similar to those of white maize. The taste of amahewu made with starter cultures were slightly liked (6.0±1.2), compared to those made without starter cultures, which were neither liked nor disliked (5.4 ±1.4). The aroma of amahewu made with starter cultures was slightly more liked (6.1±1.1), compared to their white maize counterparts (5.7±1.0). The use of starter culture thus improves the taste and aroma acceptability of amahewu. However, the preference for yellow maize to white maize is an important finding, because it has not been reported in the literature. This suggests that yellow maize has the potential to succeed as a strategy to alleviate VAD in rural communities, who are the most vulnerable to VAD.

5.1 INTRODUCTION

Vitamin A deficiency (VAD) is a major public health problem in developing regions, especially in sub-Saharan Africa. This is mainly attributed to high consumption of white maize-based foods, which are deficient in vitamin A. Biofortification of maize with provitamin A by conventional breeding has emerged as a potential long-term sustainable approach to improve vitamin A status in human beings (Howe and Tanumihardjo 2006a; Howe and Tanumihardjo 2006b; Nestel *et al.,* 2006).

Biofortification of maize with provitamin A carotenoids changes the grain colour from white to yellow/orange, as well as the aroma and flavour of the maize. These changes in colour and flavour and other social and cultural factors have been found to influence the acceptance of products processed using provitamin A maize (Stevens and Winter-Nelson 2008); De Groote *et al.* (2010). De Groote *et al.* (2010) reported a strong preference for white maize products over yellow maize products. This preference seemed to come from prejudice and negative perceptions, such as the use of yellow maize as animal feed, rather than from sensory characteristics. A study in Mozambique showed a more favourable response to orange maize, particularly the aroma (Stevens and Winter-Nelson, 2008). Pillay *et al.* (2011) found that preference for yellow maize was related to the age of the consumer. Children preferred the yellow maize, while adults preferred white maize. Another significant finding made by these authors was that the sensory acceptability of yellow maize varied across food products. They suggested that preference for white maize to yellow maize was significantly influenced by culture. Thus maize food type, consumer demographic profile and culture seem important factors to consider when researching on improving the consumer acceptance of provitamin A biofortified maize.

Amahewu, a fermented non-alcoholic cereal (predominantly maize)-based beverage is a popular drink in southern Africa. It serves as a refreshing drink for adults, preschool children and school-going age children. Amahewu is used as a weaning food for infants among low-income rural population groups (Gadaga *et al.,* 2004, Simango, 1997). Amahewu, like other maize-based products, is processed using white maize, consequently contributing to VAD among vulnerable rural communities. In KwaZulu-Natal, and most other provinces of South Africa,

amahewu made with white maize is a popular beverage, especially among rural African communities. At the same time, these communities are the most affected by VAD. Consumer acceptance of amahewu made with provitamin A biofortified maize has not been researched previously and therefore it is not known whether provitamin A biofortified amahewu could be used to deliver vitamin A to the communities affected by VAD. The objective of this study was to determine the acceptability of provitamin A biofortified maize amahewu to communities living in rural KwaZulu-Natal, South Africa.

5.2 MATERIALS AND METHODS

5.2.2 Materials

Provitamin A-biofortified maize, variety PVAH-62, was used. A white maize variety was used as a reference sample.

5.2.3 Preparation of amahewu samples

Amahewu samples were prepared according to a traditional method, which was described by persons from a community living in rural KwaZulu-Natal. The method involved adding one part of maize meal to seven parts of water and then boiling at 90^{O}C, with occasional stirring, for 15 minutes. The resulting porridge was left to cool to approximately 40^{O}C. The inocula; malted provitamin A biofortified maize (MM) and wheat bran (WB) were added at 0.5% concentration to porridges and these were allowed to ferment at 37^{O}C. The provitamin A biofortified maize meal was used to prepare the test amahewu samples, while the white maize meal was used to prepare the reference amahewu samples.

Amahewu samples with added starter culture were prepared as described above, but using *Lactobacillus* mixed starter culture at 5% concentration with either malted maize or wheat bran, each at 0.5% concentration, as inoculums. The starter culture-treated amahewu samples included those made with the provitamin A biofortified maize and their counterparts (references), in which the biofortified maize was replaced by the white maize.

Eight amahewu samples were prepared. The pH and titratable acidity (TTA) were monitored during fermentation. The final pH (pH 3.5-3.6) and TTA (percentage lactic acid) of the processed products were found to be within acceptable range for amahewu (Ayebo *et al.,* 1988; Mugocba, 2001).

5.2.4 Consumer acceptability evaluation

Consumer acceptability was carried out among available regular consumers of amahewu, between the ages of 30 and 51. 54 panelists were recruited from a rural area in eThekwini municipality in KwaZulu-Natal province. The rural area of Inanda was chosen as a site for the study, because amahewu is popular and favourably consumed among the residents of inanda and can be regarded as a low income area. To ensure reliable data, before sensory evaluation, a session was held in the community to explain to panellists the importance of the study and the evaluation procedure, including how the sensory attributes of amahewu were to be evaluated. Individual consumers (panellists) evaluated the products based on the following acceptability attributes: aroma, mouth feel, taste, colour and overall acceptability. The amahewu samples were evaluated using a nine-point hedonic rating scale (1=dislike extremely; 9=liked extremely). Amahewu samples were served in polystyrene cups. The samples were labelled with three-digit codes obtained from a table of random numbers and were served in a random order, which was determined using a table of random permutations of nine. Each panellist was provided with water to cleanse the palate between samples.

5.2.5 Statistical analysis

Mean acceptability scores were computed. One-way analysis of variance (ANOVA) was done; and the mean separation was by Fisher Least Significance Difference ($p < 0.05$). Principal component analysis (PCA) was used to determine the similarity and difference in the acceptability of amahewu products. Segmentation of consumers was carried out based on overall liking of amahewu. Hierarchical cluster analysis was used for segmentation. Panellists were allocated to clusters using the Ward Methods (Girish and Stewart, 1983). Chi-square tests were used to determine whether or not the clusters were significantly associated with demographics.

5.3 RESULTS AND DISCUSSIONS

The colour of provitamin A biofortified maize amahewu samples made with starter cultures (6.0±1.3), and those with the addition of either malted maize or wheat bran (5.8±1.3), were slightly more liked than their white maize counterparts (average overall acceptability: 5.8±1.1 and 5.5±1.1, respectively) (Table 7). Unlike in previous research, that found the colour acceptability of biofortified maize food products lower than the white maize counterparts (Pillay *et al*., 2011), the change in the colour of amahewu due to the use of the provitamin A biofortified maize seems not to have influenced the colour acceptability of the product. These are very promising findings, because previous research indicates that the unfamiliar colour was a major cause of low consumer preference for yellow maize compared to white maize (Pillay 2011; De Groote 2010; Kimenju 2008; Tshirely and Santos 1995; Stevens and Winter-Nelson 2008).

The taste acceptability of amahewu prepared using wheat bran and malted maize as inoculums were very similar, regardless of maize type used, i.e. provitamin A-biofortified maize or white maize (5.4±1.4 and 5.3±1.3, respectively) (Table 7). However, the taste of provitamin A biofortified amahewu samples with added starter culture was more acceptable (average overall acceptability: 6.0±1.2) relative to that of the biofortified amahewu samples with no added starter cultures (5.3±1.3). This could be attributed to the release of volatile compounds during fermentation. General improvement of product quality when starter cultures are used has been reported widely in the literature (e.g. Holzapfel 1997; Chelule *et al.,* 2010; Larry *et al*., 1990). Amahewu made with provitamin A had slightly higher acceptability scores for aroma compared to their white maize counterparts (Table 7). Among all amahewu samples, the aroma of provitamin A biofortified amahewu prepared with the addition of starter culture (6.1±1.1) appeared to be more liked when compared with those with no addition of starter culture. This finding is in agreement with the work of Annan *et al.* (2003) and Leroy (2004). According to these authors, the use of starter cultures improves the aroma of fermented maize due to the release of aromatic compounds. Although the fermentative micro-organisms responsible for spontaneous fermentation also produce flavour and aroma compounds, the starter culture

produced a better profile of the substances because they were deliberately developed to produce a better profile of the flavour and aroma substances. The mouth feel of provitamin A biofortified maize amahewu samples was slightly liked (6.2±1.1) and was rated the same as that of white maize amahewu counterparts (6.2±1.1). Uzogara (2010) stated that fermentation improved the textural characteristics of maize-based products.

Table 7: Consumer acceptability of provitamin A biofortified amahewu

Amahewu Products	Colour	Taste	Aroma	Mouth feel	Overall acceptability
Pvah62-wb	$5.8^{a} \pm 1.2$	$5.3^{a} \pm 1.5$	$6.2^{c} \pm 1.2$	$6.2^{b} \pm 1.3$	$7.0^{b} \pm 1.0$
Pvah62-mm+sc	$5.9^{a} \pm 1.4$	$5.6^{a} \pm 1.4$	$6.2^{c} \pm 1.0$	$6.3^{b} \pm 1.0$	$7.0^{c} \pm 1.2$
Pvah62-wb+sc	$5.7^{a} \pm 1.4$	$5.6^{a} \pm 1.4$	$6.0^{c} \pm 1.2$	$6.3^{b} \pm 1.1$	$7.0^{b} \pm 1.2$
Pvah62-mm	$5.9^{a} \pm 1.4$	$5.5^{a} \pm 1.4$	$5.9^{c} \pm 1.1$	$6.3^{b} \pm 1.1$	$7.0^{c} \pm 1.3$
Whitemaize products					
White'm-wb	$5.6^{a} \pm 1.3$	$5.2^{a} \pm 1.5$	$5.5^{a} \pm 1.2$	$5.8^{a} \pm 1.0$	$6.4^{a} \pm 1.0$
White'm-mm+sc	$5.8^{a} \pm 1.2$	$5.7^{a} \pm 1.6$	$5.8^{c} \pm 1.2$	$6.1^{b} \pm 1.1$	$6.6^{a} \pm 1.0$
White'm-wb+sc	$5.7^{a} \pm 1.0$	$5.7^{a} \pm 1.1$	$6.0^{c} \pm 0.8$	$6.1^{b} \pm 1.1$	$6.6^{a} \pm 0.7$
White'm-mm	$5.5^{a} \pm 1.0$	$5.4^{a} \pm 1.1$	$5.7^{b} \pm 1.0$	$6.0^{b} \pm 1.0$	$6.4^{a} \pm 0.7$

Mean ± SD (n=54)

Mean with different superscript letters in the same column are significantly different ($p<0.05$) according to the LSD test. Where, Pvah62= Provitamin A biofortified maize, white maize= control, wb=wheat bran, mm=malted maize, wb+sc=wheat bran + starter culture, mm+sc= malted maize+ starter culture

5.3.1 Principal component analysis (PCA)

By principal component analysis (PCA), the first two PCAs accounted for 89% of the total variation in the sensory attributes data (Figure 19). PCA 1 accounted for 71% of the total variation and differentiated the biofortified amahewu samples, to which either maize malt or wheat bran had been added from their white maize counterparts. PCA2 accounted for approx. 18% of the total variation and differentiated amahewu samples, to which starter cultures had been added, from

those without starter culture (Figure. 20). These products could have been differentiated based on taste. PCA indicates that the sensory attributes mainly influencing the overall acceptability of amahewu were taste and aroma. It appears that these two sensory attributes largely influenced the overall acceptability of amahewu because they were highly intense, and at the same time, highly acceptable, which is characteristic of fermented foods.

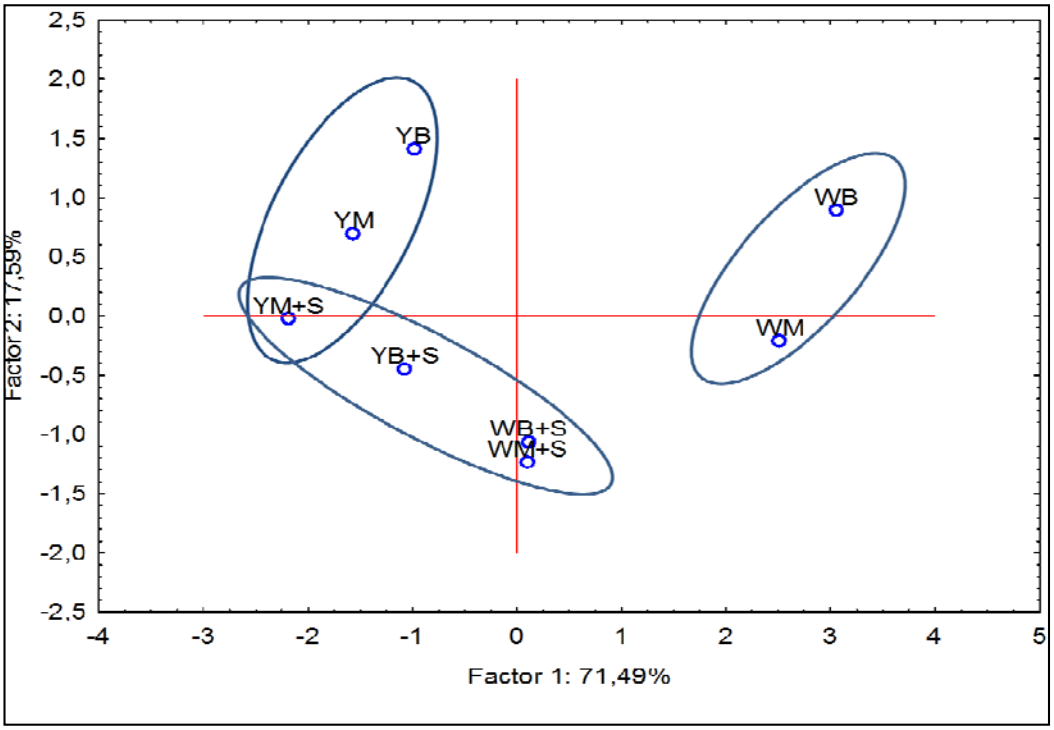

Figure 18: Principal component analysis (PCA 1) for consumer acceptability of provitamin A biofortified amahewu

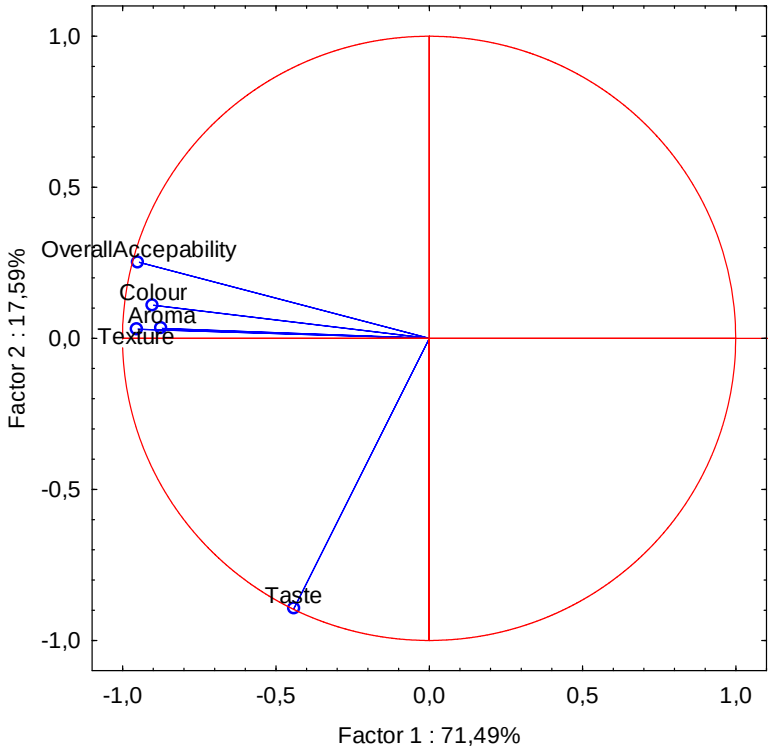

Figure 19: Principal component analysis (PCA 2) for consumer acceptability of provitamin A biofortified amahewu

5.3.2 Segmentation of consumers

Consumers were segmented into three clusters, based on their overall liking of the provitamin A biofortified maize samples (Table 8). Cluster A consisted of most consumers (43%) who liked amahewu moderately. Approximately 60% of these consumers were females and about 70% were aged between 30 and 40 years and 30% had an age range of 41 to 51 years. Cluster B consisted of most of the consumers (31%) who were undecided about their liking for the product. About 52% of the consumers in this cluster were female and 70% of them were between 30 and 40 yearsold, whilst 30% of them were 41 to 51 years old. Cluster C consisted of consumers (26%) who liked amahewu very much. Sixty-four percent of these consumers were female, with 64% of them having an age range of 30 to 40 years, and 36% of them 41 to 51 years. Age did not seem to be significantly associated with the liking of amahewu.

Pillay *et al*. (2011) observed differences in the degree of acceptability of provitamin A porridge among consumer groups, children and adults, in KwaZulu-Natal and suggested that consumer demographics may have an influence on the acceptability of provitamin A biofortified maize. These authors found that younger children preferred the biofortified maize food products, whilst the older children and adults preferred the corresponding white maize food products. However, the findings of the current study suggest that the acceptability of provitamin A biofortified maize is influenced by food type, rather than by consumer demographics. In this study, a relatively homogeneous group of consumers, that is adults, was used. Unlike in the study by Pillay *et al.*(2011), the adults indicated a high acceptance of provitamin A-biofortified maize amahewu samples. The liking was similar or slightly higher than that for the corresponding white maize amahewu samples.

Cluster B, which consisted of mostly undecided consumers, had fewer females than males, compared to the other clusters. This suggests that gender, to some extent, might have had some level of influence on the acceptability of amahewu.

Table 8: Segmentation of the consumer panel according to their overall liking of provitamin A biofortified maize samples

Clusters	**Consumers N (%)**	**Gender**		**P-values**	**Age (years)**		**P-values**
		Male	**Female**		**30-40**	**41-51**	
A	23 (43)	40	60	0.784	70	30	0.412
B	17 (31)	48	52	0.784	70	30	0.412
C	14 (26)	36	64	0.784	64	36	0.412

N=54. *P-values generated using chi-square test

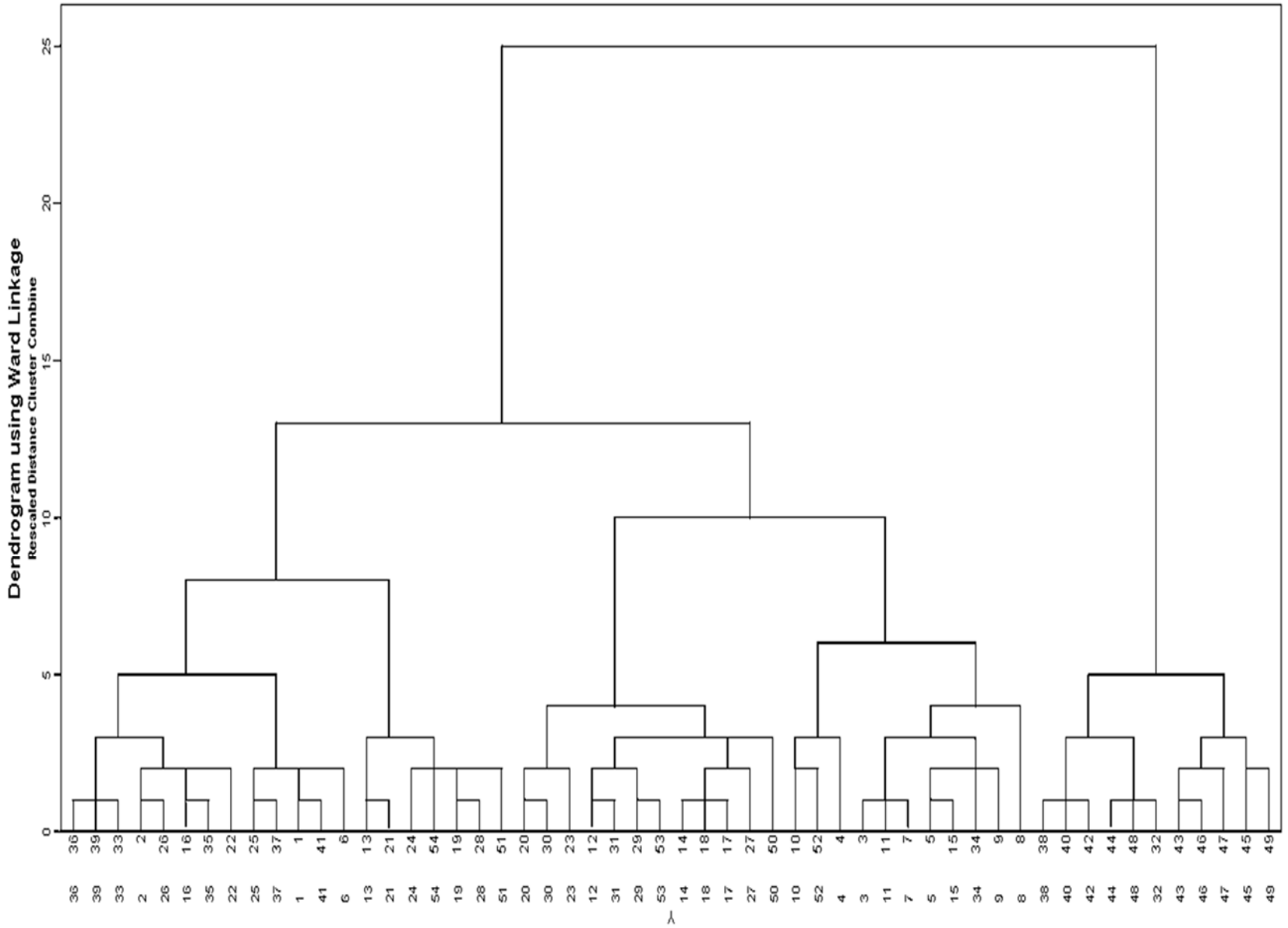

Figure 20: Dendrogram of cluster analysis

5.4 CONCLUSIONS

Provitamin A biofortified maize amahewu is highly acceptable to consumers and its acceptability appears slightly higher than amahewu made with white maize. The use of starter cultures enhances the taste, aroma and overall acceptability of amahewu. Provitamin A is substantially retained in amahewu after fermentation. Amahewu seems, therefore to be a good candidate for delivering vitamin A to VAD vulnerable populations.

CHAPTER 6

MICROBIAL QUALITY OF PROVITAMIN A BIOFORTIFIED AMAHEWU

ABSTRACT

Vitamin A deficiency is a major problem in sub-Saharan Africa where maize is a staple food. In this study, the microbial quality of processed provitamin A biofortified amahewu products was determined. Provitamin A biofortified amahewu was processed following a traditional method used to produce amahewu. Processing variables were inoculum type (malted provitamin A maize, wheat bran, and *Lactobacillus* mixed starter culture with either malted provitamin A maize or wheat bran) and inoculum concentration (0.5%, 1% and 2%). One-way analysis of variance was conducted on the results and the means compared using Fisher's Least Significant Difference Test at $p<0.05$. A total of four provitamin A-biofortified amahewu samples were subjected to different storage conditions: $4^{O}C$, $25^{O}C$ and $37^{O}C$. The pH and TTA of the amahewu samples were monitored throughout the storage period. The amahewu samples were plated and observed every day for a period of five days to assess the presence of aerobic and anaerobic spore formers, *E.coli* and moulds. The addition of starter culture substantially reduced the fermentation time of amahewu samples (6 h, pH 3.3) compared to those with no addition of starter culture (24 hr pH 3.5). The presence of aerobic spore formers and moulds were observed on day three. *E.coli* and anaerobic spore formers were not isolated throughout the storage period. Microbial counts were low at $4^{O}C$, whilst higher counts were observed at higher storage temperatures, $25^{O}C$, with $37^{O}C$ having the highest colony counts. Throughout the storage period, the pH of the amahewu samples was stable. Provitamin A biofortified amahewu samples stored under refrigerated conditions ($4^{O}C$) had better microbiological quality compared to those stored at $25^{O}C$ and $37^{O}C$. Refrigeration effectively maintains the microbiological quality of amahewu for three days.

6.1 INTRODUCTION

Amahewu is a sour, maize-based fermented beverage consumed mainly by indigenous people (Holzapfel, 1997). It is well known and appreciated throughout sub-Saharan Africa (Odunfa, 2001). Several studies have been conducted on the microbial quality of non-alcoholic gruels, such as ogi, kunun-zaki, agidi and togwa (Dike and Sanni, 2010; Ohenhen 2007; Mugula *et al.,* 2003a; Odunfa *et al.,* 1985) Ohenhen *et al.* (2007) found that ogi stored under refrigerated conditions had reduced microbial load compared with that stored at ambient temperature. Similar results have been reported by Dike and Sanni (2010) on the shelf-life of agidi. Chako (2010) observed that micro-organisms were not prevalent in fermented yoghurt stored under refrigerated conditions. Storage of kunun-zaki at 4^{O}C had an extended shelf-life, compared to those stored at 37^{O}C (Gaffa *et al.,* 2002). Dong *et al.* 2011 investigated during the storage of ginseng chicken porridge for 28 weeks at 25°C and concluded that ginseng chicken porridge could be marketable for at least 24 weeks at 25°C. A similar observation was noticed in the ogi stored at 20ºC. (Bolaji *et al.*2011) Olasupo *et al*. (1997). According to Olasupo *et al*. (1997), the shelf life of wet ogi is less than 7days at room temperature but when stored at low temperatures (-10±3 and -20±3ºC) , the shelf life was extended for longer period.

Pasteurisation has been associated with a decrease in microbial loads of non-alcoholic beverages such as kunun-zaki (Inyang, 1997). Decreases in microbial counts after pasteurisation have been reported in ogi (Abdel, 2009), kunun-zaki (Efiuvwevwere and Akoma, 1997; Inyang, 1997) and togwa (Mugula, 2007b).

Pasteurisation has been found to stabilize the pH and titratable acidity of fermented cereal foods (Mugula, 2007b). Osuntogun (2004) reported that a combination of pasteurisation and refrigeration was found most effective at reducing the microbial count in cereal beverages, thereby prolonging their shelf-life. The effects of fermentation and storage conditions on the microbial quality of provitamin A-biofortified maize amahewu are not known.

6.2 MATERIALS AND METHODS

6.2.1 EXPERIMENTAL DESIGN

One variety of provitamin A biofortified maize, PVAH-62, was used. White maize was used as a reference. Provitamin A biofortified amahewu was processed following a traditional method used to produce amahewu. Processing variables were inoculum type (malted provitamin A maize, wheat bran, and *Lactobacillus* mixed starter culture with either malted provitamin A maize or wheat bran) and inoculum concentration (0.5%, 1% and 2%). The maize varieties were grown in the same location and under the same conditions.

6.2.2 Microbial quality

The microbial quality of four provitamin A biofortified maize amahewu samples was determined. The samples of amahewu were pasteurised at 63^{O}C for 15 minutes. The samples were at different temperatures: 4^{O}C, 25^{O}C and 37^{O}C. The microbiological quality of the samples was monitored by taking aliquots, daily, from each sample and analysed for aerobic spore formers, anaerobic spore formers, *E.coli*, total coliform counts and moulds, using the standard plating technique. A 1 ml aliquot of each amahewu sample was serially diluted and inoculated onto specific differential and selective media, to analyse for specific microbial types.

6.2.2.1 Determination of aerobic and anaerobic spore-formers

Tryptone Soy Agar was prepared, sterilised and kept in a water bath at 50^{O}C until use. Amahewu samples were heated in a sterile test tube in a water bath (75^{O}C) for 20 minutes (Austin, 1998). Serial dilutions were pour plated. A set of plates were incubated aerobically at 37C for 48 hr, while the other set of plates was incubated anaerobically in an anaerobic jar with Anaerocult (Merck Ltd.,Wadeville, Gauteng, South Africa) at 37^{O}C for 48 hr. Three replicates of each sample were analysed.

6.2.2.2 Determination of *E.coli*

A 1ml aliquot of amahewu was inoculated into 10 ml double strength of Lauryl Sulfate Tryptose (LST) broth and incubated for 24hrs. After 24hrs, a 1ml aliquot was transferred into 10 ml of Brilliant Green Lactose (BGLB) broth and incubated at

35^{O}C for 24hrs. After 24hrs, 1ml aliquot was transferred into *Escherichia Coli* (EC) broth tubes in a water bath at 45°C. This was transferred into Eosin Methylene Blue (EMB) agar, which was incubated in a water bath at 45^{O}C. After 24hrs, EMB plate was incubated at 35^{O}C for 24hrs and observed for typical non-mucoid, nucleated, dark-centred colonies with or without a metallic sheen, which are indicative of E.coli (Feng *et al*., 2002). Three replicates of each sample were analysed.

6.2.2.3 Determination of total coliform count.

Using plate count agar, amahewu samples were serially diluted and inoculated into petri dishes. The plates were incubated for 24 to 48hrs. Colonies were then counted and expressed as log cfu/ml (Chako, 2010). Three replicates of each sample were analysed.

6.2.2.4 Determination of moulds

Determination of the presence of moulds was done according to the method of Beuchat (1992) and modified. Potato dextrose agar (PDA) was prepared and sterilised and kept in a water bath at 50^{O}C until use. Twenty-five ml of PDA was poured into petri dishes and allowed to set. Amahewu samples were serially diluted and inoculated onto petri dishes and swirled. The petri dishes were incubated at room temperature for 48-72 hours. Three replicates of each sample were analysed.

6.2.2.5 pH and titratable acidity

The pH and titratable acidity (TTA) of the amahewu samples were monitored at six-hourly intervals. TTA was measured by the samples against 0.1 NaOH and expressed as percentage lactic acid. Phenolphthalein was used as an indicator (Amerine *et al.*, 1967).

6.3 RESULTS AND DISCUSSIONS

The microbial quality of amahewu varied with storage time and temperature. For all the storage temperatures, moulds were not present on day 0, 1 and 2 (Table 9-11), but were present on day 3 (Table 12-13). Aerobic spore-formers were also not present on Day 0, 1 and 2 (Table 9-11). However, these micro-organisms were

isolated on day 3, with the exception of anaerobic spore-formers and *E.coli* (Table 12-13). The growth of moulds and aerobic spore-formers increased with increasing temperature, i.e. microbial growth was lowest and highest at $4^{O}C$ and $37^{O}C$, respectively. These results show that the provitamin A biofortified maize amahewu sample stored under refrigerated conditions ($4^{O}C$) was of better microbiological quality compared to those stored at $25^{O}C$ and $37^{O}C$. The findings of this study are in agreement with Osuntogun (2004), who reported that refrigeration was most effective at reducing the microbial growth in cereal beverages.

Table 9: Effect of temperature conditions on the microbial quality of provitamin A biofortified amahewu (day 0)

	Day 0	**4^{o}C**			
Amahewu products	**Mould (logcfu)**	**Total coliform count (log cfu)**	**Aerobic spore-formers (logcfu)**	**Anaerobic spore-formers (log cfu)**	***E.coli* (logcfu)**
SCM	ND	2.39^{c}	ND	ND	ND
SCWB	ND	1.06^{a}	ND	ND	ND
M	ND	2.06^{b}	ND	ND	ND
WB	ND	2.01^{b}	ND	ND	ND
		25^{o}C			
SCM	ND	2.85^{a}	ND	ND	ND
SCWB	ND	2.33^{a}	ND	ND	ND
M	ND	2.88^{a}	ND	ND	ND
WB	ND	2.71^{a}	ND	ND	ND
		37^{o}C			
SCM	ND	2.89^{a}	ND	ND	ND
SCWB	ND	2.79^{a}	ND	ND	ND
M	ND	2.89^{a}	ND	ND	ND
WB	ND	2.86^{a}	ND	ND	ND

LA: lactic acid; SCM: starter culture with malt; SCWB: starter culture with wheat bran; M: malt; WB: wheat bran; ND: not detected. Mean with different superscript letters in the same column are significantly different ($p<0.05$) according to the LSD test.

Table 10: Effect of temperature conditions on the microbial quality of provitamin A biofortified amahewu (day 1)

Amahewu products	Mould (log cfu)	Total coliform count (log cfu)	Aerobic spore-formers (log cfu)	Anaerobic spore-formers (log cfu)	*E.coli* (log cfu)
		Day 1	**4^{o}C**		
SCM	ND	2.79^{b}	ND	ND	ND
SCWB	ND	2.03^{a}	ND	ND	ND
M	ND	2.85^{b}	ND	ND	ND
WB	ND	2.81^{b}	ND	ND	ND
		25^{o}C			
SCM	ND	2.88^{a}	ND	ND	ND
SCWB	ND	2.88^{a}	ND	ND	ND
M	ND	2.89^{a}	ND	ND	ND
WB	ND	2.81^{a}	ND	ND	ND
		37^{o}C			
SCM	ND	3.09^{a}	ND	ND	ND
SCWB	ND	3.3^{b}	ND	ND	ND
M	ND	3.42^{b}	ND	ND	ND
WB	ND	3.44^{b}	ND	ND	ND

LA: lactic acid; SCM: starter culture with malt; SCWB: starter culture with wheat bran; M: malt; WB: wheat bran; ND: not detected. Mean with different superscript letters in the same column are significantly different ($p<0.05$) according to the LSD test.

Table 11: Effect of temperature conditions on the microbial quality of provitamin A biofortified amahewu (day 2)

	Day 2	**4^{O}C**			
Amahewu products	**Mould (log cfu)**	**Total coliform count (log cfu)**	**Aerobic spore-formers (log cfu)**	**Anaerobic spore-formers (log cfu)**	***E.coli* (log cfu)**
SCM	ND	3.84^{a}	ND	ND	ND
SCWB	ND	4.33^{a}	ND	ND	ND
M	ND	3.86^{a}	ND	ND	ND
WB	ND	3.86^{a}	ND	ND	ND
		25^{O}C			
SCM	ND	4.86^{a}	ND	ND	ND
SCWB	ND	5.05^{a}	ND	ND	ND
M	ND	4.85^{a}	ND	ND	ND
WB	ND	4.86^{a}	ND	ND	ND
		37^{O}C			
SCM	ND	4.85^{a}	ND	ND	ND
SCWB	ND	5.38^{a}	ND	ND	ND
M	ND	4.86^{a}	ND	ND	ND
WB	ND	4.87^{a}	ND	ND	ND

LA: lactic acid; SCM: starter culture with malt; SCWB: starter culture with wheat bran; M: malt; WB: wheat bran; ND: not detected. Mean with different superscript letters in the same column are significantly different ($p<0.05$) according to the LSD test.

Table 12: Effect of temperature conditions on the microbial quality of provitamin A biofortified amahewu (day 3)

	Day 3	**4^{o}C**			
Amahewu products	**Mould (log cfu)**	**Total coliform count (log cfu)**	**Aerobic spore-formers (log cfu)**	**Anaerobic spore-formers (log cfu)**	***E.coli* (log cfu)**
SCM	0.80^{a}	3.92^{a}	0.82^{a}	ND	ND
SCWB	0.83^{a}	4.09^{a}	0.77^{a}	ND	ND
M	0.83^{a}	3.97^{a}	0.81^{a}	ND	ND
WB	0.84^{a}	3.86^{a}	0.64^{a}	ND	ND
		25^{o}C			
SCM	0.83^{a}	4.87^{a}	0.82^{b}	ND	ND
SCWB	0.88^{a}	4.33^{a}	0.79^{b}	ND	ND
M	0.89^{a}	4.70^{a}	0.83^{b}	ND	ND
WB	0.85^{a}	4.83^{a}	0.67^{a}	ND	ND
		37^{o}C			
SCM	1.87^{b}	5.36^{a}	1.85^{b}	ND	ND
SCWB	0.89^{a}	5.32^{a}	0.79^{a}	ND	ND
M	1.88^{b}	5.08^{a}	0.94^{a}	ND	ND
WB	1.89^{b}	5.19^{a}	0.73^{a}	ND	ND

LA: lactic acid; SCM: starter culture with malt; SCWB: starter culture with wheat bran; M: malt; WB: wheat bran; ND: not detected. Mean with different superscript letters in the same column are significantly different ($p<0.05$) according to the LSD test.

Table 13: Effect of temperature conditions on the microbial quality of provitamin A biofortified amahewu (day 4)

	Day 4	**4^{o}C**			
Amahewu products	**Mould (log cfu)**	**Total coliform count (log cfu)**	**Aerobic spore-formers (log cfu)**	**Anaerobic spore-formers (log cfu)**	***E.coli* (log cfu)**
SCM	1.88^{a}	3.94^{a}	1.85^{a}	ND	ND
SCWB	1.86^{a}	4.37^{a}	1.79^{a}	ND	ND
M	1.88^{a}	3.98^{a}	1.81^{a}	ND	ND
WB	1.84^{a}	3.86^{a}	1.74^{a}	ND	ND
		25^{o}C			
SCM	1.89^{b}	4.87^{a}	1.92^{b}	ND	ND
SCWB	1.33^{a}	4.59^{a}	1.79^{b}	ND	ND
M	1.89^{b}	4.87^{a}	1.83^{b}	ND	ND
WB	1.86^{b}	4.89^{a}	1.07^{a}	ND	ND
		37^{o}C			
SCM	2.39^{b}	5.38^{a}	2.05^{b}	ND	ND
SCWB	1.06^{a}	5.37^{a}	1.07^{a}	ND	ND
M	2.06^{b}	5.11^{a}	1.04^{a}	ND	ND
WB	2.01^{b}	5.29^{a}	1.73^{b}	ND	ND

LA: lactic acid; SCM: starter culture with malt; SCWB: starter culture with wheat bran; M: malt; WB: wheat bran; ND: not detected. Mean with different superscript letters in the same column are significantly different ($p<0.05$) according to the LSD test.

Table 14: pH and titratable acidity of amahewu products during the storage period (% lactic acid)

Amahewu products	**Mould**	***Lactobacillus***	**ASP**	**ANSP**	***E.coli***
	pH/TTA 4,25,37^{O}C	pH/TTA 4,25,37^{O}C	pH/TTA 4,25,37^{O}C	pH/TTA 4,25,37^{O}C	pH/TTA 4,25,37^{O}C
	Day 0-4	Day 0-4	Day 0-4	Day 0-4	Day 0-4
SCM	3.6/0.5	3.6/0.5	3.6/0.5	3.6/0.5	3.6/0.5
SCWB	3.6/0.5	3.6/0.5	3.6/0.5	3.6/0.5	3.6/0.5
M	3.5/0.4	3.5/0.4	3.5/0.4	3.5/0.4	3.5/0.4
WB	3.6/0.5	3.6/0.5	3.6/0.5	3.6/0.5	3.6/0.5

Where ASP= aerobic spore-formers; ANSP= anaerobic spore-formers

6.4 CONCLUSIONS

Provitamin A biofortified maize amahewu is quite shelf stable. However, the presence of aerobic spore-formers and moulds were observed on the third day. This is particularly significant for the storage of the product by poor rural communities who have either no, or very limited, access to refrigeration facilities. Refrigeration was found most suitable for the storage of provitamin A biofortified amahewu. Thus amahewu appears a suitable biofortified maize food product for delivering provitamin A to the targeted communities and to contribute to alleviating food and nutrition insecurity, especially vitamin A deficiency.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

The overall results show that provitamin A biofortified maize is just as acceptable as white maize to consumers when used to produce amahewu.

- A substantial amount of provitamin A is retained in amahewu after fermentation. In addition to provitamin A content, the apparent improvement in essential amino acids and minerals, such as iron and zinc, suggest that provitamin A biofortified maize amahewu is much better than white maize amahewu in terms of its nutritional quality. The essential amino acids would be nutritionally adequate for adults and fairly adequate for age groups lower than five years.

- The findings of the study findings indicate that consumers prefer provitamin A biofortified maize amahewu over white maize amahewu. The use of starter cultures improved the taste, aroma and overall acceptability of amahewu. Females seemed to be more positive about provitamin A biofortified amahewu than males. The consumers used in this study have grown up in a cultural environment where white maize is accepted as the traditional food. The findings of this study suggest that there is an opportunity to change the cultural mind-set of preference for white maize.

- Provitamin A biofortified amahewu is shelf stable. It can be stored at room temperature for at least three days, which would be a significant advantage for poor rural communities with no, or limited, access to refrigeration facilities.

- The study has demonstrated that, in the form of amahewu, provitamin A biofortified maize has the potential to contribute to the alleviation of vitamin A deficiency among the targeted communities, especially the resource-poor rural communities who are highly vulnerable to vitamin A deficiency.

7.1 RECOMMENDATIONS

Provitamin A biofortified maize can be used to produce amahewu. However, it is important that future researchers focus on :

- Assessment of the retention of provitamin A carotenoids during the storage of amahewu.

- The effects of processing and fermentation on the bio-availability of provitamin A carotenoids.

- Sensory acceptability should be carried out with a wider range of consumer groups, to include infants, caregivers and weaning mothers. Consumer acceptability studies should be carried out using subjects from other provinces in South Africa and should include the popular maize foods consumed in the respective provinces.

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PUBLICATIONS, PRESENTATIONS AND CONFERENCES ATTENDED

Temitope D. Awobusuyi, Eric O. Amonsou, Muthulisi Siwela, Ijabadeniyi, A. O. 2014. Process optimisation and microbial quality of provitamin A biofortified amahewu, a non-alcoholic maize-based beverage. **Oral Presentation at the 6th African Nutrition Epidemiology Conference. 21-25 July 2014, Accra, Ghana.**

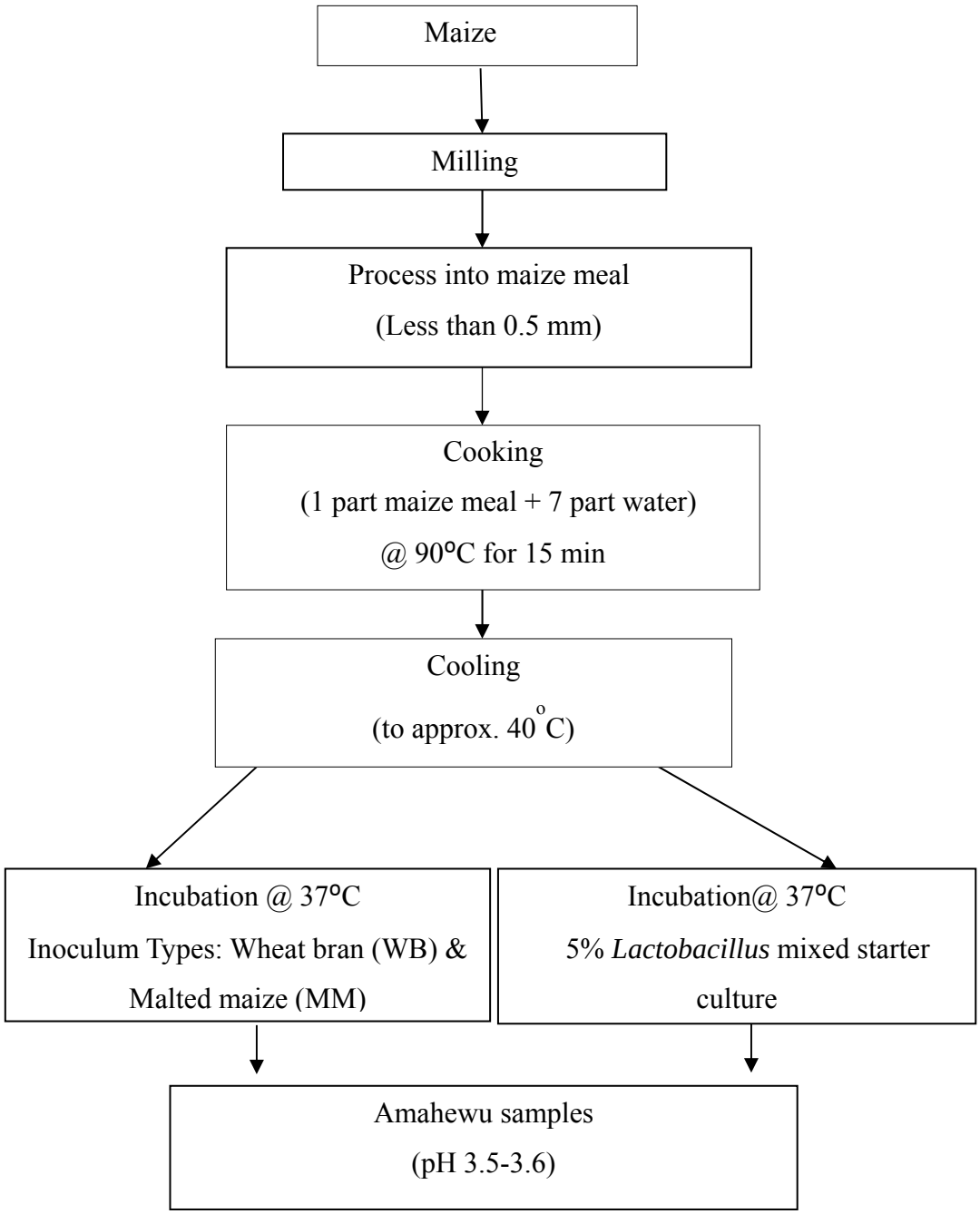
Temitope D. Awobusuyi, Eric O. Amonsou, Muthulisi Siwela and Unathi Kolanisi 2014. Provitamin A retention and sensory acceptability of amahewu, a non-alcoholic cereal-based beverage made with provitamin A biofortified maize. **Submitted to Journal of the Science of Food and Agriculture (under review, revised manuscript submitted).**

Temitope D. Awobusuyi, Eric O. Amonsou and Muthulisi Siwela. Consumer acceptability of amahewu, a non-alcoholic cereal-based beverage made with provitamin A biofortified maize. **Oral Presentation at the 17th World Congress of Food Science and Technology, (IUFosT, 2014), 17-21 August 2014, Montreal, Canada.**

Temitope D. Awobusuyi, Eric O. Amonsou and Muthulisi Siwela 2014. Nutritional composition of amahewu made with provitamin A biofortified maize. **Manuscript in preparation for submission to Food Chemistry (under review).**

APPENDICES

Appendix A



A typical flow diagram of amahewu processing

Appendix B

DURBAN UNIVERSITY OF TECHNOLOGY

DEPARTMENT OF FOOD AND BIOTECHNOLOGY

Form No……

Name……Age……

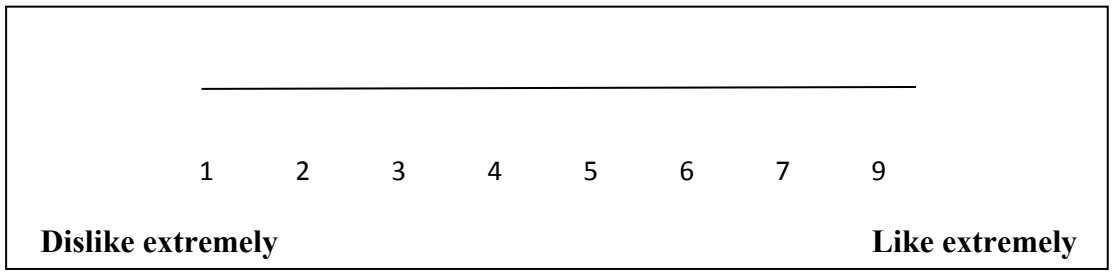
Date…… Gender…….

Sample No.……

Presented to you are a series of samples of provitamin A biofortified amahewu products. Rate these samples by the order of increasing intensity for the following characteristics. Starting from left to right using the displayed ruler scale.

Colour

Observe the samples visually and rate the intensity of the following descriptors.

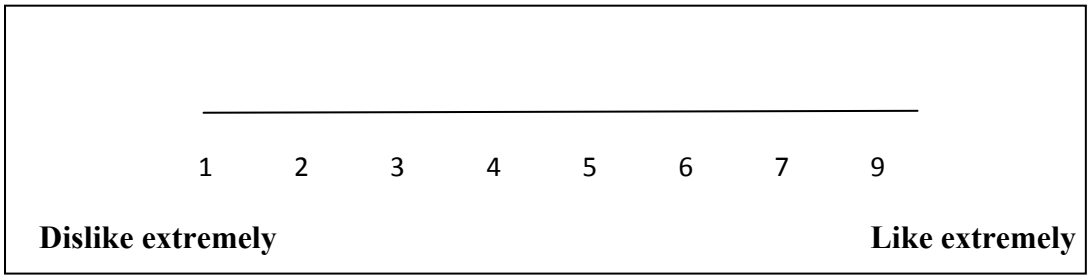


Taste

Using sips of amahewu; swirling on the tongue, rate the intensity of the following descriptors

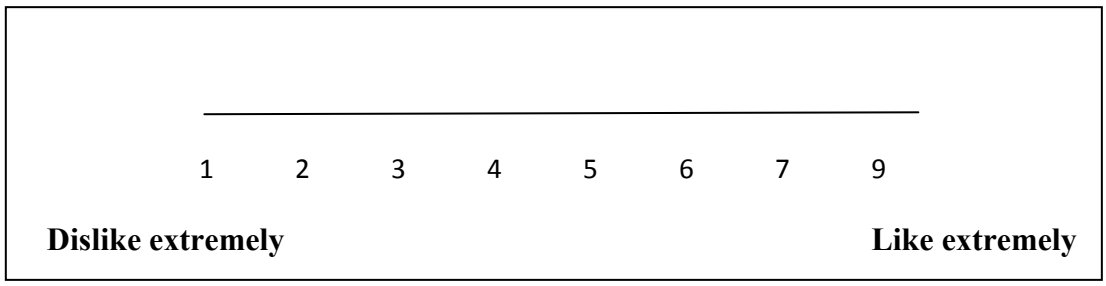
Aroma/ Smell

Smell using short sniffs. Rate the intensity of the following aroma descriptors



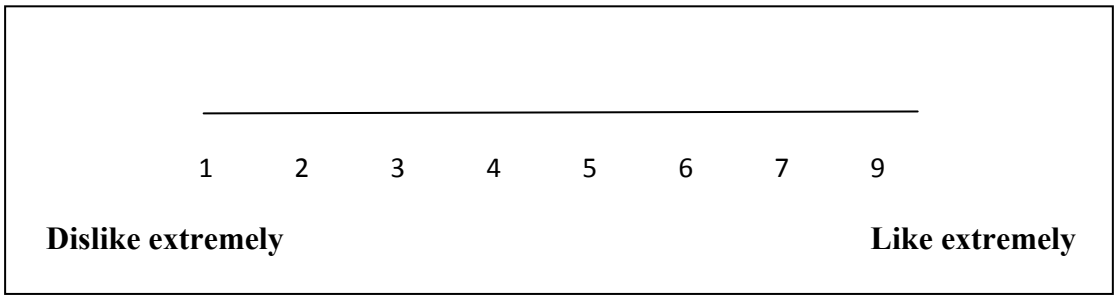
Mouth feel

Using small sips of swirling in the mouth, rate the intensity of the following descriptors



Overall acceptability

Please rate the product according to your overall assessment of the attributes



Appendix C: Descriptive, ANOVA and LSD

	Univariate Results for Each DV (Sheet1 in Fermentation) Sigma-restricted parameterization Effective hypothesis decomposition												
Effect	Degr. of Freedom	FT SS	FT MS	FT F	FT p	pH SS	pH MS	pH F	pH p	TTA SS	TTA MS	TTA F	TTA p
Intercept	1	32400,0	32400,0	630,000	0,00000	2270,52	2270,52	15231,5	0,00000	19,0677	19,0677	743,820	0,00000
Variety	2	0,0(	0,0(	0,000	1,00000	0,038	0,019	0,13	0,88068	0,0138	0,0069	0,270	0,76313
Inoculum	1	0,0(	0,0(	0,000	1,00000	0,09(	0,09(	0,60	0,43860	0,0100	0,0100	0,390	0,53338
Conc	2	0,0(	0,0(	0,000	1,00000	0,18	0,09	0,62	0,53929	0,0068	0,0034	0,132	0,87581
Variety*Inoculum	2	0,0(	0,0(	0,000	1,00000	0,06	0,03	0,21	0,81455	0,0216	0,0108	0,422	0,65626
Variety*Conc	4	0,0(	0,0(	0,000	1,00000	0,05	0,01	0,10	0,98298	0,0090	0,0022	0,088	0,98603
Inoculum*Conc	2	0,0(	0,0(	0,000	1,00000	0,02(	0,01(	0,07	0,93515	0,0579	0,0289	1,129	0,32639
Variety*Inoculum*Conc	4	0,0(	0,0(	0,000	1,00000	0,04	0,01	0,07	0,99063	0,0029	0,0007	0,028	0,99841
Error	12(	6480,0	51,4			18,78	0,14			3,2300	0,0256		
Total	14	6480,0				19,27				3,3522			

LSD test; variable pH (Sheet1 in Fermentation)
Homogenous Groups, alpha = ,05000 (Non-Exhaustive Search)
Error: Between MS = ,00215, df = 72,000

Cell No.	Variety	Inoculum	Conc	FT	pH Mean	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
72	CWhite	MaltM	5	24	3,300000	****																		
24	AYellow	MaltM	5	24	3,300000	****																		
48	BYellow	MaltM	5	24	3,300000	****																		
68	CWhite	MaltM	2	24	3,400000		****																	
64	CWhite	MaltM	1	24	3,400000		****																	
16	AYellow	MaltM	1	24	3,400000		****																	
36	BYellow	WBran	5	24	3,500000			****																
44	BYellow	MaltM	2	24	3,500000			****																
20	AYellow	MaltM	2	24	3,500000			****																
40	BYellow	MaltM	1	24	3,500000			****																
12	AYellow	WBran	5	24	3,500000			****																
56	CWhite	WBran	2	24	3,550000			****	****															
32	BYellow	WBran	2	24	3,550000			****	****															
4	AYellow	WBran	1	24	3,600000				****	****														
52	CWhite	WBran	1	24	3,600000				****	****														
8	AYellow	WBran	2	24	3,600000				****	****														
28	BYellow	WBran	1	24	3,600000				****	****														
60	CWhite	WBran	5	24	3,650000					****	****													
3	AYellow	WBran	1	18	3,700000						****	****												
51	CWhite	WBran	1	18	3,700000						****	****												
35	BYellow	WBran	5	18	3,700000						****	****												
27	BYellow	WBran	1	18	3,700000						****	****												
11	AYellow	WBran	5	18	3,750000							****	****											
31	BYellow	WBran	2	18	3,750000							****	****											
63	CWhite	MaltM	1	18	3,800000								****	****										
67	CWhite	MaltM	2	18	3,800000								****	****										
15	AYellow	MaltM	1	18	3,800000								****	****										
7	AYellow	WBran	2	18	3,800000								****	****										
23	AYellow	MaltM	5	18	3,800000								****	****										
55	CWhite	WBran	2	18	3,800000								****	****										
47	BYellow	MaltM	5	18	3,850000									****	****									
39	BYellow	MaltM	1	18	3,850000									****	****									
43	BYellow	MaltM	2	18	3,850000									****	****									
59	CWhite	WBran	5	18	3,850000									****	****									
19	AYellow	MaltM	2	18	3,900000										****									
71	CWhite	MaltM	5	18	3,900000										****									
14	AYellow	MaltM	1	12	4,050000											****								
38	BYellow	MaltM	1	12	4,100000											****	****							
62	CWhite	MaltM	1	12	4,100000											****	****							
46	BYellow	MaltM	5	12	4,100000											****	****							
26	BYellow	WBran	1	12	4,100000											****	****							
61	CWhite	MaltM	1	6	4,150000												****	****						
50	CWhite	WBran	1	12	4,200000													****	****					
70	CWhite	MaltM	5	12	4,200000													****	****					
34	BYellow	WBran	5	12	4,250000														****	****				
66	CWhite	MaltM	2	12	4,250000														****	****				
25	BYellow	WBran	1	6	4,250000														****	****				
2	AYellow	WBran	1	12	4,250000														****	****				
10	AYellow	WBran	5	12	4,250000														****	****				
30	BYellow	WBran	2	12	4,250000														****	****				
37	BYellow	MaltM	1	6	4,250000														****	****				
49	CWhite	WBran	1	6	4,300000															****	****			
6	AYellow	WBran	2	12	4,300000															****	****			
54	CWhite	WBran	2	12	4,300000															****	****			
13	AYellow	MaltM	1	6	4,300000														****	****	****	****		
42	BYellow	MaltM	2	12	4,300000															****	****			
65	CWhite	MaltM	2	6	4,300000															****	****			
22	AYellow	MaltM	5	12	4,300000															****	****			
33	BYellow	WBran	5	6	4,350000																****	****		
58	CWhite	WBran	5	12	4,350000																****	****		
18	AYellow	MaltM	2	12	4,350000																****	****		
21	AYellow	MaltM	5	6	4,400000																	****	****	
69	CWhite	MaltM	5	6	4,400000																	****	****	
41	BYellow	MaltM	2	6	4,400000																	****	****	
5	AYellow	WBran	2	6	4,400000																	****	****	
45	BYellow	MaltM	5	6	4,400000																	****	****	
29	BYellow	WBran	2	6	4,445000																		****	****
57	CWhite	WBran	5	6	4,445000																		****	****
53	CWhite	WBran	2	6	4,500000																			****
9	AYellow	WBran	5	6	4,500000																			****
17	AYellow	MaltM	2	6	4,500000																			****
1	AYellow	WBran	1	6	4,500000																			****

Descriptive statistics: wheat flour

		N	Mean	Std. Deviation	Std. Error
pH	yA yello	8	3.99	.275	.097
	BYellow	8	3.86	.262	.092
	CWHITE	8	3.91	.264	.093
	Total	24	3.92	.260	.053
TTA	yA yello	8	.34	.177	.063
	BYellow	8	.43	.128	.045
	CWHITE	8	.40	.160	.057
	Total	24	.39	.154	.031

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
pH	Between Groups	.063	2	.032	.444	.647
	Within Groups	1.496	21	.071		
	Total	1.560	23			
TTA	Between Groups	.032	2	.016	.664	.525
	Within Groups	.514	21	.024		
	Total	.546	23			

Multiple Comparisons

LSD

Dependent Variable	(I) Vasriety	(J) Vasriety	Mean Difference (I-J)	Std. Error	Sig.
pH	yA yello	BYellow	.125	.133	.360
		CWHITE	.075	.133	.580
	BYellow	yA yello	-.125	.133	.360
		CWHITE	-.050	.133	.712
	CWHITE	yA yello	-.075	.133	.580
		BYellow	.050	.133	.712
TTA	yA yello	BYellow	-.087	.078	.276
		CWHITE	-.063	.078	.433
	BYellow	yA yello	.087	.078	.276
		CWHITE	.025	.078	.752
	CWHITE	yA yello	.063	.078	.433
		BYellow	-.025	.078	.752