Cape Peninsula
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DEVELOPMENT OF A STOCK CUBE WITH FUNCTIONAL FOOD CHARACTERISTICS

by

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DECLARATION

I, Kevin Michael Swarts, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

The chronic diseases of lifestyle, tuberculosis and Human immunodeficiency virus/Acquired immunodeficiency syndrome have a high prevalence in South Africa. These diseases are characterised by oxidative stress and a chronic inflammatory state that contribute to both the development and the acceleration of these diseases. Research into the phytochemical plant food components suggest that these substances could possibly play a vital role in the prevention of such disease. Corn steep liquor (CSL) is a waste product with an exceptionally high polyphenol content and total antioxidant capacity (TAC). This led to the suggestion that it could be utilised in the development of food products with functional food characteristics. Stock cubes, due to the widespread use among consumers of different socio-economic backgrounds, were identified as vehicle for the delivery of the CSL with its phytochemical content. This lead to the development of a stock cube utilising CSL as a source of phytochemical polyphenolic antioxidants with the micronutrients zinc, selenium and copper as added support to immunonutrition, along with iron due to the wide spread prevalence of iron deficiency in the South African population. The acceptability of the developed stock cube was tested by preparing savoury rice and pea soup and having blue collar (n = 50) and white collar (n = 49) participants rate the acceptability on a 9-point hedonic scale, ranging from “dislike extremely” to “like extremely”. The savoury rice received a 41% “like very much” rating followed by a 24% “like moderately” rating. The pea soup was rated even more positively as it received a 42% “like very much” rating and a 29% “like extremely” rating. In addition, among the blue collar participants, significant ($p < 0.05$ for each) findings occurred with a greater liking of the sample dishes prepared with the developed stock cube by participants of the ethnic Black grouping, with Xhosa as home language and being married, as well as those participants who habitually prepare the meals in the household. A significant ($p < 0.05$) finding with the white collar participants was a greater liking of the sample dishes prepared with the developed stock cube among those participants who would be willing to make use of a stock cube with health benefits. The developed stock cube was also subjected to six months accelerated shelf-life stability testing, reflecting twelve months real time storage. The parameters measured were microbial growth, oxidative rancidity, antioxidant status and organoleptic changes. All microbial growths tested remained within the acceptable specification ranges. Oxidative rancidity, measured as the peroxide value, was detected analytically at the fifth and sixth samplings, but was not detectable organoleptically. The antioxidant status, measured as the TAC and the total polyphenol content, remained relatively stable during the testing period. Though no noticeable organoleptic changes were observed during the stability testing, there was a darkening of

the colour by the second sampling. The results of the acceptance testing, as well as the shelf-life stability testing, support the conclusions that the study objectives of developing a stock cube with functional food characteristics and it being received positively by consumers were achieved.

Keywords:

stock cubes; corn steep liquor; phytochemicals; consumer acceptance; new product development

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LIST OF OPERATIONAL TERMS AND CONCEPTS

Convenience foods – Processed foods in which a considerable amount of the preparation has already been carried out by the manufacturer (Bender & Bender, 1995:101).

Corn steep liquor – By-product of the starch producing industry (Sharma & Kothari, 1992:213)

Free radical – Highly reactive molecules with an unpaired electron (Bender & Bender, 1995:154).

Functional foods – Foods eaten for specified health purposes, because of their (rich) content of one or more nutrients or non-nutrient substances which may confer health benefits (Bender & Bender, 1995:157).

Organoleptic – Sensory properties, i.e. those that can be detected by the sense organs (Bender & Bender, 1995:265)

Polyphenols – Polyhydroxylated phytochemicals, of which the two main classes are the flavonoids and the phenolic acids (Lotito & Frei, 2006:1727).

Phytochemicals – Physiologically active food components from plant sources (Hasler *et al*., 2004:814).

Stock – The juice obtained by boiling meat, fish, or vegetables, used to prepare soups, stews, and gravy (Bender & Bender, 1995:346).

Stock cube – Ready-made, dried, preparations of stock (Bender & Bender, 1995:346).

Total antioxidant capacity (TAC) – The cumulative capacity of food components to scavenge free radicals (Pellegrini *et al.*, 2003:2812).

LIST OF ABBREVIATIONS

ADA ..... American Dietetic Association
BRISK ..... Black Risk Factor
CAT ..... catalase
CDL ..... chronic diseases of lifestyle
CE ..... catechin equivalents
CLA ..... conjugated linoleic acid
CPUT ..... Cape Peninsula University of Technology
CSL ..... corn steep liquor
CVD ..... cardiovascular disease
DNA ..... deoxyribonucleic acid
DRI ..... dietary reference intakes
FFRU ..... Functional Foods Research Unit
g ..... gram
GAE ..... gallic acid equivalents
GPx ..... glutathione peroxidase
GSH ..... glutathione
H_2O_2 ..... hydrogen peroxide
HIV/AIDS ..... human immunodeficiency virus/acquired immunodeficiency syndrome
HPLC ..... high-performance liquid chromatography
H-ORAC ..... hydrophilic oxygen radical absorbance capacity
L-ORAC ..... lipophilic oxygen radical absorbance capacity
meq. O_2/kg ..... milli-equivalents oxygen per kilogram
µg ..... microgram
mg ..... milligram
ml ..... millilitre
Mn-SOD ..... manganese superoxide dismutase
MRC ..... Medical Research Council
MSG ..... monosodium glutamate
NDP ..... new product development
ORAC ..... oxygen radical absorbance capacity
PV ..... peroxide value
QE ..... quercetin equivalents
RDA ..... recommended dietary allowance

RNS ..... reactive nitrogen species
ROS ..... reactive oxygen species
SOD ..... superoxide dismutase
TAC ..... total antioxidant capacity
TB ..... tuberculosis
TE ..... Trolox equivalents
THUSA ..... Transition, Health and Urbanization in South Africa
VIGHOR..... Vanderbijlpark Information Project on Gesondheid/Health
VUT ..... Vaal Triangle University of Technology

CHAPTER 1
INTRODUCTION

1.1 Rational for the study

Studies that investigated the dietary intake of adult South Africans indicated that the intake of fruits and vegetables are generally below the recommended daily servings (Langenhoven *et al*., 1995:526; Vorster *et al*., 1995:125; MacIntyre *et al*., 2002:252). A further finding is the decrease in consumption of dietary fibre with the increasing urbanisation of the population (Steyn *et al*., 2006a:13), which indicates a change in the consumption of whole grain foods to more refined grain foods. Plant foods are valuable sources of phytochemicals, in particular the flavonoids and the carotenoids (Kopsell & Kopsell, 2006:499; Tsao & Akhtar, 2005:13). It is therefore possible that the typical diet of South African adults due to the low fruit and vegetable intakes and consumption of refined grains may not provide sufficient levels of such phytochemicals. As phytochemicals provide important health benefits, it is important to consume foods that contain ample levels of phytochemicals (Grusak, 2002:508). Strategies to improve the phytochemical content of the typical South African adult diet, besides changing the dietary intake such as increasing the intakes of fruit and vegetables and consuming whole grains, must therefore be sought as it has been indicated that changing dietary intake is a difficult undertaking (Brady, 1996:17). In addition to this challenge, it has also been reported that affordability and availability present hurdles to improving the fruit and vegetable consumption of South Africans, especially for those in low income groups and people living in rural areas (Love & Sayed, 2001:S29). Vorster and Nell (2001:S19) reported that all South African population groups have a low intake of dietary fibre and should be encouraged to consume more unrefined carbohydrates, e.g. whole grains and cereals. One such a strategy would be through product development by incorporating phytochemicals within food products to provide food with added health attributes.

The increase in the prevalence of chronic diseases of lifestyle (CDL), such as cardiovascular disease, type II diabetes mellitus and hypertension, can partly be ascribed to the typical diet, alike to the Western diet, of the South African population (Steyn *et al*., 2006b:249). The growing interest in nutrition and the role that diet plays in preventing the onset or development of CDL has placed more emphasis on the positive effects of diet (Kaur & Kapoor, 2001:703) such as the consumption of plant foods. Added to CDL, South Africa also has a high rate of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) and tuberculosis (TB) (Bradshaw *et al*., 2004:24), both of which could benefit from improved nutrition. The term

“functional foods” has been used to describe foods with added attributes that contribute to health in ways other than furnishing nutrients. One such contribution is that of plant foods that provide various phytochemical substances that, amongst other attributes, act as antioxidants (Pennington, 2002:419). As the importance of phytochemicals to health becomes more apparent, it provides a challenge to the food and healthcare industries to develop and market such functional foods (Kaur & Kapoor, 2001:718).

The Cape Peninsula University of Technology (CPUT) has through the Functional Foods Research Unit (FFRU) established relationships with the food industry. One such company approached the FFRU with dried corn steep liquor (CSL), currently used in animal feed, with the possibility of utilising it in a product for human consumption. Microbiological analysis has indicated that this by-product of cereal grain processing is free from pathogenic microorganisms and that it would be safe for human consumption (Benadé, 2008). Further analysis by the Analytical Laboratory Services, Oxidative Stress Research Centre of CPUT indicated that the by-product has an exceptionally high total antioxidant capacity (TAC) and polyphenol content (Rautenbach, 2008). This would seem to indicate that the dried CSL could be useful as an ingredient in a food product that could provide the product with functional food characteristics as it would contribute to the dietary phytochemical content on consumption.

A pilot study conducted at the Vaal Triangle University of Technology (VUT) investigated the possibility of using stock cubes or stock powder as a vehicle for micronutrient fortification. This study found that these products were consumed in constant quantities throughout the year by a high proportion (97%) of the sample population (n = 802) of randomly selected males and females older than 15 years (Chen & Oldewage-Theron, 2004:174). These results indicated the suitability of stock cubes to be used for product development work incorporating functional food characteristics. Incorporation of the dried CSL in a stock cube or stock powder formulation may contribute largely to the TAC and polyphenol content of the product formulation and would provide for an added phytochemical intake which is possibly consumed in low quantities by South Africans.

1.2 Objectives of the study

The objectives of the study were as follow:

i. To develop a product that is commonly used by South African households (e.g. stock cube) that incorporated dried CSL as a source of phytochemicals and micronutrients such as iron, zinc, copper and selenium that may contribute immunosupport.

ii. To develop a stock cube that met accepted sensory attributes of stock cubes currently available as confirmed by sensory evaluation.

iii. To develop a stock cube that had functional properties (e.g. TAC/polyphenols) and an acceptable shelf-life as determined by accelerated shelf-life testing methods.

iv. To determine if the stock cube was sensorially acceptable to adult consumers when incorporated into two household dishes (e.g. a soup and a stew) where it is typically used as an ingredient.

v. To determine if any participant demographic or stock cube usage characteristic is associated with their acceptance of the two household dishes incorporating the stock cube as ingredient.

The first two objectives were addressed as part of the methodology of this study as it was necessary to first develop the new stock cube which could then be utilised in consumer acceptance testing and accelerated shelf-life testing. The last three objectives formed the results of this study as these arose from the completion of the new product development work.

CHAPTER 2
LITERATURE REVIEW

Changes in dietary habits, especially to a more “westernised” pattern, are contributing to the development of the CDL (Steyn *et al*., 2006b:1). The initial burden of disease estimates for South Africa, for the year 2000, by the South African Medical Research Council (MRC), found that cardiovascular disease (CVD) was the second leading cause of death, after HIV/AIDS (Bradshaw *et al*., 2003:v). Add to this the prevalence of the other CDL and TB in South Africa (Steyn *et al*., 2006b:65) and there seems to be a need for nutritional support to those persons suffering from these diseases, but also the general South African population. This literature review explores the disease burden situation in South Africa, and the opportunities for the development of food products that could offer health support through the contribution of nutrients, and in particular phytochemicals, to the diet of South African consumers.

2.1 South African burden of disease profile

The situation in South Africa has been described as being a quadruple burden of diseases. These four burdens are the poverty-related diseases, the CDL, mortality due to trauma (e.g. violent crime and injuries) and the HIV/AIDS epidemic (Steyn *et al*., 2006b:1). Since trauma has no link to nutrition, only the remaining three are relevant to the study and will be discussed.

2.1.1 Chronic diseases of lifestyle

The CDL are a group of diseases that share similar risk factors, such as obesity and tobacco addiction. Many years of exposure to these risk factors lead to the high mortality rate of the CDL due to amongst other, the high incidence of fatal stroke, heart attacks and cancer (Steyn *et al*., 2006b:iv). The term CDL indicates that aspects of people's daily lives contribute to the development of these diseases. The three main lifestyle factors that contribute to the development of these diseases are smoking, a lack of exercise and many years of consuming an unhealthy diet (Steyn *et al*., 2006b:1). The diet of a large percentage of South Africans generally follows the dietary pattern referred to as the "westernised diet". This diet typically consists of high levels of saturated fat (particularly animal fat), sodium (salt), cholesterol, alcohol, sugar and energy. In contrast, the westernised diet typically contains low levels of dietary fibre, vitamins and trace elements (Steyn *et al*., 2006b:1). In the westernised diet processed food products can, for example, contribute as much as 75% of the total dietary salt intake. This high

salt intake contributes greatly to the development of hypertension. Hypertension in turn, has long been known to be a contributing factor to the development of CVD (Law, 1995:34).

The South African MRC initial burden of disease estimates indicate that the estimated national mortality rates for the year 2000 due to stroke (5.8%) and ischemic heart disease (5.6%) are placed second and third, respectively. Mortality due to hypertensive heart disease (3.1%) is placed in the eighth position, diabetes mellitus (2.6%) in the tenth position and inflammatory heart disease (1.3%) in the 14th position (Bradshaw *et al*., 2004:24).

2.1.2 Tuberculosis as poverty-related disease

The poverty-related diseases are those infectious diseases related to under-development, poverty and undernutrition (Steyn *et al*., 2006a:34). In South Africa TB is probably the most important of these diseases. Tuberculosis has long been a problem disease affecting poor communities in South Africa and the link between TB and poverty is widely recognised (Steyn *et al*., 2006a:32; Escott & Newell, 2007:512; Harling *et al*., 2008:492). The association between TB and undernutrition is also well established and nutrition intervention is an important area of support to TB patients (Metcalfe, 2005:116; McInerney *et al*., 2007:169). The high incidence of HIV/AIDS in South Africa also adds to the risk of contracting TB as a result of immunosuppression (McInerney *et al*., 2007:164). The MRC initial burden of disease estimates found that TB was the fifth leading cause of death in South Africa in the year 2000, with a national mortality rate of about five per cent (5.1%) (Bradshaw *et al*., 2004:24).

2.1.3 Human immunodeficiency virus/Acquired immunodeficiency syndrome

The top ranking cause of death in South Africa for the year 2000 was HIV/AIDS, with an estimated mortality rate of 29.8% of all deaths (Bradshaw *et al*., 2004:24). In a no-change scenario the projected mortality rate due to HIV/AIDS for the year 2010 is 65.8% (Bradshaw *et al*., 2003:28). Adding to the problem, a TB infection also seems to accelerate the progression of HIV (Saltini, 2006:2091). The seriousness of the HIV/AIDS pandemic is mirrored in the Global Burden of Disease study for 2001 conducted by the World Health Organization (WHO), where the international death rate due to HIV/AIDS increased from two per cent in 1990 to 14% in 2001 (Lopez *et al*., 2006:1751).

2.2 Underlying mechanisms of the South African burden of disease profile

Inflammation and increased oxidative stress are two physiological conditions that have been observed in the development of the CDL (Gaté *et al*., 1999:169; Ceconi *et al*., 2003:217; Ferroni *et al*., 2006:222; Giugliano *et al*., 2006:682). Inflammation and increased oxidative stress are also associated with TB and HIV/AIDS (Stehbens, 2004:121; Hasan *et al*., 2006:2517; Appay & Sauce, 2008:231; Selek *et al*., 2008:143). These two conditions are, however, inter-related. Reactive oxygen species (ROS) are released from neutrophils and macrophages during the inflammatory response and could be responsible for cell injury (Conforti *et al*., 2009:587). Gill and co-workers (2010:1129) has hypothesised a pathway via Toll-like receptors and damage-associated molecular pattern molecules where the ROS produced during oxidative stress could trigger an inflammatory response leading to end-organ injury

2.2.1 Immune response and inflammation

The immune system defends the body against the entry and/or destruction of pathogens and can be divided into nonspecific and specific immunity. Nonspecific immunity includes species resistance, mechanical barriers (e.g. the skin and mucous membranes), the action of enzymes (e.g. lysozyme in tears), interferon, inflammation and phagocytosis, whereas specific immunity includes the action of specialised cells that can recognise foreign substances and respond against them. Examples of such cells are lymphocytes and macrophages (Hole, 1993:726). It is this inflammatory response of the immune system that is an underlying mechanism of many chronic diseases, as well as exacerbating diseases such as HIV/AIDS and TB (Friedland, 2008:1; Seaman, 2002:169).

Inflammation can be triggered by cellular/tissue injury or as a result of infection by pathogens and is part of the body's immune response (Issa *et al*., 2006:411). Chronic inflammation has been implicated as one of the key causes of the development of the CDL (Seaman, 2002:169). This chronic inflammation can lead to extensive tissue damage as a result of excessive ROS, reactive nitrogen species (RNS) and proteolytic enzyme production (Ohshima *et al*., 2005:111). A cluster of risk factors is associated with the development of CVD. These risk factors include abdominal obesity, insulin resistance, glucose intolerance, hypertension, atherodyslipidemia, a prothrombotic and also a pro-inflammatory state (Cefalu, 2008:208). In the development of atherosclerosis, it is hypothesised that the increased expression of certain cytokines and adhesion molecules could be the mechanism that mediates the inflammatory state of CVD

(Hennig & Toborek, 2001:280). A chronic low-grade inflammation is associated with obesity and could be an underlying mechanism in the development of insulin resistance (De Luca & Olefsky, 2007:103). Chronic inflammation is also seen in cancer patients (De Flora & Ferguson, 2005:8).

In persons suffering from HIV/AIDS a persistent and chronic inflammatory state has been observed (Gil *et al*., 2003:217). It is also the inflammation associated with TB that is responsible for the tissue damage that is seen in this disease (Friedland, 2008:1).

2.2.2 Oxidative stress

The production of ROS is unavoidable as oxygen is essential to human life (Valko *et al*., 2006:32). Consequently, ROS are a product of normal metabolic processes in the body which include mitochondrial metabolism and the activation of neutrophils. Added to this, many environmental and behavioural factors contribute to the ROS to which humans are exposed. These include visible and ultraviolet-radiation, ozone, man-made pollutants such as vehicle emissions, smoking and chronic excessive exercise (Gracy *et al*., 1999:17). As a group, ROS include free radicals such as the superoxide anion, hydroxyl, peroxyl, alkoxyl and hydroperoxyl radicals, as well as non-radical species such as hydrogen peroxide (H2O2), hypochlorous acid, hypobromous acid, peroxynitrite and ozone (Gracy *et al*., 1999:18; Fang *et al*., 2002:872). The RNS such as nitric oxide and nitrogen dioxide are examples of nitrogen containing free radicals (Fang *et al*., 2002:872).

The ROS have an important part to play in human biology. It mediates cell signalling, assists the immune system and plays an important part in cell apoptosis (Seifried *et al*., 2007:568). However, when in excess, ROS are toxic and can lead to the damage of cellular structures, components and molecules (Seifried *et al*., 2007:567). It is when ROS overwhelms the body's antioxidant protection system that oxidative stress occurs (Kannan & Jain, 2000:154). They are capable of altering cellular molecules, such as proteins, nucleic acids and lipids, causing alterations in structure and function (McCall & Frei, 1999:1034). The body utilises antioxidants in the form of antioxidant enzymes and non-enzymatic antioxidants in the form of certain nutrients to protect itself from the damaging effects of ROS (Morrissey & O'Brien, 1998:463). These antioxidant enzymes are of great importance, since even small deviations in ROS concentrations can have a dramatic effect on the cell's resistance to oxidative damage. Three of these enzymes are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). It is CAT, for example, that converts the non-radical ROS H2O2 to water and oxygen (Formigari *et al*.,

2007:447). Non-enzymatic antioxidants include, amongst others, vitamin C, vitamin E, ubiquinone and glutathione (GSH) (Dallner & Sindelar, 2000:285). It is GSH that acts as a general redox buffer inside cells (Formigari *et al*., 2007:447).

Oxidative damage has been implicated in the development of CVD (Flintoff-Dye & Omaye, 2005:2). One of the factors linked to oxidative damage that has been implicated in the development of atherosclerosis is the long term metabolic alteration of the vascular endothelium caused by pro-oxidants (Hennig & Toborek, 2001:280). Papaharalambus and Griendling (2007:52) have hypothesised that the underlying reason for the failure of antioxidant therapies in clinical studies could be because antioxidants are more effective in preventing the development of CVD rather than reversing the damage already done. Obesity, an important risk factor in the development of CVD and also a common problem associated with type 2 diabetes, is associated with a state of oxidative stress (Ozata *et al*., 2002:630; Keaney *et al*., 2003:434; Stefanović *et al*., 2008:160; Lavie *et al*., 2009:1930). In a study conducted by Stefanović and co-workers (2008:161), obese patients with diabetes were shown to be under increased oxidative stress as measured by a higher level of plasma thiobarbituric acid-reacting substances on malondialdehyde formation and superoxide anion. There is further a general consensus that persons suffering from type 2 diabetes have decreased antioxidant defences (Ble-Castillo *et al*., 2005:293). Results from studies suggest that γ-tocopherol may help reduce the risk of developing type 2 diabetes (Saldeen & Saldeen, 2005:881). Cancer is another disease that presents oxidative stress as an underlying mechanism (De Flora & Ferguson, 2005:8; Franco *et al*., 2008:6).

Persons living with HIV/AIDS can develop various nutrient deficiencies, including depleted antioxidant levels such as ascorbic acid, tocopherols, carotenoids and selenium, leading some researchers to believe that HIV/AIDS patients could benefit from antioxidant supplementation. (Romero-Alvira & Roche, 1998:172; Garland & Fawzi, 1999:1268; Fields-Gardner & Fergusson, 2004:1426). Altered indices of oxidative status have also been observed in patients with HIV/AIDS (Repetto *et al*., 1996:116). Gil and co-workers (2003:221) observed a large decrease in the plasma levels of GSH in HIV/AIDS patients. Along with these observations, they also found raised levels of total H2O2 and the by-products of lipid peroxidation (Gil *et al*., 2003:222). As has been mentioned, the risk of contracting TB is increased in HIV/AIDS patients (see 2.1.2). Tuberculosis itself is associated with a state of oxidative stress as measured by serum levels of conjugated dienes and malondialdehyde (Kwiatkowska *et al*., 1999:274; Reddy *et al*., 2004:216).

However, oxidative stress is not only seen on a systemic level, but also in the airways of TB patients (Kwiatkowska *et al*., 2007:574).

2.3 Nutrients and non-nutrients with possible antioxidant and/or immunonutrition properties

There is evidence that diet plays a major role in the development of CDL. The literature on oxidative stress also indicates that certain nutrient and non-nutrient antioxidants could be beneficial to the general adult population regarding the prevention CDL (Knekt *et al*., 2002:567). The following section considers the individual phytochemicals and micronutrients that could provide protection against the underlying mechanisms of the South African burden of disease.

2.3.1 Antioxidants

Antioxidants offer protection from oxidative stress by directly removing free radicals. Some characteristics that an antioxidant should possess are the quenching of free radicals, chelating redox metals and regenerating other antioxidants (Valko *et al*., 2006:21). In some cases the antioxidant itself scavenges free radicals and in other cases it is part of complex molecules, such as enzymes, that are involved in redox activity (Weisburger, 1999:945). It should be emphasised that antioxidants are different from each other, differing in structure, properties and target substrate (Seifried, 2007:169).

Nutrients that have recognised antioxidant activity are some of the vitamins, e.g. vitamin C, vitamin E and the pro-vitamin A ß-carotene as phytochemical, some minerals, e.g. selenium and zinc, and certain amino acids, e.g. arginine and taurine (Fang *et al*., 2002:872). It is the fruit and vegetable groups of food that are rich sources of antioxidants (Seaman, 2002:175). Conforti and co-workers (2009:587), for example, studied the effects of edible plant extracts from the Calabria region in Italy and found that all had antioxidant and topical anti-inflammatory properties. The Department of Health has published a report on the food consumption of South Africans (Nel & Steyn, 2002). This report was based on various food consumption studies conducted from 1983 to 2000. Referring to the compiled tables indicating the foods consumed by more than three per cent of the population, it can be seen that the typical South African diet does not contain a wide variety of different foods, especially from the fruit and vegetable groups (Nel & Steyn, 2002:62-64).

Antioxidants obtained from the diet have also been shown to have a beneficial effect on immunity, e.g. neutrophil mobilisation and stimulated lymphocyte proliferation (Meydani, 1999:126). One of the mechanisms that have been proposed to explain the inverse relationship observed between antioxidant intake and the development of CVD, is that antioxidants may inhibit the synthesis of pro-inflammatory cytokines (Meydani, 1999:128).

2.3.1.1 Exogenous vitamin antioxidants contributing to the antioxidant system

Vitamins C and E function as antioxidants by acting as reducing agents (Seifried, 2007:169). Vitamin antioxidants also need a network of co-antioxidants to prevent these vitamins from becoming pro-oxidants themselves (Rietjens *et al*., 2002:324). It is, for example, vitamin C that reduces the oxidised form of vitamin E back to its antioxidant form (Grimble, 1998:1302).

a) Vitamin C

Humans, unlike most animals, have lost the ability to produce their own ascorbic acid, also known as vitamin C (Nimbkar & Lateef, 2007:1129). Consumed, it is regarded as an excellent physiological antioxidant because of its ability to donate electrons (Cholewa *et al*., 2008:177). As a water soluble substance, it is an effective antioxidant in the aqueous parts of the body, e.g. blood plasma and cell cytosol (Jacob, 1995:757). Vitamin C, in the form of ascorbate, therefore acts as an antioxidant in the extra cellular environment. However, in low concentrations it may act as a pro-oxidant (Morrissey & O'Brien, 1998:465). This could indicate the importance of only consuming adequate amounts of this vitamin and not excessively low or high amounts.

b) Vitamin E

Vitamin E is a collective name of four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ), all of which are antioxidants (Reiter *et al*., 2007:668). The basic chemical structures of the two vitamin E forms are presented in Figure 2.1. The two major forms of the vitamin in humans are α- and γ-tocopherol (Reiter *et al*., 2007:669). It has been observed that α-tocopherol can also act as a pro-oxidant and that γ- and δ-tocopherol are much more potent antioxidants (Saldeen & Saldeen, 2005:880). Vitamin E is a lipophilic vitamin and this makes it useful in the protection of cell membranes against lipid peroxidation, especially the unsaturated fatty acids found in these membranes (Jacob, 1995:757; Berg *et al*., 2002:506). Gamma-tocopherol is more active in trapping RNS than α-tocopherol (Saldeen & Saldeen, 2005:879).

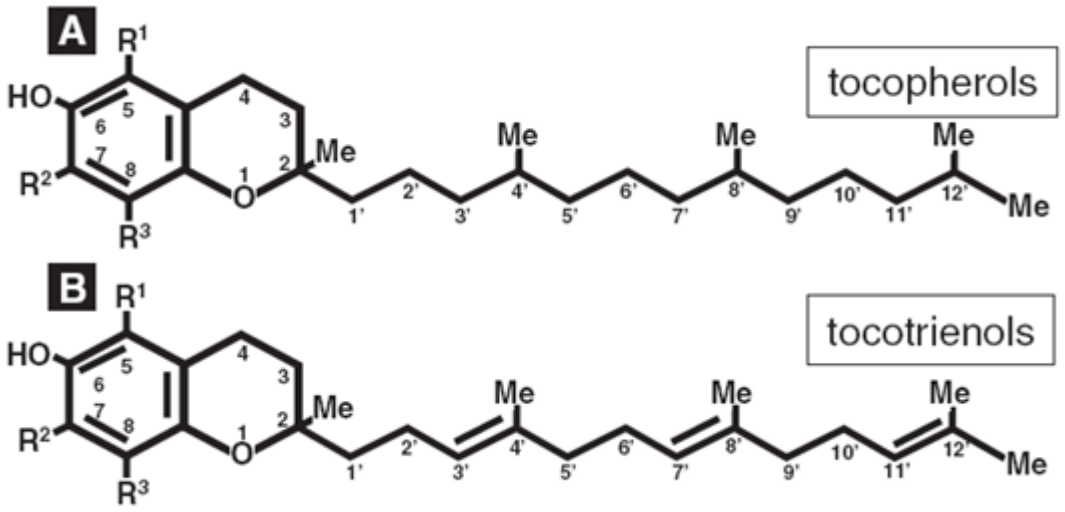

Figure 2.1: Basic structure of tocopherols and tocotrienols (obtained from Sen *et al*., 2006:2089)

Animal and human studies have also indicated that α- and γ-tocopherol have significant anti-inflammatory properties (Reiter *et al*., 2007:679). A major underlying mechanism of arteriosclerosis and hypertension is the proliferation of vascular smooth muscle cells and it appears that α-, γ- and δ-tocopherol may prevent this (Saldeen & Saldeen, 2005:881). A mixture of γ-, δ- and α-tocopherol (5:2:1) appears to be safe and possessing a greater antioxidant and anti-inflammatory capacity than α-tocopherol on its own (Saldeen & Saldeen, 2005:886). The tocotrienols, such as those found in palm oil, often exhibit properties not found in tocopherols and its anti-inflammatory and antioxidant properties could combat diseases such as cancer and CVD (Sen *et al*., 2007:692; Aggarwal *et al*., 2010:1626). Tocotrienols might also exhibit cholesterol lowering and neuroprotective properties (Sen *et al*., 2006:2088; Frank *et al*., 2012:176). Rossi and co-workers (2007:815) reported a significant correlation between the presence of α-tocotrienol and γ-tocotrienol in vegetable oil and radical scavenging activity. It has been suggested that tocotrienols' greater cellular protective properties, compared to tocopherols, are due to its ability to be readily incorporated into liposomal membranes (Yoshida *et al.*, 2007:443). Vitamin E therefore seems to improve immunity and offer some protection against the development of age-related diseases such as CVD (Meydani, 1999:129).

2.3.1.2 Trace elements involved in the enzymatic antioxidant system

A low level deficiency of trace elements may affect many of the body's systems, resulting in a gradual degeneration of these systems. The poor are especially vulnerable and trace element supplementation is a low cost method of preventing disease that could be too costly to treat once fully developed (Ames, 2004:227).

a) Selenium

The trace element selenium is essential to humans, but is also toxic in excess (Hartikainen, 2005:310). It is biologically active as inorganic salts and as amino acid and methylated compounds (Zeng & Combs, 2008:2). Selenium incorporated as selenocysteine as part of seleno-enzymes, such as GPx, acts mainly by a redox dependant mechanism in such processes as cancer cell apoptosis (Steinbrenner *et al*., 2006:1513; Valko *et al*., 2006:22, 30). Lower than normal plasma selenium levels have been observed in cancer patients and this could indicate that low selenium levels might be a risk for certain cancers (Sanz Alaejos *et al*., 2000:381). Another characteristic of GPx is a reduction of inflammation (Mishra *et al*., 2007:42). It has also been hypothesised that GPx could possibly be a first line defence against HIV infection (Foster, 2007:1279). The importance of selenium with regards to the immune response is the role that it plays in cell proliferation (Zeng & Combs, 2008:6).

b) Zinc

Zinc is widely involved in cellular metabolism and consequently even small changes in its availability could have a metabolic effect (Faa *et al*., 2008:1258). The zinc found in hundreds of enzymes such as the oxido-reductase, hydrolase and ligase families, can have a structural or a catalytical role (Maret, 2007:1; Faa *et al*., 2008:1258). The metabolic mechanisms of zinc still remain unclear despite several hypotheses. However, several researchers have demonstrated that lowered levels of cellular zinc leads to increased oxidative stress (Ho, 2004:573). Zinc containing enzymes are involved in regenerating oxidised glutathione to GSH and this reduced GSH is thought to be one of the most important intracellular defences against ROS (Faa *et al*., 2008:1263). Metallothionein is a zinc containing protein (Maret, 2008:365) and it appears that zinc and zinc-metallothionein has a protective effect against iron and copper as redox metal mediated oxidative stress (Formigari *et al*., 2007:456). Due to the important role zinc plays in cell

growth and differentiation, a zinc deficiency has a marked effect on tissues with a rapid cell turnover, such as the cells of the immune system (Faa *et al*., 2008:1258).

c) Copper

Long term excessive dietary intake of iron and zinc can compete with copper absorption and lead to a marginal copper deficiency (Uriu-Adams & Keen, 2005:271). Some populations may have marginal copper deficiencies that could be implicated in the development of certain diseases (Uriu-Adams & Keen, 2005:269). Copper deficiency can affect the body's oxidant defence system, since it is part of certain enzymes, e.g. copper-zinc-SOD and ceruloplasmin. Other enzymes of the oxidant defence system that are sensitive to copper levels are CAT and GPx, as well as non-enzymatic scavengers of ROS, such as the zinc containing protein metallothionein and GSH (Uriu-Adams & Keen, 2005:272).

The synthesis and secretion of ceruloplasmin can rise markedly during inflammation and infection, as well as in patients with diabetes, cancer and CVD (Uriu-Adams & Keen, 2005:273). Kemp and co-workers (2002:51) found a positive association between the number of T-helper cells and plasma levels of copper in elderly persons. However, copper can also be toxic and initiate the formation of ROS when there is an excessive accumulation of this trace element in the intracellular environment (Formigari *et al*., 2007:444). Copper toxicity can manifest itself when the element displaces other metal cofactors (Formigari *et al*., 2007:445).

d) Manganese

Manganese superoxide dismutase (Mn-SOD) is one of the defence enzymes that play a major role in the protection of mitochondria against oxidative damage (Ohshima *et al*., 2005:113). During inflammation, increased Mn-SOD activity could possibly be a way of minimising damage to host cells (Kuratko & Constante, 1998:192). However, a recent study measured the blood manganese values of first-grade school children in Johannesburg and Cape Town (Röllin *et al*., 2005:98). The research was conducted to determine if the manganese containing fuel additive being used in unleaded fuel has an impact on the population. A significant rise in blood manganese values was seen in the study population (Röllin *et al*., 2005:98), and for this reason, manganese fortification of the stock cube was not considered.

2.3.2 Macronutrient components

Some researchers have hypothesised that certain amino acids and fatty acids may also have antioxidant properties *in vivo* (Fang *et al*., 2002:876; Richard *et al*., 2008:454). These are discussed as the macronutrient components.

2.3.2.1 Amino acids

Amino acids are the building blocks of antioxidant enzymes and the nonenzymatic antioxidant GSH is a tripeptide consisting of glycine, glutamic acid and cysteine (Grimble, 1998:1303). There are, however, some amino acids that act as antioxidants by directly scavenging free radicals. These include arginine, citrulline, glycine, taurine and histidine (Fang *et al*., 2002:876). The amino acid proline, on the other hand, seems to be part of a mechanism that works against the formation of ROS in the intracellular spaces (Krishnan *et al*., 2008:680). An analysis of the amino acid profile of the partially dried CSL conducted by the CSL provider company indicated that proline was the most abundant amino acid in this by-product. It was found to be at levels twice as high as the second ranking amino acid, leucine (see Addendum A).

2.3.2.2 Fatty acids

Conjugated linoleic acid (CLA) possibly acts as an antioxidant and might have a protective effect against the development of arteriosclerosis in humans. It is speculated that the cis-9, trans-11 isomer, which makes up 80 to 90% of CLA found in animal fat, is the active form (Flintoff-Dye & Omaye, 2005:2). Pariza and co-workers (2001:295) have also reported that CLA appears to inhibit cancer formation in animal studies. Richard and co-workers (2008:454) also hypothesise that omega three fatty acids reduce inflammation by indirectly acting as antioxidants.

2.3.3 Phytochemicals

Numerous phytochemicals possessing health benefits are found in plant foods (Craig, 1997:S199). Two groups of natural antioxidant phytochemicals stand out, the polyphenols and the carotenoids (Tsao & Deng, 2004:85). Phytochemicals can have more than one effect, such as acting as cofactors to enzymes, inhibiting enzymatic reactions and scavenging reactive or toxic chemicals (Dillard & German, 2000:1744), to name a few. These effects can be synergistic, additive or even antagonistic with higher concentrations occasionally exhibiting pro-oxidative

characteristics in the presence of heavy metal catalysts (Issa *et al*., 2006:414). Data from various studies also indicate that phytochemicals possibly have the ability to down regulate the signalling pathways involved in the inflammatory response (Hennig *et al*., 2007:166).

2.3.3.1 Polyphenols

Polyphenols are polyhydroxylated phytochemicals of which the two main classes are the flavonoids and the phenolic acids, with the former the largest class (Lotito & Frei, 2006:1727). Flavonoids are a large group of polyphenols that are not produced by animals (Gaté *et al*., 1999:176). The flavonoids are compounds containing two aromatic rings (A-ring and B-ring) linked together with a bridge of three carbons forming an oxygenated hetero-cycle C-ring (see Figure 2.2).

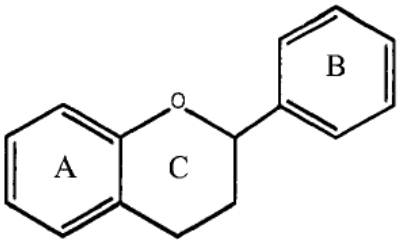

Figure 2.2: Basic structure of flavonoids (obtained from Steinberg *et al*., 2003:218)

The flavonoids can be divided into subclasses based on their individual structures and are most commonly categorised as flavan-3-ols (or catechins), flavanones, flavones, isoflavones, flavonols and anthocyanidins (Graf & Blumberg, 2004:50) (see Figure 2.3). These phytochemicals are secondary metabolites that are synthesised via the cinnamic acid pathway along with isoflavonoids, lignans and coumarins (Issa *et al*., 2006:407). They are widely found in foods of plant origin and numerous flavonoids within each of the subclasses have been identified (Hollman *et al*., 1996:43).

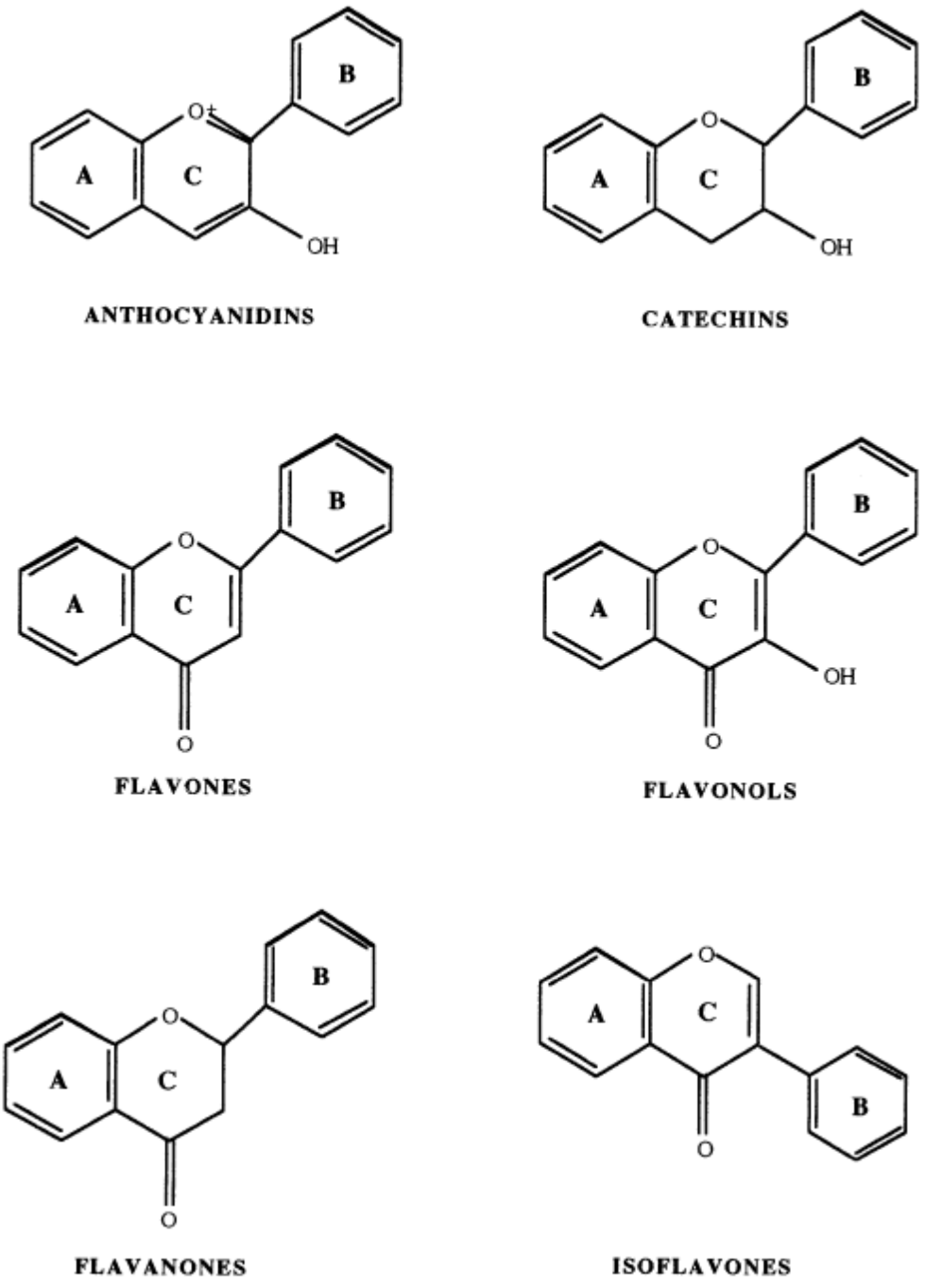

Figure 2.3: Structures of the subclasses of flavonoids (obtained from Aherne & O'Brien, 2002:76)

A Finnish study reported an inverse relation between flavonoid intake and diseases such as ischemic heart disease, cerebrovascular disease, lung and prostate cancer, type 2 diabetes and asthma (Knekt, 2002:565-566). The flavonoids that are found in tea have been greatly studied and shown to be antioxidants that are beneficial to health (Fang *et al*., 2002:877). In an Indian study (Agarwal *et al*., 2010:24), TB patients received 500 microgram (µg) green tea catechin

extract three days per week for four months. The researchers found a reduction in malondialdehyde, a marker of oxidative stress, in the patients receiving the green tea catechin extract (Agarwal *et al*., 2010:26).

The second main class of polyphenols, the phenolic acids, can be divided into the hydroxycinnamic or hydroxybenzoic groups, depending on the number and position of the hydroxyl groups on the aromatic ring (Robbins, 2003:2866). Phenolic acids make up approximately a third of all polyphenols found in plant foods (Robbins, 2003:2867). The most common phenolic acid in cereal grains is ferulic acid (Rouau *et al*., 2003:899) which could indicate that CSL might be rich in this polyphenol and would make it a valuable contributory functional ingredient in the development of new food products (see Figure 2.4). Ferulic acid is an antioxidant that has been shown to positively affect health, especially with regard to the CDL (Srinivasan *et al*., 2007:92). Besides it being an antioxidant, ferulic acid also has antimicrobial and antimutagenic activity (Ou & Kwok, 2004:1267). Itagaki and co-workers (2009:466) have hypothesised that it is ferulic acid's chain-breaking activity that may contribute to its protection against oxidative stress.

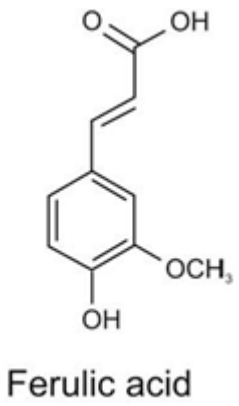

Figure 2.4: Structure of ferulic acid (obtained from Bondia-Pons *et al*., 2009:325)

2.3.3.2 Carotenoids

Another group of non-nutritive antioxidants are the carotenoids. These phytochemicals are synthesised along the isoprenoid pathway along with terpenes and saponins (Issa *et al*., 2006:407). Carotenoids are plant pigments composed of isoprene groups polymerised into large molecules, usually containing at least 40 carbon atoms with accompanying hydrogen (see

Figure 2.5). The pigment colours range from yellow to orange and some reds. The carotenoids can be subdivided into carotenes and xanthophylls. Examples of carotenes are α-carotene, β-carotene and lycopene. Lutein and zeaxanthin are examples of xanthophylls (McWilliams, 1993:229). Some carotenoids have provitamin A activity, but they are also potent antioxidants and may enhance the immune response (Bendich, 1991:127; Faulks & Southon, 1997:246). The carotenoids may also have a protective effect against certain cancers and eye diseases. In the case of eye diseases, it is thought that carotenoids in the eyes absorb damaging blue light and in doing so, protect the eyes against oxidative damage (Krinsky & Johnson, 2005:459). Farombi and Britton (1999:315) also found that α-carotene, which is found in abundance in red palm fat, is more effective against lipid peroxidation than β-carotene. Donaldson (2011:1003) has proposed a carotenoid health index with a plasma carotenoid level of 2.5 to 4 μM classified as low risk and levels higher than 4 μM as very low risk. However, the author indicated that 95% of the American population fell below these levels.

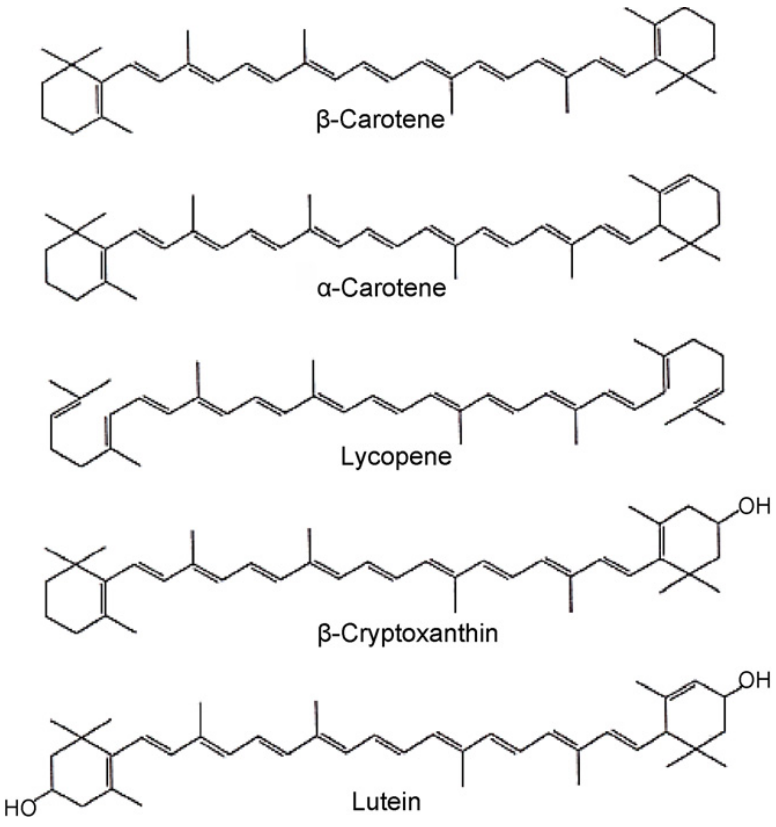

Figure 2.5: Structure of some major carotenoids (obtained from Rao & Rao, 2007:208)

2.3.4 Iron

Iron homeostasis is highly regulated by the body as an excess can directly catalyse the formation of ROS (Formigari *et al*., 2007:445). The body utilises ferritin to store iron safely as well as sequestering free, intracellular ferrous iron from Fenton-like reactions that would otherwise produce toxic ROS (MacKenzie *et al*., 2008:1762; Arosio *et al*., 2008:2) (see Equation 2.1). However, Vergnaud and associates (2007:268) found no connection between the general dietary iron intake or serum ferritin and markers of arteriosclerosis development. It seems that a deficiency of iron could cause deoxyribonucleic acid (DNA) damage similar to that caused by radiation (Ames, 2004:228).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$$

Equation 2.1: Fenton reaction (obtained from Faa *et al*., 2008:1263)

2.4 Food sources of the nutrients and non-nutrients supporting the antioxidant defence system and immunonutrition

Vitamin E is one of the fat soluble vitamins and is widely distributed in foods. Plant oils and margarine are good sources, as are wheat germ, whole grains, legumes, nuts and dark green leafy vegetables (Robinson *et al*., 1993:188). Vitamin C, on the other hand, is one of the water soluble vitamins (Wardlaw, 2003:260). It is found only in the growing part of plants. Fruits and vegetables contribute vitamin C to the diet, with citrus fruits, strawberries, guavas and pineapples being rich sources of the vitamin. Other fruits, such as peaches, apples, pears and bananas also contain vitamin C, but in substantially lower quantities. These fruits can be important sources of vitamin C if they are consumed in large quantities. In the vegetable group, dark green leafy vegetables, tomatoes and broccoli are good sources. Potatoes and sweet potatoes can make a contribution to the daily vitamin C intake if they are consumed in large amounts (Robinson *et al*., 1993:194; Rolfes *et al*., 2009:354).

Zinc is found in many foods of animal and plant origin. It is found in oysters, organ meats, muscle meats, dark meat of chicken, whole grain breads and cereals, legumes, peanuts and peanut butter (Robinson *et al*., 1993:161; Rolfes *et al*., 2009:454). Rich sources of copper are

organ meats, shellfish, whole grain cereals, legumes and nuts (Robinson *et al*., 1993:163; Rolfes *et al*., 2009:458). The trace element selenium is found in foods rich in protein, such as meats, seafood and cereals (Robinson *et al*., 1993:164). Tea, nuts, legumes, and whole grain cereals are foods that are rich in manganese (Robinson *et al*., 1993:164; Rolfes *et al*., 2009:460).

The term phytochemical refer to physiological active substances of plant origin (Hasler *et al*., 2004:814). It includes the flavonoids and the carotenoids, both of which are pigments found in the fruit and vegetable groups (Craig, 1997:202). The carotenoids are found in fruits and vegetables of a yellow, orange or red colouring (Rao & Rao, 2007:208). Tomatoes are an example of a food source of the carotenoid lycopene, which gives tomatoes their red colour (Rao & Rao, 2007:210). The flavonoids are widely found in vegetables and fruits, with red wine, tea, cocoa and herbs also containing significant amounts of flavonoids (Lotito & Frei, 2006:1730).

Protein rich foods are excellent sources of amino acids since they are the building blocks of all proteins (Robinson *et al*., 1993:105). Meat, fish and poultry are all foods that are rich in proteins, as well as eggs, dairy and legumes (Robinson *et al*., 1993:113; Rolfes *et al*., 2009:181). The food sources rich in the amino acids that may act as antioxidants include nuts and legumes (rich in arginine) (Vega-López *et al*., 2010:556), watermelon (rich in citrulline, which in turn can be metabolised to arginine) (Collins *et al*., 2007:261), shellfish and the dark meat of chicken (rich in taurine) (Wójcik *et al*., 2010:20), cheeses such as Emmental, Cheddar and Edam (rich in histidine and proline) (Weder & Belitz, 2003:4820) and tuna and pork (rich in histidine) (Weder & Belitz, 2003:4820). Glycine is produced from other amino acids in the body (Hons *et al*., 2010:106), but meats, such as beef, pork, mutton and chicken, are rich food sources (Weder & Belitz, 2003:4820). The food in which CLA is commonly found are fat-containing animal products such as beef, lamb and dairy products, such as milk and cheese, and it has also been identified in margarine due to the hydrogenation process of fat (Flintoff-Dye & Omaye, 2005:2).

2.5 South African dietary nutrient and phytochemical intakes

The terms malnutrition and undernutrition are sometimes used interchangeably. Malnutrition refers to any deviation from an optimal diet, which would include diets furnishing excessive amounts of energy that could lead to obesity. Undernutrition can be used to refer to underfeeding or to a sub-optimal diet (Shetty, 2006:524). In other words, malnutrition can be subdivided into overnutrition (excess energy) and undernutrition (inadequate energy and/or nutrients) This distinction is important, as it has been recognised that poverty is not only

associated with undernutrition, but could also lead to overnutrition and obesity (Tanumihardjo *et al*., 2007:1966). This clearly places the poor in a position of developing not only diseases of nutrient deficiencies, but also the diseases of lifestyle associated with obesity.

In developing countries micronutrient deficiencies are a cause for concern (Adelekan, 2003:473). Deficiencies of micronutrients, such as vitamin A and iron, are the cause of many cases of ill health and death among the poor, especially children and the elderly (Ramalingaswami, 1998:381). It is also recognized that deficiencies of more than one micronutrient commonly occur within poor communities (Black, 2003:79). Micronutrient deficiencies are, in addition, also implicated in the development of the CDL, such as cancer, with vitamin B12, folic acid, vitamin B6, niacin, vitamin C, vitamin E, iron or zinc deficiencies linked to DNA damage (Ames, 1998:102). Bhutta and co-workers (2008:431) reviewed various maternal and child nutritional interventions and came to the conclusion that stunting at 36 months could be reduced by a third and mortality at 36 months by a quarter on provision of adequate nutritional interventions making nutritional interventions important in securing a better overall health status.

At present there are no national data on micronutrient deficiencies of South African adults (Steyn *et al*., 2006a:23). In food consumption studies done on specific population groups some figures on dietary intake are available. The Transition, Health and Urbanization in South Africa (THUSA) study was conducted from 1996 to 1998. An African population in the North West Province was sampled across five strata of urbanisation (MacIntyre *et al*., 2002:240). MacIntyre and associates (2002:240) found that calcium and zinc intakes of the population were not meeting the Recommended Dietary Allowances / Adequate Intakes (RDA/AI) of the United States. The intake of one of the groups of men in the five strata met the Recommended Dietary Allowance (RDA) for iron. Only the intake of the upper class urban stratum met the RDA for vitamin C, and the women in the upper class stratum was the only group with an adequate vitamin A intake. Some of the sub-groups intakes for thiamine, riboflavin, niacin, and vitamin B12 were below the RDA, with folic acid the only reported B-complex vitamin that met the recommended intake levels (MacIntyre *et al*., 2002:245). The Black Risk Factor (BRISK) study was conducted in 1990 where a sample of African men and women living in the Cape Peninsula was studied (Bourne *et al*., 1993:238). Bourne and co-workers (1993:241) reported that this population experienced a low intake of calcium, iron, magnesium, zinc and copper. They also reported a low intake for thiamine, riboflavin, niacin, vitamin B6, folate and vitamin B12, with especially low intakes for vitamin A and vitamin C (Bourne *et al*., 1993:243). The Vanderbijlpark Information Project on Gesondheid/Health (VIGHOR) was conducted in 1988 using a white population as a study

sample (Vorster *et al*., 1995:120). The intakes of the 35 to 44 year olds of the sample were compared to international dietary intake recommendations. The researchers found that intakes of vitamin D, folate, calcium, magnesium, selenium, potassium, copper and iron were not meeting the recommended intake levels. The sample's intakes for thiamin, riboflavin, niacin, vitamin B6 and zinc were not at the recommended levels of the American RDA, but did meet the British recommendations (Vorster *et al*., 1995:119). It is acknowledged that these studies present a limited view of the micronutrient provision of the South African diet, but they do seem to indicate an inadequate intake of the micronutrients.

A study among South African urban black children assessing their nutritional status at ages five, seven, nine and ten years found that their intakes of calcium, iron, zinc and biotin fell below the RDA/AI (MacKeown *et al*., 2003:188). However, at age ten large percentages of the children had micronutrient intake levels below the RDA/AI, with the following percentages representing those with intakes below the RDA/AI: calcium (90%), iron (72%), potassium (79%), zinc (85%), copper (90%), vitamin A (98%), riboflavin (79%), vitamin B6 (76%), ascorbic acid (80%), pantothenic acid (98%) and biotin (98%) (MacKeown *et al*., 2003:190). Similar results were found in The National Food Consumption Survey of 1999, with calcium, iron, zinc, selenium, vitamins A, C, D and E, riboflavin, niacin, vitamin B6 and folic acid all below two thirds of the RDA/AI for South African children in general (Labadarios *et al*., 2005:540). These figures indicate that there are many areas for potential nutritional support for South African children, also especially with regard to micronutrient nutrition.

Although there are no data on the South African intakes for amino acids, CLA, polyphenols and carotenoids, it could be deduced from the food consumption studies that amino acid and CLA intakes would not be such a great nutritional concern, since the availability of meat and fat products has increased during the period from 1961 to 2001 (Steyn *et al*., 2006a:13). However, due to its presence in full-fat animal products, the consumption of CLA may need to be attended to due to the nutritional campaigns concerning the reduced consumption of high-fat food sources. The polyphenol and carotenoid dietary contents on the other hand is probably low due to the fact that the availability of vegetables did not increase over this 40 year period and remained at 205 grams per day (Steyn *et al*., 2006a:16). South Africans are generally known for not consuming and adequate daily intake of either vegetables or fruits.

2.6 Commercial opportunities for dietary support through the development of food products with functional food characteristics

There is no universally accepted definition of the term “functional food”, but the concept is usually understood as a food product that has a specific and identifiable physiological action or health benefit (Wahlqvist & Wattanapenpaiboon, 2002:1). The American Dietetic Association (ADA) made the following statement on functional foods: “It is the position of the American Dietetic Association (ADA) that functional foods, including whole foods and fortified, enriched, or enhanced foods, have a potential beneficial effect on health when consumed as part of a varied diet on a regular basis, at effective levels. The Association supports research to define further the health benefits and risks of individual functional foods and their physiological active components. Dietetics professionals will continue to work with the food industry, the government, the scientific community, and the media to ensure that the public has accurate information regarding this emerging area of food and nutrition science” (Hasler *et al*., 2004:814). Functional foods may have different benefits such as improving health in general or delaying the onset of chronic diseases (Mark-Herbert, 2004:713). Adding nutrients to food is a further way of combating diseases caused or exacerbated by deficiencies. The criteria for the choice of nutrients emphasize that it should have a proven benefit and/or that there is a deficiency of that nutrient in the population. It should also be safe and stable under storage and household conditions (Brady, 1996:12).

The food industry is being led by three global mega trends in new product development. These three trends are described as “pleasure and indulgence, convenience and health”. A product that incorporates both the convenience and health trend is seen as a “one stop nutrition” product (Maguire, 2008:5). The proposed stock cube will fulfil these two trends. The stock cube is inherently a convenience product and the inclusion of dietary components, such as phytochemicals along with micronutrients, will add a health benefit. A further health aim of this study is to reduce the sodium content of the stock cube. The traditional stock cubes available can have a sodium content as high as 608 milligram (mg)/100 gram (g) (Langenhoven *et al*., 1991:46).

In a recent article by Sloan (2008), ten functional food trends were identified, of which two are particularly relevant to this study. The “contemporary conditions” trend indicates that functional foods will become more condition specific and that polyphenols will increasingly be used in products targeting heart health (Sloan, 2008:32). In another trend, “proactive lifestyle”, the

author indicates that consumers are increasingly following a disease preventative lifestyle. This trend has led to the use of fortified products becoming a lifestyle, with consumers realising the benefits that functional foods could make in areas such as immunity (Sloan, 2008:34). These two trends support the development of the new stock cube as the CSL will provide a phytochemical rich ingredient and through the addition of micronutrients could act in a preventative manner. Currently products that could represent whole foods such as fruit juices and tea are being marketed as naturally rich sources of phytochemicals. Internationally development is taking place with waste products such as apple and mango peel being utilised as ingredients that could enhance the polyphenol content of food products (Vasantha Rupsinghe *et al*., 2008:1217; Ajila *et al*., 2010:219). Green tea extract is another substance that is showing potential as a polyphenol rich ingredient and has been used in various products, such as beverages and bread dough (Wang *et al*., 2007:470). No stock based product has yet been launched in South Africa that has been enhanced to specifically provide phytochemicals, though reduced salt stock cubes are available. However, the trend for enhanced food products is emerging, for example, tea (rooibos and green tea) being utilised as a base for beverages and yoghurts with probiotic active bacteria developed for the South African market.

CHAPTER 3
RESEARCH DESIGN AND METHODOLOGY

The methodology of this study involved the steps of the new product development (NPD) process. Ethical approval was obtained from the CPUT Faculty of Applied Sciences Research Ethics Committee (4 June 2009) for the consumer acceptance testing part of the study.

3.1 New product development

The NPD process consists of a number of developmental steps alternating with evaluation steps (Tzokas *et al*., 2004:619). These steps are as follow: generation and screening of ideas; concept development and concept testing; building business case and business analysis; product development and product testing; market testing and analysing the test market results; and finally product launch and post-launch evaluation (Tzokas *et al*., 2004:620). A simplified NPD process was used in this study and included the steps of product idea generation, idea screening, feasibility analysis (considering aspects related to business analysis and feasibility), development, testing (the consumer acceptance and shelf-life testing data obtained will be reported as results in Chapter 4), introduction and commercialisation (Rochford, 1991:288). Recommendations for the product introduction and commercialisation steps will only be made in Chapter 7 (Recommendations), as these steps are beyond the scope of the study.

3.1.1 Generation of ideas

This step of the NPD process includes techniques such as brainstorming and market research (Rochford, 1991:289-290). The co-supervisor of this study suggested the idea for the stock cube or stock powder. His work experience and involvement with the food industry and awareness of food preparation customs through fieldwork in especially disadvantaged communities led him to the suggestion in an idea generation discussion that took place.

3.1.2 Screening of ideas

The evaluation of the ideas that were generated in the first step of the NPD process takes place in this step (Tzokas *et al*., 2004:620). A database search to ascertain whether or not a similar product had been developed was conducted to screen the idea of a fortified stock cube. The search lead to an article reporting on a pilot study that was conducted by Chen and Oldewage-

Theron (2004:174) to determine the idea of using stock cubes and powders as vehicles for micronutrient fortification. Their study concluded that the product would be an ideal vehicle for fortification in South Africa as stock cubes are used regularly across a wide demographic range.

3.1.3 Feasibility analysis

It is hypothesised that true innovation in food products is held back by slow changing consumer eating habits and an aversion to too much novelty in food (Costa & Jongen, 2006:457). However, true innovation is needed if a product is to be successful, but only if it satisfies a consumer need (Stewart-Knox & Mitchell, 2003:62). Although the use of CSL for the stock cube formulation is an innovative way of adding phytochemicals to a food product, its use as an ingredient in a commonly used product, such as stock cubes, could mean that consumers would not perceive it as too novel. The addition of nutrients to food products that are regularly consumed by the target market is also a much quicker method of improving the group's dietary intake than attempting to change their eating habits (Brady, 1996:17). A pilot study conducted at the VUT investigated whether stock cubes and stock powders could be considered as vehicles for fortification. The study found that these products are excellent vehicles of fortification as they are consumed regularly by a large number of South African consumers (Chen & Oldewage-Theron, 2004:178). A similar observation was made by the co-supervisor during community based research prospects in rural KwaZulu-Natal and the Eastern Cape (Benadé, 2008). This lead to the idea of utilising a stock cube as a carrier for phytochemicals and micronutrients in support of immunonutrition and oxidative stress defence (see Chapter 2). The high salt content of traditional stock cubes presented a problem as high sodium intakes are linked to hypertension (Karppanen & Mervaala, 2006:59) and it was clear that the salt content would have to be attended to in the new stock cube (see 3.7).

In addition to the above a microbial and nutritional analysis was conducted on CSL as part of the feasibility analysis. This was done as CSL would be incorporated into the new stock cube as a source of phytochemicals.

3.1.3.1 Microbial analysis

The CSL was initially tested to determine its safety for human consumption by the CSL provider company. The analysis was conducted by Swift Micro Laboratories, Claremont, Cape Town and was found to be microbiologically safe (see Addendum B).

3.1.3.2 Nutritional analysis

An initial analysis of the CSL powder by the Analytical Laboratory Services of the institution indicated the total flavonol content to be 2.21 g quercetin equivalents (QE) / 100 g and the total flavanol content to be 0.04 g catechin equivalents (CE) / 100 g. The laboratory analysis also indicated the total polyphenol content to be 4.57 g gallic acid equivalents (GAE) / 100 g. This level of polyphenols is high compared to most fruits and vegetables, which have an average total polyphenol content of between 0.1 to 0.5 g GAE / 100 g (Rautenbach, 2008). This indicated the suitability of CSL as a source ingredient of phytochemical antioxidants in the proposed stock cube.

A further analysis was conducted by Microchem, Cape Town, to determine the nutrient profile of the CSL (see Table 3.1). The analysis indicated that CSL was exceptionally rich in biotin (430 µg / 100 g) (see Table 3.1) as the Adequate Intake (AI) for males and females 19 to 30 years of age is only 30 µg per day and 12 µg per day for children aged four to eight years (Rolfes *et al*., 2009:B). This is more than seven times the level found in organ meats (Robinson *et al*., 1993:203); for example, beef liver which is a rich source of biotin at 59.2 µg / 100 g (Langenhoven *et al*., 1991:27). The compositional contents of copper, potassium and magnesium also stand out, with copper at 1 900 µg / 100 g, potassium at 5 040 mg / 100 g and magnesium at 966 mg / 100 g (see Table 3.1). In comparison to foods that are often cited as being rich in these micronutrients, beef liver contains copper at levels approximately 2 820 µg / 100 g (Langenhoven *et al*., 1991:27; Robinson *et al*., 1993:176), bananas contain potassium at levels approximately 396 mg / 100 g (Langenhoven *et al*., 1991:87; Robinson *et al*., 1993:173) and sunflower seeds contain magnesium at levels approximately 354 mg / 100 g (Langenhoven *et al*., 1991:77; Robinson *et al*., 1993:157).

However, in contrast to these micronutrients mentioned above that occur in CSL in rather large amounts the sodium content of CSL is only 10.2 mg / 100 g (see Table 3.1). This can be considered minimal as it is lower than the sodium content of low sodium foods, such as vegetables (Robinson *et al*., 1993:172), for example broccoli (26 mg / 100 g) (Langenhoven *et al*., 1991:103). The incorporation of CSL in product development work would therefore not make an appreciable contribution to the sodium content of the product.

Table 3.1: Nutrient profile of dried corn steep liquor[1]

Nutrient	Amount	Nutrient/100g	Amount
% Moisture	14.4	Biotin (µg)[2]	430.00
% Ash	14.1	Thiamin (mg)[3]	0.02
% Total fat	0.4	Riboflavin (mg)	0.04
% Monounsaturated fatty acids	0.1	Niacin (mg)	4.33
% Polyunsaturated fatty acids	0.1	Vitamin B6 (mg)	0.03
% Saturated fatty acids	0.2	Pantothenic acid (mg)	0.64
% Trans fatty acids	0.0	Folic acid (µg)	ND[4]
Cholesterol (mg/100g)	1.0	Vitamin B12 (µg)	<20
% Protein	42.2	Aluminium (mg)	6.00
% Nitrogen	6.8	Calcium (mg)	68.10
% Total dietary fibre	8.8	Copper (µg)	1900.00
% Carbohydrates	20.1	Iron (mg)	14.50
% Total sugar	0.0	Mercury (µg)	0.30
% Fructose	0.0	Potassium (mg)	5040.00
% Glucose	0.0	Magnesium (mg)	966.00
% Sucrose	0.0	Manganese (mg)	5.25
% Maltose	0.0	Sodium (mg)	10.20
% Lactose	0.0	Lead (mg)	ND
Energy (kJ/100g)[5]	1144.0	Zinc (mg)	9.65

1 – Nutrient profile analysis by Microchem, Cape Town, July 2009
2 – Microgram per 100 gram (µg/100g)
3 – Milligram per 100 gram (mg/100g)
4 – Not detected (ND)
5 – Kilojoules per 100 gram (kJ/100g)

It should be noted that rather small amounts of CSL incorporated into the newly developed stock cube would be consumed compared to the dietary amounts consumed of foods rich in these micronutrients. The micronutrient and amino acid profiles of different CSL products have also been published in articles by DeFrain and associates (2003:82-83) and Sharma and Kothari (1992:217). The micronutrient and amino acid profiles as published by these authors are indicated in Tables 3.2 and 3.3, respectively. The micronutrient compositional analysis of Defrain and associates (2003:82-83) also reflect an abundant copper and potassium content in CSL

(see Table 3.2) as indicated above. An analysis of the amino acid profile of the partially dried CSL conducted by the CSL provider company indicated that proline was the most abundant amino acid. It was present at levels twice as high as the amino acid ranked second, leucine. In the analysis by Sharma and Kothari (1992:217) proline was ranked as the second highest amino acid (see Table 3.3). Proline has been linked to a defence mechanism against ROS formation, adding support for the use of CSL in the product development process in oxidative stress defence (Krishnan *et al*., 2008:680).

Table 3.2: Micronutrient profile of different corn steep liquor products

Nutrient (mg/100g)[1]	Wet corn steep liquor[2]	Dried corn steep liquor[2]	Condensed corn steep liquor[3]
Calcium	0.62	0.62	80
Phosphorus	0.85	0.85	2 040
Magnesium	NA[4]	NA	750
Potassium	NA	NA	2 890
Sodium	NA	NA	170
Sulphur	NA	NA	1 900
Iron	NA	NA	11.9
Zinc	NA	NA	10.1
Manganese	NA	NA	0.4
Copper	NA	NA	0.6

1 – Milligram per 100 gram
2 – Analysis of product for CSL provider company
3 – Obtained from DeFrain *et al*. (2003:82)
4 – Not available (NA)

Table 3.3: Amino acid profile of corn steep liquor

Amino acids	Percentage[1]	Ranking[1]	Gram/kilogram of dry matter[2]	Ranking[2]	Milligram/100gram[3]	Ranking[3]
Aspartic acid	5.0	7	41.5	3	NA[4]	NA
Glutamic acid	8.4	4	79.1	1	NA	NA
Serine	6.8	6	24.2	8	NA	NA
Threonine	3.9	9	18.0	10	1.78	4
Proline	12.2	2	NA	NA	4.10	1
Glycine	6.8	6	26.4	7	0.34	12
Alanine	17.4	1	36.9	4	NA	NA
Valine	7.3	5	27.4	5	2.06	3

Table 3.3: Amino acid profile of corn steep liquor (cont.)

Amino acids	Percentage[1]	Ranking[1]	Gram/kilogram of dry matter[2]	Ranking[2]	Milligram/100gram[3]	Ranking[3]
Methionine	2.6	13	10.7	15	0.55	10
Isoleucine	3.5	11	17.6	12	0.89	7
Leucine	11.9	3	42.5	2	2.1	2
Tyrosine	2.8	12	15.6	14	0.42	11
Phenylalanine	3.6	10	17.9	11	0.83	8
Histidine	NA	NA	16.6	13	0.61	9
Lysine	4.2	8	21.9	9	1.75	5
Arginine	3.6	10	27.1	6	1.71	6

1 – Obtained from Sharma & Kothari (1992:217)
2 – Obtained from DeFrain *et al*. (2003:83)
3 – Analysis of product for CSL provider company
4 – Not available (NA)

3.2 Development

A number of developmental steps were undertaken. The first step was the formulation of a prototype of the product, considering the basic ingredients to be incorporated, which then acted as a base formulation for further refinements. This was followed by considering the quantities of CSL and micronutrient fortification that would be added. Trials of different formulations were then conducted to develop a final bench formulation.

3.2.1 Basic ingredient identification

As a starting point, the ingredient lists of existing stock cubes were obtained and the ingredients screened. Beef flavoured stock cubes were chosen, as this was indicated as the most popular flavour amongst South Africans in the study conducted by Chen and Oldewage-Theron (2004:177). The two top-selling beef flavoured stock cube brands (Fourie, 2007; Van de Vyver, 2007) available on the South African market were identified. The ingredient list of three beef flavoured stock cubes (two from Brand A and one from brand B), as well as a formulation from the internet was obtained (see Table 3.4). The ingredients common to all four stock cube examples were salt, fat (vegetable), monosodium glutamate (MSG) and flavour ingredients, e.g. herbs and spices, with the basic ingredients being salt and vegetable fat.

Table 3.4: Ingredient lists of three stock cubes[1]

Ingredient ranking[1]	Brand A beef flavoured	Brand A beef flavoured (25% less salt)	Brand B beef flavoured	Beef flavour bouillon cubes[2]
1	Salt	Salt	Salt	Salt
2	Maize flour (radurized)	Potassium chloride (salt replacer)	Hydrogenated vegetable fat	Lactose monohydrate
3	Partially hydrogenated vegetable fat[3]	Partially hydrogenated vegetable fat[3]	Flavour enhancers (E621, E631)[7]	Maltodextrin
4	Vegetable fat[3]	Vegetable fat[3]	Permitted colorant (E150)[8]	Monosodium glutamate
5	Hydrolysed vegetable protein (soya, egg)	Maize flour (radurized)	Sugar	Sugar
6	Colorant (E150c)[4]	Hydrolysed vegetable protein (soya, egg)	Maize	Hydrogenated fat (melting point 45-50°C)
7	Monosodium glutamate	Colorant (E150c)[4]	Acidulants (E330, E363)[9]	PentaBase Bouillon Flavour BF 1261
8	Acidifier (E330)[5]	Monosodium glutamate	Emulsifiers (E339, E322, E435)[10]	PentaBase Bouillon Aroma BF 1262
9	Yeast extract	Acidifier (E330)[5]	Anticaking agent (E551)[11]	Dehydrated parsley
10	Parsley	Yeast extract	Eggs	
11	Flavour enhancer (E631, E627)[6]	Parsley	Herbs	
12	Beef	Flavour enhancer(E631, E627)[6]	Stabiliser (E412)[12]	
13		Beef	Spice extracts	
14			Preservative (E223)[13]	
15			Flavourants	
16			Antioxidants (E320, E321, E319)[14]	

1 – Listed in descending order of weight
2 – Obtained from Anon., n.d.
3 – Contains tertiary butylated hydroquinone (TBHQ)
4 – Ammonia caramel (E150c)
5 – Citric acid (E330)
6 – Disodium inosinate (E631) and disodium guanylate (E627)
7 – Monosodium L-glutamate (E621) and disodium inosinate (E631)
8 – Caramel (E150)
9 – Citric acid (E330) and succinic acid (E363)
10 – Sodium phosphates (E339), lecithin (E322) and polysorbate 60 (E435)
11 – Silicon dioxide (E551)
12 – Guar gum (E412)
13 – Sodium metabisulfite (E223)
14 – Butylated hydroxyanisole (E320), butylated hydroxytoluene (E321) and tertiary butylated hydroquinone (E319)

3.2.1.1 Salt and monosodium glutamate

In the four example formulations (see Table 3.4), salt was the main ingredient. According to the MRC Food composition tables, beef bouillon contains 326 mg / 100 g sodium (Langenhoven *et al*., 1991:46). In a product such as stock cubes, usually stored at room temperature, the high salt content acts as an antimicrobial preservative besides providing a salty taste (Smith, 1991:118). In the formulation of the new stock cube, one of the objectives was to lower the sodium content. The replacement of a percentage of the salt content with a salt replacer was one possibility. Potassium chloride was indicated by the literature as the most commonly used salt substitute (Desmond, 2006:192). It is often used with a masking agent as its perceived bitterness increases and saltiness decreases with ratios above 50% (Desmond, 2006:192). However, salt is a functional and more importantly, an inexpensive ingredient (Desmond, 2006:191) and its replacement had cost implications. Another method employed in reducing salt is the use of flavour enhancers which raise the perceived saltiness of other ingredients when used with salt in reduced amounts (Desmond, 2006:193). In the four example formulations (see Table 3.4) the commonly used flavour enhancer MSG (Jinap & Hajeb, 2010:1) is listed as an ingredient, but it was not considered for inclusion in the formulation as part of the sodium reduction objective. The final developmental decision made was to decrease the salt content so that salt would not be the main ingredient in the stock cube (see Table 3.4) and to exclude MSG inclusion as ingredient. The impact of the reduction in the salt content and the exclusion of MSG was evaluated on the stock cube taste through sensory analysis and its microbial safety through shelf-life testing.

3.2.1.2 Fat

The typical stock cube preparation before incorporating it into a dish involves the addition of boiling water to the cube. The use of a fat with a melting point between 45 to 50°C would be necessary for the product to be used in this way and still be storable at ambient temperatures (Anon., n.d.). Carotino® shortening, a fat derived from red palm oil, was utilised since it is also a rich source of nutrients, such as pro-vitamin A (Hekmat & Haines, 2003:1211). The Carotino® company is able to formulate fats with different melting points and two possible samples were provided. The specifications of the palm fats that were used in combination can be found in Table 3.5. Table 3.6 provides the specification of the palm fat concentrate that was also added to raise the vitamin E and carotenoid contents of the fat. The use of these palm fats with their carotenoid and vitamin E contributions would provide antioxidants to the stock cube in support of the shelf-life, in particular on the reduction of the salt content.

Table 3.5: Specifications of palm fats[1]

Product	Product code	Free fatty acids (percentage)	Moisture & Impurities (percentage)	Slip melting point (degrees Celsius)	Carotene (ppm)[2]	Vitamin E (ppm)
Carotino Shortening	CS35FV	0,1 max[3]	0,1 max	34 - 38	app[(4)] 250	app. 450
Carotino margarine	CM45BV	0,15 max	13,5 – 14,5	44 - 48	app 40	app.400

1 – Obtained from Carotino® SDN. BHD (A member of J.C. Chang Group, Malaysia)
2 – Parts per million (ppm)
3 – Maximum (max)
4 – Approximately (app.)

Table 3.6: Specification of palm fat concentrate[1]

Substance	Amount
Carotenoid content	At least 8%[2]
α-carotene	3.00%
β-carotene	4,5%
γ-carotene	0,3%
Lycopene	0,05%
Xanthophyll	0,18%
Other	0,2%
Tocopherol and tocotrienol content	At least 7%
Tocopherols	1,5%
Tocotrienols	6,5%
Phytosterols	3,9%
Squalene	1,1%
Co-enzyme Q	0,2%
Palm oil	58,6%
Lead	<0,1ppm[3]
Mercury	<0,05ppm
Ash	<0,1ppm
Microbiological activity (Total plate count, yeast and mould)	<10cfu/9[(4)]

1 – Obtained from Carotino® SDN. BHD (A member of J.C. Chang Group, Malaysia)
2 – Percentage (%)
3 – Parts per million (ppm)
4 – Colony forming units (cfu)

3.2.1.3 Flavour ingredients

A beef stew spice mix was obtained from Crown National®, Montague Gardens, Cape Town and utilised as flavour ingredient. The spice blend was made up of the following ingredients: hydrolysed vegetable protein, wheat, spices, cereal (wheat), salt, starch, dehydrated vegetables, sucrose and colorant (E150).

3.2.1.4 Corn steep liquor inclusion

CSL was used as the main source of phytochemicals in the new stock cube. The literature on oxidative stress, inflammation and phenolic compounds was used as guideline to determine the amount of CSL to be incorporated in the final stock cube to ensure an effective biological level to produce potential health effects. In a study conducted by the Federal University of Santa Catarina in Brazil, 14 healthy men undergoing resistance training were given 200 millilitre (ml) of green tea, three times a day. Their blood samples were analysed for markers of oxidative stress, such as GSH and lipid hydroperoxide, as well as total polyphenols (Panza *et al*., 2008:433). A mean intake of 4,6 µg / d green tea phenolic compounds increased the total plasma polyphenols by 27% (Panza *et al*., 2008:438). Another study conducted in Germany used two differently prepared fruit juices, labelled as juice A and juice B. Analysis by high performance liquid chromatography (HPLC) indicated that the total of 23 identified polyphenols were 714 mg / litre for juice A and 684 mg/litre for juice B (Bub *et al*., 2003:93). The participants of the study consumed 330 ml juice per day for a period of two weeks, which provided 236 mg total polyphenols per day for juice A and 226 mg total polyphenols per day for juice B (Bub *et al*., 2003:90). At these amounts the study participants showed improvement in antioxidant status and immune response, as well as reduced levels of oxidative DNA damage (Bub *et al*., 2003:95). In a study utilising grape polyphenol powder, participants received 31,3 mg / d total polyphenols for a period of one week (Young *et al*., 2000:507), but no significant changes in the study markers of oxidative stress were observed (Young *et al*., 2000:512). The findings of these studies are summarised in Table 3.7.

Table 3.7: Summary of some polyphenol feeding trials and the effects on oxidative stress and immune function

Food source of feeding trial	Total polyphenols	Participants	Time frame	Significant effect on oxidative stress and immune function
Green tea[1]	4.6 µg/day[2]	14 healthy males undergoing resistance training	7 days	Raised plasma polyphenols (by 27 %) and reduced post-exercise markers of oxidative stress
Fruit juices[3]	236 mg/day[4] (juice A) and 226 mg/day (juice B)	27 healthy males	2 weeks	Improved antioxidant status and immune function and reduced levels of oxidative DNA damage
Grape polyphenol powder[5]	31.3 mg/day	6 males and 9 females	1 week	No significant changes in markers of oxidative stress

1 – Panza *et al*. (2008)
2 – Micrograms per day (µg/day)
3 – Bub *et al*. (2003)
4 – Milligrams per day (mg/day)
5 – Young *et al*. (2000)

The total polyphenol content of the CSL is 45.7 mg / g (Rautenbach, 2008). If the average amount of the German fruit juice study (Bub *et al*., 2003:93) is taken (231 mg / day), then 5.1 g of the CSL would furnish the same amount of total polyphenols. As the stock cube would probably be utilised in dishes that would serve a minimum of four, this would indicate that the stock cube should contain approximately 20 g of CSL to be of potential benefit to all the consumers in terms of its polyphenol content provision. As commercially available stock cubes weigh only 10 g, it would implicate developing a very bulky stock cube to contain the proposed amount of CSL. However, the green tea study only furnished 4.6 µg polyphenols per day (Panza *et al*., 2008:438), which could indicate that much less CSL might be needed to have a biological effect. The grape polyphenol study at 31.3 mg/day did not show any significant biological effect (Young *et al*., 2000:512). The final level of 0.5 g of CSL per stock cube was determined in the formulation trials and was dictated by the acceptability in taste (discussed later). At this level, the stock cube would furnish 22.9 mg polyphenols per cube. If it is assumed that one cube would be used in a four portion dish, then each portion would contain 5.7 mg polyphenols. This is less than the total polyphenols of the grape polyphenol powder study that observed no significant biological effect (see Table 3.7), but 1 239 times the levels of polyphenols found in the green tea study at 0.0046 mg / day that did observe a biological effect (see Table 3.7). It should, however, be noted that the polyphenolic degradation components of green tea polyphenols in the body would predominantly be formed from catechins, which are the main polyphenolic compounds in green tea (De Mejia *et al*., 2009:722), while the polyphenolic degradation components from CSL in the body would possibly be predominantly formed from ferulic acid, which could possibly to be

the main polyphenolic compound in CSL (see 2.3.3.1). These different polyphenolic compounds that may be formed may also impact the biological effects in the body quite differently. The comparison amount of CSL inclusion is therefore only a robust estimation as to a possible biological benefit.

3.2.1.5 Micronutrient fortification in support of oxidative stress defence and immunonutrition

After investigating the literature on the micronutrient intake of South African adults and children, as well as the micronutrients that could have a beneficial effect in support of oxidative stress defence and immunonutrition, the following nutrients were identified for fortification inclusion: vitamin A, vitamin C, thiamin, riboflavin, niacin, vitamin B6, vitamin B12, folate, iron, zinc, copper, selenium and magnesium (see 2.5, pp. 22-23). The micronutrient levels and the percentage provisions of the RDA/AIs for females 31 to 50 years and for children four to eight years were calculated on a 0.5 g inclusion of CSL per stock cube furnishing four portions per stock cube. These figures are presented in the last three columns of Table 3.8. Biotin is the nutrient with the highest percentage provision to the RDA/AI, at about 1.8% for females 31 to 50 years and about 4.5% for children four to eight years, followed by vitamin B12 at about 1.25% for females 31 to 50 years and 2.5% for children four to eight years through the CSL inclusion in a stock cube. The other micronutrients as provided per one portion of the stock cube made a negligible contribution to the RDA/AIs (see Table 3.8).

Table 3.8: Micronutrient provision by dried corn steep liquor inclusion in the stock cube with reference to micronutrient intakes determined by the South African food consumption studies and the micronutrient reference intakes

Micronutrients	RDA/AI for micronutrients (females 31 – 50yrs)[1]	RDA/AI for micronutrients (children 4 – 8yrs)[1]	Upper limits of micronutrients (females 31 - 50yrs)[2]	Upper limits of micronutrients (children 4 – 8yrs)[2]	South African micronutrient intake levels			Nutrient content of dried corn steep liquor developed stock cube (one person portion)[6]	% RDA/AI provided for adults (31-50 years) by one person portion per cube[7]	% RDA/AI provided for children (4-8 years) by one person portion per cube[8]
					THUSA[3]	BRISK[4]	VIGHOR[5]			
Calcium (mg)[9]	1000	800	2500	2500	384 – 569	335 – 570	547 – 997	0.09	0.01	0.01
Chromium (µg)[10]	25	15	ND[11]	ND	-[(12)]	-	-	-	-	-
Copper (µg)	900	440	10 000	3000	-	900 – 1400	1040 – 1690	2.38	0.26	0.54
Fluoride (mg)	3	1	10	2.2	-	-	-	-	-	-
Iodine (µg)	150	90	1100	300	-	-	-	-	-	-
Iron (mg)	18	10	45	40	7.5 – 10.8	6 – 10	8,2 – 17,1	0.02	0.11	0.20
Magnesium (mg)	320	130	350	110	-	199 – 295	217 – 357	1.21	0.38	0.93
Manganese (mg)	1.8	1.5	11	3	-	-	-	0.01	0.56	0.67
Phosphorus (mg)	700	500	4000	3000	-	693 – 1111	867 – 1551	-	-	-
Potassium (mg)	4700	3800	No UL[13]	ND	-	1646 – 2212	2213 – 3559	6.30	0.13	0.17
Selenium (µg)	55	30	400	150	-	-	-	-	-	-
Sodium (mg)	1500	1200	2300	1900	-	979 – 2025	1446 – 2992	0.01	< 0.01	< 0.01
Zinc (mg)	8	5	40	12	7.1 – 11.2	6.8 – 11.8	8.1 – 14.9	0.01	0.13	0.20
Vitamin A (RAE)[14]	700	400	3000	900	533 – 1246	373 – 577	725 – 1547	-	-	-
Thiamin (mg)	1.1	0.6	ND	ND	1.04 – 1.29	0.83 – 1.1	0.86 – 1.56	0.02µg	< 0.01	< 0.01
Riboflavin (mg)	1.1	0.6	ND	ND	1.2 – 1.8	0.81 – 1.18	1.2 – 2.39	0.05µg	< 0.01	0.01
Niacin (mg)	14	8	35	15	11.0 – 18.6	9.4 – 16.1	13.1 – 24.4	0.01	0.07	0.13
Vitamin B6 (mg)	1.3	0.6	100	40	-	0.78 – 1.15	0.71 – 2.01	0.04µg	< 0.01	0.01
Folate (µg)	400	200	1000	400	181 – 244	147 – 218	157 – 280	ND	ND	ND
Vitamin B12 (µg)	2.4	1.2	ND	ND	0.80 – 1.56	3.2 – 6.9	4.0 – 8.1	0.03	1.25	2.50
Pantothenic acid (mg)	5	3	ND	ND	-	-	-	0.81µg	0.02	0.03
Biotin (µg)	30	12	ND	ND	-	-	-	0.54	1.80	4.50
Vitamin C (mg)	75	25	2000	650	21.9 – 82.9	27 – 61	46.0 – 118.3	-	-	-
Vitamin D (µg)	5	5	50	50	-	-	3.0 – 5.0	-	-	-
Vitamin E (mg)	15	7	1000	300	-	-	12.9 – 23.1	-	-	-
Vitamin K (µg)	90	55	ND	ND	-	-	-	-	-	-

1 – Recommended Dietary Allowance (RDA) / Adequate Intake (AI) for Individuals, National Academy of Science (2005) (Rolfes *et al*., 2009:B)
2 – Tolerable Upper Intake Levels (UL), National Academy of Science (2005) (Rolfes *et al*., 2009:C)
3 – MacIntyre *et al*. (2002:245-246)
4 – Bourne *et al*. (1993:241-243)
5 – Vorster *et al*. (1995:119)
6 – Values calculated on a 0.5g inclusion of CSL per stock cube (four portions per cube)
7 – Percentage contributions of RDA/AIs for females 31 – 50yrs calculated on values presented in the previous column
8 – Percentage contributions of RDA/AIs for children 4 – 8yrs calculated on values presented in the previous column
9 – Milligram (mg)
10 – Microgram (µg)
11 – Not detected (ND)
12 – Figure not provided by study (-)
13 – Upper limit
14 – Retinol activity equivalents (RAE)

Table 3.8 presents the nutrient intake ranges found in three different South African dietary consumption studies. From these values it can be seen that certain nutrients fall below the RDA/AI values, e.g. calcium, and that some have lower ends falling below the RDA/AI, but upper ends that do meet the RDA/AI, e.g. zinc. It is clear that calcium, iron, potassium and folate dietary needs are not met, as these nutrients all have upper end values that fall below the adult RDA/AI. The other nutrients that have lower end values that fall below the RDA/AI, are magnesium, phosphorus, zinc, vitamin A, thiamin, riboflavin, niacin, vitamin B6, vitamin B12, vitamin C and vitamin D. It is not the intention of this study to develop a stock cube that would furnish all the nutrients lacking in the South African diet, but specifically to add micronutrients that have antioxidant and anti-inflammatory activity and for this reason zinc, copper and selenium were selected (see Chapter 2) as support to the South African dietary intake contribution. Iron was included as has been found that iron deficiency is still a problem for the South African population (Steyn *et al*., 2006a:23).

Table 3.9 provides a summary of the commonly used forms of iron in the fortification of food products and in particular those micronutrients included in the nutrient fortification of the stock cube. In the final formulation, 32% ferrous fumarate powder (manufactured by Dr. Paul Lohmann) was utilised as a source of iron. Ferrous sulphate is commonly used in fortification mainly because it is inexpensive and being highly bioavailable (Allen *et al*., 2006:97). Ferrous fumarate is next in line as it is also inexpensive and as bioavailable as ferrous sulphate but less water soluble. The lower water solubility is in fact advantageous as it produces fewer problems with the organoleptic characteristics of a product to which it is added (Allen *et al*., 2006:97). The zinc (10%), selenium (0.2%) and copper (10%) used were all amino acid chelates (manufactured by AMT laboratories) utilising a soy protein as base and were utilised since they were made available by the FFRU. The new stock cube was fortified with the four elements to furnish 50% of the RDA/AI for females 31 to 50 years of age (see Table 3.8) per serving (four servings per stock cube). This level of fortification would contribute a reasonable amount of each element, while still keeping the amounts below the Tolerable Upper Intake Levels (ULs) for children age four to eight years (Rolfes *et al*., 2009:B).

Table 3.9: Commonly used forms of iron used in micronutrient fortification and their properties (adapted from Pearce & Dolfini, 1991: 163-164)

Micronutrient	Water solubility	Taste	Bioavailability
Ferrous sulphate	High	Metallic	Excellent
Ferrous fumerate	Moderate	Slight	Excellent

3.3 Bench formulation development

A bench formulation was developed with informal tastings by lecturing and research staff of the programme Consumer Science: Food and Nutrition. Once a final formulation was determined, it was evaluated by a panel of food professionals to compare the new stock cube with a traditional stock cube. Stock cubes were produced for the consumer acceptance testing and shelf-life testing by forming cubes in ice cube trays (see Addendum C). The cubes were then wrapped in the foiled paper typically used for stock cubes. The paper was provided by Nampak Ltd.

3.3.1 Formulation trials

The first trials were conducted utilising the basic ingredients of fat (provided by Carotino® SDN. BHD, a member of J.C. Chang Group, Malaysia), flavourant (provided by Crown National®, South Africa), fibre utilised as bulking agent (provided by the CSL provider company, another by-product of the corn starch industry), salt and CSL. Table 3.10 indicates the weight and percentage contributions of the stock cube formulation ingredients for the first trials. The fat utilised in trials one to three was a red palm fat with a slip melting point of 44 to 48 ºC (see Table 3.5). However, it was found that the fat crystallised and gave an unacceptable appearance to the stock cube. In trial two the amount of fat was decreased (see Table 3.10), but though there was less crystallisation the fat could still be observed on the surface of the stock cube. In trial four the problem was solved by using another red palm fat with a lower slip melting point of 34 to 38 ºC (see Table 3.5) by blending the two fats in a 1:1 ratio. A palm fat concentrate was also added to the fat blend to improve the carotenoid and vitamin E content (see Table 3.6). The flavour in trial one was found to be too bland and that the subsequent addition of more salt (see Table 3.10) improved the flavour. In trial three the CSL content was doubled (see Table 3.10) to determine whether the taste of the CSL would be detectable, but at this level the taste was found to be unacceptable. The CSL contributed acidity to the total flavour and a level of 4.5% or 3 g per cube (see Table 3.10) was found to be the most acceptable at a final informal evaluation by seven tasters.

Table 3.10: Ingredient weight and percentage inclusions for trial stock cube formulations one to four[1]

Ingredient	Trial 1		Trial 2		Trial 3		Trial 4	
	Weight (g)[2]	%[3]	Weight (g)	%	Weight (g)	%	Weight (g)	%
Fat	30[(4)]	43.5	24[(4)]	36.4	24[(4)]	35.3	24[(5)]	35.8
Flavourant	20	29.0	20	30.3	20	29.4	20	30.0
Fibre[6]	10	14.5	10	15.2	10	14.7	10	14.9
Salt	7	10.1	10	15.2	10	14.7	10	14.9
CSL	2	3.0	2	3.0	4	5.9	3	4.5
Total	69	100.1	66	100.1	68	100.0	67	100.1

1 – Values indicate weights and percentages of ingredients per six cubes
2 – Gram (g)
3 – Percentage (%)
4 – Fat with melting point of 44º - 48ºC
5 – Fat blend with lower melting point
6 – Added as bulking agent

The final formulation ingredient weights and percentages are presented in Table 3.11. Salt has been reduced to be only the third ingredient at 1.67 g per stock cube providing 668 mg sodium per stock cube. The flavourant used would also contribute salt (sodium) to the stock cube however, the salt content is not provided on the packaging. Although fat replaced salt as the main ingredient, it was not considered a nutritional concern as the amount of fat per cube was a mere 4 g (see Table 3.11). Each stock cube served 4 portions, contributing only 1 g of fat per portion. In addition the source of fat was a palm fat which contributes carotenoids and tocotrienols to the stock cube, which enhances its nutritional provision. According to Wilson and co-workers (2005:633) red palm oil may have plasma cholesterol lowering properties. The seven participants at the informal evaluations considered this formulation acceptable to proceed to the next step of the sensory evaluation to compare the final formulation to a traditional stock cube.

Table 3.11: Final ingredient formulation of developed stock cube

Ingredient	Weight per 12 cubes (gram)	Percentage contribution	Weight per 1 cube (gram)	Percentage contribution
Fat	48	36.4	4.00	36.4
Flavourant	38	28.8	3.17	28.8
Fibre[1]	16	12.1	1.33	12.1
Salt	20	15.1	1.67	15.1
CSL	6	4.6	0.50	4.6
Micronutrients[2]	4	3.0	0.33	3.0
Total	132	100.0	11.00	100.0

1 – Added as bulking agent
2 – Comprising iron, zinc, selenium and copper

3.3.2 Evaluation of final formulation

A sensory analysis of the stock cube was conducted on Friday, 18 September 2009, at the sensory evaluation room of the programme Consumer Science: Food and Nutrition at CPUT. This analysis was undertaken to ascertain acceptance of the final formulation of the new stock cube compared to a standard commercial beef flavoured stock cube, as seen from a consumer's perspective. The panel comprised six female staff members, all of which have specialist food knowledge and are involved in food preparation practical evaluations of first to fourth year level tertiary education students within this academic programme. Some of the panellists are also intensively involved in food product development. These panellists were to act as consumer testers. However their knowledge presents them a greater discrimination as would be expected from an average consumer. Both the new stock cube and a standard commercial beef flavoured stock cube were each dissolved in 400 ml of boiling tap water. The panel members were asked to rate each sample of the two stock liquids on a nine point hedonic scale, with one presenting an unacceptable rating and nine presenting an acceptable rating. The stocks were rated on acceptability of colour, aroma and flavour. They also rated the prepared stocks on the perceived saltiness and aftertaste (see Table 3.12). The rated averages of the new product scores were lower than those of the standard cube, but the t-test for independent samples indicated that the difference was only significant ($p < 0.05$) for the scores for aroma and saltiness (see Table 3.12). The difference in aroma was, however, not considered a concern, as the stock would not be utilised on its own, but incorporated into dishes where it would blend with the aroma of the food

itself. The difference in perceived saltiness was expected, as the new stock cube had a reduced salt content.

Table 3.12: Nine-point hedonic ratings of standard stock cube and developed stock cube by panel of staff members

Panellist	Sensory attributes of prepared stock									
	Colour		Aroma		Flavour		Saltiness		Aftertaste	
	Standard	New	Standard	New	Standard	New	Standard	New	Standard	New
1	7	8	7	4	5	6	7	2	6	3
2	4	3	6	4	7	5	4	3	6	6
3	5	2	3	2	7	6	4	4	1	2
4	6	5	4	4	6	5	6	2	4	4
5	7	6	7	6	6	5	4	1	7	8
6	8	4	6	5	6	5	6	2	7	3
Mean	6.17	4.67	5.50	4.17	6.17	5.33	5.17	2.33	5.17	4.33
SD[1]	1.47	2.16	1.64	1.33	0.75	0.52	1.33	1.03	2.32	2.25
P value		0.09		0.03		0.09		0.02		0.38

1 – Standard deviation (SD)

After the session, an informal discussion was held and the following concerns were raised by the panellists:

- Can the new stock cube be manufactured and sold at a cheaper price than the current stock cubes on the market? This might be an important consideration to gain entry into a well-established market.

- The current best sellers on the market are the beef and chicken flavours (Fourie, 2007; Van de Vyver, 2007). Any new products will have to compete with these and it might be difficult to overcome consumer loyalty.

- The level of salt in the new stock cube will probably be a purchase obstacle to the lower income market, as health benefits might not be a serious consideration in their purchases of stock cubes.

The panel made the following suggestions:

- If the new stock cube is to be developed for the lower income market, then it would be necessary to increase the salt content. It will have to be highly flavoured to gain acceptance over the current best sellers. Ways will also have to be found to manufacture the new cube more economically than those currently on the market to be provided at a competitive price.

- Instead of competing with the current best sellers of beef and chicken, the new stock cube could be developed as a vegetable flavoured product. This could then be targeted at a higher income consumer, with a higher level of education, who would respond more favourably to the health benefits.

- Increasing the salt content to a level acceptable to a lower income market could pose a problem, as an aim of this new product development process was the development of a product that could be accepted toward combating the “diseases of lifestyle”, e.g. CVD. As hypertension is one of the conditions that contributes to the development of these diseases (Steyn, 2006a:1), the reduction of the salt content that is a common feature of stock cubes was one of the aims in the development process. Steps have already been taken by the Department of Health in conjunction with industry to reduce the salt content of food products traditionally high in salt content (Charlton *et al*., 2007:1). It was suggested that marketing material could be utilised to inform consumers of the lower salt content and the benefits thereof, as hypertension is a major concern among the South African population (Steyn *et al*., 2006b:93).

The company for whom the stock cube was developed was provided with these suggestions. They made a decision at management level that the stock cube was acceptable as it was and should be pilot-tested on a group of blue collar workers to establish the acceptability of the new stock cube and the merit of the suggestions before further formulation changes were made.

3.4 Consumer sensory evaluation

Evaluation can be done of the intrinsic qualities of a product, e.g. taste, appearance, nutritional value, self-life and safety (Linnemann *et al*., 2006:185). In the development of the proposed

stock cube, it was necessary to conduct sensory evaluation to determine the acceptability of the product to the consumer as a standalone product and when utilised as an ingredient.

3.4.1 Informal group discussion evaluation

Group interviews typically consists of six to ten participants discussing a topic freely, but at the same time directed by the leader to focus on specific areas (Kotler & Armstrong, 2006:113). Group interviews are useful in that the leader can prompt the participants to obtain more detailed information and possibly the underlying motivation for initial answers (Allen, 1993:220). Two groups, one comprising blue collar workers and in addition the other white collar workers, were used as a preliminary evaluation of the acceptability of the new stock cube based on the CSL provider company suggestion before further development work and large scale consumer testing was done. Questions were also asked in the group discussion about the type of dishes that they commonly prepare using stock cubes.

The blue collar group was obtained from the contract company cleaning staff of the institution that could be relieved from work duties and was held on 9 October 2009. The group consisted of nine African women and one coloured woman. The group discussion was held in one of the lecture venues of the university and kept informal, with the women seated around the same table. The same evaluation form, short questionnaire and consent form was used as would be used in the consumer acceptance testing to determine if there was any changes to be made to improve the documents. The group was asked to taste three samples of vegetable soup prepared with three different stock cubes. Sample A was the new stock cube, sample B was prepared with the Brand A beef stock cube (25% less salt) and sample C was prepared with the Brand B beef stock cube. Sample C received seven votes, sample B and A each one for the preferred choice. However, three sample C voters indicated that sample A was their second choice. When asked why they preferred sample C, the women indicated that the stronger salty taste was the determining factor. Three women indicated that they would choose a stock cube with lowered salt content and one woman said she would specifically choose a low salt cube because she had high blood pressure. The split between those using Brand A versus Brand B in the household was about equal. All replied that they use the beef flavour, with one woman indicating that she also uses vegetable and chicken flavours. Most replied that they would like to try new products and would probably buy a new stock cube if it wasn't too expensive. Some indicated that if the new stock cube had added health benefits that they would even be prepared to pay a little more. Some of the woman indicated that they do eat for health; health problems

such as high blood pressure and constipation were mentioned. All indicated that the colour and aroma of sample A as the new stock cube was acceptable to them. A few also tasted the stock liquid made from sample A as the new stock cube on its own and described it as tasting of vegetables or chicken. A woman who described the aroma as vegetable said she did not find it unacceptable. Two women said that they liked the fact that the stock did not appear greasy.

The white collar group consisted of five white professional women (two music teachers, a clinical research associate, a bank official and a fashion designer) and was conducted on 13 October 2009. The session was held at one of the women's home and kept informal as with the blue collar workers. The same documentation was used as with the blue collar group to determine if it would be necessary to develop different forms and questionnaires for the two groups. It was found that both groups understood the documents and that it could be used for both the blue collar and white collar consumer acceptance testing. The same procedure was followed as with the blue collar group, with Sample A the new stock cube, sample B prepared with the Brand A beef stock cube (25% less salt) and sample C with the Brand B beef stock cube. In this group, one indicated sample B as her first preferred choice and four indicated sample C as their first preferred choice. As a second preferred choice, sample A received two votes and sample B three. The new stock cube prepared vegetable soup was said to taste bland after the Brand B prepared soup sample was tasted because of the higher salt content, but the new stock cube prepared soup was not perceived as unacceptable. Two women also said that even though they chose the higher salt content soup, they thought that it could become unpleasant if a whole portion of soup was consumed. Four women said that they would add more salt to the soup prepared with the new stock cube. It was also said that the soup prepared with the new stock cube tasted the most like vegetable soup. Two women indicated the chicken flavour as the one they mostly use and two said that they prefer oxtail, with the remaining woman indicating that vegetable was her favourite flavour. The group also tasted the stock made from sample A as the new stock cube on its own and found it pleasant. The appearance and colour was acceptable and the cloudiness caused by the fibre included as a bulking agent was not seen as unacceptable. One woman said that the granular stock that she normally used had the same cloudy appearance. The aroma was acceptable to all and was described similar to two minute noodles and Marmite. Some perceived a spicy aroma described as "minty and like cinnamon". One woman said that the aroma has a medicinal note, but not one that was unpleasant. It was also noted that the stock is not as greasy as traditional stock cubes.

The feedback from the two groups was positive overall with no information provided about possible improvements. It was therefore decided to continue with the stock cube as was, with the support of the CSL provider company, and continue with the acceptance and the shelf-life stability testing.

3.4.2 Consumer acceptance testing

Simple discrimination tests, e.g. triangle, duo-trio or paired comparison tests, are used to determine if any difference can be detected between a new product and a comparable reference product. These tests are also used during shelf-life testing to determine whether there are noticeable changes in sensory characteristics over time (Koeferli *et al*., 1998:409). The nine-point hedonic scale and the paired comparison test are the most commonly used in consumer acceptance testing (Stone & Sidel, 2004:251). During the informal group discussions it became evident that the saltier commercial stock cubes were preferred and could influence the acceptability of the new stock cube when using the paired comparison test. A nine-point hedonic scale (see Addendum D) was therefore chosen for the consumer acceptance testing as it was felt that comparison with traditional stock cubes would not be possible because of the different level of salt content of the new stock cube (see 3.6.1), and thus not compatible with the paired comparison test. The nine-point hedonic scale also has a number of advantages with consumer acceptance testing in that it is easy to use and produces stable results (Stone & Sidel, 2004:255).

Ethical clearance for the consumer acceptance testing was obtained from the Faculty of Applied Sciences Research Ethics Committee prior to the execution. All participants did so voluntary and the responses were anonymous. The participants were requested to sign a consent form explaining to them the purpose of the study as well as the procedure that would be followed so that they would be fully informed about the study (see Addendum E). The selected dishes incorporating the stock cube developed in this study was based on stock cube use suggestions by both blue and white collar workers as indicated by them during the informal group discussions. The type of dishes that the blue collar workers used stock cubes in, were tomato and onion “smoor”, cabbage cooked in stock, samp and mielies, rice and potatoes, stews and soups such as vegetable, split pea and bean soup. This group indicated that they typically used stock cubes in soups and that they also flavoured samp and rice with stock cubes. The white collar workers said that they tend to use stock cubes more during the winter (at least once a week) when they used it in the preparation of stews and soups (e.g. butternut, vegetable, split

pea and dried bean soup). One woman mentioned that she used stock cubes when preparing risotto. The responses from both groups lead to the decision to prepare a split pea soup and savoury rice for the consumer acceptance testing.

In consumer testing done at a central location, such as an office building, it is usual to make use of a 100 plus consumers from the general public (Stone & Sidel, 2004:265). It was decided to recruit a consumer group of 50 blue collar volunteers and 50 white collar volunteers. The contract cleaning staff at the Cape Town campus of CPUT made up the blue collar consumer group, excluding those that participated in the informal group discussion. The white collar group was drawn from the administration and lecturing staff of the Cape Town campus of CPUT. An advertisement was placed on the CPUT intranet to invite participation in the study. The response was not adequate to meet the number for the white collar group and an additional group had to be recruited from the Bellville Campus of Northlink College. The venues were each set up with spaciously placed tables and chairs, a cup of water, a napkin and a spoon set out for each participant. The participants received a pen and a sheet printed with short instructions and a nine-point hedonic scale for each of the two samples, as well as a consent form and a short questionnaire upon arriving at the venue. After the consent form (see Addendum E) was explained and signed by the participants, they each received 50 ml of each sample in a plastic cup. Each participant evaluated the savoury rice and the vegetable soup prepared utilising the new developed stock cube (see Addendums F and G for the respective recipe formulations). The samples were rated on the nine-point hedonic scale (see Addendum D). In addition each volunteer also completed the short questionnaire pertaining to their bio/demographic and stock cube usage information (see Addendum H) that comprised closed-ended questions in the multiple-choice format for ease of completion and data administration.

3.5 Shelf-life testing

The Agrifood Technology Station of CPUT was contracted to conduct the shelf-life testing. The stock cube underwent a three month accelerated shelf-life testing period to reflect a six month “real time” period. During this time the samples were kept at 30°C and 65% relative humidity. Reference standard samples were kept at -80°C to be used in the sensory analysis to determine if any changes in sensory characteristics could be detected at the end of month one, two and three. The parameters tested every four weeks were moisture content, microbial activity and lipid peroxidation. All the microbial tests were conducted using standard methodology based on

literature and industry standards (see Addendum I for the microbial standard operating procedures; *B. cereus* was analysed using the Biomerieux 150 CHB kit).

The TAC and polyphenol analysis was outsourced to the Analytical Laboratory Services of the Oxidative Stress Research Centre, CPUT. The TAC was determined by analysing both the hydrophilic oxygen radical absorbance capacity (H-ORAC) and lipophilic oxygen radical absorbance capacity (L-ORAC) utilising the ORAC assay on the Fluoroskan Ascent by Thermo Electron Corporation using the protocol described by Ou and co-workers (2001:4620). The total polyphenols were measured on the Multiskan Spektrum by Thermo Electron Corporation based on the methodology developed in the literature (Arendt *et al*., 2005; Lotito & Frei, 2004; Ruel *et al*., 2005; van het Hof *et al*., 1997; McAnlis *et al*., 1998; Wolfram *et al*., 2002; Park *et al*., 2003; Zang & Zuo, 2004) (see Addendum J for the ORAC analysis standard operating procedure and Addendum K for the polyphenol analysis standard operating procedure).

The sensory analysis forming part of the shelf-life testing was conducted with the aid of the panel of six staff members of the program Consumer Science: Food and Nutrition that assisted with the sensory analysis during the development phase (see 3.7.3). The programme has its own purpose built sensory evaluation facility, with a bank of eight tasting booths. Each booth has an area of 59 x 77 cm and is illuminated with fluorescent lighting. Optional coloured lights are provided by three 60 Watt spot lights in green, yellow and red. The booths are situated directly opposite a preparation kitchen and food samples are passed through a sliding hatch at the front of each booth. Each booth is also equipped with a red and a green light at the front to indicate when a panellist is busy tasting or ready for the following sample. A duo-trio test (Stone & Sidel, 2004:152) was used to determine if the panellist could detect a difference in flavour (see Addendum L for form used). In this test the panellist received a reference sample and two numbered samples. They were then asked to indicate which of the two numbered sample was the same as the reference sample, or indicate if they could taste no difference. However, by the second sampling (end of month two) a distinct difference in colour was seen between the two samples, and this necessitated the use of red light illumination to disguise this characteristic. The stock cubes were evaluated on flavour at the end of the first three months. A final evaluation was conducted at the end of six months.

3.6 Data analysis

All data from the consumer acceptance testing was analysed statistically using the PSAW Statistics 18 software (Release 18.0.0). The Fisher's exact test was applied to the response data, with $p < 0.05$ taken as significant, to determine associations or differences in the consumer acceptance scores and their bio/demographic and stock cube usage information provided through the short questionnaire completed (see Addendum H) and for the detection of organoleptic changes, as indicated by the duo-trio sensory testing, over the six month accelerated condition shelf-life stability testing of the new developed stock cube. The microbial growth, oxidative rancidity and antioxidant status results of the accelerated shelf-life stability testing was analysed by subjecting each to statistical process control expressed in a control chart. These charts indicate an upper and lower control limit which would indicate that the process is in control and no parameters need to be adjusted. The control chart is regarded as a basic tool of quality control.

CHAPTER 4
RESULTS

The sensory analysis of the new stock cube comprised of an analytical analysis by a sensory panel, two informal group discussions (one comprising blue collar workers and the other white collar workers) as part of the product development process, and consumer acceptance testing. The results of the sensory panel and the two informal group discussions, being part of the product development process, were therefore included in the research design and methodology chapter of this study. The results of the consumer acceptance testing of the new stock cube are provided in this chapter. It was also necessary to determine the shelf-life stability of the new stock cube. An accelerated shelf-life assessment was used to determine the product stability of the new stock cube of which the results are also discussed in this chapter.

4.1 Consumer acceptance testing

The acceptability of the new stock cube was tested on two groups of consumers. The private company contracted cleaning staff at the CPUT were used as the blue collar test subjects. Administration and lecturing staff at the CPUT, Cape Town campus and the Protea campus of Northlink College formed the white collar group. Consumer acceptance testing of the blue collar group was conducted on 29 October 2009 and the CPUT white collar group on 30 October 2009. The remaining white collar group obtained from Northlink College was conducted on 6 November 2009. Both groups completed a demographic, household and stock cube usage questionnaire (see Addendum H). The two food samples prepared with the new stock cube were then rated by the participants on a nine point hedonic scale (see Addendum D).

4.1.1 Participant sample and their demographic characteristics

The participant sample comprised 99 consumers of whom 50 were blue collar workers and 49 were white collar workers, forming the two sub-sample groups. Nearly five times as many females (82.8%) as males (17.2%) participated. The majority (38.4%) of the participants were aged 25 to 54 years (81.8%) with only a few participants 24 years and younger (8.1%) or 55 years and older (10.1%). Three different ethnic groups participated, with the coloured (39.4%) and the black (38.4%) consumers making up the majority of the participant sample. This was mirrored in the participant home languages with Afrikaans as home language at 38.4% and Xhosa as home language at 36.3%. Just under half (47.5%) of the participants were married,

while the rest (52.5%) were single, divorced or widowed. About a third (31.3%) of the consumer sample indicated that their highest education was in the grade 11 to grade 12 grouping (31.3%), while half (49.5%) of the sample had a tertiary education. The monthly income groups that were represented by most of the participants was the lowest monthly income group, earning R1 700 or less (27.3%), followed by the second lowest monthly income group, earning between R1 701 and R3 000 (21.2%) and the second highest monthly income group, earning between R10 201 and R16 250 (20.2%) (see Table 4.1).

Table 4.1: Participant sample and sub-sample demographic characteristics

Demographic characteristics		Total sample (n=99)		Blue collar workers (n=50)		White collar workers (n=49)	
		Number	%	Number	%	Number	%
Gender	Male	17	17.2	6	12.0	11	22.4
	Female	82	82.8	44	88.0	38	77.6
Age (years)	≤24	8	8.1	4	8.0	4	8.2
	25 – 34	38	38.4	22	44.0	16	32.7
	35 – 44	20	20.2	10	20.0	10	20.4
	45 -54	23	23.2	11	22.0	12	24.5
	≥55	10	10.1	3	6.0	7	14.3
Ethnicity	Black	38	38.4	33	66.0	5	10.2
	Coloured	39	39.4	17	34.0	22	44.9
	White	22	22.2	0	0	22	44.9
Home language	English	25	25.3	3	6.0	22	44.9
	Afrikaans	38	38.4	15	30.0	23	46.9
	Xhosa or other African language	36	36.3	32	64.0	4	8.2
Marital status	Married	47	47.5	21	42.0	26	53.1
	Single, widowed, divorced or separated	52	52.5	29	58.0	23	46.9
Highest level of education	Standard 8/Grade 10 or lower	19	19.2	19	38.0	0	0
	Standard 9/Grade11 to Standard 10/Grade12	31	31.3	29	58.0	2	4.1
	Certificate	5	5.1	1	2.0	4	8.2
	Diploma	12	12.1	1	2.0	11	22.4
	Degree	19	19.2	0	0	19	38.8
	Postgraduate	13	13.1	0	0	13	26.5
Monthly Income[1]	Up to R1 700	27	27.3	26	52.0	1	2.0
	R1 701 – R3 000	21	21.2	20	40.0	1	2.0
	R3 001 – R7 500	8	8.1	2	4.0	6	12.2
	R7 501 – R10 200	11	11.1	2	4.0	9	18.4
	R10 201 – R16 250	20	20.2	0	0	20	40.8
	R16 251 or more	12	12.1	0	0	12	24.5

1 – Adapted from National Treasury and South African Revenue Service, 2008

Table 4.1 also indicates the demographic characteristics of the blue collar workers and the white collar workers. The white collar worker group, compared to the blue collar worker group, had slightly more male participants (22.4% versus 12%), as well as more persons in the 55 years and older group (14.3% versus 6%). A large difference was seen in the ethnic groupings, where blacks represented two thirds (66%) of the blue collar group, but only a tenth (10.2%) of the white collar group. This was once again mirrored in the home languages, with Xhosa as home language at 64% in the blue collar group, but only eight per cent (8.2%) in the white collar group. The marital status of the two groups indicated a lower percentage of married persons in the blue collar group (42%) compared to the white collar group (53.1%). A large difference was seen in the education of the two worker groups, and this was also mirrored in the income between the blue and white collar workers. The majority (96%) of the blue collar workers only had a secondary school education, whereas the majority (87.7%) of the white collar workers had a tertiary education. The blue collar workers were spread across the two lowest monthly income groups (92% combined) with the majority of white collar workers spread across the top three monthly income groups (83.7% combined).

4.1.2 Participant sample and sub-sample acceptance of the savoury rice and pea soup prepared with developed stock cube

The acceptance of the savoury rice and the pea soup each prepared utilising the new stock cube by the total sample, as well as that of the blue collar workers and the white collar workers, are indicated in Tables 4.2 and 4.3, respectively. The majority of the participant responses were to the positive acceptance rating of the hedonic scale, represented by the responses “like slightly”, “like moderately”, “like very much” and “like extremely”, for both the savoury rice (93.8%) and the pea soup (94.9%). The highest rating for the savoury rice by the total sample (41.4%) and the blue collar worker sub-sample (56%) was for the “like very much” response. The highest rating for the savoury rice by the white collar worker sub-sample was for the “like moderately” response (30.6%), closely followed by the “like very much” response (26.5%) (see Table 4.2). Similar ratings were found for the acceptance of the pea soup by the total sample and the blue collar worker sub-sample. The highest ratings were also found to be the “like very much” response for both the total sample (42.4%) and for the blue collar worker sub-sample (52%). The highest rating by the white collar sub-sample was also for the “like very much” response (32.7%), followed closely by the “like extremely” response (26.5%) (see Table 4.3).

Table 4.2: Acceptance of savoury rice prepared with new stock cube by the total sample and the blue collar worker and white collar worker sub-samples

Acceptance rating of hedonic scale	Total sample (n=99)		Blue collar workers (n=50)		White collar workers (n=49)	
	Number	%	Number	%	Number	%
Dislike extremely	0	0.0	0	0.0	0	0.0
Dislike very much	0	0.0	0	0.0	0	0.0
Dislike moderately	0	0.0	0	0.0	0	0.0
Dislike slightly	3	3.0	0	0.0	3	6.1
Neither like nor dislike	3	3.0	2	4.0	1	2.0
Like slightly	14	14.1	5	10.0	9	18.4
Like moderately	24	24.2	9	18.0	15	30.6
Like very much	41	41.4	28	56.0	13	26.5
Like extremely	14	14.1	6	12.0	8	16.3

Table 4.3: Acceptance of the pea soup prepared with new stock cube by the total sample and the blue collar worker and white collar worker sub-samples

Acceptance rating of hedonic scale	Total sample (n=99)		Blue collar workers (n=50)		White collar workers (n=49)	
	Number	%	Number	%	Number	%
Dislike extremely	0	0.0	0	0.0	0	0.0
Dislike very much	0	0.0	0	0.0	0	0.0
Dislike moderately	1	1.0	0	0.0	1	2.0
Dislike slightly	1	1.0	0	0.0	1	2.0
Neither like nor dislike	3	3.0	2	4.0	1	2.0
Like slightly	13	13.1	3	6.0	10	20.4
Like moderately	10	10.1	3	6.0	7	14.3
Like very much	42	42.4	26	52.0	16	32.7
Like extremely	29	29.3	16	32.0	13	26.5

4.1.3 Total participant sample acceptance of the savoury rice and the associations with their demographic and stock cube usage characteristics

No significant associations / differences ($p > 0.05$) were found between the participant acceptance of the savoury rice and their gender, age, marital status or monthly income. Neither were any significant associations / differences ($p > 0.05$) found between the participants' savoury rice acceptance and the presence or not of a family member suffering from a chronic disease or the willingness to pay more or not for a stock cube with health benefits (see Table 4.4). However, a significant difference ($p < 0.05$) was found between the participant acceptance of the savoury rice and their ethnic grouping. Far more black participants (63.2%) indicated that

they liked the savoury rice “very much” compared to the white (36.4%) or coloured (23.1%) participants. This was also mirrored in the home language of the participants with far more Xhosa speaking (63.9%) than Afrikaans (34.2%) or English (20%) speaking participants liking the savoury rice “very much” ($p < 0.05$). Another significant difference ($p < 0.05$) occurred in the level of education. Double the number of participants without a tertiary education (58%) indicated that they liked the savoury rice “very much”, compared to participants with a tertiary education (24.5%). This was also mirrored in the split between the blue collar workers (56%) and white collar workers (26.5%) ($p < 0.05$) broadly representing the professions of the participant sample. A significant difference ($p < 0.05$) was also found between the savoury rice acceptance and the willingness to use a stock cube with health benefits. Nearly half (42.5%) the participants who indicated that they would use a stock cube with health benefits liked the savoury rice “very much”, with close to a quarter (23%) of the participants indicating their liking it as “moderately”. With the participants who indicated that they would not make use of a stock cube with health benefits, the opposite was found, with the majority (36.4%) of the participants indicating their liking it as “moderately”, followed by more than a quarter (27.3%) indicating their liking it as “very much”. A significant difference ($p < 0.001$) was furthermore found between those participants who usually and those who do not usually prepare the meals and their acceptance of the savoury rice with most (48.8%) of those who usually prepare the meals indicating that they liked the sample “very much”, compared to most (47.1%) of those who do not prepare the meals indicating that they liked the sample “slightly” (also see Table 4.4 for these results).

Table 4.4: Total participant sample acceptance of savoury rice and the associations with their demographic and stock cube usage characteristics

Demographic and stock cube usage characteristics	Acceptance ratings for the savoury rice by the total participant sample[1]													P value
	Total (n=99)	Dislike slightly (n=3)		Neither like nor dislike (n=3)		Like slightly (n=14)		Like moderately (n=24)		Like very much (n=41)		Like extremely (n=14)		
	n	n	%	n	%	n	%	n	%	n	%	n	%	
Gender														0.400
Male	17	1	5.9	0	0.0	4	23.5	5	29.4	4	23.5	3	17.6	
Female	82	2	2.4	3	3.7	10	12.2	19	23.2	37	45.1	11	13.4	
Age (years)														0.202
≤24	8	0	0.0	0	0.0	2	25.0	1	12.5	4	50.0	1	12.5	
25-34	38	1	2.6	1	2.6	7	18.4	13	34.2	14	36.8	2	5.3	
35-44	20	1	5.0	1	5.0	3	15.0	3	15.0	10	50.0	2	10.0	
45-54	23	1	4.3	1	4.3	1	4.3	4	17.4	7	30.4	9	39.1	
≥55	10	0	0.0	0	0.0	1	10.0	3	30.0	6	60.0	0	0.0	

Table 4.4: Total participant sample acceptance of savoury rice and the associations with their demographic and stock cube usage characteristics (cont.)

Demographic and stock cube usage characteristics	Acceptance ratings for the savoury rice by the total participant sample[1]													P value
	Total (n=99)	Dislike slightly (n=3)		Neither like nor dislike (n=3)		Like slightly (n=14)		Like moderately (n=24)		Like very much (n=41)		Like extremely (n=14)		
	n	n	%	n	%	n	%	n	%	n	%	n	%	
Ethnicity														0.016
Black	38	0	0.0	0	0.0	3	7.9	8	21.1	24	63.2	3	7.9	
Coloured	39	1	2.6	3	7.7	9	23.1	10	25.6	9	23.1	7	17.9	
White	22	2	9.1	0	0.0	2	9.1	6	27.3	8	36.4	4	18.2	
Home language														0.033
English	25	2	8.0	1	4.0	6	24.0	7	28.0	5	20.0	4	16.0	
Afrikaans	38	1	2.6	2	5.3	6	15.8	9	23.7	13	34.2	7	18.4	
Xhosa	36	0	0.0	0	0.0	2	5.6	8	22.2	23	63.9	3	8.3	
Marital status														0.212
Married	47	1	2.1	1	2.1	4	8.5	15	31.9	17	36.2	9	19.1	
Single or widowed	52	2	3.8	2	3.8	10	19.2	9	17.3	24	46.2	5	9.6	
Level of education														0.007
School	50	0	0.0	2	4.0	4	8.0	10	20.0	29	58.0	5	10.0	
Tertiary	49	3	6.1	1	2.0	10	20.4	14	28.6	12	24.5	9	18.4	
Profession														0.028
Blue collar	50	0	0.0	2	4.0	5	10.0	9	18.0	28	56.0	6	12.0	
White collar	49	3	6.1	1	2.0	9	18.4	15	30.6	13	26.5	8	16.3	
Monthly income[2, 3]														0.126
≤R1700	27	0	0.0	1	3.7	3	11.1	6	22.2	16	59.3	1	3.7	
R1701-R3000	21	0	0.0	0	0.0	2	9.5	3	14.3	11	52.4	5	23.8	
R3001-R7500	8	0	0.0	0	0.0	3	37.5	1	12.5	2	25.0	2	25.0	
R7501-R10200	11	0	0.0	1	9.1	2	18.2	4	36.4	3	27.3	1	9.1	
R10201-R16250	20	0	0.0	1	5.0	3	15.0	7	35.0	6	30.0	3	15.0	
≥R16251	12	3	25.0	0	0.0	1	8.3	3	25.0	3	25.0	2	16.7	
A member of the household suffers from a chronic diseases														0.061
Yes	41	2	4.9	0	0.0	9	22.0	13	31.7	12	29.3	5	12.2	
No	58	1	1.7	3	5.2	5	8.6	11	19.0	29	50.0	9	15.5	
Would use a stock cube with health benefits														0.029
Yes	87	2	2.3	1	1.1	14	16.1	20	23.0	37	42.5	13	14.9	
No	11	1	9.1	2	18.2	0	0.0	4	36.4	3	27.3	1	9.1	
Would pay more for a stock cube with health benefits														0.419
Yes	76	1	1.3	2	2.6	10	13.2	18	23.7	33	43.4	12	15.8	
No	23	2	8.7	1	4.3	4	17.4	6	26.1	8	34.8	2	8.7	
Usually prepares the meals														<0.001
Yes	82	2	2.4	2	2.4	6	7.3	20	24.4	40	48.8	12	14.6	
No	17	1	5.9	1	5.9	8	47.1	4	23.5	1	5.9	2	11.8	

1 – Acceptance ratings "dislike extremely", "dislike very much", and "dislike moderately' omitted due to no participant rating indications

2 – Adapted from National Treasury and South African Revenue Service, 2008

3 – Calculated utilising the Monte Carlo method based on 10000000 sampled tables with starting seed 79654295.

4.1.4 Total participant sample acceptance of the pea soup and the associations with their demographic and stock cube usage characteristics

In the total participant sample there were no significant associations / differences ($p > 0.05$) between the acceptance of the pea soup and the participant gender, age, profession, monthly income and whether any person in the household suffers from any chronic diseases or not (see Table 4.5). Among the different ethnic groups a significant difference ($p < 0.05$) was found with the majority (68.4%) of black participants indicating that they liked the pea soup “very much”, compared to a third (33.3%) of the coloured participants and slightly more than a third (36.4%) of the white participants indicating that they liked the pea soup extremely. A significant difference ($p < 0.05$) was furthermore found between the participant acceptance of the pea soup and their home language with about two-thirds (66.7%) of the Xhosa and about half (44%) of the English speakers indicating that they liked the sample “very much”, followed by about a quarter (22.2% and 24%, respectively) of the Xhosa speakers and of the English speakers indicating that they liked the sample “extremely”. In comparison slightly more than a third (39.5%) of the Afrikaans speakers indicated that they liked the sample “extremely”, followed by less than a quarter (21.1%) who indicated that they liked the sample “slightly”. Within the participant marital status a further significant difference ($p < 0.05$) occurred with half (51.9%) of the single and widowed participants indicating that they liked the pea soup “very much”, compared to a third (31.9%) of the married participants indicating that they liked the pea soup “very much” and another third (34%) of the participants indicating that they liked the pea soup “extremely”. The level of the participants’ education also presented a significant difference ($p < 0.05$) with the majority (84%) of the participants with a school education indicating that they liked the pea soup “very much” (54%) and extremely (30%), compared to just over half (59.2%) the participants with a tertiary education indicating their liking of the pea soup as “very much” (30.6%) and “extremely” (28.6%). A significant difference ($p < 0.05$) was also found between the participant acceptance of the pea soup and their willingness or not to use a stock cube with health benefits, with the majority (42.5%) of the participants answering “yes” indicating they liked the sample “very much”, followed by 31% of the participants indicating that they liked the sample “extremely”, compared to the majority (36.4%) of the participants who answered “no” indicating that they liked the sample “very much”, followed by 18.2% indicating their liking as “extremely”, with another 18.2% indicating that they neither liked nor disliked the sample. The willingness to pay more for a stock cube with health benefits, or not, and the participants’ liking of the pea soup presented another significant difference ($p < 0.05$). A third (32.9%) of the participants willing to pay more indicated their liking of the pea soup as “extremely”, compared to less than a fifth (17.4%) of the

participants not willing to pay more indicating their liking as “extremely”. A further significant difference ($p < 0.05$) emerged between the pea soup acceptance and the participant involvement in the household food preparation. Here nearly half (45.1%) of those who do prepare the food in the household indicated they liked the sample “very much”, followed by more than a quarter (28%) who liked the sample “extremely”, compared to those who do not prepare the household meals, with slightly more than a third (35.3%) indicating that they liked the sample “extremely”, followed by nearly another third (29.4%) who liked the sample “very much” (see Table 4.5 for the results).

Table 4.5: Total participant sample acceptance of pea soup and the associations with their demographic and stock cube usage characteristics

Demographic and stock cube usage characteristics	Acceptance ratings for the pea soup by the total participant sample[1]															P value
	Total (n=99)	Dislike moderately (n=1)		Dislike slightly (n=1)		Neither like nor dislike (n=3)		Like slightly (n=13)		Like moderately (n=10)		Like very much (n=42)		Like extremely (n=29)		
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Gender																0.437
Male	17	0	0.0	0	0.0	2	11.8	2	11.8	1	5.9	6	35.3	6	35.3	
Female	82	1	1.2	1	1.2	1	1.2	11	13.4	9	11.0	36	43.9	23	28.0	
Age (years)																0.740
≤24	8	0	0.0	0	0.0	0	0.0	1	12.5	0	0.0	3	37.5	4	50.0	
25-34	38	1	2.6	1	2.6	1	2.6	7	18.4	4	10.5	19	50.0	5	13.2	
35-44	20	0	0.0	0	0.0	1	5.0	2	10.0	3	15.0	7	35.0	7	35.0	
45-54	23	0	0.0	0	0.0	1	4.3	1	4.3	3	13.0	9	39.1	9	39.1	
≥55	10	0	0.0	0	0.0	0	0.0	2	20.0	0	0.0	4	40.0	4	40.0	
Ethnicity																0.005
Black	38	0	0.0	0	0.0	0	0.0	2	5.3	2	5.3	26	68.4	8	21.1	
Coloured	39	1	2.6	1	2.6	3	7.7	5	12.8	5	12.8	11	28.2	13	33.3	
White	22	0	0.0	0	0.0	0	0.0	6	27.3	3	13.6	5	22.7	8	36.4	
Home language																0.003
English	25	1	4.0	1	4.0	1	4.0	3	12.0	2	8.0	11	44.0	6	24.0	
Afrikaans	38	0	0.0	0	0.0	2	5.3	8	21.1	6	15.8	7	18.4	15	39.5	
Xhosa	36	0	0.0	0	0.0	0	0.0	2	5.6	2	5.6	24	66.7	8	22.2	
Marital status																0.036
Married	47	1	2.1	1	2.1	0	0.0	6	12.8	8	17.0	15	31.9	16	34.0	
Single or widowed	52	0	0.0	0	0.0	3	5.8	7	13.5	2	3.8	27	51.9	13	25.0	

Table 4.5: Total participant sample acceptance of pea soup and the associations with their demographic and stock cube usage characteristics (cont.)

Demographic and stock cube usage characteristics	Acceptance ratings for the pea soup by the total participant sample[1]															P value
	Total (n=99)	Dislike moderately (n=1)		Dislike slightly (n=1)		Neither like nor dislike (n=3)		Like slightly (n=13)		Like moderately (n=10)		Like very much (n=42)		Like extremely (n=29)		
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Level of education																0.047
School	50	0	0.0	0	0.0	2	4.0	3	6.0	3	6.0	27	54.0	15	30.0	
Tertiary	49	1	2.0	1	2.0	1	2.0	10	20.4	7	14.3	15	30.6	14	28.6	
Profession																0.067
Blue collar	50	0	0.0	0	0.0	2	4.0	3	6.0	3	6.0	26	52.0	16	32.0	
White collar	49	1	2.0	1	2.0	1	2.0	10	20.4	7	14.3	16	32.7	13	26.5	
Monthly income[2, 3]																0.128
≤R1700	27	0	0.0	0	0.0	0	0.0	2	7.4	1	3.7	16	59.3	8	29.6	
R1701-R3000	21	0	0.0	0	0.0	1	4.8	1	4.8	2	9.5	9	42.9	8	38.1	
R3001-R7500	8	0	0.0	0	0.0	0	0.0	2	25.0	1	12.5	4	50.0	1	12.5	
R7501-R10200	11	1	9.1	0	0.0	1	9.1	1	9.1	1	9.1	1	9.1	6	54.5	
R10201-R16250	20	0	0.0	1	5.0	0	0.0	4	20.0	3	15.0	7	35.0	5	25.0	
≥R16251	12	0	0.0	0	0.0	1	8.3	3	25.0	2	16.7	5	41.7	1	8.3	
A member of the household suffers from a chronic diseases																0.928
Yes	41	0	0.0	0	0.0	2	4.9	6	14.6	5	12.2	16	39.0	12	29.3	
No	58	1	1.7	1	1.7	1	1.7	7	12.1	5	8.6	26	44.8	17	29.3	
Would use a stock cube with health benefits																0.042
Yes	87	1	1.1	0	0.0	1	1.1	12	13.8	9	10.3	37	42.5	27	31.0	
No	11	0	0.0	1	9.1	2	18.2	1	9.1	1	9.1	4	36.4	2	18.2	
Would pay more for a stock cube with health benefits																0.049
Yes	76	0	0.0	0	0.0	1	1.3	9	11.8	7	9.2	34	44.7	25	32.9	
No	23	1	4.3	1	4.3	2	8.7	4	17.4	3	13.0	8	34.8	4	17.4	
Usually prepares the meals																0.043
Yes	82	0	0.0	1	1.2	1	1.2	10	12.2	10	12.2	37	45.1	23	28.0	
No	17	1	5.9	0	0.0	2	11.8	3	17.6	0	0.0	5	29.4	6	35.3	

1 – Acceptance ratings “dislike extremely” and “dislike very much” omitted due to no participant rating indications
2 – Adapted from National Treasury and South African Revenue Service, 2008
3 – Calculated utilising the Monte Carlo method based on 10000000 sampled tables with starting seed 1993510611.

4.1.5 Blue collar worker sample acceptance of savoury rice and the associations with their demographic and stock cube usage characteristics

The blue collar worker results showed no significant associations / differences ($p > 0.05$) between their acceptance of the savoury rice and their gender, age, level of education, monthly

income, presence of households with persons suffering from chronic diseases or not and willingness to use or pay more or not for a stock cube with health benefits (see Table 4.6). A significant difference ($p < 0.05$) was found for their racial grouping with more than two thirds (69.7%) of the black participants indicating that they liked the savoury rice “very much”, followed by slightly more than a fifth (21.2%) who indicated that they liked the sample “moderately”, compared to the more than a quarter (29.4% respectively) of coloured participants who indicated that they either liked the sample “very much” or “extremely”. This was mirrored in the language groups, with more than two thirds (68.8%) of the Xhosa speakers indicating that they liked the savoury rice “very much”, followed by slightly more than a fifth (21.9%) who indicated that they liked the sample “moderately”, compared to the third (33.3%) of Afrikaans speakers who indicated that they liked the sample “very much” and slightly more than a quarter (26.7%) who indicated that they liked it “extremely” and the third (33.3% respectively) of English speakers who indicated their liking as either “very much” or “extremely”. A significant difference ($p < 0.05$) was also found for their marital status, with the majority (62.1%) of single or widowed participants indicating that they liked the savoury rice “very much”, followed by nearly a fifth (17.2%) indicating that they liked it “slightly”, compared to nearly half (47.6%) of the married participants indicating their liking as “very much”, followed by more than a quarter (28.6%) who liked the sample “extremely”. A significant difference ($p < 0.001$) was furthermore found between those participants who prepare the meals in the household and those who do not, with two thirds (66.7%) indicating that they liked the savoury rice “very much”, followed by a much smaller group (16.7%) who liked it “moderately”, compared to those participants who do not prepare any meals in the household, with half (50%) of them indicating that they only liked the savoury rice “slightly”, followed by a quarter (25%) who liked it “moderately” (also see Table 4.6 for the results).

Table 4.6: Blue collar worker sample acceptance of savoury rice and the associations with their demographic and stock cube usage characteristics

Demographic and stock cube usage characteristics	Acceptance ratings for the savoury rice by the blue collar worker participant sub-sample[1]											P value
	Total (n=50)	Neither like nor dislike (n=2)		Like slightly (n=5)		Like moderately (n=9)		Like very much (n=28)		Like extremely (n=6)		
	n	n	%	n	%	n	%	n	%	n	%	
Gender												0.075
Male	6	0	0.0	2	33.3	2	33.3	1	16.7	1	16.7	
Female	44	2	4.5	3	6.8	7	15.9	27	61.4	5	11.4	
Age (years)												0.171
≤24	4	0	0.0	1	25.0	1	25.0	3	75.0	0	0.0	
25-34	22	0	0.0	3	13.6	5	22.7	13	59.1	1	4.5	
35-44	10	1	10.0	1	10.0	2	20.0	6	60.0	0	0.0	
45-54	11	1	9.1	0	0.0	1	9.1	4	36.4	5	45.5	
≥55	3	0	0.0	0	0.0	0	0.0	3	100.0	0	0.0	
Ethnicity												0.002
Black	33	0	0.0	2	6.1	7	21.2	23	69.7	1	3.0	
Coloured	17	2	11.8	3	17.6	2	11.8	5	29.4	5	29.4	
White	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
Home language												0.011
English	3	0	0.0	1	33.3	0	0.0	1	33.3	1	33.3	
Afrikaans	15	2	13.3	2	13.3	2	13.3	5	33.3	4	26.7	
Xhosa	32	0	0.0	2	6.3	7	21.9	22	68.8	1	3.1	
Marital status												0.002
Married	21	0	0.0	0	0.0	5	23.8	10	47.6	6	28.6	
Single or widowed	29	2	6.9	5	17.2	4	13.8	18	62.1	0	0.0	
Level of education												0.078
School	48	2	4.2	4	8.3	9	18.8	28	58.3	5	10.4	
Tertiary	2	0	0.0	1	50.0	0	0.0	0	0.0	1	50.0	
Monthly income[2]												0.334
≤R1700	26	1	3.8	3	11.5	6	23.1	15	57.7	1	3.8	
R1701-R3000	20	0	0.0	1	5.0	3	15.0	11	55.0	5	25.0	
R3001-R7500	2	0	0.0	1	50.0	0	0.0	1	50.0	0	0.0	
R7501-R10200	2	1	50.0	0	0.0	0	0.0	1	50.0	0	0.0	
R10201-R16250	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
≥R16251	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
A member of the household suffers from a chronic diseases												0.133
Yes	20	0	0.0	4	20.0	3	15.0	9	45.0	4	20.0	
No	30	2	6.7	1	3.3	6	20.0	19	63.3	2	6.7	
Would use a stock cube with health benefits												0.214
Yes	41	1	2.4	5	12.2	6	14.6	24	58.5	5	12.2	
No	8	1	12.5	0	0.0	3	37.5	3	37.5	1	12.5	
Would pay more for a stock cube with health benefits												0.391
Yes	38	2	5.3	3	7.9	5	13.2	23	60.5	5	13.2	
No	12	0	0.0	2	16.7	4	33.3	5	41.7	1	8.3	
Usually prepares the meals												≤0.001
Yes	42	1	2.4	1	2.4	7	16.7	28	66.7	5	11.9	
No	8	1	12.5	4	50.0	2	25.0	0	0.0	1	12.5	

1 – Acceptance ratings “dislike extremely”, “dislike very much”, “dislike moderately” and “dislike slightly” omitted due to no participant rating indications

2 – Adapted from National Treasury and South African Revenue Service, 2008

4.1.6 Blue collar worker sample acceptance of pea soup and the associations with their demographic and stock cube usage characteristics

No significant associations / differences ($p > 0.05$) were found between the acceptance of the pea soup by the blue collar workers and their gender, age, level of education, monthly income, households with members suffering from chronic diseases or not, willingness to use a stock cube with health benefits or not or willingness to pay more or not for a stock cube with health benefits and usually preparing the household meals or not (see Table 4.7). As with the acceptance of the savoury rice, there were significant differences ($p < 0.05$ for both) between the racial groupings and home language of the blue collar workers and their acceptance of the pea soup. The majority (69.7%) of black participants indicated that they liked the pea soup “very much”, followed by more than a fifth (21.2%) indicating their liking as “extremely”, compared to more than half (52.9%) of the coloured participants indicating they like the sample “extremely”, followed by nearly a fifth (17.6%) who liked the sample “very much”. This was mirrored in the home language groupings, with the majority (68.8%) of Xhosa speakers indicating they liked the sample “very much”, followed by more than a fifth (21.9%) who liked it “extremely”, compared to more than half (53.3%) of Afrikaans participants and a third (33.3%) of English participants who liked the sample “extremely”, followed by a fifth (20%) of Afrikaans speakers and a third (33.3%) of English speakers who liked the sample “very much”. Marital status also presented a significant difference ($p < 0.05$), with double the number of single or widowed participants (65.5%) indicating that they liked the pea soup “very much”, followed by a fifth (20.7%) who liked it “extremely”, compared to nearly half (47.6%) of married participants who liked the sample “extremely”, followed by a third (33.3%) who liked it “very much”. A significant difference ($p < 0.05$) was also found with participants who prepare the meals of their households and those who do not indicating a greater acceptance of the pea soup (57.1% “like very much”; 31% “like extremely”) among those participants who prepare the meals, compared to those participants who do not prepare the meals. The acceptance ratings of the latter participants were spread across “neither like nor dislike” (12.5%), “like slightly” (25%), “like very much” (25%) and “like extremely” (37.5%) (see Table 4.7).

Table 4.7: Blue collar worker sample acceptance of pea soup and the associations with their demographic and stock cube usage characteristics

Demographic and stock cube usage characteristics	Acceptance ratings for the pea soup by the blue collar worker participant sub-sample[1]											P value
	Total (n=50)	Neither like nor dislike (n=2)		Like slightly (n=3)		Like moderately (n=3)		Like very much (n=26)		Like extremely (n=16)		
	n	n	%	n	%	n	%	n	%	n	%	
Gender												0.246
Male	6	1	16.7	1	16.7	0	0.0	2	33.3	2	33.3	
Female	44	1	2.3	2	4.5	3	6.8	24	54.5	14	31.8	
Age (years)												0.448
≤24	4	0	0.0	1	25.0	0	0.0	2	50.0	1	25.0	
25-34	22	1	4.5	2	9.1	2	9.1	14	63.6	3	13.6	
35-44	10	0	0.0	0	0.0	1	10.0	5	50.0	4	40.0	
45-54	11	1	9.1	0	0.0	0	0.0	4	36.4	6	54.5	
≥55	3	0	0.0	0	0.0	0	0.0	1	33.3	2	66.7	
Ethnicity												0.001
Black	33	0	0.0	2	6.1	1	3.0	23	69.7	7	21.2	
Coloured	17	2	11.8	1	5.9	2	11.8	3	17.6	9	52.9	
White	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
Home language												0.008
English	3	1	33.3	0	0.0	0	0.0	1	33.3	1	33.3	
Afrikaans	15	1	6.7	1	6.7	2	13.3	3	20.0	8	53.3	
Xhosa	32	0	0.0	2	6.3	1	3.1	22	68.8	7	21.9	
Marital status												0.013
Married	21	0	0.0	1	4.8	3	14.3	7	33.3	10	47.6	
Single or widowed	29	2	6.9	2	6.9	0	0.0	19	65.5	6	20.7	
Level of education												1.000
School	48	2	4.2	3	6.3	3	6.3	25	52.1	15	31.3	
Tertiary	2	0	0.0	0	0.0	0	0.0	1	50.0	1	50.0	
Monthly income[2]												0.351
≤R1700	26	0	0.0	2	7.7	1	3.8	16	61.5	7	26.9	
R1701-R3000	20	1	5.0	1	5.0	2	10.0	8	40.0	8	40.0	
R3001-R7500	2	0	0.0	0	0.0	0	0.0	2	100.0	0	0.0	
R7501-R10200	2	1	50.0	0	0.0	0	0.0	0	0.0	1	50.0	
R10201-R16250	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
≥R16251	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
A member of the household suffers from a chronic diseases												0.976
Yes	20	1	5.0	1	5.0	1	5.0	10	50.0	7	35.0	
No	30	1	3.3	2	6.7	2	6.7	16	53.3	9	30.0	
Would use a stock cube with health benefits												0.463
Yes	41	1	2.4	2	4.9	3	7.3	21	51.2	14	34.1	
No	8	1	12.5	1	12.5	0	0.0	4	50.0	2	25.0	
Would pay more for a stock cube with health benefits												0.188
Yes	38	1	2.6	1	2.6	2	5.3	20	52.6	14	36.8	
No	12	1	8.3	2	16.7	1	8.3	6	50.0	2	16.7	
Usually prepares the meals												0.057
Yes	42	1	2.4	1	2.4	3	7.1	24	57.1	13	31.0	
No	8	1	12.5	2	25.0	0	0.0	2	25.0	3	37.5	

1 – Acceptance ratings “dislike extremely”, “dislike very much”, “dislike moderately” and “dislike slightly” omitted due to no participant rating indications

2 – Adapted from National Treasury and South African Revenue Service, 2008

4.1.7 White collar worker sample acceptance of savoury rice and pea soup and the associations with their demographic and stock cube usage characteristics

The acceptance of both the savoury rice and the pea soup by the white collar workers showed no significant association / difference ($p > 0.05$) with any of their demographic and stock cube usage characteristics investigated besides for the willingness or not to make use of a stock cube with health benefits, as well as the presence of a family member suffering from a chronic disease. The willingness or not to make use of a stock cube with health benefits provided a significant difference within the white collar participant acceptance of both the savoury rice ($p < 0.05$) and the pea soup ($p < 0.001$). In the case of the savoury rice, the participants who answered “yes” leaned more to the positive side of the acceptance rating scale, with nearly a third (30.4%) who indicated that they liked the sample “moderately”, followed by 28.3% who liked it “very much” and 17.4% who liked it extremely, compared to the participants who answered “no” who leaned more to the negative side of the scale, with a third (33.3%) indicating they liked the sample “moderately”, another third (33.3%) indicating they neither liked nor disliked the sample and the final third (33.3%) indicating that they disliked the sample “slightly”. The same tendency was seen in the case of the pea soup with just over a third (34.8%) of the participants who answered “yes” indicated their liking as “very much” followed by 28.3% who liked the sample “extremely”, compared to the participants who answered “no” who were distributed equally (33.3% respectively) between “like moderately”, “neither like nor dislike” and “dislike slightly”. The only other significant difference ($p < 0.05$) found was between the presence or not of a family member suffering from a chronic disease and the participants’ liking of the savoury rice. Here nearly half (47.6%) the participants who answered “yes” indicated their liking the savoury rice as “moderately” with a further quarter (23.8%) of the participants indicating their liking as “slightly”. Of those participants who answered “no” to the question, a third (35.7%) indicated their liking of the savoury rice as “very much”, with a further quarter (25.0%) indicating their liking as “extremely” (see Table 4.8 for the savoury rice acceptance and Table 4.9 for the pea soup acceptance).

Table 4.8: White collar worker sample acceptance of savoury rice and the associations with their demographic and stock cube usage characteristics

Demographic and stock cube usage characteristics	Acceptance ratings for the savoury rice by the white collar worker participant sub-sample[1]													P value
	Total (n=49)	Dislike slightly (n=3)		Neither like nor dislike (n=1)		Like slightly (n=9)		Like moderately (n=15)		Like very much (n=13)		Like extremely (n=8)		
	n	n	%	n	%	n	%	n	%	n	%	n	%	
Gender														1.000
Male	11	1	9.1	0	0.0	2	18.2	3	27.3	3	27.3	2	18.2	
Female	38	2	5.3	1	2.6	7	18.4	12	31.6	10	26.3	6	15.8	
Age (years)														0.313
≤24	4	0	0.0	0	0.0	1	25.0	0	0.0	2	50.0	1	25.0	
25-34	16	1	6.3	1	6.3	4	25.0	8	50.0	1	6.3	1	6.3	
35-44	10	1	10.0	0	0.0	2	20.0	1	10.0	4	40.0	2	20.0	
45-54	12	1	8.3	0	0.0	1	8.3	3	25.0	3	25.0	4	33.3	
≥55	7	0	0.0	0	0.0	1	14.3	3	42.9	3	42.9	0	0.0	
Ethnicity														0.561
Black	5	0	0.0	0	0.0	1	20.0	1	20.0	1	20.0	2	40.0	
Coloured	22	1	4.5	1	4.5	6	27.3	8	36.4	4	18.2	2	9.1	
White	22	2	9.1	0	0.0	2	9.1	6	27.3	8	36.4	4	18.2	
Home language														0.801
English	22	2	9.1	1	4.5	5	22.7	7	31.8	4	18.2	3	13.6	
Afrikaans	23	1	4.3	0	0.0	4	17.4	7	30.4	8	34.8	3	13.0	
Xhosa	4	0	0.0	0	0.0	0	0.0	1	25.0	1	25.0	2	50.0	
Marital status														0.655
Married	26	1	3.8	1	3.8	4	15.4	10	38.5	7	26.9	3	11.5	
Single or widowed	23	2	8.7	0	0.0	5	21.7	5	21.7	6	26.1	5	21.7	
Level of education														1.000
School	2	0	0.0	0	0.0	0	0.0	1	50.0	1	50.0	0	0.0	
Tertiary	47	3	6.4	1	2.1	9	19.1	14	29.8	12	25.5	8	17.0	
Monthly income[2]														0.747
≤R1700	1	0	0.0	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0	
R1701-R3000	1	0	0.0	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	
R3001-R7500	6	0	0.0	0	0.0	2	33.3	1	16.7	1	16.7	2	33.3	
R7501-R10200	9	0	0.0	0	0.0	2	22.2	4	44.4	2	22.2	1	11.1	
R10201-R16250	20	0	0.0	1	5.0	3	15.0	7	35.0	6	30.0	3	15.0	
≥R16251	12	3	25.0	0	0.0	1	8.3	3	25.0	3	25.0	2	16.7	
A member of the household suffers from a chronic diseases														0.042
Yes	21	2	9.5	0	0.0	5	23.8	10	47.6	3	14.3	1	4.8	
No	28	1	3.6	1	3.6	4	14.3	5	17.9	10	35.7	7	25.0	
Would use a stock cube with health benefits														0.018
Yes	46	2	4.3	0	0.0	9	19.6	14	30.4	13	28.3	8	17.4	
No	3	1	33.3	1	33.3	0	0.0	1	33.3	0	0.0	0	0.0	
Would pay more for a stock cube with health benefits														0.188
Yes	46	1	2.2	0	0.0	7	15.2	13	28.3	10	21.7	7	15.2	
No	3	2	66.7	1	33.3	2	66.7	2	66.7	3	100.0	1	33.3	
Usually prepares the meals														0.287
Yes	38	2	5.3	1	2.6	5	13.2	13	34.2	12	31.6	7	18.4	
No	11	1	9.1	0	0.0	4	36.4	2	18.2	1	9.1	1	9.1	

1 – Acceptance ratings “dislike extremely”, “dislike very much” and “dislike moderately” omitted due to no participant rating indications

2 – Adapted from National Treasury and South African Revenue Service, 2008

Table 4.9: White collar worker sample acceptance of pea soup and the associations with their demographic and stock cube usage characteristics

Demographic and stock cube usage characteristics	Acceptance ratings for the pea soup by the white collar worker participant sub-sample[1]															P value
	Total (n=49)	Dislike moderately (n=1)		Dislike slightly (n=1)		Neither like nor dislike (n=1)		Like slightly (n=10)		Like moderately (n=7)		Like very much (n=16)		Like extremely (n=13)		
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Gender																0.552
Male	11	0	0.0	0	0.0	1	9.1	1	9.1	1	9.1	4	36.4	4	36.4	
Female	38	1	2.6	1	2.6	0	0.0	9	23.7	6	15.8	12	31.6	9	23.7	
Age (years)																0.779
≤24	4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	25.0	3	75.0	
25-34	16	1	6.3	1	6.3	0	0.0	5	31.3	2	12.5	5	31.3	2	12.5	
35-44	10	0	0.0	0	0.0	1	10.0	2	20.0	2	20.0	2	20.0	3	30.0	
45-54	12	0	0.0	0	0.0	0	0.0	1	8.3	3	25.0	5	41.7	3	25.0	
≥55	7	0	0.0	0	0.0	0	0.0	2	28.6	0	0.0	3	42.9	2	28.6	
Ethnicity																0.697
Black	5	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0	3	60.0	1	20.0	
Coloured	22	1	4.5	1	4.5	1	4.5	4	18.2	3	13.6	8	36.4	4	18.2	
White	22	0	0.0	0	0.0	0	0.0	6	27.3	3	13.6	5	22.7	8	36.4	
Home language																0.406
English	22	1	4.5	1	4.5	0	0.0	3	13.6	2	9.1	10	45.5	5	22.7	
Afrikaans	23	0	0.0	0	0.0	1	4.3	7	30.4	4	17.4	4	17.4	7	30.4	
Xhosa	4	0	0.0	0	0.0	0	0.0	0	0.0	1	25.0	2	50.0	1	25.0	
Marital status																0.796
Married	26	1	3.8	1	3.8	0	0.0	5	19.2	5	19.2	8	30.8	6	23.1	
Single or widowed	23	0	0.0	0	0.0	1	4.3	5	21.7	2	8.7	8	34.8	7	30.4	
Level of education																0.577
School	2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	100.0	0	0.0	
Tertiary	47	1	2.1	1	2.1	1	2.1	10	21.3	7	14.9	14	29.8	13	27.7	

1 – Acceptance ratings “dislike extremely” and “dislike very much” omitted due to no participant rating indications

Table 4.9: White collar worker sample acceptance of pea soup and the associations with their demographic and stock cube usage characteristics (cont.)

Demographic and stock cube usage characteristics	Acceptance ratings for the pea soup by the white collar worker participant sub-sample[1]															P value
	Total (n=49)	Dislike moderately (n=1)		Dislike slightly (n=1)		Neither like nor dislike (n=1)		Like slightly (n=10)		Like moderately (n=7)		Like very much (n=16)		Like extremely (n=13)		
	n	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Monthly income[2]																0.765
≤R1700	1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	100.0	
R1701-R3000	1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	100.0	0	0.0	
R3001-R7500	6	0	0.0	0	0.0	0	0.0	2	33.3	1	16.7	2	33.3	1	16.7	
R7501-R10200	9	1	11.1	0	0.0	0	0.0	1	11.1	1	11.1	1	11.1	5	55.6	
R10201-R16250	20	0	0.0	1	5.0	0	0.0	4	20.0	3	15.0	7	35.0	5	25.0	
≥R16251	12	0	0.0	0	0.0	1	8.3	3	25.0	2	16.7	5	41.7	1	8.3	
A member of the household suffers from a chronic diseases																0.842
Yes	21	0	0.0	0	0.0	1	4.8	5	23.8	4	19.0	6	28.6	5	23.8	
No	28	1	3.6	1	3.6	0	0.0	5	17.9	3	10.7	10	35.7	8	28.6	
Would use a stock cube with health benefits																0.001
Yes	46	1	2.2	0	0.0	0	0.0	10	21.7	6	13.0	16	34.8	13	28.3	
No	3	0	0.0	1	33.3	1	33.3	0	0.0	1	33.3	0	0.0	0	0.0	
Would pay more for a stock cube with health benefits																0.080
Yes	46	0	0.0	0	0.0	0	0.0	8	17.4	5	10.9	14	30.4	11	23.9	
No	3	1	33.3	1	33.3	1	33.3	2	66.7	2	66.7	2	66.7	2	66.7	
Usually prepares the meals																0.148
Yes	38	0	0.0	1	2.6	0	0.0	9	23.7	7	18.4	13	34.2	10	26.3	
No	11	1	9.1	0	0.0	1	9.1	1	9.1	0	0.0	3	27.3	3	27.3	

1 – Acceptance ratings “dislike extremely” and “dislike very much” omitted due to no participant rating indications
2 – Adapted from National Treasury and South African Revenue Service, 2008

4.2 Shelf-life stability characteristics of stock cube at accelerated conditions over a six month period

The AgriFood Technology Station of CPUT conducted a six month accelerated shelf-life stability testing, equalling a 12 month period in ambient temperatures and normal storage conditions. The analysis of the TAC was conducted on these storage samples by the Analytical Laboratory Services, Oxidative Stress Research Centre of CPUT utilising the same time frame as the microbial growth and oxidative rancidity status testings.

4.2.1 Microbial growth

The microbial growth test results are provided in Table 4.10. An acceptable specification range for each organism, expressed as a number of colony forming units per gram, was supplied by the AgriFood Technology Station, which is the applied specification range in their shelf-life testing. The suggested specification for the total viable count is less than 1 million. The highest total viable count of the stock cube was 8350/g at the first sampling. The total viable count starts slightly above the upper control limit, but then stays within the control limits for the duration of the six month shelf-life period (see Figure 4.1). No coliforms were detected. The highest average yeasts and moulds count was 2 500/g, again at the first sampling. The yeasts and moulds count remained within the control limits for the duration of the six months accelerated shelf-life stability testing (see Figure 4.2). The tests for all of the specific microorganisms, namely *E. coli*, *S. aureus*, *B. cereus*, the *Clostridium* species and the *Salmonella* species, were negative for all the samplings (see Table 4.10).

Table 4.10: Microbial growth in new developed stock cube at accelerated conditions over six month period

Six month accelerated shelf-life	Average number of colony forming units per gram			Confirmation test			Detection test	
	Total viable count (average)	Coliforms (average)	Yeasts and moulds (average)	*E. coli*	*S. aureus*	*B. cereus*	*Clostridium* spp.[1]	*Salmonella* spp.[1]
	Acceptable specification range for test parameter[2]							
	< 1 000 000/ gram	< 1 000/gram	<10 000/gram	negative	negative	negative	negative	negative
Sampling 1	8350	0	2500	negative	negative	negative	negative	negative
Sampling 2	3000	0	1750	-	-	-	-	-
Sampling 3	1800	0	1850	negative	negative	negative	negative	negative
Sampling 4	800	0	300	-	-	-	-	-
Sampling 5	100	0	200	-	-	-	-	-
Sampling 6	1950	0	1850	negative	negative	negative	negative	negative

1 – Species (spp.)
2 – Acceptable specification range for microorganisms (supplied by AgriFood Technology Station, CPUT)

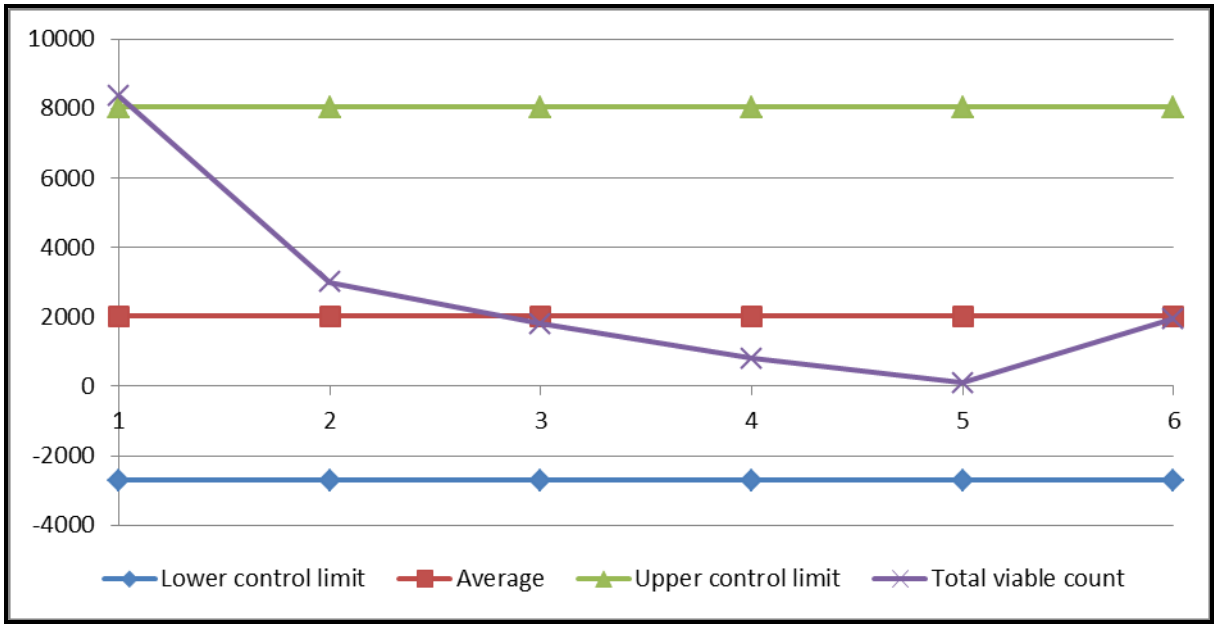

Figure 4.1: Control chart for the total viable count in the accelerated shelf-life stability testing of new developed stock cube over six month period

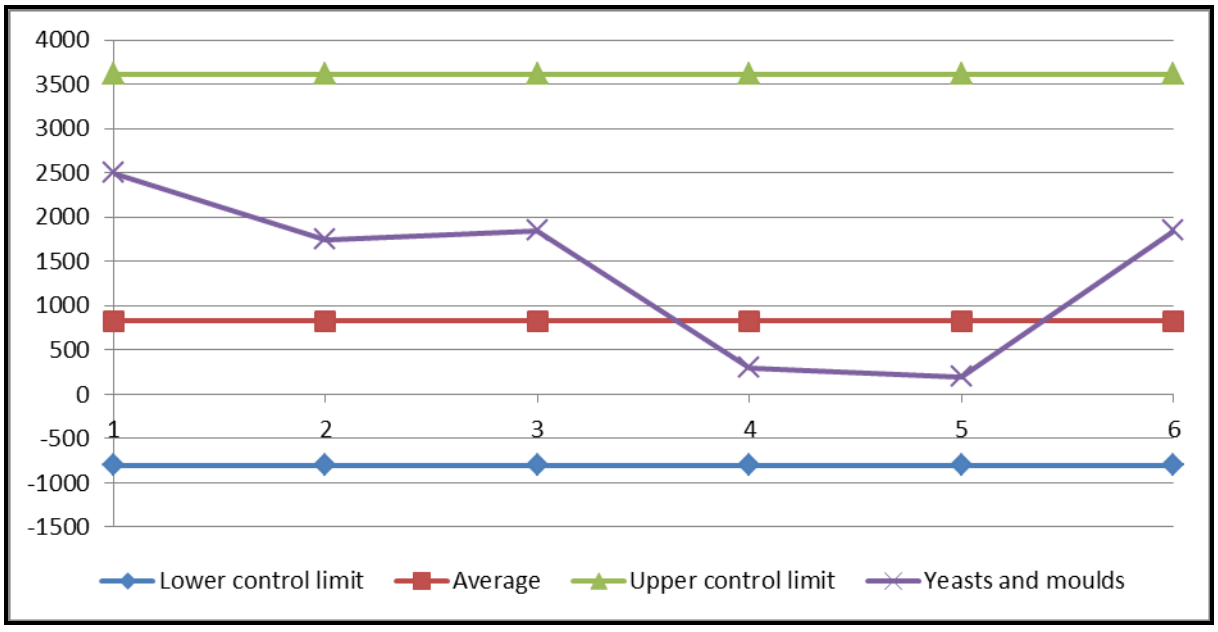

Figure 4.2: Control chart for yeasts and moulds count in the accelerated shelf-life stability testing of new developed stock cube over six month period

4.2.2 Oxidative rancidity

Oxidative rancidity is the process whereby unsaturated fatty acids take up oxygen molecules. This leads to the development of unpleasant flavours and aromas in food products due to the formation of peroxides and hydroperoxides (McWilliams, 1993:274). This accumulation of peroxides is expressed as the peroxide value (PV) and measured in milli-equivalents oxygen per kilogram (meq. O_2/kg). According to the AgriFood Technology Station fresh oils have a PV of less than 10 meq. O_2/kg, with values between 30 to 40 meq. O_2/kg having a rancid taste. Using the PV as an indication of rancidity, the stock cube is acceptable for up to four months at accelerated conditions (see Table 4.11), equalling eight months real time. However, even though the PV indicates that the stock cube had become rancid by the last two samplings, it did not develop a rancid taste (see Addendum M for PV report). The PV stayed within the control limits for the duration of the six months accelerated shelf-life stability testing (see Figure 4.3).

Table 4.11: Peroxide value of new developed stock cube at accelerated conditions over six month period

Six month accelerated shelf-life	Peroxide value (meq. O2/Kg[1])
Sampling 1	3.49
Sampling 2	7.79
Sampling 3	15.31
Sampling 4	25.97
Sampling 5	36.59
Sampling 6	36.87

1 – Milli-equivalent oxygen per kilogram

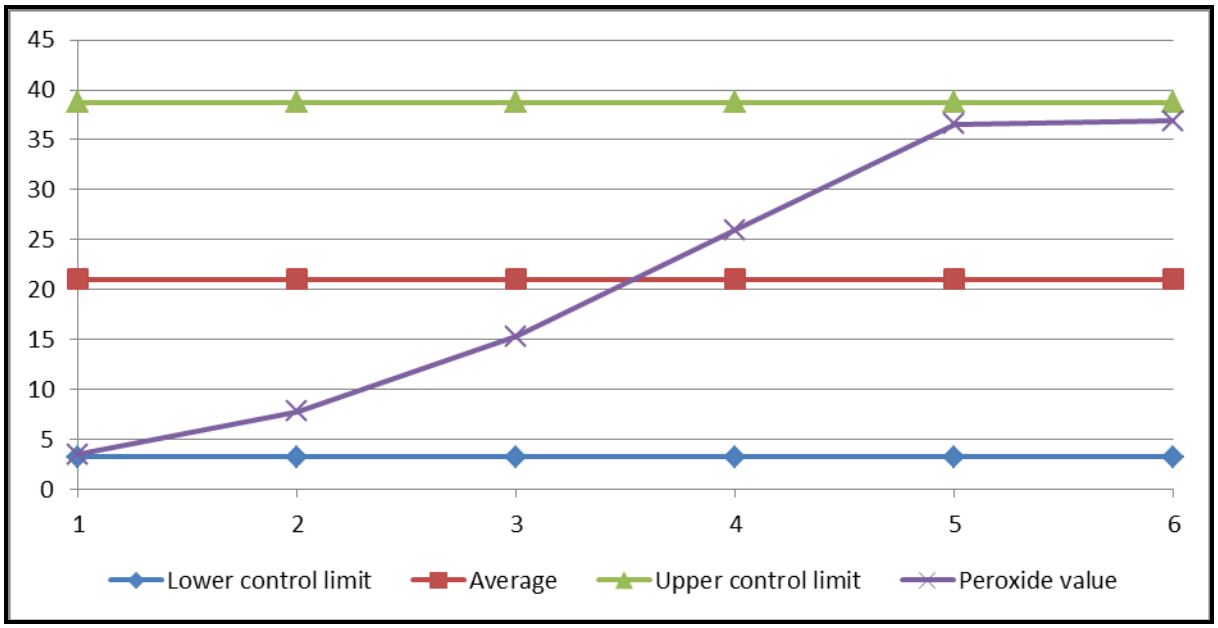

Figure 4.3: Control chart for the peroxide value in the accelerated shelf-life stability testing of new developed stock cube over six month period

4.2.3 Antioxidant status

The TAC is provided by the H-ORAC and the L-ORAC and is expressed in Trolox equivalents per gram (TE/g). Samples of the new developed stock cube, kept by AgriFoods Technology Station at accelerated shelf-life testing conditions, were analysed over the six month period by the Analytical Laboratory Services, Oxidative Stress Research Centre of CPUT to determine the stability of the H-ORAC, L-ORAC and polyphenol content over the time period. The results (see Table 4.12) indicate that the TAC and polyphenol levels remained relatively stable over the six month period at accelerated conditions. In addition the H-ORAC (see Figure 4.4), L-ORAC (see Figure 4.5) and total polyphenols (see Figure 4.6) all remained within the control limits of each for the duration of the six months accelerated shelf-life stability testing.

Table 4.12: Antioxidant status and total polyphenols of new developed stock cube at accelerated conditions over six month period

Six month accelerated shelf-life	Total antioxidant capacity				Polyphenols (mg GAE[4]/g)	SD[5]
	H-ORAC[1] (umole TE[3]/g)	SD[5]	L-ORAC[2] (umole TE[3]/g)	SD[5]		
Sampling 1	87.82	± 4.70	2.29	± 0.52	1.11	± 0.18
Sampling 2	96.48	± 6.07	3.27	± 0.78	1.12	± 0.25
Sampling 3	90.45	± 2.45	2.16	± 0.63	1.22	± 0.12
Sampling 4	92.34	± 3.87	2.34	± 0.50	1.19	± 0.18
Sampling 5	88.14	± 5.08	2.69	± 0.41	1.29	± 0.22
Sampling 6	86.96	± 1.49	2.54	± 0.43	1.21	± 0.19

1 - Hydrophilic oxygen radical absorbance capacity
2 - Lipophilic oxygen radical absorbance capacity
3 - MicromoleTrolox equivalents
4 - Milligram Gallic acid equivalents
5 – Standard deviation

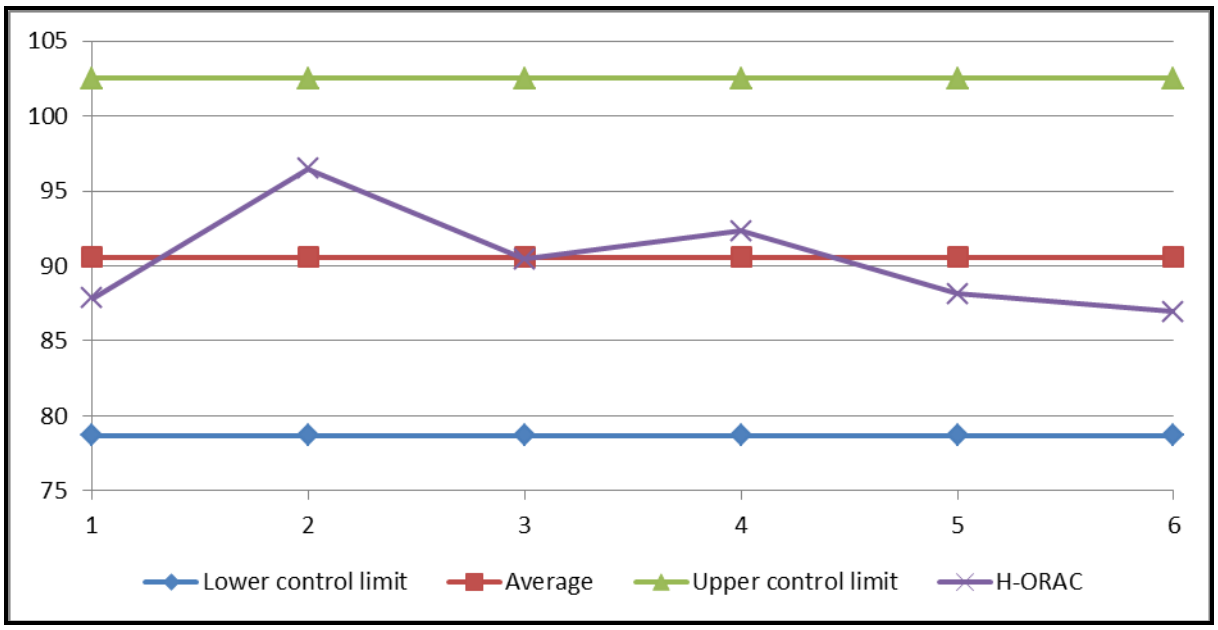

Figure 4.4: Control chart for the hydrophilic oxygen radical absorbance capacity in the accelerated shelf-life stability testing of new developed stock cube over six month period

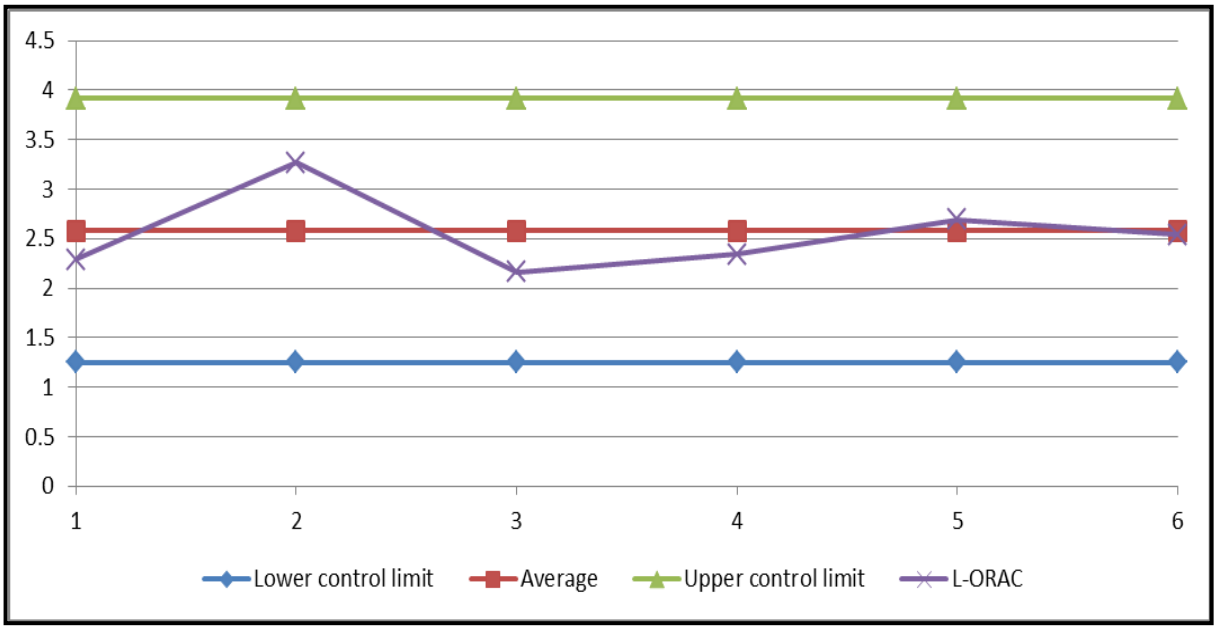

Figure 4.5: Control chart for the lipophilic oxygen radical absorbance capacity in the accelerated shelf-life stability testing of new developed stock cube over six month period

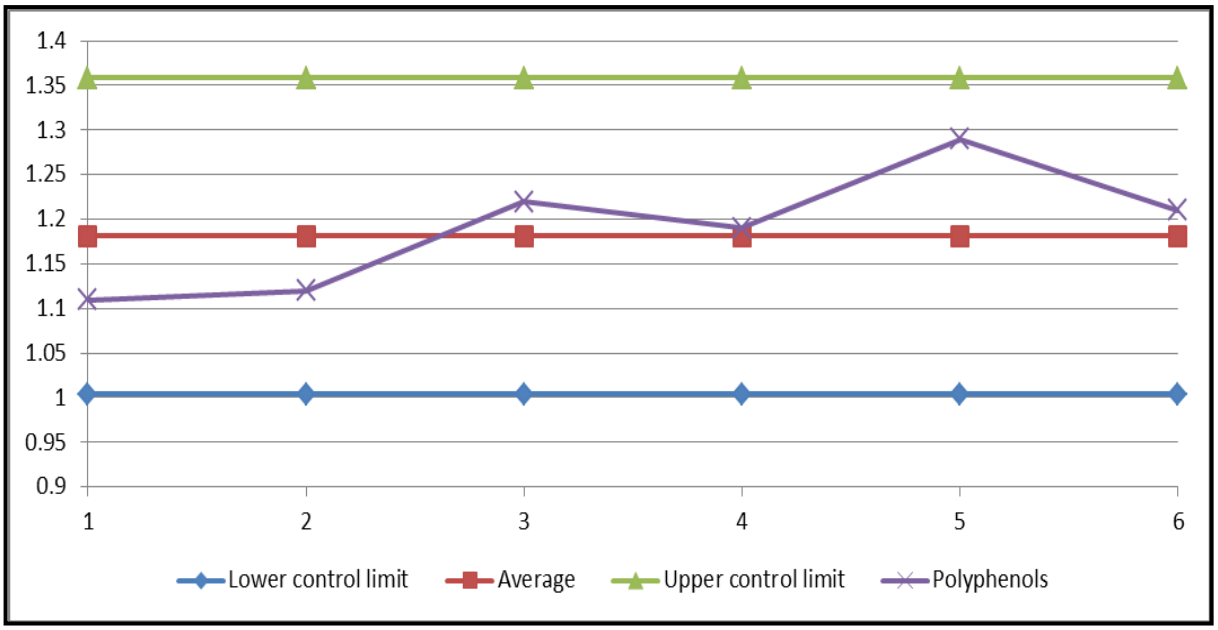

Figure 4.6: Control chart for the total polyphenols in the accelerated shelf-life stability testing of new developed stock cube six month period

4.2.4 Detection of organoleptic changes

During the shelf-life testing period, the duo-trio sensory test was used to determine if any organoleptic changes were detectable in the stock cubes over time. The sensory panel consisted of six staff members of the Programme: Consumer Science: Food and Nutrition. The duo-trio sensory test was conducted at the first three samplings of the shelf-life testing and at the final sampling due to the availability of all the panellists at these test times. No significant difference / association ($p > 0.05$) was found between the number of correct answer versus the incorrect/no difference perceived answers to the duo-trio test results presented in Table 4.13.

Table 4.13: Sensory evaluation of new developed stock cube at accelerated conditions over six month period

Six month accelerated shelf-life[1]	Score dichotomy		Total
	Correct matching with reference sample	Incorrect matching or unable to perceive difference between samples	
Sampling 1	1	5	6
Sampling 2	5	1	6
Sampling 3	3	3	6
Sampling 6	4	2	6
P Value			0.118

1 – Four samplings were analysed at months one, two, three and six due to unavailability of sensory panel between sampling three and sampling six

CHAPTER 5
DISCUSSION

The results of the study are discussed in three parts. The first part relates to the participant acceptance of the new stock cube, the second part to the participant characteristic associations with the acceptance of the new stock cube and the last part to its shelf-life stability.

5.1 Participant acceptance of the new stock cube

In this study CSL as by-product of cereal grain processing was utilised as a source of phytochemicals in a new developed stock cube. The overall acceptance of both the savoury rice and the pea soup, that were chosen as typical dishes that utilise stock cubes, was towards the positive acceptance rating of the hedonic scale that represented a range of “liking” responses. This is an encouraging finding, as McEwan (1997:183) found that consumers are surer of their likes, compared to their dislikes. In the development of a new stock cube that could possibly provide health benefits, a reduction of the salt content was one consideration which could have a negative impact on the acceptability of a dish. A reduced salt stock cube could reduce the salty taste of the dish in comparison to when a traditional stock cube was used as ingredient. Salt is one of the basic seasonings used in cooking and it seems that the human taste for salt is mostly learned (Leshem, 2009:13). However, Adams and co-workers (1995:452) also found that the acceptability of salt levels was dependent on the type of food consumed. This observation may have impacted the findings of this study in the use of the savoury rice and the pea soup as the chosen food vehicles for the use of the new developed stock cube.

There has been some interest in the use of by-products of the food industry and non-traditional food ingredients utilised as functional ingredients in product development work that could add phytochemicals and/or increase the TAC of the new food products. Researchers have, for example, made use of apple fruit skins as source of fibre and polyphenols (e.g. quercetin glycosides and catechins) in muffins (Vasantha Rupasinghe *et al*., 2008:1217), ginger powder as source of shogaols in bread (Balestra *et al*., 2011:700) and green tea as source of catechins in sponge cake (Lu *et al*., 2010:1090) in attempting to increase the phytochemical content and the TAC of the traditional counterparts. This type of new product development of foods with functional food characteristics presents the food industry with a marketing strategy to deliver new value-added food products to the consumer (Falguera *et al*., 2012:276). In this study the majority of the participants indicated that they would use (88%) and would pay more (77%) for a

stock cube with health benefits which further presents support to the food industry for such product development work.

5.2 Participant characteristic associations with the acceptance of the new stock cube

In none of the participant groups, either the total, blue collar or white collar, any significant associations / differences ($p > 0.05$) were found between the sample gender and age and the acceptance of the two sample dishes. These findings supports the results of a study conducted in Belgium which reported that the acceptance of functional foods with specific health and nutritional claims were not greatly influenced by demographic factors such as age and gender (Verbeke *et al*., 2009:690). There was also no significant association ($p > 0.05$) between the total participant sample or the two sub-groups' acceptance of the two sample dishes and the presence of a chronically ill member of the household, with the exception of the white collar participants and their liking of the savoury rice ($p = 0.042$). This finding was also encountered in a study conducted to determine the acceptability of functional foods (Verbeke, 2005:51).

The participant demographic difference trends of the total sample provided by the study findings were a difference between the ethnic groupings and home language in the acceptance of the two different sample dishes incorporating the new stock cube. In the acceptance of both the savoury rice and the pea soup significant differences ($p < 0.05$ for each) were found in the ethnic and language groupings, with a higher percentage of the black and Xhosa speaking participants indicating liking of the two sample dishes incorporating the new stock cube. According to Karanja and co-workers (2007:1534) differences observed between different racial and language groupings are due to the differences in culture. This was also reflected in the significant differences ($p < 0.05$ for each) for the level of education and profession (blue collar versus white collar) in the acceptance findings of the savoury rice as sample dish. There was in other words a higher acceptability of the savoury rice prepared with the new stock cube towards the lower socio-economic groups and the blue collar workers. The blue collar sub-group comprised mostly of the black and coloured racial groupings. This was, however, not the case for the pea soup.

The blue collar worker findings presented a significant difference ($p < 0.05$) between the acceptance of the savoury rice and the participant being the person who prepares the meals at home or not. More participants preparing the meals in the household accepted the savoury rice than those not involved in the household food preparation. This finding was nearly significant ($p = 0.057$) for the pea soup acceptance. This finding within the blue collar worker sub-group

was also encountered in the total sample for the acceptance of both the sample dishes. This could possibly be because the participant who prepares the meals at home was less critical of other's food, being familiar with their cooking being judged by others. A significant difference ($p < 0.05$) was also found between the participant marital status and the acceptance of the savoury rice and the pea soup. In this finding the ratings indicated by the married participants were spread across the top three ratings of "like moderately", "like very much" and "like extremely", compared to the single participants who were centred in the "like very much" rating. This could possibly be because married participants have to cater to a wider range of tastes as they are not the only person in the household.

The acceptance findings of the white collar workers as a sub-group presented a significant difference ($p < 0.05$) with a greater acceptance of both sample dishes by those participants willing to use a product with possible health benefits. This overall significant ($p < 0.05$) finding within the white collar worker sub-group for the savoury rice and the pea soup was also encountered in the total sample for the acceptance of the two sample dishes. The finding of the study by Verbeke (2005:54) that there is a greater acceptance of functional foods that are perceived as beneficial to health, is mirrored in the significant difference ($p < 0.05$) between the acceptance of both the savoury rice and the pea soup by the white collar workers with the greater acceptance among those willing to use a stock cube with health benefits.

5.3 Shelf-life stability of the new stock cube

The parameters analysed during the shelf-life stability testing included the microbial growth, oxidative rancidity, antioxidant status and organoleptic changes. The samples all tested negative for coliforms, *E. coli*, *S. aureus*, *B. cereus*, *Clostridium* species and *Salmonella* species. It is assumed that lower microbial loads would be possible in a more controlled factory environment. The fact that the total viable count decreased during the first five months could indicate that the formulation of the stock cube has an inhibiting effect on microbial growth. The averages of the total viable count and yeast and moulds were all within the acceptable specification range. Both these test parameters declined over the first five months of the accelerated time period and then increased in the final month of the accelerated test period. The new stock cube also measured an increase in PV, indicating oxidative rancidity, after the fourth sampling. However, no rancid taste developed. The antioxidant status of the new stock cube measured as the H-ORAC, L-ORAC and total polyphenol content stayed relatively stable over the accelerated testing period. It would therefore appear as if the polyphenol content of the new stock cube had little effect in

preventing an increase in the PV of the stock cube. This observation questions the suitability of polyphenols as an agent for preventing oxidation of fats in foods. The final parameter was the changes in organoleptic characteristics. These were measured by means of a duo-trio test. There was no significant ($p < 0.05$) change in the organoleptic properties of the new stock cube; however, there was a darkening of the colour by the second sampling which may reflect the initiation of oxidative rancidity which was subsequently measured.

5.4 Possible nutritional contribution of new stock cube to South African population

A stock cube was chosen as the vehicle for the CSL, as a source of phytochemicals, and of the added micronutrients, iron, zinc, copper and selenium, as it was found that stock cubes were consumed regularly by a large number of South African consumers (Chen & Oldewage-Theron, 2004:178). The fat base utilised in the new stock cube was a red palm fat, which is rich in carotenoids (provitamin A) and vitamin E (see 3.2.1.2) and which could further contribute to the functional food characteristics of the new stock cube. Even though the stock cube was developed to be used by a wide range of consumers, it does have the potential to contribute to the nutritional status of particularly vulnerable segments of the South African population.

The first and fifth ranking causes of death in South Africa for the year 2000 was HIV/AIDS, with an estimated mortality rate of 29.8% of all deaths, and TB, with a national mortality rate of about five per cent (5.1%) (Bradshaw *et al*., 2004:24). Both of these infectious diseases are associated with increased levels of inflammation and oxidative stress (Stehbens, 2004:121; Hasan *et al*., 2006:2517; Appay & Sauce, 2008:231; Selek *et al*., 2008:143). Other leading causes of mortality for the year 2000 were the CDL, with stroke (5.8%) and ischemic heart disease (5.6%) in second and third place, respectively, and hypertensive heart disease (3.1%) in the eighth position, diabetes mellitus (2.6%) in the tenth position and inflammatory heart disease (1.3%) in the 14th position (Bradshaw *et al*., 2004:24). It is the inflammatory response of the immune system that is an underlying mechanism of many CDL, as well as exacerbating diseases such as HIV/AIDS and TB (Seaman, 2002:169; Friedland, 2008:1). The CSL added to the stock cube as a source of phytochemicals, in particular polyphenols, along with the added micronutrients, will provide it with functional food characteristics as having anti-inflammatory and antioxidant properties (as supported in the literature section). In a Brazilian study, participants were provided green tea to drink for a period of seven days, resulting in a mean intake of 4,6 µg phenolic compounds per day. The study observed an increase in the plasma total polyphenols by 27% (Panza *et al*., 2008:438). At the daily per capita polyphenol provision level of the stock cube at 5.7 mg phenolic

compounds, an increase in the total plasma polyphenols could thus be expected with regular use with even possibly resultant health protective effects against the CDL and infectious diseases. A German study utilising fruit juice as source of the polyphenolic phytochemicals found an improvement in antioxidant status and immune response, as well as reduced levels of oxidative DNA damage (Bub *et al*., 2003:95).

South African children and women also face specific nutrition-related disease challenges. The Birth-to-Ten Study determined the nutrient intakes of urban black children at four different age interceptions, namely five, seven, nine and ten years of age (MacKeown *et al*., 2003:185). This study found that more than half the children at all four age interceptions had iron and vitamin A intakes below the RDA, with the exception of vitamin A intakes for the five years age group, where a third had vitamin A intakes below the RDA (MacKeown *et al*., 2003:188). It is estimated that a vitamin A deficiency under South African children between the ages of zero and four was a contributing factor to mortality rates due to diarrhoeal diseases (28% of total deaths), measles (23%) and malaria (21%) in the year 2000 (Nojilana *et al*., 2007a:748). Another risk group in South Africa is pregnant women. In a ranking of risk factors leading to death, iron deficiency anaemia was placed in 16th place for the year 2000, accounting for 0.4% of total South African deaths (Norman *et al*., 2007:638). Nojilana and co-workers (2007b:744) reported that nine to twelve per cent of pregnant South African women had iron deficiency anaemia, compared to the average of four per cent found in industrialised countries, with approximately seven per cent of perinatal deaths and five per cent maternal deaths attributed to the iron deficiency anaemia in the year 2000. Another South African study also found that iron deficiency anaemia had a negative impact on postpartum mother-child interaction, as well as on the development of the baby (Perez *et al*., 2005:854). Fortified food products could be one of the possible solutions to overcome these nutrient deficiencies.

Besides the phytochemicals (in the form of polyphenols) provided by the CSL and the provitamin A and vitamin E provided by the inclusion of red palm fat in the new stock cube, micronutrients (iron, zinc, copper and selenium) were added to provide 50% of the RDA/AI for women of child-bearing age, and approximately 100% of the RDA/AI for children per serving in a single stock cube providing four servings. One intervention study among primary school children utilised fortified biscuits as vehicle for iron, iodine and β-carotene at 50% RDA micronutrient contributions per three biscuits serving (van Stuijvenberg *et al*., 1999:498). The results showed a significant improvement in the iron, iodine and vitamin A status and overall health of the children consuming the biscuit during school days for six months (van Stuijvenberg *et al*., 1999:497). The

new stock cube therefore has a similar potential to contribute nutritional value to the dietary intakes of particularly children and women in its regular household use when incorporated in dishes requiring fluid addition for cooking purposes, where the fluid is either absorbed forming part of the dish (such as in rice and pasta) or consumed as part of the dish (such as in soups and stews).

5.5 Limitations of study

One of the limitations of the study is the absence of a final nutritional analysis of the new stock cube which was not conducted due to financial constraints. Neither was a theoretical nutritional composition analysis possible due to the non-existence of nutritional information on the flavouring agent used in the development of the new stock cube. This formed a major obstacle with regard to the salt/sodium content, as the flavouring agent itself contributed salt to the final stock cube formulation. However, the new stock cube was developed as a prototype and further work should include a detailed nutritional content analysis.

Another limitation may possibly be the use of English as only language in the supporting documentation (taste testing sheet, informed consent form and participant demographic and stock cube usage questionnaire) of the consumer acceptance testing (see Addenda D, E and H), particularly the differentiation of the acceptance ratings in the taste testing sheets. These documents were first used during the informal group discussions to determine whether or not any changes had to be made. No problems were experienced and it was assumed that non-English first language speakers would have an acceptable level of English language proficiency to complete the questionnaire. There is nonetheless the possibility that the acceptance rating differentiations could have been grasped better if also indicated in a home language for the Black participants such as Xhosa.

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The development of the new stock cube was set out with five objectives in mind (see Introduction). The following conclusions can be drawn with regards to these objectives:

i. The development of a product with functional food characteristics utilising a stock cube for this purpose was an appropriate choice, as it is a product used by a wide spectrum of consumers. The CSL was successfully incorporated as a source of phytochemicals. The micronutrients zinc, copper, selenium and iron were also successfully incorporated to provide added immunonutritional support. The consideration of being successful is based on the participant acceptance of the two sample dishes incorporating the new stock cube, though only the product TAC and total polyphenol content, and not the content of the micronutrients added, were analysed. However, the new developed stock cube had a TAC approximately 17 times that of a traditional stock cube (Rautenbach, 2010). The salt content of the new developed stock cube was also reduced compared to a traditional stock cube, particularly in that it was the third and not the first ingredient in the product ingredient listing, which is in line with proposed regulations with regards to the reduction of sodium in foodstuffs, specifically stock cubes, to levels of 5 500 mg sodium / 100 g foodstuff by 30 June 2014 and 3 500 mg sodium / 100 g foodstuff by 30 June 2017 (Milk South Africa, 2012). As indicated previously, the sodium content of the new stock cube as provided by its salt ingredient inclusion, and not considering the sodium contribution from the flavourant ingredient inclusion, approximates 668 mg sodium per 11 g (as stock cube) and 6 073 mg sodium per 100 g, compared to 25 900 mg sodium per 100 g in traditional stock cubes (nutritional information on packaging of Imana beef flavoured cubes).

ii. The new developed stock cube was judged by a sensory panel of food professionals on its organoleptic characteristics to be comparable to stock cubes currently available on the market.

iii. The accelerated shelf-life testing indicated that the product would be stable for 12 months. No microbial growth exceeded the standard parameters in this time frame.

Although there was an initial darkening in the colour and a measurable development of rancidity after eight months according to the PV measurement, the taste was not adversely affected in the remaining period as based on the results of the sensory evaluation. These organoleptic characteristics were possibly not greatly reduced as the TAC and polyphenol content stayed relatively stable over the accelerated testing period.

iv. The acceptance ratings by the participants of the two dishes were mostly to the positive acceptance rating end of the hedonic scale with the majority of the participants indicating ratings ranging from “like slightly” to “like extremely” and few or none indicating the ratings of “dislike slightly” to “dislike extremely”. The consumer acceptance testing utilising the two sample dishes incorporating the new developed stock cube, i.e. savoury rice and pea soup, also found that the majority of the participants would use and would pay more for a stock cube with health benefits. During informal discussions with the participants on completion of the questionnaire incorporating the acceptance ratings it was also echoed that both the blue collar and white collar participants would be interested in using the product.

v. The consumer acceptance testing indicated that the dishes prepared with the new developed stock cube were to a larger extent accepted by those participants who were willing to make use of a product with possible health benefits and those who usually prepare the meals. The willingness to make use of a product with possible health benefits and acceptance of the two sample dishes was specifically a significant finding encountered within the white collar worker sub-group, while the acceptance of the two sample dishes among those who usually prepare the meals was a significant finding encountered within the blue collar worker sub-group.

6.2 Recommendations

The new developed stock cube has the potential to be used as the foundation for the development of a range of products in the same category. Stock cubes come in a variety of different flavours and the basic formulation of the new stock cube could easily be utilised to expand the flavour variants.

During the shelf-life testing phase, there was a darkening of the colour of the stock cube, as well as some rancidity development towards the end of the testing period. Though the darker colour

was not found to be an appreciable problem, improved packaging to better exclude air, as would be possible in a factory setting, and formulations utilising different flavour ingredients could overcome this. It would also be advisable to explore the addition of a stabilising ingredient to inhibit the development of rancidity, even though the new developed stock cube did not develop an unacceptable taste.

The results of the consumer acceptance testing indicated that the participants did not find the inclusion of CSL unacceptable in products that were well known to them. It is recommended that further development work be undertaken to explore other possible uses for CSL as an ingredient in food products, especially savoury food products, as the flavour of the CSL itself has an affinity with these types of products. This will expand its use as by-product and as functional ingredient in new food products.

It would be difficult to develop foods considering possible future health claims without undertaking clinical intervention trials to determine the efficacy to deliver the main functional ingredient to, for instance, blood levels. As no product health claims can be made at the present time, as to any benefits that CSL or products containing it may have, it could be advantageous to submit CSL or products containing CSL to clinical trials. With more consumers becoming aware of functional foods, it could present the food industry with a substantial area of growth when especially a by-product can be used. However, the provision of health claims for products will only be possible with substantial backing of scientific evidence. There is also scope for the inclusion of additional fortificants alongside the use of CSL that could be tailored to people with specific health needs as done in this study through the use of carotenoid rich red palm fat.

No analytical analysis of the micronutrients incorporated in the new stock cube formulation was conducted during the accelerated shelf-life stability testing. This is acknowledged as a limitation of the study. However, there is no reason to believe that the micronutrient content would greatly be reduced over the shelf-life testing time span, as minerals are inorganic substances that are not destroyed through processing (Robinson *et al*., 1993:150).

CHAPTER 7

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Addendum A: Amino acid profile of partially dried corn steep liquor

Table 2: Amino acid analysis of partially dried corn steep liquor

	Sample 3
Arginine	1.71
Glycine	0.34
Threonine	1.78
Tyrosine	0.42
Proline	4.10
Methionine	0.55
Valine	2.06
Phenylallanine	0.83
Isoleucine	0.89
Leucine	2.10
Histidine	0.61
Lysine	1.75
Methionine	0.55

Addendum B: Microbial analysis of partially dried corn steep liquor

Dry Sample - Original

Orac : 105 / mmole FE/g

SANAS ACCREDITED LABORATORY T0058

MICRO REPORT
REPORT 2

Swift MICRO LABORATORIES (Pty) Ltd
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E-mail: info@swift.co.za • Web: www.swift.co.za

PSS OILS
P.O. BOX 36534
CHEMPET
7441

ATTENTION: SHERIFFA MOHAMED

DATE: 09/10/07
DATE RECEIVED: 04/10/07
DATE TESTED: 04-09/10/07

REQ NO: CT 83258/07

SAMPLE TYPE: PRODUCTS
METHOD NO: SWJM 17; 23; 32; 35; 42; 45; 50; 53; 54

SAMPLE TYPE	TEST TYPE	DET TIME (HRS)	BACT. COUNT CFU/gram
Products			
CSL	TMA		500
	Escherichia coli	No Growth	No Growth
	Yeast & Mould		20 mould
	Clostridium perfringens	To follow	
	Salmonella		Absent/25g
	Staphylococcus aureus		No Growth
	Bacillus cereus		100
	Listeria	To follow/25g	
SG	TMA		9 600
	Escherichia coli	No Growth	No Growth
	Yeast & Mould		60 mould
	Clostridium perfringens	To follow	
	Salmonella		Absent/25g
	Staphylococcus aureus		No Growth
	Bacillus cereus		100
	Listeria	To follow/25g	

SEAN SWATTON
LABORATORY MANAGER

BRENDA DU TOIT
LABORATORY MANAGER

ANAS
ACCREDITED LABORATORY 1005B

MICRO REPORT
REPORT 2

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7441

ATTENTION: **SHERIFFA MOHAMED** Zuet

DATE: 14/11/07
DATE RECEIVED: 09/11/07
DATE TESTED: 10-14/11/07

REQ NO: CT 84444/07

PAGE 1 OF 1

SAMPLE TYPE: PRODUCT
METHOD NO: SWJM 17; 23; 32; 35; 42; 45; 50; 53; 54

SAMPLE TYPE	TEST TYPE	DET TIME (HRS)	BACT. COUNT CFU/gram
Product CSL	TMA		3 400
	Escherichia coli	No Growth	No Growth
	Yeast & Mould	To follow	
	Clostridium perfringens	To follow	
	Salmonella		Absent/25g
	Staphylococcus aureus		No Growth
	✓ *Bacillus cereus*		No Growth
	Listeria	To follow/25g	

SEAN SWATTON
LABORATORY MANAGER

BRENDA DU TOIT
LABORATORY MANAGER

- TMA = Total Microbial Activity/Total Viable Plate Count
- Limit of detection of Conventional Plate Count Methods = 10 CFU, unless otherwise specified.
- A test report relates only to the specific item submitted for testing. It furnishes or implies no guarantee whatsoever in respect of a similar item that has not been tested.
- Method numbers refer to in-house methods. Standard test method references available on request.
- Detection times only relevant to certain test methods, where Malthus Systems are applicable.
-

SANAS
ACCREDITED LABORATORY T0050

MICRO REPORT
ADDENDUM

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ATTENTION: SHERIFFA MOHAMED

DATE: 16/11/07
DATE RECEIVED: 09/11/07
DATE TESTED: 10-16/11/07

REQ NO: CT 84444/07

PAGE 1 OF 1

SAMPLE TYPE: PRODUCT
METHOD NO: SWJM 17; 23; 32; 35; 42; 45; 50; 53; 54

SAMPLE TYPE	TEST TYPE	DET TIME (HRS)	BACT. COUNT CFU/gram
Product			
CSL	TMA		3 400
	Escherichia coli	No Growth	No Growth
	Yeast & Mould		No Growth
	Clostridium perfringens		No Growth
	Salmonella		Absent/25g
	Staphylococcus aureus		No Growth
	Bacillus cereus		No Growth
	Listeria		No Growth

SEAN SWATTON
LABORATORY MANAGER

BRENDA DU TOIT
LABORATORY MANAGER

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- Method numbers refer to in-house methods. Standard test method references available on request.
- Detection times only relevant to certain test methods, where Malthus Systems are applicable.
- The test report shall not be reproduced except in full without written approval of Swift Micro Laboratories.

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T0055

MICRO REPORT
ADDENDUM

Swift
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Dry Sample

ATTENTION: **SHERIFFA MOHAMED**

DATE: 20/12/07
DATE RECEIVED: 13/12/07
DATE TESTED: 13-20/12/07

REQ NO: CT 85888/07

PAGE 1 OF 1

SAMPLE TYPE: **PRODUCT**
METHOD NO: **SWJM 17; 23; 32; 35; 42; 45; 50; 53; 54**

SAMPLE TYPE	TEST TYPE	DET TIME (HRS)	BACT. COUNT CFU/gram
Product			
CSL	TMA		20
10.12.07	*Escherichia coli*	No Growth	No Growth
	Yeast & Mould		10 yeast
	Clostridium perfringens		No Growth
	Salmonella		Absent/25g
	Staphylococcus aureus		No Growth
	Bacillus cereus		No Growth
	Listeria		Absent/25g

SEAN SWATTON
LABORATORY MANAGER

BRENDA DU TOIT
LABORATORY MANAGER

- TMA = Total Microbial Activity/Total Viable Plate Count
- Limit of detection of Conventional Plate Count Methods = 10 CFU, unless otherwise specified.
- A test report relates only to the specific item submitted for testing. It furnishes or implies no guarantee whatsoever, in respect of a similar item that has not been tested.
- Method numbers refer to in-house methods. Standard test method references available on request.
- Detection times only relevant to certain test methods, where Malthus Systems are applicable.
-

Addendum C: New stock cube bench formulation

Stock cube recipe formulation

Yield: 132 g
Portions: 12 x 11 g

Ingredients:

24 g Carotino shortening (CS35FV)
24 g Carotino margarine (CM45BV)
20 g Salt
6 g Corn steep liquor
16 g Fibre
38 g Flavour mix (Crown National, Beef Stew)

Method:

1. Bring 500ml water to a simmer (80°C) in a saucepan.
2. Place fat in a heat resistant bowl (Pyrex 500ml) and melt fat in simmering water.
3. Add dry ingredients to fat and mix well. Remove from waterbath.
4. Weight 11g portions into the compartments of an ice-cube tray.
5. Leave stock cubes to cool for 30 minutes before removing before removing from ice-cube tray.

Addendum D: Acceptance testing participant taste testing sheet (nine-point hedonic scale)

TASTE TESTING SHEET

Instructions:

Rinse you mouth with water before tasting each sample. Taste the sample you received and mark the box that most describes your opinion of the sample.

Sample no 1 (Savoury rice)

- ☐ Like extremely
- ☐ Like very much
- ☐ Like moderately
- ☐ Like slightly
- ☐ Neither like nor dislike
- ☐ Dislike slightly
- ☐ Dislike moderately
- ☐ Dislike very much
- ☐ Dislike extremely

Sample no 2 (Split pea soup)

- ☐ Like extremely
- ☐ Like very much
- ☐ Like moderately
- ☐ Like slightly
- ☐ Neither like nor dislike
- ☐ Dislike slightly
- ☐ Dislike moderately
- ☐ Dislike very much
- ☐ Dislike extremely

Addendum E: Acceptance testing participant informed concent form

INFORMED CONSENT

Title of Research Study

Development of a convenience stock cube with functional food characteristics

Principal Investigator
Mr Kevin Swarts (Tel. 083 763 9835)
Email: kmswarts@hotmail.com
M Tech: Consumer Science: Food and Nutrition
Department: Agricultural and Food Sciences, Faculty of Applied Sciences,
Cape Peninsula University of Technology: Cape Town campus

Research study supervisors
Ms I Venter and Prof S Benadé,
Cape Peninsula University of Technology: Cape Town campus

Introduction and Purpose of Study:

Chronic diseases such as heart disease (high blood cholesterol), diabetes (high blood sugar) and hypertension (high blood pressure) are on the increase in South Africa. These diseases all have a link to the foods that we eat and also do not eat in large enough quantities. Studies have found that South Africans typically do not eat enough fruits and vegetables. Fruits and vegetables are rich in a group of nutrients called "phytonutrients" that help prevent the development of these chronic diseases. A study found that stock cubes are regularly used by many South Africans. To help South Africans add phytonutrients to their diet, a new stock cube was developed that contain phytonutrients.

Procedure:

If you take part in this study, you will be asked to:

1. Taste food samples that were prepared using the new stock cube and regular stock cubes.
2. Indicate which food sample you prefer. The tasting of the food samples and indicating how much you like each food sample on the tasting sheet will take about 30 minutes of your time.

Benefits:

The information obtained from you will help to find out if the new stock cube if used in food is accepted by consumers. If it is accepted by the persons who have tasted the food samples a food company can produce the new stock cube which can then become available for use by consumers.

Risks:

The new stock cube has been tested for its safety to consume and there are no known risks at this time, but you should not take part in this study if you suffer from any food allergies and/or food intolerances. You will be given a list of the ingredients in the food samples you have to taste on which you can check for foods that you do not eat. The food samples are prepared in a normal kitchen. You therefore also do not have to take part if the food preparation does not meet the dietary rules of your religion.

Voluntary Participation/Withdrawal:

You do not have to take part in this study. If you do decide to take part, you may also change your mind and withdraw at any time.

Costs:

There will be no costs to you during this study. All the necessary utensils to taste the food and the tasting sheet and short questionnaire will be given to you.

Compensation:

You will not be paid to take part in this study.

Confidentiality:

Information from this study may be published, but your identity will be kept confidential in any publication.

Questions:

If you have any questions, please contact Kevin Swarts: Tel 083 763 9835

……

Consent to Participate in this Research Study:

I agree to take part in this study and I retain the right to withdraw from the study at any time. My signature below indicates that I have read this entire consent form, including the details of the study, the risks and benefits, and have had all my questions answered. This consent form will be kept on file and be available on the day which the research project is conducted.

_____
Signature of Study Subject Date:

_____
Printed name of Study Subject Date:

_____
Signature of Investigator Date:

Addendum F: Recipe formulation of savoury rice sample dish utilising new stock cube

Savoury rice recipe formulation

Yield: 758 g
Portions: 24 x 30 g

Ingredients:

1 l water
1 stock cube
220 g white rice

Method:

1. Bring water to a boil. Add stock cube and stir to dissolve.
2. Add rice and simmer for 20 minutes.
3. Raise heat and stir to evaporate any remaining water.

Addendum G: Recipe formulation of pea soup sample dish utilising new stock cube

Pea soup recipe formulation

Yield: 5771 g
Portions: 50 x 115 g

Ingredients:

660 g	split peas
3 l	water
270 g	carrots, grated
530 g	potatoes, grated
120 g	celery, grated
6	stock cubes
1.125 l	water

Method:

1. Soak split peas in 1.5 litres of water for one hour.

2. Dissolve six stock cubes in 1.125 litres of boiling water.

3. Place vegetables, stock, 1.5 liters water, split peas and soaking water in a pressure cooker. Bring pressure up to 30 kpa and cook for 15 minutes.

4. Remove pressure cooker from heat and allow to cool.

5. Purée soup in a food prosessor.

Addendum H: Acceptance testing participant demographic and stock cube usage questionnaire

				Office use
1)	What is your gender?	Male	1	
		Female	2	1
2)	In which age group do you fall?	24 years or younger	1	
		25-34 years	2	
		35-44 years	3	
		45-54 years	4	
		55-60 years	5	
		61 years and older	6	2
3)	With which racial group do you identify the most?	Black	1	
		Coloured	2	
		Indian	3	
		White	4	
		Other (Specify).....	5	3
4)	What is your home language?	English	1	
		Afrikaans	2	
		Xhosa	3	
		Other (Specify).....	4	4
5)	What is your marital status?	Married	1	
		Single	2	
		Widowed / separated / divorced	3	5
6)	How many persons are in your household? (Indicate the number of persons)			6
7)	How many persons in your household are older than 18 years? (Indicate the number of persons)			7
8)	How many persons in your household are 18 years or Younger? (Indicate the number of persons)			8
9)	What is your highest level of education?	Standard 5/Grade7 or lower	1	
		St 6/Gr 8 to St 8/Gr 10	2	
		St 9/Gr 11 to St 10/Gr 12	3	
		Certificate	4	
		Diploma	5	
		Degree	6	
		Postgraduate	7	9
10)	What do you do for a living? (Please print clearly)	_____		10
11)	In which group does your **monthly** income fall?	R 0 – R 1 000	1	
		R 1 001 – R 1 700	2	
		R 1 701 – R 3 000	3	
		R 3 001 – R 7 500	4	
		R 7 501 – R10 200	5	
		R10 2001 – R16 250	6	
		R16 251 – R22 500	7	
		R22 501 and higher	8	11

12) Does anybody in your household suffer from any chronic diseases? (chronic diseases include cardiovascular disease, cancer, diabetes, arthritis and obesity which are permanent or persisiting that require long term supervision, observation and care)

Yes	1
No	2

12

13) Do you normally prepare the meals consumed in your household?

Yes	1
No	2

13

If you answered "no" to question 13, do not answer question 14, 15 & 16.

14) How many stock cubes do you typically make use of in a month? (Indicate the number)

_____stock cubes / month

14

15) What type of dish you would typically prepare using stock cubes? (More than one dish may be indicated)

Soups	1
Stews	2
Sauces	3
Other (Specify).....	4

15

16) Which flavour of stock cube do you use?

Beef	1
Chilli Beef	2
Chicken	3
Oxtail	4
Mutton	5
Vegetable	6
Other (Specify).....	7

16

17) Would you make use of a stock cube with health benefits more often than your current usage?

Yes	1
No	2

17

18) Would you pay more for a stock cube with health benefits?

Yes	1
No	2

18

Thank you for your participation in the study

Addendum I: Microbial growth testing standard operation procedure

1. **Title**

   The determination of the Yeast and Mould count by means of the Pour Plate Technique

2. **Scope**

   Foods samples

3. **Background**

   Yeasts are microscopic fungi that grow as single cells while Moulds are fungi that grow in the form of multicellular filaments, called hyphae. Yeast and mould are a common contamination of food. Most often, yeast does not result in food poisoning but it causes food spoilage. Some species of yeast are opportunistic pathogens where they can cause infection in people with compromised immune systems. Both yeast and mould are chemoorganotrophs since they use organic compounds as a source of energy and do not require sunlight to grow. Yeast obtain their carbon mostly from hexose sugars such as glucose and fructose, or disaccharides such as sucrose and maltose while mould obtain theirs from complex biopolymers such as starch, cellulose and lignin. Yeasts are able to grow in foods with a low pH, (5.0 or lower) and in the presence of sugars, organic acids and other easily metabolized carbon sources, many molds can begin growing at 4 °C, the temperature within a typical refrigerator, or less. Moulds can produce mycotoxins, some of which can be harmful to humans. Mould spores can be carried by the wind, and hence can have easy entry to a food production facility. On the petri plates, yeast colonies will show blue-green or off-white colour with small-defined colonies while mould colonies tend to be larger and more diffused and are usually dark blue or greenish in colour.

4. Principle

A selective nutrient agar is used to suppress the growth of bacteria whilst encouraging the growth of a broad range of yeasts and moulds. Selectivity is usually based on low media pH (less than 5.0), low A_w values or the use of antibiotic supplements, e.g. chloramphenicol, gentamicin and oxytetrcycline.

5. Media and reagents

Ringers' solution

Potato Dextrose Agar (PDA)

6. Special apparatus

Colony counter

7. Test procedure

7.1 Aseptically weigh 10g of the sample into stomacher bag.

7.2 Pour 90ml of sterile Ringers' solution into the stomacher bag and allow to run at medium speed for 60 seconds and at high speed for 60 seconds.

7.3 Prepare dilutions of the sample (10^{-1} to 10^{-6}).

7.4 Carefully add 1.0 ml aliquots of the sample dilutions each into the base of sterile labelled Petri plates.

7.5 Pour the PDA, enough to allow for growth, into the Petri plates.

7.6 Mix carefully by rotating gently in an 8 sign direction.

7.7 Allow to solidify and incubate inverted at 25°C for 120 h (5 days).

7.8 Count all typical colonies.

**NB: Always pour the PDA into a clean Petri plate that serves as the control.

8. Safety precautions

8.1. Make use of Autoclave gloves when taking agar out of the Autoclave

8.2. Do not allow decimal dilutions to stand for longer than 10 minutes before pouring the plates.

8.3. Agar medium must be cooled to approximately 44 – 46°C. Do not use hot or overcooled lumpy agar medium.

8.4. Use Ringers' solution as a diluent.

8.5. Minimum opening of the Petri plate when adding sample to the dish and hold the pipette at a 45°C angle with a tip touching at the bottom of the Petri plate

8.6. Do not place sterile pipettes down on the bench top should this happen then make use of another sterile tip.

8.7. Flame the mouth of the Agar bottle when pouring the media and open the Petri dish lid high enough to pour in the media, do not put down the lid on the bench.

8.8. Mix carefully and thoroughly (making a swell in the direction of 8) and prevent the mixture from splashing over the edges of the Plate.

8.9. After solidification of the plates, place promptly in the incubator in the inverted position and the plates should reach incubation temperature within 2 hours.

8.10. Examine all doubtful objects with maximum magnification when counting the plate to distinguish between foreign objects and colonies.

9. Results and Interpretation

9.1 Counting of colonies

Make use of the magnifier and the counting grid/marker (guide plate) of the colony counter to obtain an accurate number of all typical colonies. Examine doubtful objects with maximum magnification when counting the plate to distinguish between foreign objects and colonies.

9.2. Determining the Yeast ad Mould counts

9.2.1 Take the average of the duplicate plates of the dilution used.

9.2.2 Multiply the average by the reciprocal of the dilution.

9.2.3 Record the results in a table as Cfu/ml or per gram of a sample.

10. References

The above method is based on the following references but does not necessary reflect the exact method

ICMSF(1989). Micro-organisms in foods 1 – their Significance and Methods of Enumeration. 2nd Edition. Blackwell Scientific Publications, University of Toronto Press. pp 157-159.

Pitt, J.I. and Hocking, A.D. (1985). Fungi and Food Spoilage, Academic Press.

Vanderzant, C. and Splittstoeseer, F. (Eds) (1992). Compndium of Methods for the Microbiological Examination of Foods. 3rd Edition, American Public Health Association. Washington.

1. Title

The determination of the total viable count (TVC) on the samples provided by means of the pour plate technique.

2. Scope

Food (including dairy) and animal feeds
Environmental samples
Sugars and syrups
Soft drinks
Not normally used for water

3. Background

The total viable count is also known as the aerobic plate count (APC), standard plate count, mesophilic count or total plate count. The test is based upon the assumption that each cell will form a visible colony when mixed with agar containing the appropriate nutrients. The total viable count can be used successfully to gauge sanitary quality, organoleptic acceptability, adherence to good manufacturing practices (GMP) and to a lesser extent an indicator of safety. The results can give the food processor information regarding the quality of raw materials, processing conditions, storage conditions and the handling of the product. The TVC can thus be a valuable tool to evaluable food quality. Microbiological specifications for various foods have been set by manufacturers and various organizations such as the WHO, SABS, and the ICMSF e.g. the TVC for pasteurized market milk is set at not more than 20 000 CFU/ml and the WHO allow a maximum APC of 1000/gram for dried instant products.

4. Principle

A non-selective nutrient medium is used to culture a wide range of micro-organisms, collectively referred to as the standard plate count, total viable count or total colony count. It is important to recognize that only those micro-organisms capable of forming colonies under the conditions of the test (time, temperature, nutrients etc) will be enumerated. This method also includes media and conditions used by certain sectors of the food industry (e.g. soft drinks manufacturers) for the enumeration of micro-organisms from particular products or ingredients.

5. Media and reagents

Ringers' solution
Plate Count Agar (PCA)

6. Special apparatus

Colony counter

7. Test procedure

1. Aseptically weigh 10g of product sample into a stomacher bag, add 90 ml sterile ringer's blank. Mix using the Homogenizer (Normal for 60sec, then High for 60sec); aseptically transfer the solution into the Scott bottle. This represents a 10^{-1} dilution. Add 1ml of this dilution to the next 9.0ml blank for a 10^{-2} dilution. Prepare diltutions up to 10^{-6}.
2. Carefully add 1.0ml aliquots of the sample and dilutions each into the base of sterile labeled Petri dishes. (The Petri dishes must be labeled on the base).
3. Pour approximately 15ml of pre-cooled sterile plate count agar (approximately 46^0C) into the base of the dish.
4. Plate count agar is poured into a Petri dish without the addition of sample. This serves as control check for the sterility of the growth media and Petri dish.
5. Mix carefully by rotating gently in a clockwise direction and then in a counterclockwise direction. Take care not to splash onto lid.
6. Allow to solidify, and then carefully invert the plates.
7. Incubate the plates in the inverted position in the incubator at 37^oC for 24 hours.

**NB: Always pour the PCA into a clean Petri plate that serves as the control.

8. Safety precautions

1. Agar medium must be cooled to approximately 44 – 46^oC. Do not pour with hot or overcooled lumpy agar medium.
2. Do not allow the decimal dilutions to stand for longer than 10 minutes before pouring the plates.
3. Make use of ¼ strength ringer's solution or 1.0% buffered peptone water as a diluent.
4. Minimum opening of the Petri dish lid when adding the sample to the dish. Hold the pipette at a 45^oC angle with the tip touching the bottom of the Petri plate.
5. Keep the pippete canister horizontal and flame the opening when removing a sterile pipette
6. Do not place the sterile pipette down on the bench top.
7. When pouring the media, flame the mouth of the container and open the petri dish lid just high enough to pour in the medium. Do not put the lid on the bench top.
8. Mix carefully and thoroughly taking care not to splash the mixture over the edge.
9. After solidification of the plate, place promptly in the incubator in the inverted position. The plates should reach incubation temperature within 2 hours.
10. Examine doubtful objects with maximum magnification when counting to distinguish between foreign objects and colonies.

9. Results and Interpretation

9.1 Counting of the colonies

. Select plates containing 25 – 250 colonies for counting.
. Make use of the colony counter to determine the number of colonies on the plates selected for counting. Use the magnifier and the counting grid (guide plate) of the colony counter to obtain an accurate count.

9.2. Determining the total viable count:

9.2.1 Multiply the number of colonies counter by the reciprocal of the dilution used.
9.2.2 Record the results in a table as CFU/ml or per gram. (CFU colony forming units)

10. References

The above method is based on the following references but does not necessary reflect the exact methods.

BS 4285: Microbiological examination of food and animal feeding stuffs. Part 5: 1981: Enumeration of micro-organisms: Colony count at 30^{o}C (surface plate technique)

Baylis, C.L. (2006).The catalogue of rapid microbiological methods (5th edition.). CCFRA Review No. 1.

Micro-organisms in foods 1: Their significance and methods of enumeration. 2nd edition, 1978. ICMSF. University of Toronto Press, pp 112-124

1. Title

Detection of Presumptive *E coli* species by means of Colony Count Technique

2. Scope

2.1 Food samples

2.2 Personnel and surface swabs

3. Introduction

Escherichia coli (E. coli) is a Gram negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most E. coli strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls (Vogt & Dippold 2005). The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 and by preventing the establishment of pathogenic bacteria within the intestine (Hudault et al., 2001; Reid et al., 2001). It should be noted that, E. coli are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination (Feng et al., 2002; Thompson & Andrea 2007). It can live on a wide variety of substrates and uses mixed-acid fermentation in anaerobic conditions to produce lactate, succinate, ethanol, acetate and carbon dioxide. Optimal growth of E. coli occurs at 37°C but some laboratory strains can multiply at temperatures of up to 49°C (Fotadar et al., 2005). E. coli was discovered by a German paediatrician and bacteriologist Theodor Escherich in 1885 (Feng et al., 2002) and is now classified as part of the Enterobacteriaceae family.

4. Principle

This technique relies on the use of bile salts or lauryl sulphate as selective agents and the fermentation of lactose to produce acid and or gas at 37^0C to indicate the presence of coliform organisms. Subsequent sub-culture into a second selective medium containing bile salts and then a third medium containing tryptophan are used as the diagnostic system to reveal the presence of *E.coli* by production of gas and indole at 44^0C, respectively. This procedure will not detect lactose negative, non gas-producing, or indole negative strains of *E.coli* and *s*trains which do not grow at 44^oC, e.g. E.coli 0157, will not be detected.

4. Equipments

4.1 Sterile test tubes (swabs)
4.2 Sterile McCartney bottles (product samples)
4.3 Weighing balance
4.4 Sterile utensils for sample handling (**s**patula, twizer and Scalper)
4.5 Permanent marker
4.6 Incubator at 37^0C
4.7 Incubator at 44^0C

5. Reagents

5.1 Tryptone Soy broth (enrichment)
5.2 Brilliant green lactose bile (BGLB) broth Tryptone Water (selective enrichment)
5.3 Gram stain reagents
5.4 Tryptone water
5.5 Kovacs reagent.
5.6 Durham's tube.

6. Test procedure

6.1 Aseptically weigh 1 g of sample into a sterile McCartney bottle using a spatula.

6.2 Transfer 5 ml of Tryptone Soy Broth (TSB) into the sample to prepare enrichment

6.3 Incubate at 37°C for 24 hrs.

6.4 Sterilely transfer 1 ml of the enriched sample into 5ml Brilliant green lactose bile broth (prepared with Durham's tubes). Then, transfer 0.1ml of the mixture into 5ml tryptone water

6.5 Then incubate both tubes at 44^0C for 24hrs.

6.6 After incubation check for bubbles, if present

6.7 Add few drops of Kovac's reagent and observe for positive results.

6.8 A red colour change will indicate positive reaction (Positive *E.coli*)

7. References

The above method is based on the following references but does not necessary reflect the exact method

Manual of Microbiological Methods for the food and drinks industry (2007). Campden & Chorleywood Food Research Association Group. 5th editions. C.L. Bsylis (Editor).

Vogt RL, Dippold L (2005). "*Escherichia coli O157:H7* outbreak associated with consumption of ground beef, June–July 2002". *Public Health Rep* **120** (2): 174–8. PMID 15842119.

Hudault S, Guignot J, Servin AL (July 2001). "*Escherichia coli* strains colonising the gastrointestinal tract protect germfree mice against *Salmonella typhimurium* infection". *Gut* **49** (1): 47–55. doi:10.1136/gut.49.1.47. PMID 11413110.

Reid G, Howard J, Gan BS (September 2001). "Can bacterial interference prevent infection?". *Trends Microbiol.* **9** (9): 424–8. doi:10.1016/S0966-842X(01)02132-1. PMID 11553454.

Feng P, Weagant S, Grant, M (2002-09-01). "Enumeration of *Escherichia coli* and the Coliform Bacteria". *Bacteriological Analytical Manual (8th ed.)*.

FDA/Center for Food Safety & Applied Nutrition. http://www.cfsan.fda.gov/~ebam/bam-4.html. Retrieved 2007-01-25.

Thompson, Andrea (2007-06-04). "E. coli Thrives in Beach Sands". Live Science. http://www.livescience.com/health/070604_beach_ecoli.html. Retrieved 2007-12-03.

Fotadar U, Zaveloff P, Terracio L (2005). "Growth of Escherichia coli at elevated temperatures". *J. Basic Microbiol.* **45** (5): 403–4. doi:10.1002/jobm.200410542. PMID 16187264.

1. Title

Enumeration of coliforms by means of Pour Plate Technique.

2. Scope:

Food samples

3. Background:

Coliforms are rod-shaped Gram-negative non-spore forming organisms. They are a group of aerobic, lactose-fermenting bacteria, of which *Escherichia coli* is the most important member. Coliforms can ferment lactose with the production of acid and gas when incubated at 35-37°C. They are abundant in the faeces of warm-blooded animals, but can also be found in the aquatic environment, in soil and on vegetation. They are easy to culture and their presence is used to indicate that other pathogenic organisms of fecal origin may be present. Most coliforms are not harmful, but since they arise from faeces, they are useful as a test of faecal contamination, and particularly as a test for water pollution.

4. Principle:

This technique relies on the use of crystal violet and bile salt as selective agents, and the fermentation of lactose as the diagnostic system.

5. Media and Reagents

Ringers' solution

Violet Red Blood Agar (VRBA)

6. Special apparatus

Colony counter

7. Test Procedure

7.1. Aseptically weigh 10g of the sample into stomacher bag.

7.2 . Pour 90ml of sterile Ringers' solution into the stomacher bag and allow to run at medium speed for 60 seconds and at high speed for 60 seconds.

7.3 . Prepare dilutions of the sample (10^{-1} to 10^{-6}) by first transferring 1 ml aliquot of the 100ml solution into the first dilution (10^{-2}). One ml of the sample solution represents a dilution of 10^{-1}.

7.4 Carefully add 1.0 ml aliquots of the sample dilutions each into the base of two labeled sterile Petri plates per dilution.

7.5 Pour the prepared sterile VRBA, enough to allow for growth, mix carefully by rotating gently in an 8 sign direction and allow to solidify.

7.6 Once the plates have solidified, pour an overlay of the VRBA into the plates and allow to solidify again.

7.7 Incubate the plates inverted at 37°C for 48 h (2 days).

7.8 Count all typical colonies. Coliform colonies are pink to purple in colour.

**NB: Always pour the VRBA into a clean Petri plate that will represent the control.

8. Safety precautions

8.1. Make use of Autoclave gloves when taking agar out of the Autoclave

8.2. Do not allow decimal dilutions to stand for longer than 10 minutes before pouring the plates.

8.3. Agar medium must be cooled to approximately 44 – 46°C. Do not use hot or overcooled lumpy agar medium.

8.4. Use Ringers' solution as a diluent.

8.5. Minimum opening of the Petri plate when adding sample to the dish and hold the pipette at a 45°C angle with a tip touching at the bottom of the Petri plate

8.6. Do not place sterile pipettes down on the bench top should this happen then make use of another sterile tip.

8.7. Flame the mouth of the Agar bottle when pouring the media and open the Petri dish lid high enough to pour in the media, do not put down the lid on the bench.

8.8. Mix carefully and thoroughly (making a swell in the direction of 8) and prevent the mixture from splashing over the edges of the Plate.

8.9. After solidification of the plates, place promptly in the incubator in the inverted position and the plates should reach incubation temperature within 2 hours.

9. Results and Interpretation

9.1. Counting of colonies

Make use of the magnifier and the counting grid/marker (guide plate) of the colony counter to obtain an accurate number of all typical colonies. Examine doubtful objects with maximum magnification when counting the plate to distinguish between foreign objects and colonies.

9.2. Determining the Coliforms count

9.2.1 Take the average of the duplicate plates of the dilution used.

9.2.2 Multiply the average by the reciprocal of the dilution.

9.2.3 Record the results in a table as presumptive coliforms per gram or ml of a sample.

10. Reference:

The above method is based on the following reference but does not necessarily reflect the exact method

BS 5763: Microbiological examination of food and animal feeding stuffs. Part 3: 1991. Enumeration of coliforms: most probable number technique.

Addendum J: Oxygen radical absorbance capacity analysis standard operation procedure

ANTIOXIDANT RESEARCH LABORATORY

STANDARD OPERATING PROCEDURE
FOR THE OXYGEN RADICAL ABSORBANCE CAPACITY ASSAY (ORAC)
VOLUME VI
NARU 6.04

Prepared by: _____ **Date:**

Reviewed by: _____ **Date:**

Approved by: _____ **Date:**

Rev. No.	Date Revised	Revision Summary
1.	2006/03/09	Original SOP

Contents

1.) Scope and Application

1.1) This fluorescence-based method was first developed by Glazer *et al.* in 1989 and is based on the discovery that the fluorescence of phycoerythrin (PE) changes with respect to time upon damage caused by peroxyl or hydroxyl radical attack. The ORAC assay as published by Cao *et al.* in 1993 differs from the Glazer method in that the reaction is driven to completion in the ORAC method while the Glazer method looks at what is reported as the flat period. The ORAC method was modified in 1995 by Prior *et al*. by automation using the COBAS FARA II centrifugal analyzer. The ORAC was further modified by Prior *et al*. in 2001 using a fluorescein salt instead of β-phycoerythrin. β-phycoerythrin (β-PE) is approximately 30% pure due to the isolation process and is inconsistent from lot to lot. It was determined that due to variable reactivity to peroxyl radicals, β-PE also produces an inconsistency from lot to lot. Also, β-PE can be photo-bleached after exposure to excitation light for a certain time. It has also been noticed that, due to non-specific protein binding, β-phycoerythrin interacts with polyphenols. β-phycoerythrin is also much more expensive than fluorescein. For these reasons fluorescein was used in place of β-PE.

The ORAC method is a simple, sensitive, and reliable way to measure the peroxyl radical absorbing capacity (with AAPH) of antioxidants and serum or other biological fluids. Hydroxyl radical absorbing capacity of serum has been performed successfully using the ORAC method with H_2O_2-Cu^{2+}. The method can be used with a fluorometry microplate reader using a 96-well plate to perform simultaneous kinetic analysis of many samples and to reduce the amount of sample required. Although the ORAC method was originally developed for plasma samples, it has been successfully applied in other fields of study.

2.) Summary of Method

2.1) The ORAC method is performed using a fluorescence spectrophotometer until zero fluorescence occurs. The results are reported as the ORAC value, which refers to the net protection area under the quenching curve of β-PE (fluorescein) in the presence of an antioxidant. The ORAC value is calculated by dividing the area under the sample curve by the area under the Trolox curve with both areas being corrected by subtracting the area under the blank curve. One ORAC unit is assigned as being the net protection area provided by 1 µM Trolox in final concentration. When the area under the curve for the sample is compared to the area under the curve for Trolox, the result is given in Trolox equivalents.

The ORAC method was adapted to be able to analyze the lipid-soluble antioxidant samples by introducing randomly methylated beta-cyclodextrin

(RMCD) in 50% acetone:water mixture. This mixture made lipid-soluble antioxidants soluble in phosphate buffer. The ORAC method is unique in its analysis in that it takes into account the inhibition time and degree of inhibition into a single quantity by measuring the area under the curve. The ORAC method is not affected by dilution.

3.) Health and Safety Warnings

3.1) Standard laboratory protective clothing and eye covering is required.

3.2) Dispose of all disposable materials (tubes, pipette tips etc.) into a Biohazard bag.

3.3) Wipe all work surfaces clean with 70% ethanol before and after completion of the procedure.

4.) Interference's

4.1) Since very slight amount of turbidity interfere with the determination, samples showing visible turbidity should be clarified by centrifugation. Alternately, samples may be filtered.

4.2) Plasma/serum samples that show signs of haemolysis should be discarded, unless otherwise requested.

5.) Cautions

5.1) Reagents and standards must be prepared fresh on the day of analysis unless otherwise stated.

5.2) Plasma/serum samples should be stored at -40°C until the day of analysis.

5.3) Keep plasma/serum samples on ice when not handling them during analysis.

5.4) Before pipetting each reagent, equilibrate the pipet tip and do not expose the pipet tip to the reagent(s) already in the well.

6.) Personnel Qualifications

6.1) Technicians should be trained at least one week in the method before initiating the procedure alone.

7.) Equipment and Chemicals Required

7.1) Equipment

Centrifuge 5810R
Balance (4 decimal places)
15ml Conical tubes with screw cap
Eppendorf pipettes and tips
Multichannel pipette and solution basis
Eppendorf tubes (1.5 mL)
Fluorescence plate reader
Black 96-well plate
pH meter
Media bottle (1 L, 50 mL, 100 mL, 250 mL)
Tube rotator
Sample concentrator
Gilson pipetting aid with 10 mL disposable serological pipettes

7.2) Chemicals

Hexane (SAARCHEM Cat nr.: 2868040 LC): Store at room temperature.

AWA solution: In a 1 L media bottle add the following:
700 mL Acetone (SAARCHEM Cat nr.: 1022040 LC)
295 mL Distilled water
5 mL Glacial acetic acid (SAARCHEM Cat Nr.: 1021000)
Store @ room temperature for up to one month.

*Phosphate buffer: 75mM, pH 7.4
Weigh 1.035 g sodium di-hydrogen orthophosphate-1-hydrate ($NaH_2PO_4.H_2O$) in a 100 mL media bottle, add 100 mL ddH_2O and mix until dissolved.
Weigh 1.335 g di-sodium hydrogen orthophosphate dehydrate ($Na_2HPO_4.2H_2O$ Merck Cat nr.: 5822880EM) in a 100 mL media bottle, add 100 mL ddH_2O and mix until dissolved.
Mix 18 mL of 1st solution with 82 mL of 2nd solution.
Check pH and adjust with either phosphate buffer if required. Store @ 4°C. Recheck pH before each assay.

*Fluorescein sodium salt (Sigma Cat nr.: F6377): Stock solution (Fridge).
Dissolve 0.0225 g $C_{20}H_{10}Na_2O_5$ in 50 mL Phosphate buffer. Store @ 4 °C in dark container which can be reused for 1 year.

*Peroxyl radical: AAPH (2,2'-Azobis (2-methylpropionamidine) dihydrochloride (Aldrich Cat nr.: 440914): 25 mg/mL.
Weigh 150 mg (0.150g) of AAPH (Reagent rack) into a 15 mL screw cap tube. Prepare fresh every day. Do not add any solution until step 9.5 of sample analysis.

PCA (70% Perchloric acid, SAARCHEM Cat nr.: 494612): 0.5M
In 250 mL Media bottle add the following:
195 mL distilled water
15 mL 70% perchloric acid
Store at room temperature for up to 6 months.

*Trolox (standard): 500 µM Stock solution.
Weigh 0.00625 g 6-Hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid (Aldrich Cat nr.: 238831) (Reagent rack) in 50 mL screw cap tube, add 50 mL phosphate buffer with Gilson pipetting aid, mix until dissolved. Check: Dilute 2x. This solution should give an absorbance of 0.670 ±0.015 at 289nm.

*Trolox (control): 250 µM Stock solution.
Weigh 0.00312 g 6-Hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid (Aldrich Cat nr.: 238831) (Reagent rack) in 50 mL screw cap tube, add 50 mL phosphate buffer with Gilson pipetting aid, mix until dissolved.
This solution should give an absorbance of 0.670±0.015 at 289nm.

* The chemicals required for each ORAC assay, all other chemicals depend on the type of sample.

8.) Sample Collection, Handling and Preservation

8.1) Serum/Plasma: For plasma sample, blood should be taken in a heparin- or EDTA containing tube. Blood must be centrifuged at 4000 rpm at 4°C for 30 minutes within an hour of collection. Treat the serum/plasma with equal volume 0.5M PCA in eppendorf tube. Centrifuge the mixture at 4000 rpm for 3 minutes and transfer the supernatant to new eppendorf tube. This can then be stored at -40°C and thawed at 4°C on the day of the analysis. Serum/plasma samples send by post should be stored in a sealed polystyrene container packed with dry ice in eppendorf tubes.

8.2) Tissue samples: Tissue samples should immediately be snap frozen in a cryovial in liquid nitrogen after biopsy taken and transferred to a -40°C freezer until used. Tissue samples by post should be stored in a sealed

polystyrene container packed with dry ice and should be delivered overnight to the Antioxidant Research Laboratory for analysis.

Frozen tissue samples are homogenized (1:4 for liver, kidney, lung and 1:2 for brain W/V) in 75 mM phosphate buffer (pH 7.0) using a Potter-Elvehjem Teflon pestle and glass tube tissue homogenizer (10 strokes). The homogenate is then centrifuged at 12 000 g for 10 min at 4^{o}C. The supernatant is then deproteinized with 0.25M PCA and centrifuged at 14 000 rpm for 15 min. The resultant supernatant is stored at -40^{o}C until analyzed.
NOTE: Preparation of the sample should be done on ice throughout the whole procedure.

8.3) Food: Weigh approximately 1.0 g (note the exact weight) of the sample in a 50 mL screw-cap tube. IMPORTANT: The sample weight of 1.0 g is not fixed and may need to be changed depending on the nature of the sample. This weight should be decreased if it is suspected that the sample contains high amount of antioxidants or increased if it is suspected to contain few antioxidants.

Lipophilic extract: Add 5 mL of hexane to the screw-cap tube and homogenise the sample for 30 seconds in the polytron. Extract the sample further by placing it on the tube rotator for 15 minutes. Centrifuge the sample at 4000 rpm for 3 minutes and transfer the supernatant to a 15 mL screw cap tube. Repeat the extraction with 5 mL fresh hexane (homogenising step not necessary). Combine the two hexane fractions in 15 mL crew cap tube. Protect from light. Dry the hexane extract with nitrogen in sample concentrator. Dissolve the residue in 1000 µl acetone and transfer to 1.5 mL eppendorf tube. Store @ -40°C until analysis.

Hydrophilic extract: Evaporate any residual hexane remaining in the original 50 mL screw-cap tube with nitrogen. Extract the residue twice with 5 mL of acetone/water/acetic acid (70:29:5:0.5, v/v/v, AWA) as explained for the lipophilic extract (homogenising step not necessary). Centrifuge after each extraction (4000 rpm for 3 minutes) and transfer supernatant each time to new tube. Combine the two fractions. Protect from light. Store @ -40 °C until analysis.

8.4 Wine, juices and herbal teas: Can be used directly after a suitable dilution. If there are obvious precipitates centrifuge the sample for 3 minutes at 4000 rpm and use supernatant in analysis.

8.5) Crude powder extract: Weigh approximately 50 mg (0.050 g) (note exact weight) of the sample in a 50 mL screw-cap tube. IMPORTANT: The sample weight of 50 mg is not fixed and may need to be changed

depending on the nature of the sample. This weight should be decreased if it is suspected that the sample contains high amount of antioxidants or increased if it is suspected to contain few antioxidants. Add 50 mL water or phosphate buffer with the Gilson pipetting aid. Mix until dissolved. Sonicate if necessary. Centrifuge at 4000 rpm for 5 minutes. Supernatant can be used directly after a suitable dilution.

8.6) All samples must be held in storage (-40^{o}C) for a period of 30 days after completion of the analysis before being discarded, unless otherwise requested.

9.) Sample Analysis

9.1) Switch the computer and the fluoroskan plate reader on. IMPORTANT: The fluoroskan should be switched on at least 30 minutes before starting the assay to allow the machine to reach a temperature of 37°C.
Click on “Fanie”.
Double click on “Fluoroskan”.
Click on “Open session” 
Select “ORAC”
Click on “Open”
Click on the “General” tab and on “layout” (see Appendix A for illustration). Ensure that the plate layout corresponds to the SOP layout.
Click on the “Measure” tab (see Appendix B for illustration). Ensure that the excitation wavelength is set at 485nm and the emission wavelength set at 530nm.

9.2) Trolox standard series: Take 6 Eppendorf tubes and mark them A-F. Add the amount of standard stock solution and diluents to each tube as described in the table below.

Tube	Standard concentration µM	Trolox stock solution µL	Phosphate Buffer µL	Well number
A	0	0	750	A1-A3
B	83	125	625	A4-A6
C	167	250	500	A7-A9
D	250	375	375	A10-A12
E	333	500	250	B1-3
F	417	625	125	B4-6

9.3) Trolox standard wells – add 12 µl of standard (tubes A-E) per well in the designated wells in a clear well plate.

9.4) Control wells – add 12 µl of the control to the wells (B7-B12).

9.5) Sample wells – add 12 µl of sample IN TRIPLICATE to the wells (C1-H12).

9.6) From the fluorescein stock solution add 10 µl in 2 mL phosphate buffer (in eppendorf tube) and then dilute 240 µl of this solution in 15 mL Phosphate buffer using a 15 mL screw cap tube. Add 138 µl of this solution with a multichannel pipette into each well of a black 96-microwell plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank_Ass Assay	Blank_Ass Assay	Blank_Ass Assay	Cal_0001 Assay 50 mg/L	Cal_0001 Assay 50 mg/L	Cal_0001 Assay 50 mg/L	Cal_0002 Assay 100 mg/L	Cal_0002 Assay 100 mg/L	Cal_0002 Assay 100 mg/L	Cal_0003 Assay 200 mg/L	Cal_0003 Assay 200 mg/L	Cal_0003 Assay 200 mg/L
B	Cal_0004 Assay 400 mg/L	Cal_0004 Assay 400 mg/L	Cal_0004 Assay 400 mg/L	Cal_0005 Assay 800 mg/L	Cal_0005 Assay 800 mg/L	Cal_0005 Assay 800 mg/L	Ctrl_0001 Assay	Ctrl_0001 Assay	Ctrl_0001 Assay	Ctrl_0002 Assay	Ctrl_0002 Assay	Ctrl_0002 Assay
C	Un_0001 Assay 1:1	Un_0001 Assay 1:1	Un_0001 Assay 1:1	Un_0002 Assay 1:1	Un_0002 Assay 1:1	Un_0002 Assay 1:1	Un_0003 Assay 1:1	Un_0003 Assay 1:1	Un_0003 Assay 1:1	Un_0004 Assay 1:1	Un_0004 Assay 1:1	Un_0004 Assay 1:1
D	Un_0005 Assay 1:1	Un_0005 Assay 1:1	Un_0005 Assay 1:1	Un_0006 Assay 1:1	Un_0006 Assay 1:1	Un_0006 Assay 1:1	Un_0007 Assay 1:1	Un_0007 Assay 1:1	Un_0007 Assay 1:1	Un_0008 Assay 1:1	Un_0008 Assay 1:1	Un_0008 Assay 1:1
E	Un_0009 Assay 1:1	Un_0009 Assay 1:1	Un_0009 Assay 1:1	Un_0010 Assay 1:1	Un_0010 Assay 1:1	Un_0010 Assay 1:1	Un_0011 Assay 1:1	Un_0011 Assay 1:1	Un_0011 Assay 1:1	Un_0012 Assay 1:1	Un_0012 Assay 1:1	Un_0012 Assay 1:1
F	Un_0013 Assay 1:1	Un_0013 Assay 1:1	Un_0013 Assay 1:1	Un_0014 Assay 1:1	Un_0014 Assay 1:1	Un_0014 Assay 1:1	Un_0015 Assay 1:1	Un_0015 Assay 1:1	Un_0015 Assay 1:1	Un_0016 Assay 1:1	Un_0016 Assay 1:1	Un_0016 Assay 1:1
G	Un_0017 Assay 1:1	Un_0017 Assay 1:1	Un_0017 Assay 1:1	Un_0018 Assay 1:1	Un_0018 Assay 1:1	Un_0018 Assay 1:1	Un_0019 Assay 1:1	Un_0019 Assay 1:1	Un_0019 Assay 1:1	Un_0020 Assay 1:1	Un_0020 Assay 1:1	Un_0020 Assay 1:1
H	Un_0021 Assay 1:1	Un_0021 Assay 1:1	Un_0021 Assay 1:1	Un_0022 Assay 1:1	Un_0022 Assay 1:1	Un_0022 Assay 1:1	Un_0023 Assay 1:1	Un_0023 Assay 1:1	Un_0023 Assay 1:1	Un_0024 Assay 1:1	Un_0024 Assay 1:1	Un_0024 Assay 1:1

Plate layout

9.5) Add 6 mL of the phosphate buffer to the AAPH weighed of earlier. Mix well until dissolved. Transfer, with a multichannel pipette, 50 µl of this solution to each well.

9.6) The final volume of the assay is 200 µl. It is not necessary to use all the wells on the plate at one time.

9.7) Insert the multiwell plate into the fluorometer by pressing  to open the door of the fluorometer. Place the plate with the A1 well facing top and left. Press  to close the door. Press 

to begin the analysis.

9.8) After the analysis is complete, discard the recorded standards/samples down the drain with plenty of distilled water.

9.9) Save the date by clicking on “sheet“ and “save sheet as” (See appendix C for illustration) to the flash drive (E) with a XLS extension.

10.) Data Analysis and Calculations

10.1) Start the Microsoft excel program.

10.2) Open the ORAC_gram (for solid samples) or ORAC_mL (for liquid samples) file in the "My documents/Accreditation/Completed SOP" directory.

10.3) When prompted if the links should be updated, select "Don't Update".

10.4) Click on "Edit", select "Links" and then select "Change Source".

10.5) Select the data sheet saved on the fluoroskan computer on the "E" drive and press "OK".

10.6) Click on the "Input" tab. Fill in the appropriate values for each well.

10.7) Click on the "Graph" tab. Fill in the values for a, b and c according to the equation given inside the standard graph.

10.8) Click on the "Results" tab. The ORAC values are calculated using a regression equation ($Y = a + bX + cX^2$) between Trolox concentration (Y) (μM) and the net area under the fluorescence decay curve (X). Data are expressed a micromoles of Trolox equivalents (TE) per liter or per milligram of sample (units, μmole Trolox/mL for blood or μmole Trolox/g wet weight for food). The area under the curve (AUC) is calculates as:
$AUC = (0.5 + f_2/f_1 + f_3/f_1 + f_4f_1 + \ldots + f_i/f_1) \times CT$
Where f_1 = initial fluorescence reading at cycle 1, f_i = fluorescence reading at cycle i, and CT = cycle time in minutes.

10.9) If any of the ORAC values falls outside of the range of the standard curve, repeat assay after sample was diluted. Pipette 100 μl of sample supernatant into a new eppendorf. Add 900 μl of phosphate buffer to effect a 10-fold dilution.

11.) Data Records and Management

11.1) All laboratory records must be maintained in the proper file designated for the method.

12.) Quality Control and Quality Assurance

12.1) Trolox is used as a control sample. The calculated value after analysis should be 15 ± 1.5 (13.5 to 16.5) μmol TE/mL.

13.) References

13.1) Prior RL, Hoang H, Gu L, Wu X, Bacchiocca M, Howard L, Hampsch-Woodill M, Huang D, Ou B, Jacob R. 2003. Assays for hydrophilic and lipophilic antioxidant capacity ($ORAC_{FL}$) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry,* 51:3273-3279.

13.2) Cao G, and Prior RL. 1998. Measurement of oxygen radical absorbance capacity in biological samples. *Methods in Enzymology*, 299:50-62.

By signing this document you have indicated that you are familiar with its contents and will adhere to these procedures:

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Addendum K: Total polyphenol analysis standard operation procedure

ANTIOXIDANT RESEARCH LABORATORY

STANDARD OPERATING PROCEDURE FOR MEASUREMENT OF PHENOLICS ON PLATE READER VOLUME VI NARU 6.01

Prepared by: _____ **Date:**

Reviewed by: _____ **Date:**

Approved by: _____ **Date:**

Rev. No.	Date Revised	Revision Summary
1.	2006/03/09	Original SOP

Contents

1.) Scope and Application

Polyphenols are a diverse class of chemicals produced by plants that is characterized by the presence of more than one phenol ring. It is subdivided into acetophenones, benzofurans, chromones, coumarins, flavonoids, phenolic acids, phenylacetic acids, phenylpropanoids, quinones, stilbenes and xanthones. Polyphenols in vegetables, fruits, and teas can prevent degenerative diseases including cancers through antioxidative action and/or the modulation of several protein functions. The number of natural polyphenols has been estimated to be over one million, because they generally occur as a glycoside, and the sugar species and binding forms show great variety. However, the bioactivity is attributed to aglycon structures, not to sugar moieties. The antioxidative potency is due mainly to the orthodiol structure in aglycons. Aglycons of polyphenols can be classified into polycyclic types such as flavonoids, anthraquinones and others, and simple polyphenols.

2.) Summary of Method

The analysis makes use of the Folin Ciocalteu reagent with gallic acid as the standard to measure total polyphenols in a sample.

3.) Health and Safety Warnings

3.1) Standard laboratory protective clothing and eye covering is required.

3.2) Dispose of all disposable materials (tubes, pipette tips etc.) into a Biohazard bag.

3.3) Wipe all work surfaces clean with 70% ethanol before and after completion of the procedure.

4.) Interference’s

4.1) Since very slight amount of turbidity interfere with the determination, samples showing visible turbidity should be clarified by centrifugation. Alternately, samples may be filtered.

4.2) Plasma/serum samples that show signs of hemolysis should be discarded unless otherwise requested.

5.) Cautions

5.1) Reagents and standards must be prepared fresh on the day of analysis unless otherwise stated.

5.2) Keep all plasma/serum on ice when not handling them during analysis.

5.3) Before pipetting each reagent, equilibrate the pipet tip and do not expose the pipet tip to the reagent(s) already in the container.

5.4) The accuracy of the results will be greatly affected by the accuracy of the volumetric measurements. Make sure that any volumetric flasks or pipets used for obtaining the appropriate dilutions are calibrated correctly.

6.) Personnel Qualifications

6.1) Technicians should be trained at least one week in the method before initiating the procedure alone.

7.) Equipment and Chemicals Required

7.1 Equipment

Centrifuge 5810R
Balance (4 decimal places)
15 mL and 50 mL Conical tubes with screw cap
Eppendorf pipettes and tips
12 Channel pipette and solution basis
Eppendorf tubes (1.5mL)
Media bottle (100mL, 1L)
96 well clear plate (visible range)
Multiskan plate reader
Polytron homogeniser
Gilson pipetting aid with 10 mL disposable serological pipettes

7.2) Chemicals

Ethanol (EtOH): 10%: In a 1L media bottle, add 100 mL of ethanol (Saarchem Cat Nr: 2233540LP) to 900 mL H_2O.

Folin Reagent: In a 15 mL screw cap tube, add 1 mL Folin-Ciocalteus phenol reagent (Merck Cat Nr: 109001) (Reagent rack) to 9 mL H_2O and mix well. Prepare fresh on day of analysis. This mix should have a bright yellow colour, if otherwise discard.

Sodium Carbonate: 7.5%: In a 100 mL media bottle, weigh 7.50 g Na_2CO_3 (Aldrich Cat Nr: 223530) (Reagent rack) and add 100 mL

H_2O. Mix until dissolved. Store @ room temperature for up to a month.

Gallic acid standard: In a 50 mL screw cap tube, dissolve 40 mg (0.040 g) gallic acid (Sigma Cat Nr: G7384) (Reagent rack) in 50 mL 10% Ethanol to give a stock standard concentration of 800mg/L. Prepare fresh. Use this solution as the stock standard. Check: When diluted 50x with 10% EtOH this solution should give an absorbance of 0.509 ±0.010 at 280 nm.

Control: In a 50 mL screw cap tube, dissolve 10 mg (0.010 g) gallic acid (Sigma Cat Nr: G7384) (Reagent rack) in 50 mL 10% EtOH (200mg/L). Prepare fresh. Use this solution as the control.
Check: When diluted 12.5x with 10% EtOH this solution should give an absorbance of 0.509±0.010 at 280 nm.

8.) Sample Collection, Handling and Preservation

8.1) Food: Weigh approximately 1.0 g (note the exact weight) of the sample in a 50 mL screw-cap tube. IMPORTANT: The sample weight of 1.0 g is not fixed and may need to be changed depending on the nature of the sample. This weight should be decreased if it is suspected that the sample contains high amount of antioxidants or increased if it is suspected to contain few antioxidants.
Add 5 mL of water to the screw-cap tube and homogenise the sample for 30 seconds in the polytron homogeniser. Extract the sample further by placing it on the tube rotator for 15 minutes. Centrifuge the sample at 4000 rpm for 3 minutes and transfer the supernatant to a 15 mL screw cap tube. Repeat the extraction with 5 mL fresh water (homogenising step not necessary). Combine the two water fractions in 15 mL crew cap tube. Protect from light. Store @ -40°C until analysis.

8.2) Wine, juices and herbal teas: Can be used directly after a suitable dilution. If there are obvious precipitates centrifuge the sample for 3 minutes at 4000 rpm and use supernatant in analysis.

8.3) Crude powder extract: Weigh approximately 50 mg (0.050 g) (note exact weight) of the sample in a 50 mL screw-cap tube. IMPORTANT: The sample weight of 50 mg is not fixed and may need to be changed depending on the nature of the sample. This weight should be decreased if it is suspected that the sample contains high amount of antioxidants or increased if it is suspected to contain few antioxidants. Add 50 mL water

or ethanol with the Gilson pipetting aid. Mix until dissolved. Sonicate if necessary. Centrifuge at 4000 rpm for 5 minutes. Supernatant can be used directly after a suitable dilution.

8.4) All samples must be held in storage (-40°C) for a period of 3 months after completion of the analysis before being discarded, unless otherwise requested.

9.) Sample Analysis

9.1) Switch the computer and the multiskan spectrum plate reader on.
IMPORTANT: This must be done 30 minutes before taking a reading.
Click on “Fanie”.
Double click on “SkanIt RE for MSS 2.2”.
Click on “OK”
Click on “close”
Click on “Open session” 
Select “Folin”
Click on “Open”
Under the Session Structure window (see solid circle in Appendix A for illustration). Click on “Plate Layout”. Ensure that the plate layout corresponds to the SOP layout.
Click on “Results” in the Session Structure window.

Click on “Save” in the Results window (see stipple dash circle in Appendix A for illustration). Change the filename (see line dash circle in Appendix A for illustration).

9.2) Preparation of standard series – Take 6 Eppendorf tubes and mark them A-F. Add the amount of standard stock solution and diluents to each tube as described in the table below.

Tube	Gallic acid stock solution (µl)	10% EtOH (µl)	Concentration (mg/L)	Well number
A	0	1000	0	A1-A3
B	25	975	20	A4-A6
C	62.5	937.5	50	A7-A9
D	125	875	100	A10-A12
E	312	688	250	B1-3
F	625	375	500	B4-6

9.3) Gallic acid standard wells – add 25 µl of standard (tubes A-E) per well in the designated wells in a clear well plate.

9.4) Control wells – add 25 µl of the control to the wells (B7-B12).

9.5) Sample wells – add 25 µl of sample IN TRIPLICATE to the wells (C1-H12).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank_Ass Assay	Blank_Ass Assay	Blank_Ass Assay	Cal_0001 Assay 50 mg/L	Cal_0001 Assay 50 mg/L	Cal_0001 Assay 50 mg/L	Cal_0002 Assay 100 mg/L	Cal_0002 Assay 100 mg/L	Cal_0002 Assay 100 mg/L	Cal_0003 Assay 200 mg/L	Cal_0003 Assay 200 mg/L	Cal_0003 Assay 200 mg/L
B	Cal_0004 Assay 400 mg/L	Cal_0004 Assay 400 mg/L	Cal_0004 Assay 400 mg/L	Cal_0005 Assay 800 mg/L	Cal_0005 Assay 800 mg/L	Cal_0005 Assay 800 mg/L	Ctrl_0001 Assay	Ctrl_0001 Assay	Ctrl_0001 Assay	Ctrl_0002 Assay	Ctrl_0002 Assay	Ctrl_0002 Assay
C	Un_0001 Assay 1:1	Un_0001 Assay 1:1	Un_0001 Assay 1:1	Un_0002 Assay 1:1	Un_0002 Assay 1:1	Un_0002 Assay 1:1	Un_0003 Assay 1:1	Un_0003 Assay 1:1	Un_0003 Assay 1:1	Un_0004 Assay 1:1	Un_0004 Assay 1:1	Un_0004 Assay 1:1
D	Un_0005 Assay 1:1	Un_0005 Assay 1:1	Un_0005 Assay 1:1	Un_0006 Assay 1:1	Un_0006 Assay 1:1	Un_0006 Assay 1:1	Un_0007 Assay 1:1	Un_0007 Assay 1:1	Un_0007 Assay 1:1	Un_0008 Assay 1:1	Un_0008 Assay 1:1	Un_0008 Assay 1:1
E	Un_0009 Assay 1:1	Un_0009 Assay 1:1	Un_0009 Assay 1:1	Un_0010 Assay 1:1	Un_0010 Assay 1:1	Un_0010 Assay 1:1	Un_0011 Assay 1:1	Un_0011 Assay 1:1	Un_0011 Assay 1:1	Un_0012 Assay 1:1	Un_0012 Assay 1:1	Un_0012 Assay 1:1
F	Un_0013 Assay 1:1	Un_0013 Assay 1:1	Un_0013 Assay 1:1	Un_0014 Assay 1:1	Un_0014 Assay 1:1	Un_0014 Assay 1:1	Un_0015 Assay 1:1	Un_0015 Assay 1:1	Un_0015 Assay 1:1	Un_0016 Assay 1:1	Un_0016 Assay 1:1	Un_0016 Assay 1:1
G	Un_0017 Assay 1:1	Un_0017 Assay 1:1	Un_0017 Assay 1:1	Un_0018 Assay 1:1	Un_0018 Assay 1:1	Un_0018 Assay 1:1	Un_0019 Assay 1:1	Un_0019 Assay 1:1	Un_0019 Assay 1:1	Un_0020 Assay 1:1	Un_0020 Assay 1:1	Un_0020 Assay 1:1
H	Un_0021 Assay 1:1	Un_0021 Assay 1:1	Un_0021 Assay 1:1	Un_0022 Assay 1:1	Un_0022 Assay 1:1	Un_0022 Assay 1:1	Un_0023 Assay 1:1	Un_0023 Assay 1:1	Un_0023 Assay 1:1	Un_0024 Assay 1:1	Un_0024 Assay 1:1	Un_0024 Assay 1:1

Plate Layout

9.6) Add 125 µl Folin reagent to each well using a multichannel pipette and a solution basis.

9.7) Wait for 5 minutes before adding 100µl Na_2CO_3 to each well using a multichannel pipette and a solution basis.

9.8) Leave the plate for 2 hours at room temperature before taking a reading.

9.9) Click on “Run plate out”.  Insert the 96-well plate with the A1 well positioned to the top and left.

9.8) Press “Start Execution”  . Press “Start”

9.10) After the analysis is complete, discard the recorded standards/samples down the drain with plenty of distilled water.

9.11) Transfer the data by copying the saved text file to the flash drive (E drive) using Microsoft explorer.

10.) Data Analysis and Calculations

10.1) Start the Microsoft excel program.

10.2) To open the data text file on the E drive: Click on “open”. Select the E drive. Change “Files of type” to “text files” and select the data text file saved from the multiskan spectrum computer. Click on “Next”, Select “space” and then click on “Finish”. Save the text file as a Microsoft Excel workbook by clicking on “File”, click on “Save as” and then change the “Files of type” to Microsoft Excel workbook. Click “save” Close the workbook.

10.3) Open the Phenolics_gram (for solid samples) or Phenolics_mL (for liquid samples) file in the “My documents/Accreditation/Completed SOP” directory.

10.4) When prompted if the links should be updated, select “Don’t Update”.

10.5) Click on “Edit”, select “Links” and then select “Change Source”.

10.6) Select the text data sheet that has been save as a Microsoft Excel workbook and press “OK”.

10.7) Click on the “Input” tab. Fill in the appropriate values for each well.

10.8) Click on the “Results” tab. Data are expressed as mg Gallic acid equivalents per liter or per gram of sample (units, mg Gallic acid/L for blood or mg Gallic acid/g wet weight for food).

10.9) If any of the total phenol values are greater than the standard curve range, dilute the samples by pipetting 100 µl of the sample supernatant into a new eppendorf and adding 900 µl of 10% ethanol to effect a 10-fold dilution. Now repeat the Phenolics assay with diluted sample.

11.) Data Records and Management

11.1) All laboratory records must be maintained in the proper file designated for the method.

12.) Quality Control and Quality Assurance

12.1) Gallic acid is used as a control sample. The calculated control sample after the assay should give a value of 200±20 mg/L (180 to 220 mg/L).

13.) References

Waterhouse, A. 2005. Folin-Ciocalteau method for total phenol in wine. Department of Viticulture & Enology, University of California, Davis.

By signing this document you have indicated that you are familiar with its contents and will adhere to these procedures:

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Addendum L: Duo-trio test sheet

TASTE TESTING SHEET
Shelf-life sensory testing

Instructions:

Rinse you mouth with water before tasting each sample. Taste the control sample, which is prepared from a fresh stock cube. After tasting sample A and then sample B, indicate which of the two samples is the same as the control sample. If no difference can be tasted between sample A and sample B, indicate so by marking the third option.

Please indicate your choice by marking the appropriate box.

- ☐ Sample A is the same as the control sample
- ☐ Sample B is the same as the control sample
- ☐ I cannot taste a difference between sample A or sample B

Addendum M: Peroxide value analysis standard operating procedure

DETERMINATION OF PEROXIDE VALUE

1. INTRODUCTION

Oils gradually react with oxygen at or near the double bonds to form peroxides. The oils dissolve in acetic - chloroform reagent and react with potassium iodide. The liberated iodine is titrated with sodium thiosulphate to obtain an index of the degree of peroxide formation in the oil. PV indicates the current status of the sample.

There are other ways to quantitate oxidative stability of a product viz peroxide value (PV) which determines oxygenated fatty compounds which are unstable and precursors of volatile short chain compounds recognisable by taste and smell as rancidity. These form when the double bonds of an unsaturated fatty acid react with chemically with oxygen. This is called Oxidative rancidity.

2. HEALTH AND SAFETY

2.1 HAZARDS

a) Sodium thiosulphate – may be harmful if swallowed or inhaled.

b) Hexane is highly flammable and volatile.

2.2 SAFETY REQUIREMENTS

c) Work in fume cupboard when boiling mixture

2.3 PRECAUTIONS

d) Avoid eye and skin contact with concentrated sodium thiosulphate

3. APPARATUS

e) Burette

f) 250 ml conical flasks

g) Flat evaporating dish

3.2 PREPARATION REQUIRED

a) None

3.3 MAINTENANCE

a) Ensure fume cupboard and glass beakers are clean before and after use

3.4 CALIBRATION

a) Calibrate balance using standard weights kit regularly

3.5 STORAGE

a) Standard procedures.

4. CHEMICALS

4.1 CHEMICALS REQUIRED

a) Hexane.

b) Glacial acetic acid (120ml)/chloroform (80ml) (ratio 3:2)

4.2 REAGENT PREPARATION

a) 0.01 M sodium thiosulphate ($Na_2S_2O_3$) - freshly prepared daily by dilution of a stock solution of 0.1 M $Na_2S_22O_3$.

b) Saturated potassium iodide (KI) solution (15 g KI: 10 g H_2O) – prepare fresh.

c) 1% starch solution – freshly prepared

4.3 STANDARDIZATION

a) None

4.4 DISPOSAL

a) Dispose organic chemicals in the organic waste container

5. METHOD

5.1 SAMPLING

a) According to type of sample and a specific sampling plan.

5.2 SAMPLE PREPARATION

a) Solid samples are homogenised by milling or grinding into a fine pulp.

5.3 TEST METHOD

5.3.1 Oil Extraction

* Work in fume cupboard at all times.

a) Homogenise sample in blender.

b). Extract oil from product using pure Hexane.

c) Filter oil residue through filter paper in fume cupboard into a flat evaporating dish.

d) Evaporate Hexane from oil in fume cupboard.

5.3.2 Titration

* Work in fume cupboard at all times.

a) Weigh 5 g of extracted oil into a 250 ml clean glass beaker.

b) Add 50 ml of the glacial acetic acid/chloroform reagent.

c) Add 1 ml of the potassium iodide solution.

d) Swirl for 5 seconds.

e) Allow the mixture to stand for 1 minute in a dark place.

f) Add 100 ml distilled water then starch and immediately titrate the iodine liberated, with sodium thiosulphate until clear.

g) A blank control sample should be prepared and treated in the same way.

h) Use fresh pure sunflower oil as a control.

Record the titration volume.

5.4 CALCULATIONS

$$PV = \frac{V \times M \times 1000}{m}$$

Where:

V = Titration Volume of thiosulphate solution (ml)

M = Molarity of thiosulphate solution (mol/L)

m = Mass of sample (g)

6. REFERENCES

AOAC 965.33 (2005) 18th Edition, Chapter 41, pg. 11-12

7. VERIFICATION

a) Using reference standard.

b) Error survey against accredited laboratory.