Micronisation of cowpeas: The effects on sensory quality, phenolic compounds and bioactive properties

By

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Submitted in partial fulfilment of the requirements for the degree

PhD

Food Science

In the

Department of Food Science
Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria
South Africa

April 2013

DECLARATION

I Eugénie Kayitesi hereby declare that the thesis submitted at the University of Pretoria for the award of PhD degree is my work and has not been submitted by me for a degree at any other university or institution of higher learning.

Signature:…….

Date:……

DEDICATION

To God almighty who has made all this possible.

To my family, with love, without you all I would not have succeeded.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to the following for their assistance with this research:

My supervisor Prof A. Minnaar and co-supervisors Prof H.L. de Kock and Prof K.G. Duodu for their excellent guidance, constructive criticism and encouragement throughout my study.

Prof Megan Bester and Miss. J.C. Serem of the Department of Anatomy (University of Pretoria) for their assistance with the biological assays.

Dr Marietjie Stander and Fletcher Hiten from the Central Analytical Facility, Stellenbosch University for their assistance with the UPLC/PDA/MS analyses.

Prof Taylor, Prof Buys and Dr Emmambux for their useful advice at different stages during this programme.

Thanks to staff and fellow students in the Department of Food Science of the University of Pretoria for their constructive discussions and sharing of ideas.

University of Pretoria and USAID funded Dry Grain Pulse CRSP for financially supporting my research.

Kigali Institute of Science and Technology for giving me the opportunity for further studies.

My family and friends for offering me support, encouragement and prayers during the course of this research.

ABSTRACT

Micronisation of cowpeas: The effects on sensory quality, phenolic compounds and bioactive properties

By

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Cowpeas (Vigna unguiculata L. Walp) are legumes recognised as a good source of proteins in developing countries. Cowpeas are mostly utilised as cooked whole seeds. This is often achieved only after boiling for up to 2 hours, resulting in high energy consumption and a long time for food preparation. Micronisation of pre-conditioned cowpeas (± 41 % moisture at 153 °C) reduces their cooking time. During micronisation, cowpea seeds are exposed to electromagnetic radiation with a wavelength range of 1.8 to 3.4µm. For biological materials, the penetration of infrared rays into the food material causes intermolecular vibration, this result in a rapid increase in temperature and water vapour pressure within the seed. Micronisation changes physico-chemical properties of cowpea seeds that may affect sensory properties of cooked cowpeas. Micronisation may also affect cowpea bioactive components such as phenolic compounds and hence their antioxidant properties and bioactive properties. This study aimed at (1) determining the effects of micronisation of pre-conditioned cowpeas on sensory properties of cooked cowpeas and (2) determining the effects of mironisation of pre-conditioned cowpeas on the phenolic compounds, radical scavenging properties and their protective effects against oxidative damage of biomolecules (i.e. low density lipoproteins (LDL), deoxyribonucleic acid (DNA) and red blood cells (RBC).

Micronisation significantly reduced cowpea cooking time by 28 to 49 %, depending on cowpea type. There were significant ($P<0.05$) increases in roasted aroma and flavour, mushy texture and splitting in all micronised samples. Bechuana white, a light brown cowpea type, was more mushy and split than others. There were significant decreases in firmness, mealiness and coarseness after micronisation for all cowpea types. Micronised cowpeas were darker (lower L^* values) than unmicronised cooked cowpeas. Darkening was more evident in light coloured than dark coloured cowpea types. Although micronisation reduces cowpea cooking time, it also affects sensory properties of cowpeas. This might have an influence on consumer acceptance of micronised cowpeas.

Twenty seven phenolic compounds were identified in the cowpea types studied: 6 phenolic acids, 14 flavonols and 7 flavan-3-ols. Protocatechuic acid, *p*-coumaric acid, 4-hydroxybenzoic acid and ferulic acid were the major phenolic acids in cowpeas. Catechin, catechin-3-*O*-glucoside, myricetin, rutin, quercetin and its mono and diglycosides were present in all cowpea types analysed. Dr Saunders (701.7−849.2 µg/g) (red in colour) and Glenda (571.9−708.1 µg/g) (dark brown in colour) contained the highest total phenolic contents, followed by Bechuana white (361.5−602.3 µg/g) (light brown in colour) and Blackeye (152.0−224.5 µg/g) (cream in colour). More of the flavonols were identified in red and dark brown compared to light brown and cream cowpea types. The red cowpea type contained all the dimers and oligomeric flavan-3-ol species identified in this study.

In all cowpea types, extracts from unmicronised (uncooked) cowpeas inhibited copper-induced LDL oxidation in a dose dependent manner. Extracts from all samples analysed exhibited protective effects against AAPH (2, 2'-azobis (2-amidinopropane) hydrochloride) induced RBC haemolysis and DNA damage. Extracts from more pigmented cowpeas, i.e. Dr Saunders, Glenda and Bechuana white, had significantly ($P<0.05$) higher levels of total phenolics, total flavonoids and radical scavenging properties than Blackeye (less pigmented). Extracts from more pigmented cowpeas also offered higher protection against AAPH-induced DNA and copper-induced LDL oxidation damage than extracts from less pigmented cowpeas. These results indicate protection of biomolecules e.g. DNA, LDL and RBC) from oxidative damage and have a potential to reduce oxidative stress implicated in the development of chronic diseases. This is because cowpea phenolic compounds possess the ability to reduce oxidative damage associated with development of these diseases.

Pigmented cowpea types may be recommended for health applications as they show more potential as source of antioxidants compared to the less pigmented cowpeas.

Extracts from micronised (uncooked and cooked) samples of Dr Saunders and Glenda cowpeas had significantly higher concentrations of ferulic acid and *p*-coumaric acid compared with unmicronised samples. *Para*-coumaric acid concentrations were higher in all micronised samples of Blackeye cowpeas than in unmicronised samples. The micronisation process could release cell wall bound ferulic acid and *p*-coumaric, increasing their concentrations in micronised samples. On the contrary, extracts from all micronised samples of Bechuana white and Glenda cowpeas had lower concentrations of catechin than unmicronised samples. Results indicated that total extractable phenolics were lower in micronised samples of cowpea types than unmicronised samples. Futhermore, extracts from micronised samples of all cowpea types showed less protective effect against LDL oxidation than extracts from unmicronised samples.

However, for most cowpea types there was no significant difference in total flavonoid contents (TFC) and Trolox equivalent antioxidant capacity (TEAC) values of cooked samples of both micronised and unmicronised. Micronisation did not affect the protective effects of cowpeas against AAPH-induced RBC haemolysis and oxidative DNA damage. Micronisation, followed by cooking, may have generated heat-induced antioxidants such as Maillard reaction products contributing to radical scavenging properties in micronised (cooked) cowpea samples. Though micronised samples had lower concentrations of some phenolic compounds and total extractable phenolics than unmicronised samples, micronised cowpea samples still exhibited radical scavenging properties and offered protective effects against oxidative damage of LDL, DNA and RBC and therefore may offer potential health benefits to consumers.

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1. INTRODUCTION

Cowpea (Vigna unguiculata L. Walp) is an annual, warm-season, herbaceous legume originating in Africa and widely grown in Africa, Latin America, Southeast Asia and in the southern United States (Davis, Oelke, Oplinger, Doll, Hanson and Putnam, 1991). The total worldwide production of cowpea is estimated at 3.3 million tonnes of dry grain, of which 64% is produced in Africa (IITA, 2012). Many poor families in Africa suffer from protein-energy malnutrition, especially among children, while diet-related chronic diseases have become a common phenomenon among urban African populations (FAO, 2006). Cowpeas are legumes recognised as a good source of protein and bioactive compounds e.g phenolic compounds known to exert health benefits (Cardador-Martinez, Loarca-Pina and Oomah, 2002).

Cowpeas are cooked before consumption to improve their nutritional quality but also to obtain a softer texture and develop desirable flavours (Aremu, 1991). Cooking of cowpea seeds is often achieved after boiling for up to 2 h and even longer (Akinyele, Onigbinde, Hussain and Omololu, 1986), resulting in high energy consumption and requiring a long time for food preparation. This is a challenge to rural consumers who cannot afford the energy costs, and for urban consumers whose busy life styles make convenience an important factor in food choices. Micronisation, an infrared heat treatment, applied to pre-conditioned legumes, has been reported to reduce the cooking time by 30–44% in cowpeas (Mwangwela, Waniska & Minnaar, 2006; Phadi, 2004). During micronisation, a food material is exposed to electromagnetic radiation with a wavelength range of 1.8–3.4 μm (Fasina, Tyler, Pickard and Zheng, 1999). When infrared waves strike the food material a part of the energy is absorbed. This causes the constituent molecules to vibrate at a frequency of 60 000–150 000 MHz (Fasina, Tyler, Pickard, Zheng and Wang 2001). During vibration, intermolecular friction among the molecules occurs and results in heat generation and a rise in water vapour pressure in the food (Sharma, 2009). The changes that occur within the seed during micronisation may affect the sensory quality of micronised cowpea samples. Sensory properties are an important element of quality characteristics of food products, determining consumer reaction and satisfaction (Abbott, 1999).

Mwangwela (2006) observed dark colour development and increased splitting of cowpeas upon micronisation. However no information is available on how the micronisation process affects the other sensory characteristics such as aroma and flavour of cooked cowpeas.

Mwangwela (2006) studied the effects of micronisation on physicochemical properties of cowpeas with emphasis on how their macro components, e.g. protein and starch behaved during micronisation. Micronisation however, may affect other cowpea components such as phenolic compounds known for their health promoting properties. Cai, Hettiarachchy and Jalaluddin (2003) reported the presence of phenolic acids, such as p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid, caffeic acid, cinnamic acid and ferulic acid in cowpeas. Ojwang, Dykes and Awika (2012) reported the presence of mono-, di-, and tri (acyl) glycosides of quercetin, myricetin and kaempferol in black, red, green, white, light brown, and golden brown cowpea phenotypes. Phenolic compounds are primarily of interest to human health because of their antioxidant activities. Reactive oxygen species (ROS) generated in biological systems through endogenous metabolic systems and exogenous sources are capable of oxidising cellular proteins, nucleic acids and lipids (Heim, Tagliaferro and Bobilya, 2002). ROS have been implicated in the mechanism of the damage of the red blood cells (RBCs) in sickle cell anemia (Tedesco, Russo, Nazzaro, Russo and Palumbo, 2001). ROS also contribute to cellular aging, mutagenesis, carcinogenesis and coronary heart diseases possibly through destabilisation of membranes, deoxyribonucleic acid (DNA) damage and oxidation of low density lipoprotein (LDL) (Heim et al., 2002). Phenolic compounds, as antioxidants, may limit these damages by acting directly on ROS or by stimulating endogenous defence systems (Scalbert, Manach, Morand and Rémésy, 2005). Siddhuraju and Becker reported that extracts from light brown and dark brown cowpeas exhibited up to 84 % hydroxyl radical scavenging activities. Even though cowpea seeds are increasingly consumed as human food, their potential health benefits remain largely unexplored. This study will evaluate sensory characteristics of micronised and cooked cowpeas, as well as determining the effects of micronisation on cowpea phenolic compounds and bioactive properties.

2. LITERATURE REVIEW

In this review, focus is placed on the effects of heat processing including micronisation on cooking quality and sensory properties of cowpeas. Phenolic compound structure and antioxidant activity relationship, as well as health promoting properties related to plant food phenolic compounds are also discussed. This review also discusses effects of heat processing on cowpea bioactive properties.

2.1 Importance and utilisation of cowpeas

Cowpeas grow well under a wide variety of soil conditions under both irrigated and non-irrigated regimes but respond more positively under irrigated conditions (Ehlers and Hall, 1997). The fact that cowpeas are more drought resistant than common beans make them an important crop in many developing parts of the world where irrigation is still a problem. Nigeria is the world's largest producer with 2.1 million tonnes, followed by Niger with 650 000 tonnes and Mali with 110 000 tonnes. The total production area of cowpeas is estimated at 9.8 million hectares; about 9.3 million hectares of these in West Africa (IITA, 2012). About two-thirds of the production and more than three-quarters of the area of production is spread over the Sudan Savanna and Sahelian zones of sub-Saharan Africa (Ehlers and Hall, 1997).

Cowpeas are an important source of energy and nutrients in developing countries of Africa, Latin America, and Asia. Cowpeas are a good source of dietary protein, which complements cereals, starchy roots and tubers (Phillips, McWatters, Chinnan, Hung, Beuchat, Sefa-Dedeh, Sakyi-Dawson, Ngoddy, Nnanyelugo, Enwere, Komey, Liu, Mensa-Wilmot, Nnanna, Okeke, Prinyawiwatkul and Saalia, 2003). They provide an alternative source of protein where meat and meat products are limited or expensive. The dry grain of cowpea is the principal product used for human consumption. Leaves (mostly in eastern Africa), pea seeds (the southern US and Senegal) and the green pods (humid regions of Asia and the Caribbean) are also consumed (Taiwo, Akanbi and Ajibola, 1997b). The crop is used for green manure in south eastern US and Australia (Taiwo *et al*., 1997b).

One of the major forms in which cowpea is utilised is as cooked whole seeds. Cooking of cowpea seeds, as with most legumes, is often achieved after boiling for up to 2 h and even more (Akinyele *et al.,* 1986) resulting in high energy costs an increased food preparation time. The changing socio-economic conditions and demand bring the pressure on the food industries to develop convenience foods which are easy to prepare, have excellent taste and flavour, nutritious and wholesome in nature with long shelf life (Sharma, 2009). To achieve these goals, various processing operations are employed and may considerably affect the overall quality of the processed foods (Sharma, 2009). Micronisation, an infrared heat treatment applied to pre-conditioned legumes has been reported to reduce the cooking time of legumes such as cowpeas (Mwangwela *et al.,* 2006) and lentils (Arntfield, Scanlon, Malcolmson, Watts, Ryland and Savoie, 1997).

2.2 Micronisation of pre-conditioned legumes

Foods can be heat processed in any of the three ways of transfer of heat i.e. by conduction, convection and radiation. In the case of radiation, heat is directly transferred from source to the object being heated. The electromagnetic spectrum encompasses radiation from short to long wavelength, i.e. gamma rays, x-rays, ultraviolet, visible light, infra-red, microwaves, and radio waves (Sharma, 2009). According to Fasina *et al.* (2001), micronisation involves short time exposure of a material to electromagnetic radiation in the wavelength region of 1.8 to3.4 µm (1800 to 3400 nm). The word "micronisation" is derived from the short wavelength unit "micron" used in this processing method (Sharma, 2009). This micron size wavelength has been found to be highly efficient in achieving high temperatures (750-930˚C) in a very short time (Sharma, 2009). When infrared waves strike the food material, a part of the energy is absorbed, making the constituent molecules vibrate (Sadeghi, Nikkhah, Fattah and Chamani, 2010). During the vibration, inter-molecular friction occurs among the molecules and results in heat generation and changes in molecular structures (Sadeghi *et al*., 2010). For example proteins are denatured and starch is gelatinised (Mwangwela, 2006). Micronisation of moisture-conditioned seeds has generally been reported to reduce the cooking time of legumes by 50 % at most (Arntfield *et al*., 1997). Arntfield *et al.* (1997) reported that softening of lentils upon cooking increased with increase in tempering moisture. Thus the micronisation process includes moisture conditioning of legumes grains to increase moisture content (Mwangwela *et al*., 2006).

2.2.1 Effect of micronisation of pre-conditioned of legumes on their seed components in relation to their cooking characteristics

Cooking time is one of the food quality criterions that are used to evaluate the quality of cooked whole cowpea seeds (Ehlers and Hall, 1997). Cooking time is defined as the time required for cowpeas to attain a level of softness that is acceptable for consumption (Proctor & Watts, 1987). Micronisation of moisture-conditioned seeds has been reported to reduce the cooking time of cowpeas (Mwangwela *et al*., 2006). Reduction in cooking time of legumes has been related to improved hydration observed in micronised legume seeds during cooking (Cenkowski and Sosulski, 1997; Arntfield, Scanlon, Malcolmson, Watts, Cenkowski, Ryland and Savoie, 2001). Arntfield *et al*. (2001) reported that cell walls of lentils had a more open microstructure after micronisation. In cowpeas, micronisation caused fissuring of seed coat, cotyledon, and parenchyma cell wall of micronised seeds (Mwangwela *et al*., 2006). These changes in physical structure improve the hydration rate which in turn resulted into cooked cowpeas with a softer texture (Mwangwela *et al*., 2006). Softening of legumes upon cooking has also been attributed to the disintegration of the middle lamella between cotyledon parenchyma cells, protein denaturation and starch gelatinisation within the cotyledon parenchyma cells (Sefa-Dedeh and Stanley 1979a).

Solubilisation of the middle lamella is one of the factors that contribute towards the softening of texture during the cooking of dry cowpea seeds (Sefa-Dedeh and Stanley, 1979a). Comparing scanning electron micrographs (SEM) of micronised cowpeas (41% moisture, 153 ºC) with untreated samples, Mwangwela (2006) found that micronised cowpeas showed marked cell separation along the middle lamella. Similar results were reported by Arntfield *et al*. (2001) who found that cotyledon cells of micronised lentils (33 % moisture, 138 ºC) separated along the cell wall upon fracture during sample preparation for SEM, an indication of middle lamella disintegration. In addition, Arntfield *et al.* (1997) reported a significant reduction in pectic substances for micronised (29 % moisture, 88 °C) lentils. The middle lamella holds the individual parenchyma cells together giving a fixed structure to the cotyledon (Mwangwela, 2006). When the middle lamella is solubilised, parenchyma cells are separated thereby contributing to a soft texture (Mwangwela, 2006).

Gelatinisation of starch during cooking of legumes is another important phenomenon that has a positive correlation with texture of cooked seeds (Arntfield *et al.,* 2001). Studies have shown that micronisation of moisture-conditioned seeds increases the level of enzyme-susceptible starch in cowpeas (Mwangwela, 2006) and lentils (Arntfield *et al.,* 1997). Increased starch susceptibility to α-amylase digestion is generally used as an indication of starch gelatinisation (Arntfield *et al*., 1997). This indicates that pre-gelatinisation of starch may occur more in micronised legume seeds than in unmicronised seeds. Micronised seeds would therefore require less time and energy for cooking compared to unmicronised seeds.

Denaturation of protein during micronisation is as a result of high temperatures at which the legume seeds are processed. The temperatures of seeds during micronisation are greater than 90 °C, which is above the denaturation temperature for most plant storage proteins including cowpeas (Horax, Hettiarachchy, Chen and Jalaluddin, 2004a). Protein denaturation has been studied by monitoring physicochemical properties such as nitrogen solubility. Reduction in nitrogen solubility of thermally treated legume seeds may result from the unfolding of protein molecules to expose hydrophobic sites leading to reduction in solubility (Zheng, Fasina, Sosulski and Tyler, 1998). Mwangwela (2006) reported significant reduction in nitrogen solubility in micronised cowpea seeds. Fasina *et al*. (2001) reported that reduction in protein solubility was accompanied by a reduction in cooking time of legumes.

2.3 Sensory quality of legumes

Legume-based foods are becoming increasingly important components of the human diet. However, their effective utilisation in the diet depends, to a large degree, upon consumer acceptance of the legume sensory characteristics. Sensory charateristics e.g. flavour and aroma of legumes cannot be analysed unless they are cooked. Legumes e.g. cowpeas are cooked to obtain a softer texture and develop desirable flavours for consumption (Aremu, 1991). Legumes are also cooked before consumption to inactivate anti-nutritive components and improve the nutritional quality (Balamaze, Muyonga, Kyamugangire, Kikafunda, Nakimbugwe and Ugen, 2008; Shiga, Cordenunsi and Lajolo, 2009). Sensory properties are an important element of quality characteristics of food products, determining consumer reaction and satisfaction (Abbott, 1999).

Many varieties of cowpea seeds exist and are known by their differences in sizes, shapes and colour shades. Regional preferences of cowpeas occur for the different seed size, texture and colour of seed coat. For example, Ghanaians are willing to pay a premium for Blackeye cowpeas, while Cameroonians would lower their prices for them (IITA, 2012). A study by Penicela (2010) reported that cowpea samples described as having a cooked cowpea flavor and sweet flavours were the most preferred to consumers. Differences in seed size, colour and textural properties of cooked dry beans have been reported to influence their acceptability (Sanzi Calvo and Attienza Del Rey 1999). Mkanda, Minnaar and De Kock (2007) found that large seeded bean varieties were the most preferred, whereas small-sized beans were the least preferred. In the same study bean varieties described with sweet, cooked bean flavours, and soft textures were most preferred by consumers. Apart from small seed size, bean varieties with a bitter taste, soapy and metallic mouth feel and hard texture were least preferred (Mkanda *et al*., 2007). Mkanda *et al.* (2007) also reported a positive correlation found between splitting, thickness of broths and seed coat peeling. Beans that had a high number of splits also had a high amount of seed coat peeling and thicker broth. The authors suggested that cooking might have induced leaching of solutes from the cotyledon into the cooking media, thickening the broths. Another study by Koehler, Chang, Scheier, and Burke (1987) found that bean cultivars described with sweet, nutty, and rubbery tastes were the most acceptable. The least acceptable cultivars beans, on the other hand, were judged to taste predominantly green, astringent, and with hay-like, flavour characteristics.

Chung and Hwang (1996) reported that beaniness was not a positive flavour characteristic in soya beans, and beaniness was negatively correlated with sweetness. In a study by Anyango, De Kock and Taylor, (2011) reported that cowpea-fortified porridges were associated with an intense cooked cowpea flavour, aroma and aftertaste. This beany flavour, described by the panellists as cowpea flavour, appeared to be the most important attribute characterising cowpea-fortified porridges. This may be a limiting factor for application of raw cowpeas based products e.g. flours into other food systems such as porridges. Many chemical compounds produced by lipid oxidation are associated with beany flavours (Scott 1975). Beany flavour is attributed to the action of lipoxygenase enzyme, which catalyses the formation of odorous carbonyl compounds (pentyl furans) from components containing *cis*-1, 4-pentadiene system (reviewed by Okaka and Potter, 1979).

Brown, Senn, Dollear and Goldblatt (1973) referred to raw Spanish peanuts as having green-beany flavours most likely caused by hexanal, octanal, and possibly nonanal, 2-pentenal and 2-nonenal. According to Vara-Ubol, Chambers and Chambers (2004) 3-methyl-1-butanol; pentanol; 1-octen-3-ol; 4-heptadienal; acetophenone; 1-octen-3-one; and 3-isopropyl-2 methoxypyrazine were described as beany compounds by a trained sensory panel. Other chemicals, such as hexanal, trans-2-hexenal, trans-2-octenal, and pentanal, commonly found in volatiles of soya products were not considered beany at any concentrations (Vara-Ubol *et al.,* 2004). However, these chemical compounds do not occur individually in volatile fractions of soya based products. Some evaluations of chemical combinations have resulted in aromas that were considered beany and described as more characteristic of actual beany aroma found in foods than individual chemicals (Ang and Boatwright, 2003).

Apart from beaniness, other undesirable sensory characteristics reported in legumes are bitterness and astringency. Legumes such as beans were perceived to be bitter (Mkanda *et al.,* 2007) and astringent (Koehler *et al*., 1887). These sensory attributes have been associated in part with polyphenols in plant foods (Lesschaeve and Noble, 2005). Bitterness and astringency are well known for eliciting negative consumer reactions when present at high intensity (Lesschaeve and Noble, 2005). Penicela (2010) reported that cowpea samples that tasted bitter were the least acceptable to consumers. Simple phenols like phenolic acids are associated with sensory characteristics such as sweetness, sourness, bitterness and astringency (Peleg and Noble, 1995). Peleg and Noble (1995) studied the sensory properties of phenolic acids (benzoic acid derivatives). These included salicylic acid (2-hydroxy benzoic acid), *m*-hydroxy benzoic acid (3-hydroxy benzoic acid), gentisic acid (2, 5-hydroxy benzoic acid), protocatechuic acid (3, 4-hydroxy benzoic acid) and gallic acid (3, 4, 5-trihydroxy benzoic acid) in water. Each of these compounds elicited multiple sensations including sweetness, sourness, bitterness and astringency. However the degree of perception of these sensory characteristics varied in each of the phenolic acids. Phenolic acids such as gallic acid, protocatechuic acid, ferulic acid sinapic acid, 4-hydroxybenzoic acid and *p*-coumaric acid were identified in cowpeas (Cai *et al.,* 2003).

Higher-molecular-weight polymers such as tannins are more likely to be astringent (Nobel, 1994). Astringency, defined as a drying or puckering mouth feel detectable throughout the oral cavity, may be due to a complexing reaction between dietary polyphenols and proteins of the mouth and saliva (Nobel, 1994). The tactile sensations of astringency are elicited primarily by flavonoid phenols, including flavanols and flavonols (Nobel, 1994). In fruits and beverages, the tactile sensations of astringency are elicited primarily by flavonoid phenols, including flavanols and flavonols. Of these, the flavan-3-ol monomers (catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate) and their oligomers and polymers, which are called proanthocyanidins or condensed tannins, are the most abundant in wine and tea. Both procyanidins (polymers of epicatechin and catechin) and prodelphinidins (polymers of epigallocatechin) have been detected in grapes (Czochanska, Foo and Porter, 1979). Ojwang, Dykes and Awika (2012) reported that flavon-3-ols e.g. mono and diglycosides, of quercetin and anthocyanins, such as delphinidins and cyanidin 3-O-glucosides were the predominant flavonoids in cowpeas and therefore depending on their concentration may elicit bitterness and astringency in cowpea based products.

Chemical structure of phenolic compounds has been reported to contribute to flavour perception of both bitterness and astringency (Lesschaeve and Noble, 2005). For example, variations in proanthocyanidin composition, such as polymer size, extent of galloylation, and formation of derivatives, affect both bitterness and astringency (Lesschaeve and Noble, 2005). Astringency increases and bitterness decreases with the degree of polymerisation. In addition, small differences in flavonoid configurations can produce significant differences in sensory properties. Epicatechin is more bitter and astringent than its chiral isomer catechin (Thorngate and Noble, 1995). Similarly, the bond location and the identity of the monomeric units influence the astringency and bitterness of synthesised dimers and trimers (Peleg, Gacon, Schlich and Noble, 1999. Although the trimers and two of the dimers were more astringent than the monomers, dimer B6 (catechin-4, 6-catechin) was more bitter and astringent than dimer B3 (catechin-4, 8-catechin) and dimer B4 (catechin-4, 8-epicatechin) (Peleg *et al*., 1999). Vidal, Cheynier, Waters and Noble (2003) studied the effects of tannin structure on bitterness and astringency. They showed that modifying the molecular structure by introducing an ethyl bridge decreased astringency but increased bitterness.

2.3.1 Effects of micronisation on sensory properties of legumes

Micronisation, a high-intensity infrared-heat process may result in development of new flavour development via Maillard reaction, caramelisation, and lipid–Maillard product interactions when heating cowpeas as described by Sacchetti, Pinnavaia, Guidoline and Rosa (2004) for other legumes. This may change the inherent sensory characteristics of cowpeas and can have an influence on consumer acceptability of micronised cowpea samples.

Dark colour development in micronised cowpea was reported by Mwangwela (2006). Infra-red heat-treated lentils were also found to be darker than raw lentils (Arntfield *et al*., 2001). The change in colour on the surface of ground oat groats and ground oat flakes was significant for the micronised oat groats (Cenkowski, Ames and Muir, 2006). Generally, the a and b colour values for the whole and ground oat groats increased after micronisation indicating toasting and the enhancement of the red and yellow colour, respectively. Arntfield *et al*. (2001) and Phadi (2004) suggested that the browning observed in moisture-conditioned and micronised lentils and cowpeas at higher temperature (>160 °C) was possibly due to Maillard browning reaction.

The Maillard reaction is a non-enzymatic reaction that takes place on heating and is related to aroma, flavour and colour development in food products such as roasted coffee and cocoa beans and many baked cereal products (Martins, Jongen and Van Boekel, 2001). Mwangwela (2006) reported a significant increase in splitting of micronised cowpea seeds compared with untreated samples. Extensive splitting of micronised cowpeas during cooking could be as a result of infrared heat-induced cracking and microscopic fissuring of cowpea seed coats, cotyledon and cell walls (Mwangwela, 2006).

2.3.2 Descriptive sensory analysis

Different methods are used to study the sensory characteristicts of food products including legume based foods. Descriptive sensory analyses are distinguished from other sensory testing methods in that they seek to profile a product on all of its perceived sensory characteristics (Murray, Delahunty and Baxter, 2001). Descriptive sensory tests involve the detection and description of both the qualitative and quantitative sensory components of a product by trained panels of judges (Meilgaard, Civille and Carr, 1991).

The qualitative aspects of a product include all aroma, appearance, flavour, texture, aftertaste and sound properties of a product, which distinguish it from others. Sensory judges then quantify these product aspects in order to facilitate description of the perceived product attributes (Murray *et al.*, 2001). Descriptive sensory tests are useful in analysing relationships between descriptive sensory and instrumental or consumer preference measurements of food products (Murray *et al*., 2001). Descriptive sensory analyses are also used for quality control, for the comparison of product prototypes to understand consumer responses in relation to products (Gacula, 1997). It may also be used to track product changes over time with respect to understanding shelf life and packaging effects, to investigate the effects of ingredients or processing variables on the final sensory quality of a product (Murray *et al*., 2001).

Descriptive sensory analysis requires a panel with some degree of training or orientation. Panellists are also required to have a reasonable level of sensory acuity (Murray *et al*., 2001). To achieve this panellists are screened and those selected should perform well in a variety of tests to achieve the study objectives. Many studies have discussed the selection of sensory panellists, which screening tests to perform and how panellist performance may be monitored (Issanchou and Lesschaeve, 1995; Lawless and Heymann, 1998; and Piggott and Canaway., 1981). The training phase of descriptive sensory analysis techniques begins with the development of a common language which comprehensively and accurately describes the product attributes (Murray *et al*., 2001). For example in a study by Penicela (2010) panelists developed a lexicon for attributes describing sensory properties of cooked whole and dehulled cowpeas (Table 2.1). At this stage of analysis, a new panel develops the sensory language themselves with input from an experienced panel leader. An existing language may also be adopted from the literature to ensure that full definitions and standards are available to demonstrate the sensory attributes (Hunter and McEwan, 1998).

During term selection, the panel is exposed to a wide range of products in the category under test. Selecting the descriptors for inclusion in the final language is generally a consensus procedure. Murray (1999) suggested that a less subjective method for descriptor selection could be to quantitatively rate the appropriateness of different terms that represent similar sensory concepts.

Table 2.1 Terms developed by a descriptive panel for cooked whole and dehulled cowpeas (Penicela, 2010)

Aroma	Flavour/after taste	Texture
Grassy aroma	Cooked cowpea flavour	Firmness
Cooked cowpea aroma	Raw cowpea flavour	Chewy/rubbery texture
Raw cowpea aroma	Sweet taste	Mushiness
Meaty aroma	Boiled egg York flavour	
Earth aroma	Bitterness	
Nutty aroma		
Spicy aroma		
Dry cooked maize aroma		

The final descriptive language should be precisely defined and contain enough terms to include all attributes likely to be encountered, yet should not be so large as to be cumbersome in use (Piggott and Canaway, 1981). After selection of terms, the panel is trained to use a common ‘‘frame of reference’’ to illustrate/define the product attributes and their intensity in the products under test (Murray *et al*., 2001). This is achieved by exposing the panel to the range of products in the category under test. A common ‘‘frame of reference’’ has been defined as ‘‘the background information and reference points (frame of comparison) that assessors mentally refer to when evaluating products (Munoz and Civille, 1998). Many authors have recommended the use of reference standards to achieve concept alignment in sensory panels (Murray and Delahunty, 2000; Nielson and Zannoni, 1998; Rainey, 1986), which are both quantitative as well as qualitative (Meilgaard *et al*., 1991). Reference standards have been defined as ‘‘any chemical, ingredient, spice or product’’ (Rainey, 1986). This definition could be extended to include non-food related materials which demonstrate the sensory stimuli. However, there is some evidence that for complex attributes, assessors may be unable to generalise sensory concepts to products during evaluation (Murray and Delahunty, 2000).

2.4 Phenolic compounds in legumes

Generally, legumes play an important role in the traditional diets of many regions throughout the world (Phillips *et al*., 2003). They are consumed not only because they are excellent sources of protein and a variety of micronutrients but also bioactive compounds such as polyphenols (Anderson, Smith, and Washnock, 1999). Polyphenolic constituents of various legume seeds have been reported to contain potential medicinal properties (Mazur, Duke, Wähälä, Rasku, and Adlercreutz, 1998 and Shahidi, Chavan, Naczk, and Amarowicz, 2001). Dietary phenolic compounds exert their health benefits by various biological effects such as free radical scavenging, reducing potential (Rice-Evans, Miller, and Paganga, 1996), chain breaking (Reiners, Clift and Mathieu, 1999) as well as alteration of signal transduction pathways (Cheng, Dai, Zhou, Yang and Liu, 2007).

Phenolic compounds are defined as chemical substances that consist of an aromatic ring bearing a hydroxyl substituent including functional derivatives such as esters, methyl ethers and glycosides (Harborne, 1989; Harborne, Baxter, and Moss, 1999; Shahidi and Naczk, 1995). They are secondary plant metabolite derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants (Randhir, Lin, and Shetty, 2004). Phenolic compounds are one of the most widely occurring groups of phytochemicals, and are of considerable physiological and morphological importance in plants. These compounds play an important role in growth and reproduction, providing protection against pathogens and predators (Bravo, 1998), besides contributing towards the colour and sensory characteristics of plant foods (Alasalvar, Grigor, Zhang, Quantick, and Shahidi, 2001). Plant phenolics are universally distributed through-out the plant but their concentration varies within the different tissues (Harborne, 1989). Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerised compounds (Bravo, 1998). Total phenolic content (TPC) in leguminous seeds is one of the main parameters indicating the potential antioxidant capacity of seeds (Amarowicz, Troszynska, Barylko-Pikielna, and Shahidi, 2004). Siddhuraju and Becker (2007) reported 13.3 and 16. 4 % total phenolics in light and dark brown cowpea types respectively. Cai *et al.* (2003) reported 0.04 - 0.40 % in 17 cowpea types analysed (Table 2.2). The reported values for the TPC in cowpeas varies due to factors such as the assay method and condition e.g. type of extraction solvent and type of cowpea sample (Nwokolo and Ilechukwu, 1996).

Table 2.2 Total phenolic and phenolic acid contents of 17 varieties of cowpeas (mg per 100 g) (Cai *et al.*, 2003)

Variety name	PCA	HBA	CA	CMA	FA	DMBA	CNA	TPC
MS Silver	0.8	0.4	0.3	1.0	1.3	trace	trace	143.6
AR 91-135	0.7	0.7	trace	0.4	0.7	0.3	trace	78.4
AR 91-245	trace	Trace	0.6	trace	trace	trace	trace	34.6
AR 95-105	1.0	1.5	0.3	1.2	1.6	trace	trace	42.7
AR 95-104	0.2	2.8	0.6	0.9	1.2	trace	trace	326.2
AR 92-552	1.3	2.2	0.4	1.3	2.5	trace	trace	68.4
CT Pinkeye	2.5	3.1	0.6	0.9	2.4	1.6	0.6	38.1
Early Scarlet	2.0	2.3	0.5	2.8	2.4	1.9	trace	109.1
Arkansas Blackeye	trace	1.4	trace	0.3	0.6	0.3	trace	306.8
AR 91-333	1.0	Trace	0.4	0.8	1.3	0.4	trace	61.8
Excel	0.6	2.7	0.4	0.7	1.1	2.1	trace	103.5
AR 91-285	0.4	2.2	0.4	0.9	1.8	0.4	0.3	151.9
AR 92-574	2.8	3.1	1.0	3.4	2.5	1.1	0.3	92.8
Early Acre	0.4	3.0	0.6	0.9	1.8	0.3	trace	59.9
Texas Pinkeye	2.9	2.2	0.1	2.4	2.1	1.3	1.2	131.2
Black Crowder	1.0	0.6	0.6	1.1	trace	trace	trace	376.6
Louisiana Purple hull	3.6	3.5	0.8	4.2	6.2	2.5	1.0	347.7

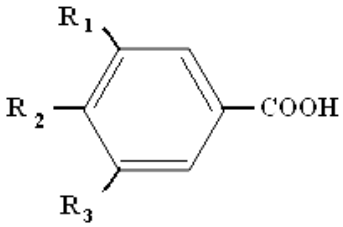
All values are dry basis. PCA, protocatechuic acid; HBA, *p*-hydroxybenzoic acid; CA, caffeic acid; CMA, *p*-coumaric acid; FA, ferulic acid; DMBA, 2,4-dimethoxybenzoic acid; CNA, cinnamic acid; trace (<0.3 mg/100 g) ; TPC, total phenolic content.

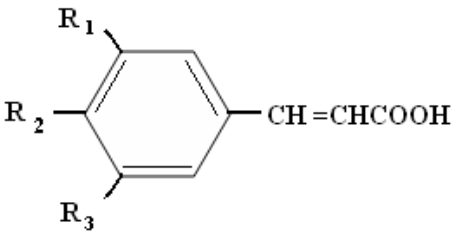
Cowpea seeds with dark-coloured seed coat (for example, black, purple or dark red) contain high levels of phenolic compounds such as flavonoids which lead to high antioxidant activity (Nzaramba, Hale, Scheuring, and Miller, 2005). In legumes, including cowpeas, the main phenolic compounds are phenolic acids, flavonoids and tannins.

2.4.1 Phenolic acids

Phenolic acids are distinguished according to two underlying structural compounds: the cinnamic and benzoic acids derivatives (Figure 2.1). Hydroxyl (OH) and methoxyl (OCH_3) groups are substituted at various positions on the aromatic ring (Madhujith and Shahidi, 2005). Common benzoic acids are vanillic, p-hydroxybenzoic, syringic, protocatechuic, salicylic and gallic acids. They may be present in soluble form, conjugated with sugars or organic acids. The four common cinnamic acid derivatives are: *p*-coumaric, caffeic, ferulic and sinapic acids (Shahidi and Naczk, 1995). Cinnamic acid derivatives are mainly found in several conjugated forms, mainly esters of hydroxyl acids such as tartaric acid and sugar derivatives (Shahidi and Naczk, 1995).

Benzoic acid derivatives **Cinnamic acid derivatives**





Acid	R1	R2	R3
p- Hydroxybenzoic	H	OH	H
Protocatechuic	OH	OH	H
Vanillic	OCH_3	OH	H
Syringic	OCH_3	OH	OCH_3
Gallic	OH	OH	OH

Acid	R1	R2	R3
p- Coumaric	H	OH	H
Caffeic	OH	OH	H
Ferulic	OCH_3	OH	H
Sinapic	OCH_3	OH	OCH_3

Figure 2.1 Phenolic acids of benzoic and cinnamic acid families (Shahidi and Naczk, 2004)

Cai *et al*. (2003) analysed 17 cowpea varieties and identified seven phenolic acids namely protocatechuic acid, *p*-hydroxybenzoic acid, caffeic acid, *p*coumaric acid, ferulic acid, 2, 4-dimethoxybenzoic acid, and cinnamic acid. They also observed that protocatechuic acid and ferulic acid were the most abundant bound phenolic acids (Table 2.2). Troszyńska, Amarowicz, Lamparski, Wolejszo and Barylko-Pikielna (2005) reported the presence of vanillic, caffeic, ferulic, *p*-coumaric and sinapic acids in pea cotyledons. Phenolic acids such as hydroxybenzoic, protocatechuic, syringic, gallic, *p*-coumaric and ferulic acids have been reported in legumes such as mung bean, field pea, lentil, faba bean, pigeon pea, navy bean, lupine, lima bean, chickpea and cowpea (Sosulski and Dabrowski, 1984). Luthria and Pastor-Corrales (2006) found that ferulic acid is the most abundant phenolic acid in common beans followed by sinapic acids and p-coumaric. Long-Ze, Harnly, Pastor-Corrales, and Luthria (2008) reported ferulic acid as the most abundant phenolic acid in common beans followed by sinapic acids and p-coumaric respectively. Xu and Chang (2008) reported that gallic acid, vanillic acid, sinapic, protocatechuic acid and *trans*-cinnamic acid were detected in yellow and black soybeans.

2.4.2 Flavonoids

Flavonoids constitute the largest group of plant phenolics, accounting for over half of the eight thousand naturally occurring phenolic compounds (Harborne, Baxter and Moss, 1999). Flavonoids are low molecular weight compounds, consisting of fifteen carbon atoms, arranged in a C6–C3–C6 configuration. Essentially the structure consists of two aromatic rings A and B, joined by a 3-carbon bridge, usually in the form of a heterocyclic ring, C (Figure 2.2). The aromatic ring A is derived from the acetate/ malonate pathway, while ring B is derived from phenylalanine through the shikimate pathway (Merken and Beecher, 2000). Variations in substitution patterns to ring C result in the major flavonoid classes, i.e., flavonols, flavones, flavanones, flavanols (or catechins), isoflavones, and anthocyanidins (Figure. 2.2) (Hollman and Katan, 1999), of which flavones and flavonols are the most widely occurring and structurally diverse (Harborne *et al*., 1999). Substitutions to rings A and B give rise to the different compounds within each class of flavonoids (Pietta, 2000). These substitutions may include oxygenation, alkylation, glycosylation, acylation, and sulfation (Hollman and Katan, 1999).

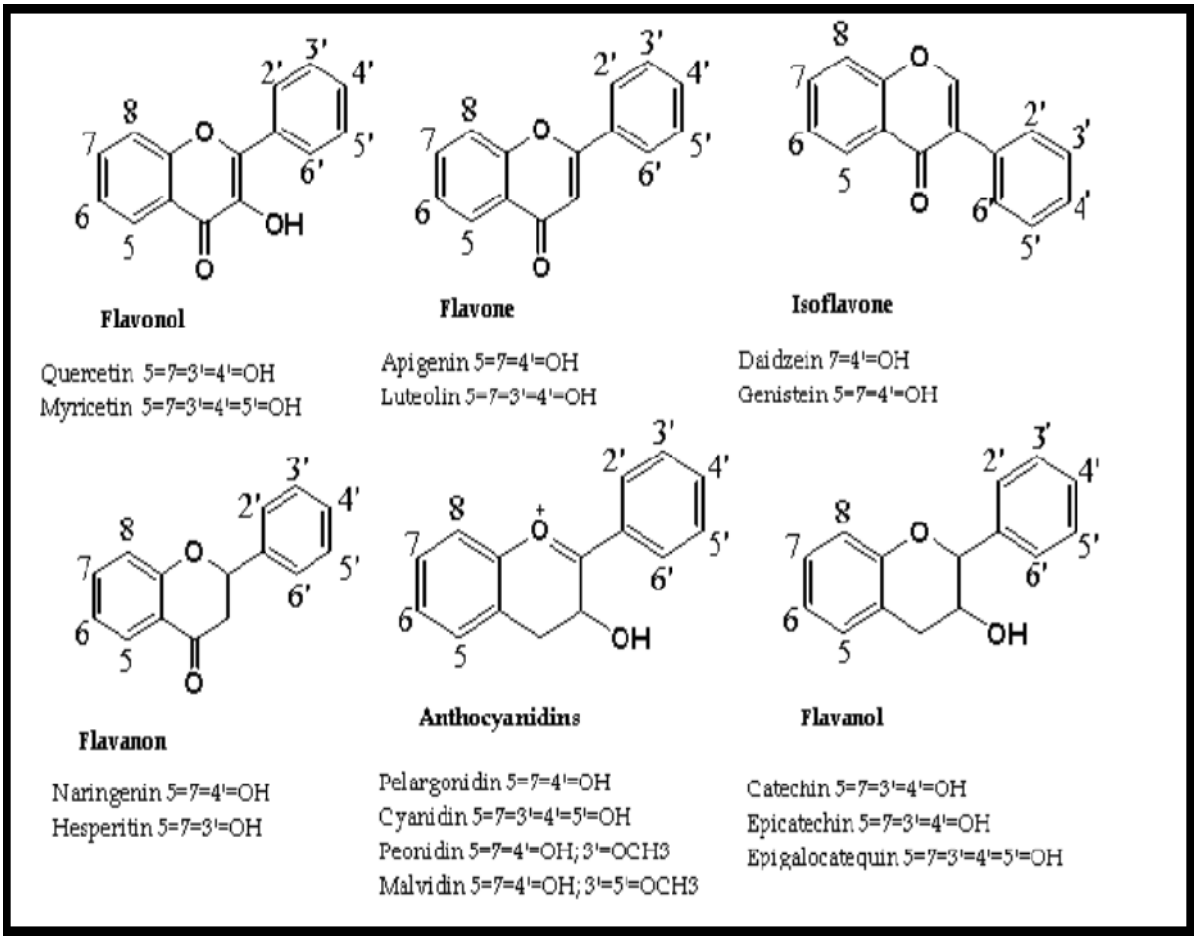


Figure 2.2 Generic structure of major classes of flavonoids. Adapted from Vinson, Dabbagh, Serry and Jang (1995)

Flavonoids present in leguminous seeds belong to flavanols, flavan-3-ols, flavones, and anthocyanidins (Dueñas, Fernández, Hernández, Estrella and Munoz, 2005). The majority of flavonoids are present as glycosides in the seeds (Díaz-Batalla, Widholm, Fahey, Castaño-Tostado and Paredes-López, 2006). Ojwang *et al.* (2012) identified flavonols such as quercetin mono, di and tri (acyl) glycosides, myricetin mono and di-glycosides, kaempferol-3-*O*-diglucoside in cowpeas. The same authors reported the prsences of anthocyanins such as delphinidins and cyanidins and their glycosides in cowpeas. Another study by Dueñas, Fernández, Hernández, Estrella, and Munoz (2005) reported quercetin diglycoside, myricetin 3- *O* -glucoside, quercetin 3- *O* -galactoside, quercetin 3- *O* -glucoside and quercetin feruloyl-diglycoside in cowpea (*Vigna sinensis* L). Wang, Gillaspie, Morris, Pittman, Davis and Pederson (2008) also reported that quercetin and myricetin were the most abundant flavonoids in cowpeas.

The presence of flavonol glycosides such as kaempferol and quercetin derivatives has been reported in other legumes such as common beans (Ranilla, Geovese and Lajoro 2007). Xu and Chang (2007) reported that black beans contained primarily the 3-O-glucosides of delphinidin, petunidin, and malvidin, while kaempferol and its 3-O-glycosides were present in pinto beans. Light red kidney bean had traces of quercetin 3-O-glucoside and its malonates, but pink and dark red kidney beans contained the diglycosides of quercetin and kaempferol. Small red beans contained kaempferol 3-O-glucoside and pelargonidin 3-O-glucoside (Xu and Chang, 2007).

2.4.3 Tannins

Tannins are high molecular weight compounds and have many phenolic groups (Hagerman, Riedl, Jones, Sovik, Ritchard, Hartzfeld and Riechel, 1998). Tannins are widely distributed in plants and are capable of precipitating proteins from aqueous solutions. Depending on their structures, tannins are defined as hydrolysable or condensed (Caballero *et al.*, 2003; Shahidi and Naczk, 2004). The name proanthocyanidin is used alternatively for condensed tannins because upon treatment with hot acid, anthocyanidin monomers are released (Harborne, 1998). Hydrolysable tannins are esters of gallic acids (Heim *et al*., 2002) while condensed (non-hydrolysable) tannins are oligomers and polymers of flavonoids specifically flavan-3-ols (Shahidi and Naczk, 2004).

The whole cowpea seeds have been reported to contain about 0.18–0.59% tannins (Reddy, Pierson, Sathe, and Salunkhe, 1985). Siddhuraju and Becker (2007) also reported (0.6%) and (0.4%) tannins in light and dark brown cowpeas, respectively. Ojwang *et al.* (2012) reported high level of monomeric anthocyanin contents in cowpeas (1676 -2094 µg/g). Cowpea anthocyanins were predominatly delphinidin-3-O-glucoside, cyanidin-3-O-glucoside and petunidin-3-O-glucoside (Ojwang *et al.,* 2012). Earlier, Ha, Lee, Park, Pae, Shim, Ko, Shin, Baek and Park (2010) reported that glycosides of delphinidin cyanidin, pelargonidin, malvidin and peonidin in seed coats of the black yard-long beans.

2.4.4 Structure-antioxidant activity relationships of phenolic compounds

Although the biological functions of polyphenols and/or metabolism in the human body are not completely established, two mechanisms are commonly proposed to explain the antioxidant role of phenolic compounds.

These are metal chelation and free radical scavenging properties (Khokhar and Apenten, 2003). Both the metal chelating and radical scavenging properties of phenolic compounds are directly related to their structure (Dueñas *et al.*, 2005). Siddhuraju and Becker (2007) found that extracts from light and dark brown cowpeas exhibited radical scavenging activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis -3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radicals. Siddhuraju and Becker (2007) also found a positive correlation between total phenolics and radical scavenging activities. The chemical structure of polyphenols gives them the ability to act as free radical scavengers. The type of compound, the degree of methoxylation and the number of hydroxyl groups are some of the parameters that determine the antioxidant activity. In the case of phenolic acids, the antioxidant activity depends on the numbers and positions of the hydroxyl groups in relation to the carboxyl functional group (Rice-Evans *et al*., 1996 and Robards, Prenzler, Tucker, Swatsitang, and Glover, 1999). Monohydroxy benzoic acids with the –OH moiety at the ortho- or para-position to the –COOH show no antioxidant activity, though the same is not true for the hydroxybenzoic acid (Rice-Evans *et al*., 1996).

The antioxidant activity of phenolic acids increase with increasing degree of hydroxylation, as is the case of the trihydroxylated gallic acid, which shows a high antioxidant activity. However, substitution of the hydroxyl groups at the 3- and 5-position with methoxyl groups as in syringic acid reduces the activity (Rice-Evans *et al*., 1996). Hydroxycinnamic acids exhibit higher antioxidant activity compared to the corresponding hydroxybenzoic acids (Andreasen, Landbo, Christensen, Hansen, and Meyer, 2001). The higher activity of the hydroxycinnamic acid could be because of the CH=CH–COOH group, which ensures greater H-donating ability and radical stabilisation than the –COOH group in the hydroxybenzoic acids (Rice-Evans *et al*., 1996). Flavonoids are generally higher in antioxidant activity than phenolic acids (Fukomoto and Mazza, 2000). This is attributed to the complexity of the flavonoid molecule compared to phenolic acids. Some of the structural features and nature

of substitutions on rings B and C which determine the antioxidant activity of flavonoids include the following (Figure 2.3)

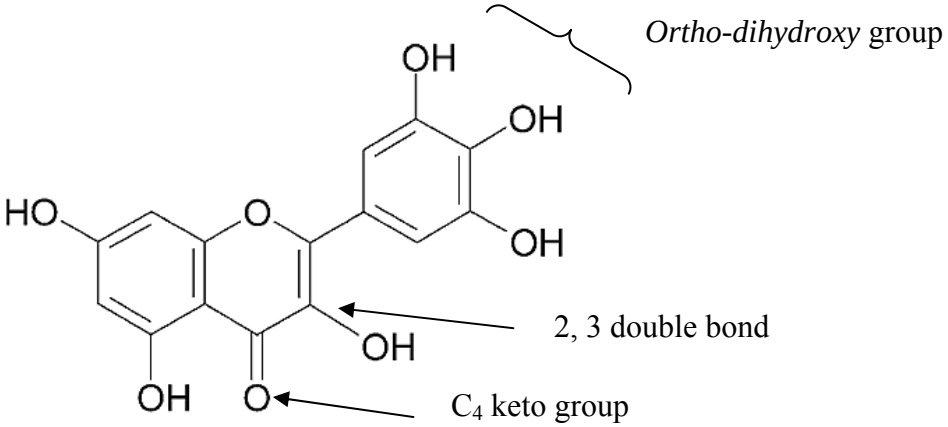


Figure 2.3 Structure of a flavonoid (myricetin) showing features important for antioxidant activity of phenolic compounds (Manach, Scalbert, Morand, Remesy and Jimenez, 2004)

(1) The degree of hydroxylation and the positions of the –OH groups in the B ring, in particular an ortho-dihydroxyl structure of ring B (catechol group) results in higher activity as it confers higher stability to the aroxyl radical by electron delocalisation (Van Acker, Van den Berg, Tromp, Griffoen, Van Bennekom, Van der Vijgh and Bast, 1996.), or acts as the preferred binding site for trace metals (Pietta, 2000). (2) A double bond between C-2 and C-3, combined with a 3-OH, in ring C, also enhances the active radical scavenging capacity of flavonoids (Van Acker *et al*., 1996). (3) A double bond between C-2 and C-3, conjugated with a C_4 keto group in the C ring enhances the radical scavenging capacity of flavonoids (Pietta, 2000) and (4) Substitution of hydroxyl groups in ring B by methoxyl groups alters the redox potential, which affects the radical scavenging capacity of flavonoids (Pietta, 2000; Seeram and Nair, 2002). Tannins have free radical-scavenging activities that are influenced by the structure of the monomers and the degree of polymerisation (Hatano, Miyatake, Natsume, Osakabe, Takizawa, Ito, and Yoshida, 2002). They are also chelators of metal ions such as copper and iron (Lopes, Schulman & Hermes-Lima, 1999; Khokhar and Apenten, 2003). Hagerman *et al.* (1998) reported that tannins, which are highly polymerised and have

many phenolic hydroxyl groups, are very effective antioxidants. They found that both condensed and hydrolysable tannins scavenge radicals very effectively. High molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for free radical scavenging by tannins than specific functional groups (Hagerman *et al*., 1998).

2.5 Health-promoting properties of phenolic compounds in plant foods

Phytochemicals e.g. phenolic compounds are bioactive non-nutrient compounds in plant foods thought to promote health (Cardador-Martinez *et al*., 2002). Phenolic compounds are primarily of interest to human health because of their antioxidant activity (Cardador-Martinez *et al*., 2002). Phenolic compounds can have complementary and overlapping mechanisms of action, including modulation of detoxifying enzymes, stimulation of the immune system, regulation of lipid and hormone metabolism, antioxidant, antimutagen, and antiangiogenic effects, reduction of tumor initiation, and promotion and induction of apoptosis (Campos-Vega, Loarca-Pina and Oomah, 2010). It has been known for a long time that a number of diseases are related to reactive oxygen species (ROS) (Dewanto, Wu, Adom and Liu, 2002). ROS, including superoxide anion ($O_2^{\bullet-}$), peroxide anion (O_2), hydroxyl radical (OH•) and singlet oxygen ($^1O_2$), that are highly reactive molecules, formed from molecular oxygen (O_2) by gaining or losing electrons. ROS are produced spontaneously during biological processes come from electron leakages from cell membranes and inadequately coupled reactions. The released electrons reduce molecular oxygen to superoxide anion or peroxide (Dewanto *et al.*, 2002). ROS could also be from external sources (e.g. pollution, smoking, and carcinogens in the environment).

Cancer is one of the leading causes of morbidity and mortality throughout the world. Biological oxidative damage of DNA, lipids and proteins in the human body is normally considered to be one of the causes of cancer (Jacob and Burri, 1996; Jacobs, Meyer, Kushi and Folsom, 1998). Reactive oxygen species appear to be involved in all steps of cancer development (Willcox, Ash and Catignani, 2004). Coronary heart diseases are the leading cause of death in most developed countries. Low density lipoprotein is believed to play a significant role in the formation and progression of early atherosclerotic lesions linked to cardiovascular diseases (Steinberg, 1997). In its native form LDL does not form atherosclerotic plaques. However oxidised LDL is responsible for the pathogenesis of

atherosclerosis that may lead to build up of plaque in the arteries (Astadi, Astudi, Santoso and Nugraheni, 2009).

Many studies have been performed to demonstrate the role of antioxidants in health promotion and prevention of chronic diseases, such as certain cancers, cardiovascular diseases and other aging-related diseases (Thompson, 1994). Although no information is available on health promoting properties of cowpea, Table 2.3 summarises some research work that was done on health promoting properties of dietary phenolic compounds. All phenolic compounds presented in Table 2.3 have been previously identified in cowpeas. Phenolic compounds have received increased attention because of their potential antioxidant activities that may exert cardioprotective effects in humans (Kinsella, Frankel, German and Kanner, 1993). Antioxidants suppress the formation of free radicals, quench the existing radicals, and reduce the availability of oxygen in biological system to prevent the oxidative damage of DNA, proteins and lipids in human body (Jacob and Burri, 1996).

Dietary phenolic compounds exert their health benefits by various biological effects such as free radical scavenging, metal chelation, reducing potential, chain breaking, and modulation of enzymatic activity as well as alteration of signal transduction pathways (Rice- Evans et al. 1996; Reiners et al. 1999; Cheng *et al*.2007). Phenolic compounds are associated with a wide range of biological activities, including antioxidant (Tsuda, Horio and Osawa,, 2003), anti-inflammatory (Wang and Mazza, 2002; Youdim, McDonald, Kalt and Joseph, 2002) and anticancer (Bomser, Madhavi, Singletetary and Smith, 1996). For this reason, the food and medicinal industries have become increasingly interested foods with high contents of e.g. phenolic compounds for the manufacture of supplements with preventative and therapeutic uses. Studies have found a significant inverse relationship of per capita consumption food rich in dietary antioxidants with coronary heart disease mortality (Zhou, Laux and Yu, 2004; Adom, Sorrells and Liu, 2003). Table 2.4 summarises some work done on pulses and their main potential health benefits.

Table 2.3 Evidences for health-promoting properties of dietary phenolic compounds

Phenolic compounds	Health-promoting properties	References
Ferulic acid	-Free radical scavenging properties against hydroxyl and peroxynitrite radicals	Yu *et al.* (1999); Kikuzaki *et al.* (2002)
	-Inhibition of LDL oxidation	Ogiwara *et al.* (2002); Andreasen *et al.* (2001)
	- Reduced free radical damage in neuronal cell systems	Kanski *et al.* (2002)
	-Demonstrated chemopreventive activity against Alzheimer's disease	Yan *et al.* (2001)
	-Decreased systolic blood pressure in a dose-dependent manner in hypertensive rats	Suzuki *et al.* (2002)
	-Elevated the activities of detoxifying enzymes, namely glutathione S-transferase and lower incidences of colonic carcinomas induced by azoximethane	Kawabata *et al.* (2000)
Feruloyl oligosaccharides	-Inhibited haemolysis of erythrocytes	Yuan *et al.* (2005)
Caffeic & Ferulic acids	-Exerted protection to the human skin against ultraviolet radiation-induced erythema	Saija *et al.* (2000)
Caffeic acid	Inhibition of copper-catalyzed human LDL oxidation	Meyer *et al.* (1998)
	Inhibits oxidation of LDL, suppressed the growth of HepG2 tumor xenografts in nude mice	Nardini *et al.* (1995); Chung *et al.* (2004)
Caffeic acid	-Showed high antioxidant activity by inhibiting AAPH and copper-catalyzed human LDL peroxidation	Cheng *et al.* (2007)
p-Coumaric acid	-Inhibited the peroxynitrite-induced protein modification using the anti-3-nitrotyrosine antibody	Niwa *et al.* (1999)
	-Inhibited LDL oxidation and reduced LDL cholesterol levels by 33% in rat serum	Zang *et al.* (2000)
Caffeic acid & Ferulic acid	-Inhibited tongue carcinogenesis induced by 4-nitroquinoline-l-oxide when they were administered to rats concurrently with the carcinogen	Tanaka *et al.* (1993)
Quercetin	-Inhibited the expression & function of the androgen receptor in LNCaP prostate cancer cells	Xing *et al.* (2001)
	-Inhibited colon cancer in rats and mice induced by azoxymethanol	Avila *et al.* (1994)
	-Inhibited the growth of malignant cells, encouraged apoptosis of cancerous cells	Hedges and Lister (2006)
	Inhibited mitogen activated protein (MAP) kinase in human epidermal carcinoma cells and human breast cancer cells	Bird *et al.* (1994); Choi *et al.* (2001)
Quercetin and myricetin	Inhibited the tumorigenicity of BP-7,8-diol-9, 10-epoxide-2 on mouse skin and in the newborn mouse	Chang *et al.* (1985)
Quercetin and Rutin	Exerted protective effect against *tert* butylhydroperoxide and menadione induced DNA single strand breaks in Caco-2 cells	Aherne and O'Brien (2000)
Catechin	Reduced cholesterol absorption from rat intestine	Ikeda *et al.* (1992)
	Inhibited oxidation of LDL induced by the mouse transformed macrophage cell line	Mangiapane *et al.* (1992)

Table 2.4 A summary of research done on pulses and their reported potential health beneficial effects (Source: Campos-Vega *et al*. 2010)

Source	Involved metabolism	Beneficial effect	Reference
Legumes e.g.common beans. Split peas and lentils	Cardiovascular Cardiovascular and Diabetes Endometrial cancer Breast cancer Colon cancer Legumes Type II diabetes	Cardiovascular 22% lower risk of coronary heart disease, and an 11% lower risk of cardiovascular disease Modulation of glucose, insulin, and homocysteine concentrations and lipid peroxidation in coronary artery disease patients Low risk of endometrial cancer Low breast cancer risk Low risk of colorectal adenoma Risk reduction to develop T2DM in the order of 20–30% Low average body mass index (BMI) and low risk of obesity	Bazzano *et al*. (2001) Jang *et al*. (2001) Tao *et al*. (2005) Velie *et al*. (2005) Agurs-Collins *et al*. (2006) Venn and Mann (2004) Greenwood *et al*. (2000)
Azuki bean juice	Hypertriglyceridemia	-Decreased triglyceride concentrations by inhibited pancreatic lipase activity	Maruyama *et al*. (2008)
Mung bean	Glucose and lipid metabolism	Modify glucose and lipid metabolism favourably in rats	Lerer-Metzger *et al*. (1996)
Beans	Obesity	Low body mass index and waist circumference (WC)	Haveman-Nies *et al*. (2001)
Common beans	Lymphoblastic Leukemia	Low risk of lymphoblastic leukemia	Petridou *et al*. (2005)
	Colon cancer	Inhibition of aberrant foci crypt development in rat colon	Feregrino-Perez *et al*. (2008)
Chickpeas	Hypertriglyceridemia	Reductions in serum total and low-density lipoprotein cholesterols	Pittaway *et al*. (2006)

2.6 Effect of heat processing on phenolic composition and antioxidant activity of legumes

2.6.1 Effect of cooking on phenolic composition and antioxidant activity of legumes

Studies have found mixed results on the effects of cooking on phenolic composition and antioxidant activity. The effects of cooking on phenolic composition and antioxidant activity may vary based on the type of cooking employed such as pressure cooking, steaming and boiling. Results may also varey based on differences in cooking parametes/conditions e.g. cooking time and temperature. Siddhuraju and Becker (2007) reported a reduction in extractable total phenolics, tannins and condensed tannins of autoclaved cowpea samples. They also reported a reduction in scavenging activity on the superoxide radicals of cowpea extracts. Barroga, Laurena and Mendoza (1985) also found that boiling and steam cooking reduced the quantity of phenolic compounds in mung bean (*Vigna radiata*) by 73%. Similar results were reported by Rocha-Guzman, Gonzales-Laredo, Ibarra-Perez, Nava-Berumen, and Gallegos-Infante (2007) when phenolic content in common beans reduced drastically after pressure cooking. Granito, Britto and Torres (2007) also observed significant losses in condensed tannin concentrations (84%) in beans following the cooking process at 100°C. The authors suggested that significant reduction in tannins during thermal treatment either due to leaching of these compounds into the soaking and cooking water or due to the breakdown of phenolics during processing.

Rocha-Guzman *et al.* (2007) on the other hand reported a significant increase in antioxidant capacity assessed by the DPPH method in beans (Phaseolus vulgaris L.) cooked at 121°C without soaking and not draining the cooking water, independent of the evaluated cultivar. Ranilla *et al.*(2007) reported that kaempferol and quercetin derivatives in Brazilian and Peruvian beans were reduced significantly (>70%) in cooking treatments with a soaking and draining step following thermal treatment, independent of cooking temperature. Conversely, in treatments without soaking and without draining of boiling water, flavonol contents increased (up to 25%) and phenolic acids such as ferulic and p-coumaric acids also increased in beans. Rakic, Petrovic, Kukic, Jadranin, Tesevic, Povrenovic and Siler-Marinkovic (2007)

observed that following thermal treatment of oak acorns from Serbia, non-tannin phenolics, including gallic acid, increased significantly, whereas tannin contents decreased, indicating that during thermal treatment hydrolysable tannins were degraded resulting in an increase of simple phenolics such as gallic acid. According to Bunea, Andjelkovic, Socaciu, Bobis, Neacsu, Verhe and Van Camp (2008), the increase in concentrations of certain phenolic compounds after thermal treatment may be explained by their better release from the food matrix as a result of the breakdown of supramolecular structures e.g. cell wall containing phenolic groups.

2.6.2 Effect of infrared heat processing and other dry heat processing technologies on phenolic composition and antioxidant activity of grains

The changes that occur during micronisation as mentioned earlier may affect the phenolic composition and antioxidant activity of cowpeas. Generally, the outer layers of plant such as peel, shell, and hull contain large amount of polyphenolic compounds to protect inner materials. A number of phenolic acids such as p-coumaric acid and ferulic acid are linked to various cell wall components such as arabinoxylans and proteins (Durkee, and Thivierge, 1977; Hartley, Morrison, Himmelsbach and Borneman, 1990). Breakdown of cell structures due to heat processing may increase the bio-availability of antioxidant compounds but their degradation may also take place (Bryngelsson, Dimberg and Kamal-Eldin, 2002).

Lee, Jeong, Kim, Park, Nam and Ahn (2006) reported that infrared heat treatment of peanut hulls resulted in increased phenolic contents from water extracted peanut hulls. Similarly Lee, Kim, Jeong, Kim, Ha and Nam (2003) reported a significant increase in the radical scavenging activity and total phenol contents of rice hull extracts. Furthermore Lee *et al*. (2003) reported that infra-red heated rice hulls contained more phenolic compounds such as 3-vinyl-1-oxybenzene, p-hydroxybenzaldehyde, vanillin p-hydroxybenzoic acid, 4,7 dihydroxyvanillic acid, and isoferulic acid, in addition to the phenolic compounds detected in intact rice hull extracts. Only a few phenolic compounds (o-methoxycinnamic acid, p-coumaric acid, and N-indolyl acetate) had been detected in intact rice hull extracts. Ferulic and p-coumaric acids in plants may be linked to cell wall polysaccharides, ligin, suberin, and cutin, and diferulic acid serves as a cross-link between pentosan chains (Herrmann, 1989). Lee *et al*. (2003) indicated that infrared radiation liberated polyphenols in rice hulls, which

were either covalently bound to high molecular weight compounds or part of repeating subunits of high molecular weight polymers such as tannins. Siddhuraju and Becker (2001) observed an increase in total phenolics after cooking and roasting of common beans.

Earlier research in cereals had indicated that a major portion of phenolics is present as soluble conjugated or insoluble bound forms (Sosulski, Kryger, and Hogge, 1982). Dewanto *et al*. (2002) explained that thermal processing might release more bound phenolic acids from the breakdown of cellular constituents. Randhir, Kwon, and Shetty (2008) suggested that the dissociation of conjugated phenolic forms due to thermal processing may increase total extractable phenolics.

2.7 Methodologies to measure health promoting properties of phenolic compounds

Based on the chemical reactions involved, the major antioxidant capacity assays can be roughly divided into two categories (Huang, Ou and Prior, 2005): (1) Single electron transfer (SET) reaction based assays and (2) hydrogen atom transfer (HAT) reaction based assays. SET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes colour when reduced. The degree of colour change is correlated with the sample's antioxidant concentrations (Huang *et al*., 2005). SET-based assays include the Trolox equivalence antioxidant capacity (TEAC). HAT-based assays apply a competitive reaction scheme, in which antioxidant and substrate compete for generated peroxyl radicals through the decomposition of azo compounds (Huang *et al*., 2005). These assays include the oxygen radical absorbance capacity (ORAC) assay.

2.7.1 Trolox equivalence antioxidant capacity or the ABTS assay

This assay measures the ability of antioxidant to scavenge free radicals (Awika, Rooney, Wu, Prior and Cisneros-Zevallos, 2003). This spectrophotometric technique measures the relative ability of hydrogen-donating oxidants to scavenge the 2, 2'-azinobis (3-ethyl-benzothaizoline-6-sulphonic acid) radical cation chromogen ($ABTS^{\cdot+}$) in comparison with Trolox, the water soluble vitamin E analogue. The reduced $ABTS^{\cdot+}$ concentration by a certain amount of antioxidant is related to that of Trolox and this gives the TEAC value of the antioxidants (Awika *et al*., 2003). Samples react with preformed ABTS (2, 2'-azinobis (3-ethyl-benzothaizoline-6-sulphonic acid) free radical. The ability of the sample to quench the free radical is measured by monitoring colour at 734 nm and trolox is used as a standard. The antioxidants reduce the radicals depending on the antioxidant activity, concentration of the antioxidants and the duration of the reaction.

2.7.2 The oxygen radical absorbance capacity (ORAC)

This method was developed by Cao, Alessio and Culter (1993), and measures the ability of antioxidants to protect protein from damage by free radicals. It is a method that measures ability of a compound to competitively inhibit peroxyl radical-initiated damage to a substrate. Ability of the sample to protect fluorescein from free radical attack by AAPH (2,2'-azobis (2-amidinopropane) hydrochloride) is monitored for 90 min at 37^{o}C using a fluorescence spectrophotometer (excitation 485 nm, emission 528 nm). A major advantage of ORAC is that the method is automated and largely standardised.

2.7.3 Inhibition of oxidative DNA damage (agarose gel electrophoresis method)

In this assay, the ability of the antioxidants in the sample analysed to protect the DNA from oxidative damage. Supercoiled plasmid DNA strand damage is induced by addition of AAPH (Aronovitch, Godinger, Israeli, Krishna and Goldstein, 2007), a thermolabile radical starter that generates peroxy radicals. Supercoiled plasmid DNA under oxidative stress is converted into relaxed circular and linear forms. The migration rates of supercoiled (unnicked), relaxed circular (nicked) and linear degraded plasmid DNA are studied by agarose gel electrophoresis. The three forms can be separated by agarose gel electrophoresis due to their different electrophoretic mobility. After electrophoresis the gels are illuminated with UV light and photographed. DNA subjected to electrophoresis in the absence of an antioxidant under identical conditions serves as the control. The gel electrophoretic mobility of the various forms of DNA is compared with the control (Aronovitch *et al*., 2007).

2.7.4 Inhibition of red blood cell (erythrocytes) haemolysis assay

Erythrocytes are vulnerable to lipid peroxidation due to their high content of polyunsaturated lipids, their rich oxygen supply, and the presence of transition metals (Zhu, Holt, Lazarus, Orozco and Keen, 2002). ROS can attack erythrocyte membranes, inducing oxidation of lipids and proteins. This triggers disruptions in the membrane eventually leading to haemolysis (Miki, Tamia, Mino, Yamamoto and Niki, 1987). ROS have been implicated in the mechanism of the damage of the red blood cells in sickle cell anemia and other haemoglobinopathies (Tedesco, Russo, Russo, Iacomino, Russo, Carraturo, Faruolo, Moio and Palumbo, 2000). The erythrocytes assay measures the ability of the antioxidants in the sample analysed to protect the erythrocyte cells from haemolysis.

In this assay isolated erythrocytes are suspended in a phosphate buffer solution (PBS) and oxidative stress is induced by incubation of red blood cells with AAPH which generate the peroxyl radical (Tang & Liu, 2008) in the presence or absence of the phenolic compound or extract at 37 °C. The mixture is centrifuged and the degree of haemolysis is measured by taking absorbance readings of the supernatant at 570 nm.

2.7.5 Inhibition of LDL Oxidation assay

Oxidized LDL has been shown to accelerate several steps in atherosclerosis including endothelial damage, uptake of LDL by foam cells, monocyte/macrophage recruitment, and alteration in vascular tone, induction of growth factors, and production of antibodies (Yu, Haley, Perret and Harris, 2002). The inhibition of LDL oxidation assay is a method that measures the ability of the antioxidants in the sample analysed to inhibit LDL. Oxidation is induced *in vitro* by incubating LDL with an oxidant (either copper ions or AAPH) can be measured spectrophotometrically by continuous monitoring of conjugated dienes at 234 nm (Abuja, Murkovic and Pfannhauser., 1998) or measuring the final products of lipid oxidation known as the thiobarbituric acid reactive species (TBARS) at 532 nm (Xu, Yuan and Chang, 2007). In the TBARS assay, oxidised LDL is incubated at 37 °C with or without an antioxidant for a period of time (3-4 hours). The reaction is stopped by the addition of EDTA solution, proteins are precipitated with trichloroacetic acid and the late stage products of lipid peroxidation reacts with thiobarbituric acid to form pink chromophores which are measured spectrophotometrically at 532 nm (Xu *et al.*, 2007).

2.8 Gaps in knowledge

Research has been done on the phenolic composition of cowpeas. However the potential health promoting properties of cowpeas are largely unexploited. These include the protective effect of cowpea phenolic compounds against LDL oxidation, red blood cell hemolysis and oxidative DNA damage. This would provide information on whether the consumption of cowpea would offer some protection against chronic diseases such as coronary heart disease and cancer which are a result of LDL oxidation, red blood cell hemolysis and oxidative DNA damage.

One of the major forms in which cowpea is utilised is as cooked whole seeds. Cooking of cowpea seeds, as with most legumes takes up to 2 h and more. Research has been directed at reducing the cooking time of legumes using various processing techniques such as pre-soaking of seeds and treatment of pre-conditioned legume seeds with infrared energy (micronisation). Studying of the effect of micronisation processing on the phytochemical quality and potential health promoting properties of cowpea would be important to understand health benefits of micronised cowpeas to the consumer. No data is available on how such processing techniques may affect cowpea phenolic compounds and their potential health benefits.

Potential utilisation of cowpeas will not only depend on its potential health benefits, but also on consumer acceptance of micronised cowpeas based on their sensory properties. Micronisation like any other heat processing technology may affect the sensory properties of cooked cowpea. Limited information is available on how micronisation affects sensory characteristics more especially flavour and aroma of cooked cowpea.

3. HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

3.1.1 Micronised cooked cowpeas will have toasted flavour notes and be darker than unmicronised cooked cowpeas. This is because of the production of Maillard reaction products during infra-red heating that results in darkening of cowpeas as suggested by Mwangwela (2006) and Phadi (2004). Maillard reaction products also result in the development of toasted flavours (Chua and Chou, 2005) which would be detected in cooked cowpeas. The Maillard reaction is a non-enzymatic reaction that takes place on heating and is related to aroma, flavour and colour development in food products (Martins et al., 2001).

3.1.2 Extracts from micronised cowpea samples will have lower total phenolic content, total phenolic acids, total flavonoids and antioxidant activity, as well as lower protective effect against erythrocyte haemolysis, LDL oxidation and oxidative DNA damage initiated by radicals compared to unmicronised samples. This is because heat processing results in complexation of seed phenols with its macromolecules such as proteins thus reducing the extractability of phenolic compounds (Awika, Dykes, Gu, Rooney and Prior, 2003a) decreasing their radical scavenging properties and protection against oxidative damage.

3.2 Objectives

3.2.1 To determine the effects of micronisation of pre-conditioned cowpeas on cooking time and sensory characteristics in terms of appearance, aroma, flavour and texture of cooked cowpeas.

3.2.2 To determine the effects of micronisation of pre-conditioned cowpeas on their total phenolic compounds (total phenolic content, total flavonoids, phenolic compounds as determined by HPLC–MS) and in vitro free radical scavenging properties determined by using TEAC and ORAC assays.

3.2.3 To determine the effects of micronisation of pre-conditioned cowpeas on their protective effects against copper-induced human LDL oxidation, AAPH-induced oxidative DNA damage and AAPH-induced human red blood cell haemolysis.

4. RESEARCH

The research, which tested the hypotheses stated in Chapter 3, was devided into four research chapters, each chapter presented as intended for publication.

4.1 Effect of micronisation of pre-conditioned cowpeas on cooking time and sensory properties of cooked cowpeas (published in part in the Journal of the Science of Food and Agriculture).

4.2 Effects of micronisation of pre-conditioned cowpeas on phenolic composition of uncooked and cooked cowpeas.

4.3 Effects of micronisation of pre-conditioned cowpeas on total phenolic content, total flavonoids and in vitro free radical scavenging properties of uncooked and cooked cowpeas.

4.4 Effects of micronisation on cowpea protective effects against Low Density Lipoprotein (LDL) oxidation, oxidative DNA damage and red blood cell haemolysis.

4.1 Effect of micronisation of pre-conditioned cowpeas on cooking time and sensory properties of cooked cowpeas.

ABSTRACT

Cowpea is mostly utilised as cooked whole seeds. This is often achieved only after boiling for up to 2 hours, resulting in high energy consumption and a long time for food preparation. Micronisation of pre-conditioned cowpeas reduces their cooking time. Micronisation changes physico-chemical properties of cowpea seeds that may affect sensory properties of cooked cowpeas. Consumer acceptance and utilisation of micronised cowpeas depends on their sensory properties. Micronised and unmicronised samples of Blackeye (cream), Bechuana white (light brown) Glenda (dark brown) and Dr. Saunders (red) cowpeas were subjected to cooking time, descriptive sensory and colour analyses.

Micronisation significantly reduced cowpea cooking time by 28 to 49 %. There were significant ($p<0.05$) increases in roasted aroma and flavour, mushy texture and splitting in all micronised samples. Bechuana white was more mushy and split than others. There were significant decreases in firmness, mealiness and coarseness after micronisation for all cowpea types. Micronised cowpeas were darker (lower L^* values) than unmicronised cooked cowpeas. Darkening was more evident in less pigmented cowpeas than in more pigmented cowpeas. Micronisation reduces cowpea cooking time, but also affects sensory properties of cowpeas such as introducing roasted flavours that may not be familiar to consumers. This might have an influence on consumer acceptance of micronised cowpeas.

4.1.1 INTRODUCTION

Starchy legumes also known as pulses, including cowpea (*Vigna unguiculata* L. Walp) have been consumed by humans since the earliest practice of agriculture (Phillips and McWatters, 1991). Cowpeas are an important source of energy and protein in developing countries of Africa, Latin America and Asia (Phillips *et al*., 2003). One of the major forms in which cowpea is utilised is as cooked whole seeds. Cooking of cowpea seeds, as with most legumes, is often achieved after boiling for up to 2 hours and even longer (Akinyele *et al*., 1986; Demooy and Demooy, 1990) resulting in high energy consumption and requiring long time for food preparation. Micronisation, an infrared heat treatment applied to pre-conditioned legumes, has been reported to reduce the cooking time by 30 to 44% in cowpeas (Mwangwela *et al*., 2006; Phadi, 2004). During the vibration, inter-molecular friction among the molecules occurs and results in heat generation and rise in water vapour pressure in the food (Sharma, 2009). The increase in vapour pressure and temperature results in changes in structure and physico-chemical properties e.g. starch gelatinisation and protein denaturation of cowpea seeds (Mwangwela *et al*., 2006). These changes may affect the sensory properties of cooked cowpeas. Utilisation of cowpeas depends on consumer acceptance of the sensory properties. Sensory properties are an important element of quality characteristics of food products, determining consumer reaction and satisfaction (Abbott, 1999).

Quantitative descriptive sensory analysis is applied for detailed description of sensory characteristics of a food product. In this method an assumption is made that sensory quality is a complex of many descriptors, which can be individually estimated by consumers (Meilgaard *et al*., 1999). Dark colour development and increase in splitting of micronised cowpeas has been previously reported (Mwangwela *et al*., 2006; Phadi, 2004). However no information is available on how the micronisation process affects the other sensory characteristics such as aroma and flavour of cooked cowpeas. The aim of this study was to determine the effect of micronisation of pre-conditioned cowpeas on cooking time and sensory characteristics of cowpeas.

4.1.2 MATERIALS AND METHODS

4.1.2.1 Cowpea samples

Four cowpea types, different in colour and seed size (grams per 100 seeds) were used in the study (Table 4.1.1). Cowpea seed size was determined by a method described by Mwangwela (2006). Bechuana white, Glenda and Dr Saunders were supplied by AGRICOL, Potchefstroom, while Blackeye was supplied by Premier Seeds International, Pretoria, South Africa. The choice of cowpea types used in the study was directed by the larger research project that studies the effects of micronisation on potential health benefits of cowpeas. Two dark and two light coloured cowpea types were selected for comparison. The first group was expected to have higher levels of phenolic compounds compared to the second group. All cowpea samples were cleaned, packed and stored at 4 °C until used. Pictures of cowpeas samples used presented in Figure 4.1.1.

Table 4.1.1 Description of cowpea types used in the study

Cowpea type	Seed coat colour	Cowpea seed size (g/100 seeds)
Blackeye	Cream	22.2 (0.0)
Bechuana white	Light brown	15.9 (0.1)
Glenda	Dark brown	14.6 (0.7)
Dr Saunders	Red	10.1 (0.2)

Standard deviation given in parentheses

4.1.2.2 Micronisation of cowpeas

Micronisation of cowpeas was perfomed as described by Mwangwela *et al.* (2006). They reported that cowpeas pre-conditioned to a final moisture content of 41 % and infrared heating to final surface temperature of 153 °C resulted in 36 % reduction in cooking time. The amount of water required to achieve the targeted moisture content for pre-conditioning was calculated as described by Arntfield *et al.* (1997). Cowpea seeds were conditioned to 41 % moisture by steeping the seeds in deionised water for 6 h and holding for 12 h at ambient temperature (± 22 °C) for the moisture to equilibrate throughout the seeds as described by Mwangwela *et al.* (2006).

Blackeye

Bechuana white

Glenda

Dr Saunders

Figure 4.1.1 Four cowpea (*Vigna unguiculata* L. Walp) types used in the study

Two hundred grams of pre-conditioned cowpea seeds were exposed to infrared heating using a table-top microniser (Technilamp Pty, Johannesburg, South Africa) operating at 66.7 % output. The microniser was preheated for 20 min before micronising the cowpeas in a single layer (21 cm from energy source) for 6 min to a final surface temperature of 153 °C. Micronised cowpeas were cooled, packed and stored at 4 °C until used within 2 days. The moisture contents of cowpeas are presented in Table 4.1.2. Cowpea moisture contents were determined by a method described by Mwangwela (2006).

Table 4.1.2 Moisture contents (%) of dry cowpeas seeds before pre-conditioning, after preconditioning and after micronisation

Cowpea type	Original moisture content of cowpea seeds	Moisture content of pre-conditioned cowpeas	Moisture content of micronised cowpeas
Blackeye	10.2 (0.9)	40.7 (0.1)	14.5 (0.9)
Bechuana white	9.2 (0.1)	40.9 (1.3)	13.5 (0.1)
Glenda	10.6 (0.2)	39.2 (0.8)	12.4 (1.0)
Dr Saunders	11.3 (0.3)	39.7 (0.6)	13.7 (1.6)

Standard deviation given in parentheses

4.1.2.3 Cooking time of cowpeas

The Mattson Bean Cooker (rod weight: 50 g) was used to determine the cooking time of cowpeas as described by Mwangwela *et al*. (2006) Cowpea seeds (n=25) were positioned in the perforation zones of the cooker, and then cooked in a heavy aluminium pan with 1500 ml of deionised water. The cooking time was recorded as the time required for 80% of the pins to fall through the cooked seed.

4.1.2.4 Colour measurements

Colour of unmicronised and micronised cooked cowpeas was measured using a Chroma Meter CR-400 (Konica Minolta Sensing, Inc. Japan).

The colour measurements were expressed in terms of lightness (L^*), red/green characteristics (a^*) and blue/yellow characteristics (b^*).

4.1.2.5 Descriptive sensory evaluation

Students from the University of Pretoria, who were willing to evaluate cowpeas and had experience of descriptive sensory evaluation, were screened for sensory acuity. A panel of ten judges was selected based on sensory acuity and experience. They were trained for 12 h following the generic descriptive analysis method as described by Einstein (1991). Descriptive terms and scale anchors were developed, defined and agreed upon for evaluation (Table 4.1.3). Four micronised and four unmicronised cowpea samples (150 g per sample for each session) were individually placed in BB4L 300 mm × 450 mm plastic bags (Cryovac, Johannesburg, South Africa) containing 600 mL deionised water. Cowpeas were cooked in the bags, in boiling water using 6 L aluminium saucepans (four samples per saucepan) on a two plate industrial stove (Anvil, Johannesburg, South Africa, 220–240 V, 50 Hz and 3200 W). The bags were pierced at the top with steel rods, allowing them to hang in the saucepan. Cowpea samples were cooked following the cooking time pre-determined using the Matson Cooker for each cowpea variety. Cooked cowpeas (20 g portions) were served in 125 mL polystyrene cups with lids. The sensory evaluation of cowpeas was conducted in a sensory evaluation laboratory with individual booths equipped with computers for direct data entry using Compusense Five ® release 5.0 (Compusense Inc., Guelph, Canada). Panellists evaluated all samples in triplicate during three days with one session per day. Each panellist received a tray, with samples to evaluate, carrots and water for rinsing the mouth before and between tasting the samples. To avoid fatigue, two cowpea samples were presented and tasted one after the other followed by a 5 min break before the next two samples were presented. The order of sample presentation was randomised over the panel. The panel used 32 descriptive terms (Table 4.1.3) grouped under the headings of aroma, appearance, flavour and texture. Aroma was evaluated immediately after removing the lid using short sniffs, followed by appearance evaluation. Then a spoon full of cowpeas was chewed in the mouth to evaluate for flavour and texture properties. All sample attributes were rated on a line scale where 0=minimum score and 10 = maxmum score

Table 4.1.3 Terminology used by descriptive sensory panel to describe the sensory attributes of cooked cowpeas

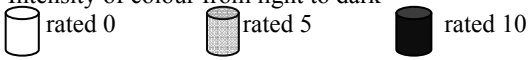
Attributes	Definition	References	Rating scale
Appearance & Aroma			
Mushiness	The extent to which cowpeas visually resemble a soft and pulpy texture.	Black-eyed cowpeas boiled for 150 min (overcooked) rated 10	Not mushed = 0, Very mushed =10
Splitting	The visual assessment of the number of seeds that were transversely or longitudinally cracked after cooking	Overcooked cowpeas with all seeds split (100 % splitting) rated 10	Not split = 0 Very split = 10
Seed size	The visual assessment of seed size	Uncooked lentils (Small) rated 0 and speckled sugar beans (Large) rated 10	Small = 0 Large = 10
Colour intensity	The degree of colour intensity ranging from light to dark	Intensity of colour from light to dark  rated 0 rated 5 rated 10	Not dark = 0, Very dark = 10
Overall aroma intensity	The intensity of the overall aroma of cooked cowpeas	No reference	No aroma = 0 Very intense = 10
Sweet aroma	The intensity of a sweet aroma	Boiled sweet corn rated 10	Not Sweet = 0, Very sweet = 10
Earthy	The intensity of the characteristic smell of wet soil	Distilled water rated 0, Wet Soil rated 10	Not earthy = 0, Very earthy = 10
Beany aroma	The intensity of beany aroma characteristic of cooked beans and other legumes	Speckled sugar beans boiled for 120 min rated 10	Not beany = 0, Very beany = 10
Fermented aroma	The intensity of aroma associated with fermented products such as sour cabbages	Distilled water rated 0, Langeberg Co-operation's canned sauerkraut rated 10	No fermented aroma = 0, Very intense fermented aroma = 10
Acetone aroma	The intensity of aroma associated with acetone also referred to as fruity smell	Distilled water rated 0, Acetone (100 %) rated 10	No acetone aroma = 0, Very intense acetone aroma = 10
Roasted	The intensity of aroma associated with roasted cooked legumes	Oven dried/roasted (150°C, 20 min) cooked (97 min) black-eyed cowpeas rated 10	Not burnt = 0, Very intense roasted aroma = 10
Hay aroma	The intensity of smell associated with dried grass	Distilled water rated 0, Dry hay rated 10	No hay aroma = 0, Very intense hay aroma = 10

Table 4.1.3 Terminology used by descriptive sensory panel to describe the sensory attributes of cooked cowpeas (continued)

Attributes	Definition	References	Rating scale
Flavour			
Overall flavour intensity	The intensity of the overall flavour of cooked cowpeas	No reference	No flavour = 0 ,Very intense = 10
Beany flavour	The intensity of beany flavour characteristic of cooked beans and other legumes	Speckled sugar beans boiled for 120 min rated 10	Not beany = 0,Very beany = 10
Sweet	The intensity of the basic sweet taste associated with sucrose	Dry adsorbent paper rated 0, dry adsorbent paper soaked in 5% sucrose in spring water rated 10	Not sweet = 0, Very sweet = 10
Sour	The intensity of the basic sour taste associated with acidic solutions like citric acid	Dry adsorbent paper rated 0, dry adsorbent paper soaked in 0.08% tartaric acid in spring water rated 10	Not sour = 0, Very sour = 10
Bitter	The intensity of the basic bitter taste associated with caffeine or quinine	Blank paper rated 0, dry adsorbent paper soaked in 0.15% caffeine in spring water rated 10	Not bitter = 0, Very bitter = 10
Fermented flavour	The intensity of flavour associated with fermented products such as sour cabbages	Distilled water rated 0, KOO canned sauerkraut rated 10	No fermented flavour , = 0, Very intense fermented flavour = 10
Starchy flavour	The intensity of flavour associated with chalky flavour	Dry maize starch rated 10	Not starchy = 0, Very starch = 10
Off flavour	The intensity of unpleasant flavour associated with enzymatic oxidation of legumes	No reference	No off flavours = 0, Very intense off flavours = 10
Roasted	The intensity of flavour associated with roasted cooked legumes	Oven dried/roasted (150°C, 20 min) cooked (97 min) black-eyed cowpeas rated 10	Not roasted = 0, Very intense roasted flavour = 10

Table 4.1.3 Terminology used by descriptive sensory panel to describe the sensory attributes of cooked cowpeas (continued)

Attributes	Definition	References	Rating scale
Nutty flavour	The intensity of flavour associated with raw nuts	Raw cashew nuts rated 10	Not nutty = 0, Very nutty = 0
Plastic	The intensity of flavour associated with plastics	No reference	No plastic flavour = 0, Very intense plastic flavour = 10
Woody	The intensity of flavour associated with dry wood flavour perceived when lightly chewing dry wood	Tooth picks rated 10	Not woody = 0, Very woody = 10
Texture			
Coarseness	The extent to which graininess of cooked cowpeas caused by small particles could be perceived in the mouth	Smooth peanut butter rated 0 Roasted peanuts crushed (30 s in a blender) rated 10	Not coarse = 0, Very coarse = 10
Moistness	The moistness of mass or amount of moisture/ wetness in chewed cowpeas bolus	No reference	Not watery = 0, Very watery = 10
Mealiness	The chalky and floury texture that is felt in the mouth as cowpeas are chewed	No reference	Not mealy = 0, Very mealy = 10
Firmness	The force required to compress cowpeas between the teeth	Roasted peanuts without skins rated 10	Not firm = 0, Very firm = 10
Chewiness	The length of time required to chew cowpeas before they are ready for swallowing	Roasted peanuts with skins rated 10	Not chewy = 0, Very chewy = 10
Seed coat residues	The extent to which the seed coat remains in the mouth after swallowing	Roasted peanuts without skins rated 0, Roasted peanuts with skins rated 10,	No residue in the mouth = 0, Much residues in the mouth = 10
Astringency	The sensation that lingers and coats, dries and numbs the mouth, palate and tongue when eating cowpeas	Black tea (two bags soaked in 750 mL hot boiled water) rated 10	Not astringent = 0, Very astringent = 10

4.1.3 STATISTICAL ANALYSES

One way analysis of variance (ANOVA) was used to analyse data for cooking time and instrumental colour measurements. Means for all analyses were compared at $p < 0.05$ using Fisher's least significant difference (LSD) test. Two way ANOVA including cowpea type and micronisation treatment and their interaction effect as independent variables on the dependent sensory properties was performed. Significant differences were noted for the main variables. However, no significant interactive effect of cowpea type and micronisation was noted. Since the focus of the study was on the effect of micronisation on cowpea sensory properties, cowpeas type differences in sensory properties were not explored further. The student t-test was therefore used to compare sensory properties of micronised and unmicronised versions of individual cowpea types using Statistica software version 10.0 (Statsoft, Tulsa, USA). Principal Component Analysis (PCA) was used to evaluate the multivariate factors distinguishing the sensory profiles of cooked cowpeas.

4.1.4 RESULTS

Table 4.1.4 shows the cooking time of the four cowpea types. Blackeye (unmicronised) cooked longer than other cowpea types, followed by Dr Saunders (unmicronised).

Table 4.1.4 Effect of micronisation of pre-conditioned cowpeas on the cooking time of the four cowpea types

Cowpea type	Cooking time (min)	Reduction in cooking time (%) due to micronisation
Blackeye		34
Unmicronized	97 (4.2)^{f}	
Micronized	64 (5.0)^{c}	
Bechuana white		49
Unmicronized	74 (5.0)^{d}	
Micronized	38 (5.2)^{a}	
Glenda		35
Unmicronized	78 (6.5)^{d}	
Micronized	51 (3.3)^{b}	
Dr Saunders		28
Unmicronized	86 (5.6)^{e}	
Micronized	62 (4.0)^{c}	

Mean values within a column with different letters $^{(a\text{-}f)}$ differ significantly at $p<0.05$, Standard deviation given in parentheses.

There was no significant difference in cooking time of unmicronised samples of Bechuana white and Glenda. Micronisation significantly ($p < 0.05$) reduced cowpea cooking time. Bechuana white had the highest reduction (49%) in cooking time following micronisation (Table 4.1.4). Micronisation of pre-conditioned cowpeas affected the appearance, aroma, flavour and texture of cooked cowpeas (Figure 4.1.2 A, B, C and D). There was a significant increase in splitting and mushiness after micronization in all cowpea varieties. Descriptive sensory analysis showed that micronisation of pre-conditioned cowpeas resulted in darkening of cooked cowpeas except for Blackeye cowpea. This was confirmed by a significant reduction in lightness (L* values) in micronised Blackeye, Bechuana white and Glenda cooked cowpeas (Table 4.1.5). There was a significant increase in redness (a* values) in micronised samples of Dr Saunders and Bechuana white.

Table 4.1.5 Effect of micronisation of pre-conditioned cowpeas on the colour (*L, *a** and *b** values)[a] of cooked cowpeas**

Cowpea samples[b]	L*	a*	b*
Blackeye			
Unmicronized	50.2 (2.1)^{f}	5.5 (0.6)^{a}	2.6 (0.8)^{e}
Micronized	45.9 (2.1)^{e}	5.7 (0.7)^{a}	2.8 (0.3)^{e}
Bechuana white			
Unmicronized	42.2 (2.6)^{d}	8.0 (1.1)^{b}	-1.5 (1.6)^{d}
Micronized	32.6 (1.3)^{c}	11.7 (0.5)^{cd}	-8.7 (0.8)^{c}
Glenda			
Unmicronized	29.4 (1.9)^{b}	12.8 (0.6)^{de}	-13.5 (0.9)^{b}
Micronized	26.7 (1.6)^{a}	12.4 (0.8)^{d}	-14.8 (0.9)^{a}
Dr Saunders			
Unmicronized	28.6 (0.6)^{ab}	11.1 (1.0)^{c}	-14.3 (0.6)^{ab}
Micronized	26.5 (0.5)^{a}	13.5 (0.5)^{e}	-15.4 (0.9)^{a}

Mean values within a column with different letters $^{(a\text{-}f)}$ differ significantly at $p<0.05$, Standard deviation given in parentheses

a *L**, lightness (0 = black, 100 = white); +*a**, red; −*a**, green; +*b**, yellow; −*b**, blue.

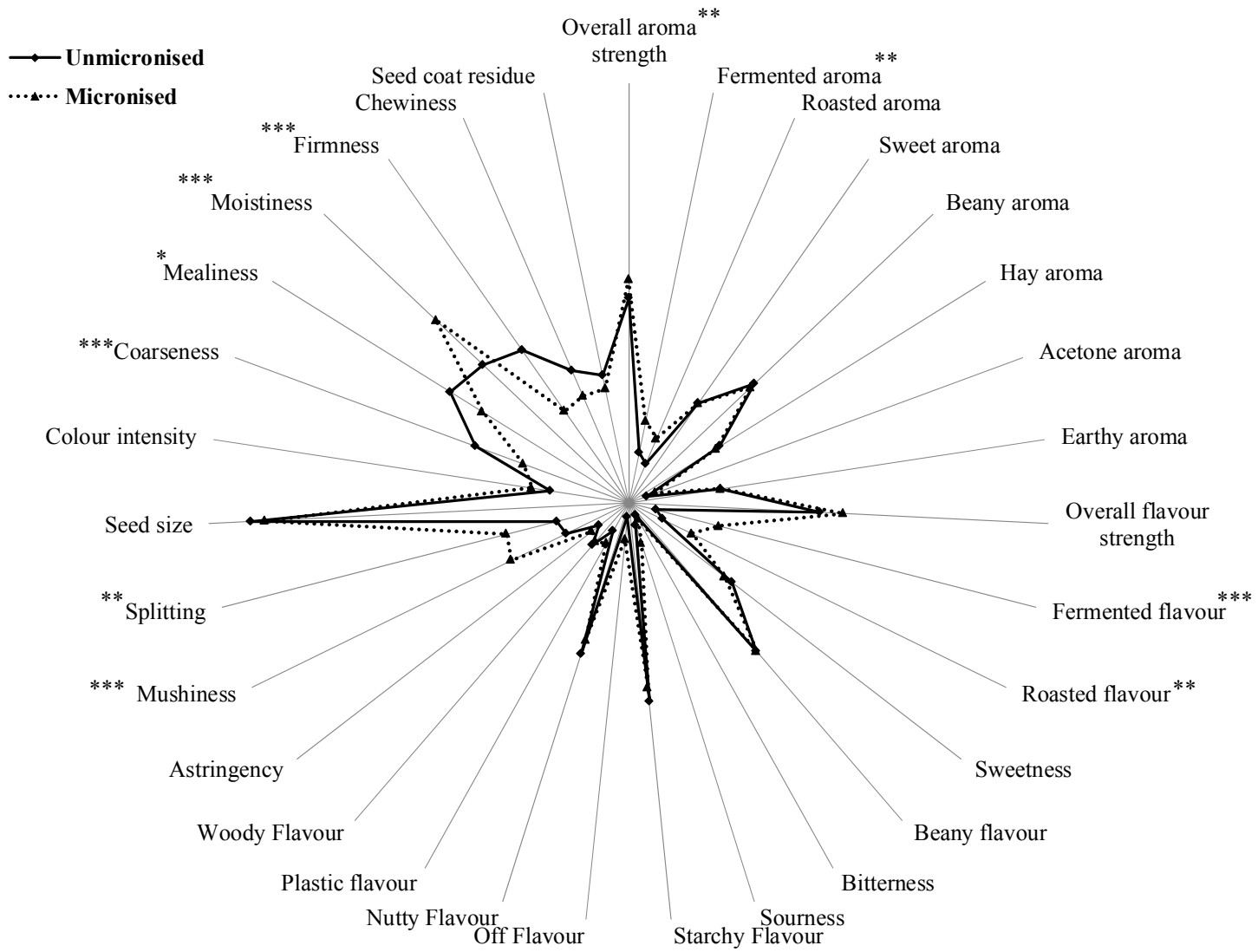


Figure 4.1.2 A: Effect of micronisation of pre-conditioned (41% moisture) Blackeye cowpeas on sensory properties of cooked cowpeas
Micronised and unmicronised cooked cowpeas differ significantly at *** p <0.001, **p <0.01, *p <0.05

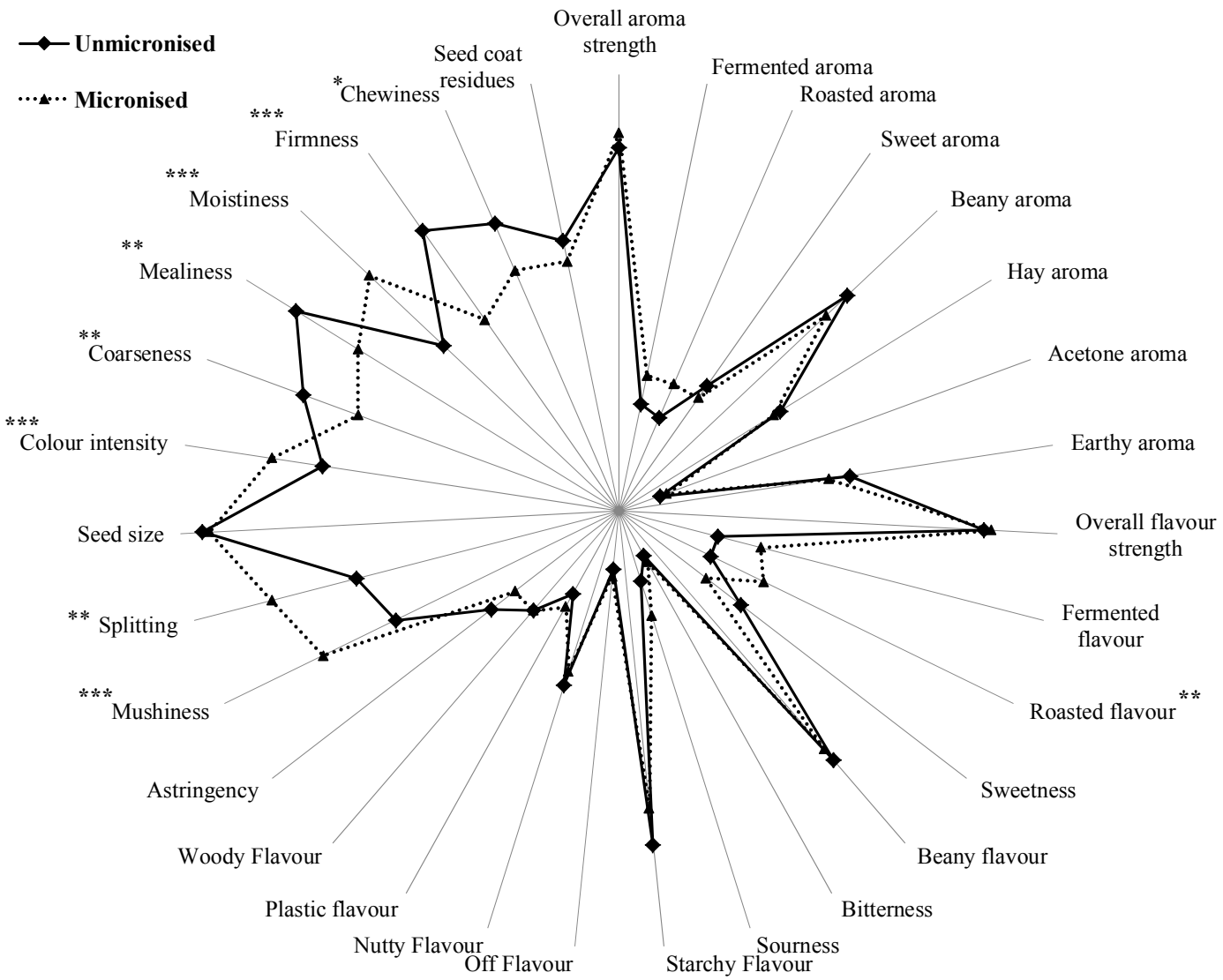


Figure 4.1.2 B: Effect of micronisation of pre-conditioned (41% moisture) Bechuana white cowpeas on sensory properties of cooked cowpeas. Micronised and unmicronised cowpeas differ significantly at *** p <0.001, **p <0.01, *p <0.05

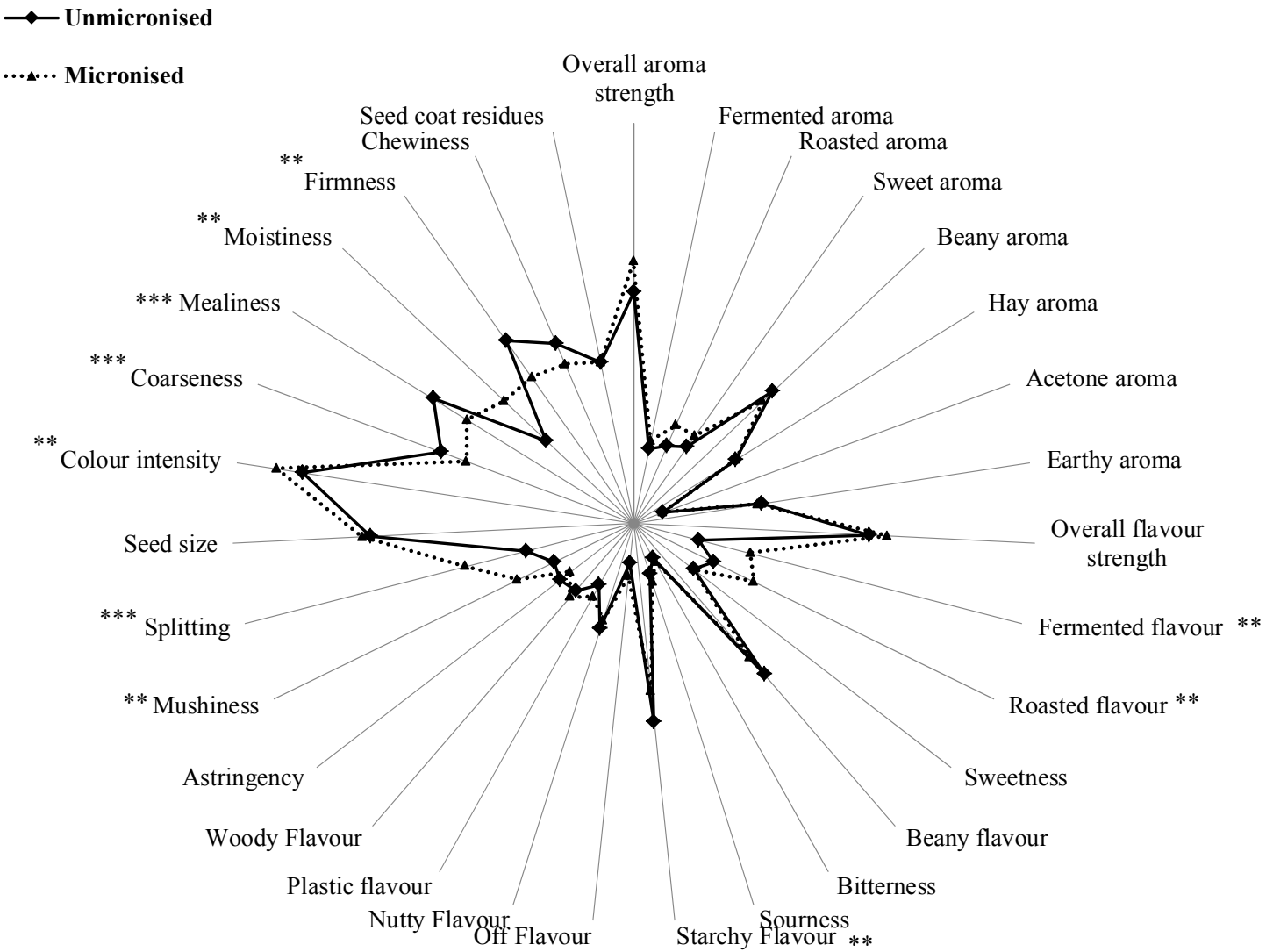


Figure 4.1.2 C: Effect of micronisation of pre-conditioned (41% moisture) Glenda cowpeas on sensory properties of cooked cowpeas
Micronised and unmicronised cowpeas differ significantly at *** p <0.001, **p <0.01, *p <0.05

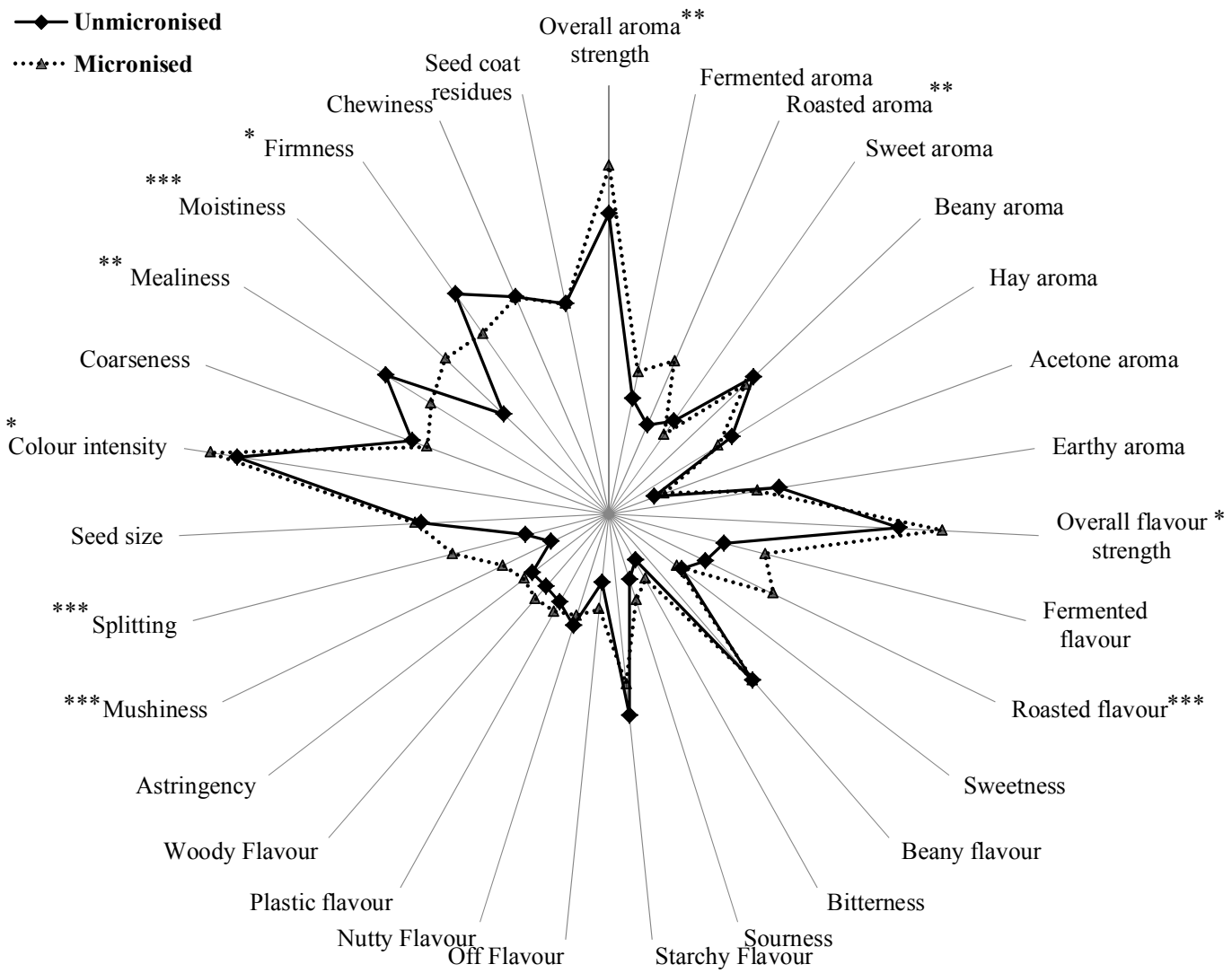


Figure 4.1.2 D: Effect of micronisation of pre-conditioned (41% moisture) Dr Saunders cowpeas on sensory properties of cooked cowpeas. Micronised and unmicronised cowpeas differ significantly at *** p <0.001, **p <0.01, *p <0.05

For all cowpea types, micronised samples had significantly higher ($p < 0.05$) intensities of roasted flavour than unmicronised samples. Overall aroma strength was enhanced in micronised samples of Blackeye and Dr Saunders. There was a significant increase in fermented flavour in micronised samples of Blackeye and Glenda (Figure 4.1.2 A and C). Micronisation of pre-conditioned cowpeas affected the textural properties of cooked cowpeas. Figure 4.1.1 (A, B, C and D) shows a significant reduction in mealiness, firmness and coarseness in micronised samples compared to unmicronised sample of all cowpea types. A significant decrease in chewiness was further observed in micronised Bechuana white sample. On the other hand micronised samples of all cowpeas types were more moist than unmicronised samples.

A multivariate data analysis model PCA was used to summarise the variation in the descriptive sensory attributes of cowpea samples. Figure 4.1.3 (A) shows the projection of scores of cowpeas and illustrates loading projections of the sensory attributes. The first two principal components described 82 % of the total variation in sensory attributes of cowpea samples. The first principal component (PCA 1) explained 56 % of the total variation. PCA 1 explained variations between cowpea types. Blackeye on the right side of the plot was differentiated from all other cowpea types on the left side of the plot. Blackeye is large seeded and lighter in colour compared to the other cowpea types. It was characterised by a more nutty flavour, sweet aroma and flavours. Dr Saunders the smallest in size as well as Glenda, both darker in colour are on the far left side of the plot.

The second principal component (PCA 2) added 26 % to the explanation of variation and separated micronised cowpeas namely: Blackeye micronised (BLKM), Bechuana white micronised (BCHM), Glenda micronised (GLM) and Dr Saunders micronised (DSRM) on the top/upper side of the plot from the unmicronised cowpeas samples on the bottom/lower side of the plot. The samples on the top/upper side of the plot were perceived with more roasted, fermented aroma and flavour, overall aroma and flavour strength, mushiness, split seeds and moistness attributes. The scores for Bechuana white on PCA 1 and PCA 2 were close to zero indicating that these two PC's did not adequately differentiate this cowpea type from the others. The third principal component (Figure 4.1.3 B) explained an additional 12 % making the total of 93 % of the variation. The third principal component differentiated Bechuana white from other cowpea varieties. Bechuana white is described as more mushy and split than other cowpea varieties.

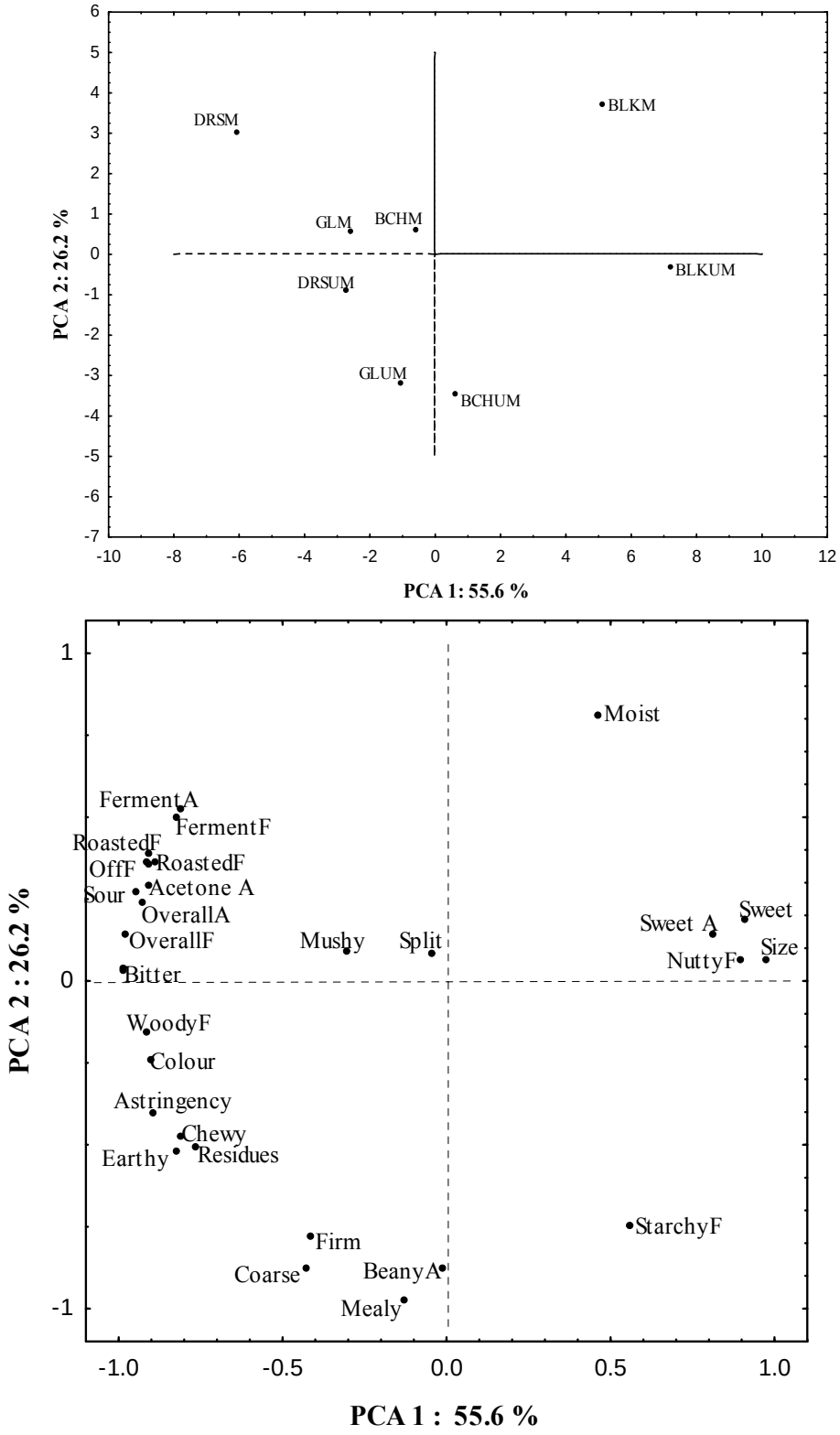


Figure 4.1.3 A: Principal Component Analysis of sensory properties of four types of cowpeas, plot of the first two principal component scores of cowpea samples and loading projections of the sensory attributes. BLKUM=Blackeye unmicronised, BLKM=Blackeye micronised, BCHUM = Bechuana white unmicronised, BCHM = Bechuana white micronised, GLUM=Glenda unmicronised, GLM=Glenda micronised, DRSUM=Dr Saunders unmicronised, and DRSM= Dr Saunders micronised, F= Flavour and A= Aroma

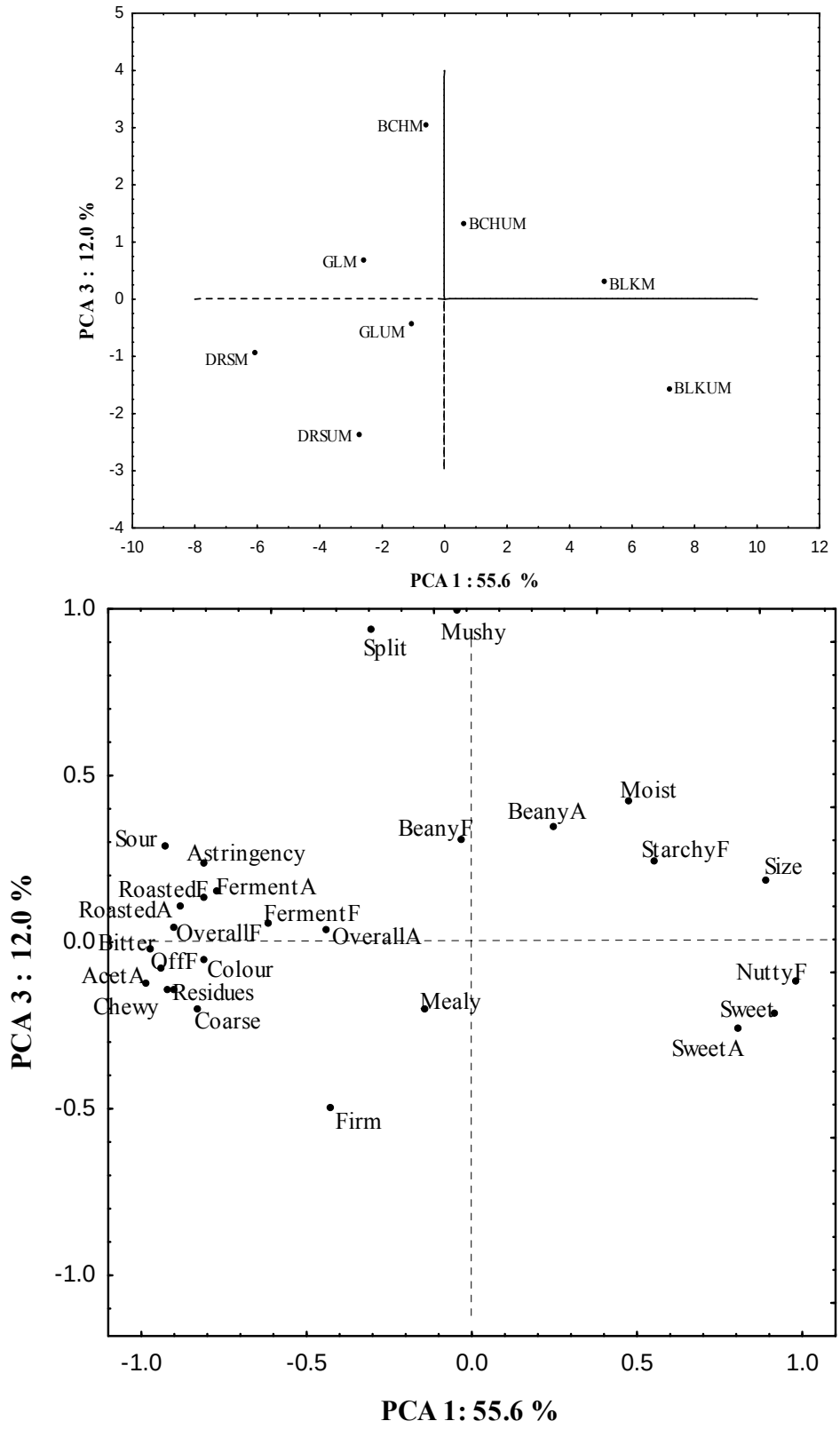


Figure 4.1.3B: Principal component analysis of sensory properties of four types of cowpeas, plot of the first and third principal component scores of cowpea samples and loading projections of the sensory attributes. BLKUM=Blackeye unmicronised, BLKM=Blackeye micronised, BCHUM = Bechuana white unmicronised, BCHM = Bechuana white micronised, GLUM=Glenda unmicronised, GLM=Glenda micronised, DRSUM=Dr Saunders unmicronised, and DRSM= Dr Saunders micronised, F= Flavour, A= Aroma and AcetA= Acetone aroma

4.1.5 DISCUSSION

Cowpea types used in the study were different in cooking time and sensory properties. This is possibly because of their inherent differences in physical and chemical properties. The cooking time values of cowpeas used in the study are within the ranges reported by Akinyele *et al.* (1986) in 18 cowpea cultivars**.** Blackeye cowpea was differentiated from other cowpeas due to its nutty and sweet flavour characteristics. Our work on cowpea phenolic compounds showed that Blackeye has significantly lower levels of phenolic compounds compared to the other cowpeas (Chapter 4.3). Phenolic compounds in legumes have been associated with undesirable flavours such as bitterness and astringency (Drewnowski and Gomez-Carneros, 2000). The presence of phenolic compounds may mask nuttiness and sweetness in cooked cowpeas. Previous reports found that consumers preferred bean (Mkanda *et al.,* 2007) and cowpea (Penicela, 2010) varieties with more nutty and sweet flavours.

Micronisation of pre-conditioned cowpeas significantly reduced the cooking time of all cowpea varieties. Reductions in cooking time of pre-conditioned and micronised legumes have been previously reported for cowpeas (Phadi, 2004; Mwangwela, 2006), lentils (Arntfield *et al*., 1997; Arntfield *et al*., 2001; Cenkowski and Sosulski, 1997) and beans (Bellido, Arntfield, Scanlon and Cenkowski, 2003). The reduction in cooking due to micronisation has been attributed to its ability to disintegrate the middle lamella between cotyledon parenchyma cells, denaturation of protein and pre-gelatinisation of starch within the cotyledon parenchyma cells that result in quicker softening of cooked cowpeas (Mwangwela, 2006).

Micronisation of pre-conditioned cowpeas increased splitting in all cowpea types. Increased cowpea splitting following micronisation was previously reported by Mwangwela (2006). During micronisation there is an increase in molecular vibrations of water that lead to rapid increase in temperature and vaporisation of water molecules that increases the volume and pressure within the seed (Fasina *et al*., 2001). The increase in pressure resulted in visible cracks on the seed coat before cooking thus increasing splitting in cooked cowpeas. Because of splitting the cowpeas seed constituents, mostly starch, leaches out in the cooking resulting in a "mash-like" paste that was described as mushiness. Bechuana white was the more split and mashed than other cowpeas types before and after micronisation. Similar results have been reported by Mwangwela (2006).

Penicela (2010) demonstrated that compactness cotyledon and thickness of seed coat limited water permeability and heat transfer. The high temperature and pressure during cooking disrupts the seed coat and cotyledon structural arrangement leading to more splitting in Bechuana white (with a thick seed coat and compact cotyledon). Consumers consider excessive splitting of cooked cowpeas as an undesirable characteristic (Afoakwa, Yenyi and Sakyi-Dawson, 2006). Selection of cowpea types that are less prone to splitting for micronisation is important to obtain more acceptable micronised cowpeas, if cowpeas are to be consumed as cooked whole seeds. However cowpea types that are more prone to splitting can be used in other food applications such as soups, sauces and pastes.

Pre-conditioned and micronised cowpeas after cooking were darker than the unmicronised samples. Darkening of pre-conditioned micronised cowpeas has been previously reported by Mwangwela (2006). Infrared heat-treated lentils were also found to be darker than raw lentils (Arntfield *et al*., 2001). Arntfield *et al*. (2001) and Phadi (2004) suggested that the browning observed in moisture-conditioned and micronised lentils and cowpeas was possibly due to Maillard browning reactions. Micronisation of pre-conditioned cowpeas introduced roasted aroma/flavour notes in all cowpea types. This could be attributed to flavours compounds such as pyrazines from Maillard reaction produced during infra-red dry heating (Sacchetti *et al*., 2004).

Fermented flavour notes were perceived in Glenda and Blackeye micronised samples. Pre-conditioning cowpeas to 41 % moisture for 18 h at ambient temperature may have resulted into activity of microorganisms naturally present in the seeds, leading to production of microbial enzymes or metabolic end products with fermented flavours e.g. volatile acids. Njoku and Okemadu (1989) reported an increased production of α-amylase and proteolytic enzymes after African oil beans were steeped at room temperature for 10-12 h. Beal, Niven, Brooks and Gill (2005) found that incubating barley or wheat grains at 30 °C for 24 h resulted in production of lactic, acetic, and butyric acids. Fermented flavour compounds produced at the pre-conditioning stage may have been carried over even after infrared heating of cowpeas. In future, modification of the pre-conditioning temperature (lower temperatures recommended) to eliminate these flavours is recommended

Micronisation of pre-conditioned cowpeas reduced coarseness, chewiness and firmness of cooked cowpeas. This indicates that micronised cowpeas had softer texture than unmicronised samples. Micronisation causes fissuring of seed coat, cotyledon, and parenchyma cell wall and reduces the bulk density of micronized seeds (Mwangwela *et al*., 2006). These changes in physical structure improve the hydration rate which in turn result in cooked cowpeas with a softer texture (Mwangwela *et al*., 2006). The desired levels of softness preferred by cowpea consumers is not known, the advantage of micronisation is that micronised cowpeas seeds attain a softer texture faster than unmicronised seeds.

Results from this study indicate that micronisation of pre-conditioned cowpeas presents an opportunity to improve the utilisation cowpeas, by reducing cooking time to almost 50% in some cowpea types. On the other hand micronization affects sensory properties e.g. increased splitting, slight darkening and introduction of uncharacteristic aroma/flavour notes in cooked cowpeas that may be unfamiliar to cowpeas consumers. These changes may elicit negative consumer reaction to micronised cowpeas. Several studies have linked consumer preference to familiarity (Hetherington, Bell and Rolls, 2000; Hetherington, Pirie and Nabbi, 2002). Porcherot and Issanchou (1998) found that familiar flavours are usually also the most preferred. However increased liking was reported with repeated exposure of unfamiliar food products (Pliner, 1982). It is expected that repeated exposure to sensory properties of micronised cowpeas together with the knowledge of reduced cooking time advantage would positively drive consumer acceptance of these cowpeas. Consumer acceptability studies and further studies on the effect of repeated exposure of micronised cowpeas to the consumers should be carried out in the future.

4.1.6 CONCLUSION

Reduction in cooking time of cowpeas as a result of micronisation implies that less energy and time will be required for preparation. Such advantages could improve utilisation of cowpeas. However, micronisation affects the sensory characteristics of cooked cowpeas e.g. increasing mushiness and seed splitting. Some cowpea types are more prone to splitting and mushiness than others, thus should rather be used in food applications like pastes or soups where intact cooked seeds are not necessary. Micronisation results in darker cowpeas with roasted flavour notes on cooking. This may or may not be unfamiliar or undesirable to cowpea consumers. Consumer acceptability studies are required to determine how to effectively market micronised cowpeas with shorter cooking times but varying sensory properties to target consumers.

4.2 Effects of micronisation of pre-conditioned cowpeas on phenolic composition of uncooked and cooked cowpeas.

ABSTRACT

Micronisation, an infrared heat treatment applied to pre-conditioned cowpeas reduces their cooking time. Micronisation may affect cowpea bioactive components such as phenolic compounds and hence the antioxidant properties and potential health benefits of cowpeas. This study determined the effects of micronisation of pre-conditioned cowpeas on phenolic acids and flavonoids of uncooked and cooked cowpeas using Ultra Performance Liquid Chromatography (UPLC), coupled with diode array detection and mass spectrometry (UPLC/ PDA /MS). Four cowpea types namely Blackeye, Bechuana white, Glenda and Dr Saunders were used in the study.

Twenty seven phenolic compounds were identified: 6 phenolic acids, 14 flavonols and 7 flavan-3-ols. Protocatechuic acid, *p*-coumaric acid, 4-hydroxybenzoic acid and ferulic acid were the most predominant phenolic acids in cowpea types analysed. Catechin, catechin-3-*O* -glucoside, myricetin, rutin, quercetin and its mono and diglycosides were present in all samples analysed. Dr Saunders (701.7−849.2 µg/g) and Glenda (571.9−708.1 µg/g) contained the highest content of total phenolics quantified, followed by Bechuana white (361.5−602.3 µg/g) and Blackeye (152.0−224.5 µg/g). Ferulic acid and *p*-coumaric acid concentrations were significantly higher in all micronised samples of Dr Saunders and Glenda compared with the unmicronised samples. *Para*-coumaric acid concentrations were higher in all micronised samples of Blackeye cowpeas than in unmicronised samples. The micronisation process could release cell wall bound ferulic acid and *p*-coumaric, increasing their concentrations in micronised samples. On the other hand, extracts from all micronised samples of Bechuana white and Glenda cowpeas had lower concentrations of catechin than unmicronised samples. Heat generated within cowpea seeds during micronisation and subsequent cooking may result in complexation of seed phenols with its macromolecules such as proteins reducing their extractability.

4.2.1 INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is an annual, warm-season, herbaceous legume widely grown in tropical countries (Davis *et al*., 1991). Legumes including cowpeas play an important role in the traditional diets of many regions throughout the world (Phillips *et al*., 2003). Consumption of legumes has been linked to reduced risk of diabetes, obesity and coronary heart disease (Bazzano *et al*., 2001). The beneficial physiological effects of legumes are attributed to the presence of bioactive components such as phenolic compounds (Cardador-Martinez *et al*., 2002). Cai *et al*., (2003) identified phenolic acids such as protocatechuic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid and 4-dimethoxybenzoic acid in 17 cowpea types. Cai *et al* (2003) found that protocatechuic acid was the most abundant phenolic acid in cowpeas. Ojwang *et al*. (2012) reported that Mono-, di-, and tri (acyl) glycosides of quercetin were predominant in most cowpea phenotypes, Dueñas *et al*. (2005) reported that quercetin diglycoside, myricetin 3- *O* -glucoside, quercetin 3- *O* -galactoside, quercetin 3- *O* -glucoside and quercetin feruloyl-diglycoside were present in cowpeas.

Cooking of dried cowpea seeds is often achieved after boiling for up to 2 h, resulting in high energy consumption and long cooking time (Mwangwela, 2006). Micronisation, an infrared heat treatment has been reported to reduce the cooking time by up to 50 % in cowpeas (Chapter 4.1). Mwangwela *et al*. (2006) reported the disruption of cell structure e.g. disintegration of the middle lamella between cotyledon parenchyma cells due to micronisation of cowpeas. The changes that occur during micronisation may affect phenolic compounds and hence the potential health benefits of cowpeas. This study aimed at determining effects of micronisation of pre-conditioned cowpeas on their phenolic composition.

4.2.2 MATERIALS AND METHODS

4.2.2.1 Cowpea samples

Four cowpea types, Bechuana white, Glenda, Dr Saunders and Blackeye were used in this study. The cowpea types were selected based on their differing shades of colour and expected influence on their phenolic contents for comparison purposes.

Glenda (dark brown) and Dr Saunders (red) could be classified as dark coloured types and were expected to have higher phenolic content. Bechuana white (light brown) and Blackeye (cream) could be classified as light coloured types with expected lower phenolic content. All cowpea samples were cleaned, packed and stored at 4 °C until use.

4.2.2.2 Preparation of pre-conditioned/micronised and cooked cowpea samples

Micronised and cooked cowpea samples were prepared as described in Chapter 4.1. The cooked samples were freeze dried, packed and stored at 4 °C until use.

4.2.2.3 Preparation of cowpea extracts

Extracts from raw and cooked/freeze dried cowpea samples (milled to pass through a 500 µm mesh) were prepared using acidified methanol (1% conc. HCl in methanol). Each sample (3 g) was extracted with 30 ml solvent in three phases as follows: 10 ml solvent was added to 3 g of the sample in a conical flask and completely covered with aluminium foil. The sample was stirred for 3 h, transferred to a 40 ml plastic centrifuge tube, centrifuged at 3500 rpm for 10 min (25ºC) and decanted, keeping the supernatant. The sample residue was rinsed again with 10 ml of the solvent, stirred for 20 min centrifuged again as above and decanted, keeping the supernatant. The residue was re-extracted as in the second extraction step. The supernatants were combined and stored in a glass bottle covered with aluminium foil and kept at 4 °C until analysed.

4.2.2.4 Identification and quantification of cowpea phenolic compounds using Ultra UPLC/PDA/MS.

A Waters Acquity UPLC/MS system (Waters Corp., Milford, MA) was used. The UPLC was equipped with a binary solvent manager, sample manager, column heater, and photodiode array detector and interfaced with a tandem quadrupole (TQD) mass spectrometer equipped with an electrospray ionisation (ESI) source. Chromatographic analysis of predominant phenolic acids and flavonoids was done by a method described by Rogachev and Aharoni

(2012). Cowpea methanolic extracts were filtered through a 0.45 µm PTFE syringe filter prior to HPLC injection. For quantification, standards of phenolic compounds were purchased from Sigma Aldrich. The standards were phenolic acids (gallic, ferulic, *p*-coumaric, protocatechuic acid, sinapic and 4-hydroxybenzoic) and flavonoids (catechin, quercetin, and rutin). Phenolic compound standards were prepared in DMSO (dimethyl sulphoxide, HPLC grade) at concentrations of 400, 200, 100, 10, 0.1 and 0.01 ppm (mg/l).

UPLC/ PDA /MS *conditions*: A UPLC BEH C18 column (Waters Acquity), 100 x 2.1 mm i.d., 1.7 µm, with a column pre-filter was used. Solvent system consisted of mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile) delivered at 0.3 ml/min in a linear gradient. Solvents were delivered in a linear gradient as follows:
0 % B in 1 min, 28 % B in 21 min, 40 % B in 1 min, 100 % B in 2 min and 0 % B in 1 min. Sample injection volume was 4 µl, auto sampler temperature was set to 12ºC and column temperature was 35ºC. The Acquity UPLC PDA detector was set to acquire spectra data in the range of 210 to 500 nm. A Synapt HDMS detector with an ESI source was used for mass spectrometry. MS spectra were acquired in a negative mode from 50 till 1500 Da with scan duration of 0.4 s. and an inter-scan delay of 0.02 s, in the centroid mode. Acquisition in the centroid mode is essential for further data treatment with peak picking programs. Argon was used as a collision gas and leucine enkaphalin for lock mass calibration. The following MS parameters were used for analysis of phenolic compounds in cowpea samples: capillary voltage - 2.4 kV; cone voltage - 28 eV; source temperature - 125 °C; desolvation temperature - 275 °C; desolvation gas flow - 650 L/h.; collision energy - 4 eV. For the acquisition of MS/MS spectra, collision energies were set from 10 to 50 eV. Specific data for phenolic compounds was acquired using MassLynx 4.1 instrument software (Waters, Milford, USA).

4.2.3 STATISTICAL ANALYSES

The experiments were repeated at least twice and analyses were performed in duplicate. MANOVA including cowpea type, micronisation and cooking and their interactive effects as independent variables on cowpea phenolic acids and flavonoids was performed. Means for all analyses were compared at $P < 0.05$ using Fisher's least significant difference (LSD) test.

Principal component analysis (PCA) was used to evaluate the multivariate factors distinguishing the phenolic profile of uncooked (unmicronised and micronised) and (unmicronised and micronised). Statistica software version 10.0 (Statsoft, Tulsa, OK, USA) was used for all data analysis.

4.2.4 RESULTS AND DISCUSSION

Figure 4.2.1 shows the UPLC chromatograms of uncooked/unmicronised Glenda extracts (used as an example) in which all 37 peaks were identified. Identification of phenolic compounds was achieved by comparing their elution profile, UV-VIS spectra, molecular and fragmentation ions to standards and/or literature information on phenolic compounds. Phenolic compounds identified by UPLC/PDA/MS in cowpea methanolic extracts are presented in Table 4.2.1. Six phenolic acids were identified as gallic acid, 4-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, protocatechuic acid and sinapic acid. All these phenolic acids were detected in all cowpea samples analysed (Table 4.2.2). Cai *et al*. (2003) and Sosulski and Dabrowski (1984) reported the presence of phenolic acids such as *p*-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid and ferulic acid in cowpeas. These phenolic acids were also reported in other legumes. Troszyńska *et al*. (2005) reported the presence of *p*-coumaric and sinapic acids in field pea cotyledons. Phenolic acids such as 4-hydroxybenzoic, protocatechuic, gallic, ferulic acids and *p*-coumaric have been reported in chickpea, pigeon pea, navy bean and mung beans (Sosulski and Dabrowski, 1984).

Seven flavanols or flavanol derivatives were identified (Figure 4.2.1). Peaks 10 and 17 showed molecular ion $[M-H]^-$ at *m/z* 289 and these were identified as catechin and epi-catechin, respectively (Amarowicz and Pegg, 2008). Peak 8 showed molecular ion at *m/z* 451 and was identified as (+)-catechin 3-*O*-glucoside (Amarowicz and Pegg, 2008). Peak 28 with molecular ion at *m/z* 737 was identified as (-)-epicatechin-(2a-7) (4a-8)-epicatechin 3-*O*-galactoside (Neveu, Perez-Jiménez, Vos, Crespy, Du Chaffaut, Mennen, Knox, Eisner, Cruz, Wishart and Scalbert, 2010). Peaks 7 and 15 showed molecular ion at *m/z* 593 and were identified as prodelphidin dimers (Amarowicz *et al*., 2010). Peaks 9, 14, 19 and 20 showed molecular ion at *m/z* 577 and were identified as procyanidin dimers (Amarowicz *et al.*, 2010). Peak 12 showed molecular ion at 865 and was identified as a procyanidin trimer (Amarowicz *et al.*, 2010).

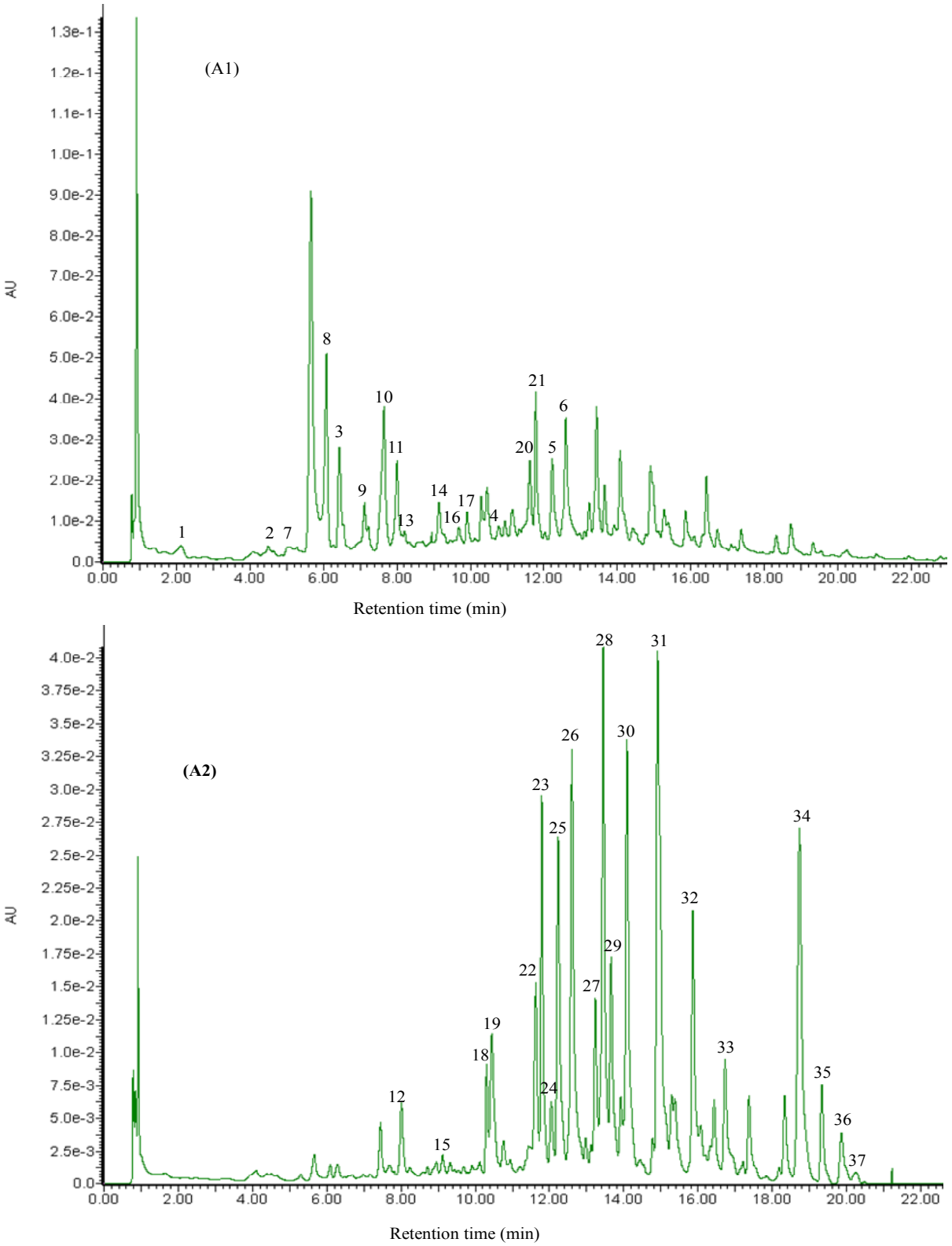


Figure 4.2.1 UPLC chromatograms of acidified methanol extracts of uncooked Glenda cowpeas showing phenolic compound peaks. (A1: extracted at 280 nm and A2: extracted at 312 nm). See Table 4.2.2 for peak identities

Table 4.2.1 Mass spectral and UV absorption data for phenolic compounds identified in all cowpea samples analysed

Pk.	* tR	PDA (λ_{max}nm)	Parent ions *m/z* [M-H]$^-$	MS/MS fragments *m/z*	Proposed compound identification
1	2.6	280	169	143(38)	Gallic acid
2	4.8	280, 296	153	109(70)	Protocatechuic acid
3	6.6	208, 256	137	93(45)	4-Hydroxybenzoic acid
4	10.7	222, 310	163	119(50)	*p*-Coumaric acid
5	12.2	216, 322	193	134(45)	Ferulic acid
6	12.7	212, 278	223	170 (80)	Sinapic acid
7	5.0	274	593	287 (100), 305 (45)	Prodelphinidin dimer (1)
8	6.2	278	451	289(100)	(+)-Catechin 3-*O*-glucoside
9	7.3	264, 295	577	407(100), 161(30)	Procyanidin dimer B1
10	7.7	279	289	300(100),301(50),	Catechin
11	8.1	285	465	303(50), 285(100)	Dihydromyricetin 3-*O*-rhamnoside
12	8.3	280, 312	865	287(100), 443(17)	Procyanidin trimer
13	8.4	282,308	451	289(100), 137(56)	(+)-Catechin 3-*O*- glucoside
14	9.0	283	577	287(100), 126 (30)	Procyanidin dimer B2
15	9.2	274	593	287(100), 289(100)	Prodelphinidin dimer (2)
16	9.3	280	465	303(100), 285(50)	Dihydromyricetin 3-*O*-rhamnoside
17	9.8	279, 310	289	245 (50), 213(60)	Epicatechin
18	10.4	254,355	641	316(100)	Myricetin-3-*O*- glucoside
19	10.5	284,312	577	287 (100), 125(50)	Procyanidin dimer B3
20	11.3	221, 282	577	287 (100), 127(50)	Procyanidin dimer B4
21	11.4	275,355	625	300(100)	Quercetin-3-*O*-dihexoside
22	11.6	265,355	625	300(100),301 (50)	Quercetin-3-*O*-dihexoside
23	11.7	262,358	479	317(30), 316(100)	Myricetin-3-*O*- glucoside
24	11.8	265,355	625	300(100)	Quercetin-3-*O*-dihexoside
25	12.1	254,355	641	316(100)	Myricetin-3-*O*-diglucoside
26	12.7	268, 344	609	284(100)	Kaempferol-3-*O*-diglucoside
27	12.8	280,326	609	284(100), 145(50)	Rutin
28	13.7	327, 353	463	301(100)	Quercetin-3- *O*- glucoside
29	14.5	378	737	394(100), 271(50)	(-)-Epicatechin-(2a-7)(4a-8)-epicatechin 3-*O*-galactoside
30	15.4	286,338	801	625(100), 301(60)	Quercetin-3-feruloyl-diglucoside
31	15.6	353	463	301 (100), 300(20)	Quercetin-3-*O*-glucoside
32	16.2	278	709	625(100), 300 (60)	Quercetin-3-diacetoyl-diglucoside
33	16.5	258, 355	563	463(100), 300 (30)	Quercetin-3-succinoyl-glucoside
34	18.8	255, 373	301	179 (40), 151(100)	Quercetin
35	19.0	330, 354	549	301(100), 505 (15)	Quercetin malonyl-glucoside
36	20.3	280	317	268(100)	Myricetin
37	21.9	278	285	279(100), 255(40)	Kaempferol

Pk: Peak ***tR**: Retention time **[M−H]**$^-$ molecular weight recorded in negative mode

Currently, there are no reports in the literature of identification of catechin-3-*O*-glucoside, prodelphinidin dimers, procyanidin dimers and trimers in cowpeas specifically. However, flavan-3-ols, their glucosides and oligomeric derivatives have been reported in other legumes such as lentils (Amarowicz *et al.*, 2010) and adzuki bean extracts (Ariga and Hamano, 1990; Amarowicz and Pegg, 2008). The monomeric flavan-3-ols (catechin, catechin-3-*O*-glucoside and epicatechin were present in all cowpea samples (Table 4.2.2). However, micronised Blackeye samples (uncooked and cooked) did not contain epicatechin. The flavan-3-ol dimers and oligomers were not detected in all Blackeye samples. Cooked (unmicronised and micronised) of Bechuana white and Glenda (all samples) did not contain procyanidin trimers, prodelphinidin dimers and epicatechin dimer. Uncooked (unmicronised and micronised) samples of Dr. Saunders contained all the dimers and oligomeric flavan-3-ol species identified in this study. These results indicate that the occurrence of dimeric or oligomeric flavan-3-ol species (tannins) in cowpeas is dependent on cowpea type. More pigmented cowpea types such as Glenda and Dr. Saunders are more likely to contain dimeric or oligomeric flavan-3-ol species compared with non-pigmented cowpea types such as Blackeye as observed in this research.

Fourteen flavonols including rutin, quercetin, myricetin and kaempferol were identified (Figure 4.2.1). Mono-, di-, and tri (acyl) glycosides of quercetin, mono- and diglycosides of myricetin as well as dihydromyricetin-3-*O*-rhamnoside and kaempferol-3-*O*-diglucoside were identified in cowpeas (Figure 4.2.1). All flavonols detected in cowpeas were previously reported in a study by Ojwang et al. (2012) in cowpeas. Table 4.2.2 shows that flavonols and their glycoside derivatives were the major flavonoids in cowpeas. These results are in agreement with those reported by Ojwang *et al.* (2012). Quercetin, its glucosides and dihexoside derivatives, myricetin and rutin could be identified in all cowpea samples. More of the flavonols in Table 4.2.3 could be identified in Glenda and Dr Saunders compared to Blackeye and Bechuana white. Most flavonols identified in this study have also been previously reported in cowpeas (Dueñas *et al*., 2005; Wang *et al.*, 2008; Ojwang *et al.*, 2012) and in other legumes such as common beans (Ranilla *et al*., 2007).

Table 4.2.2 Presence of phenolic compounds in all uncooked and cooked cowpea samples analysed (with or without micronisation)

Compounds identified	Blackeye				Bechuana white				Glenda				Dr Saunders			
	Raw		Cooked		Raw		Cooked		Raw		Cooked		Raw		Cooked	
	Umc	*Mc*	*Umc*	*Mc*	*Umc*	*Mc*	*Umc*	*Mc*	*Umc*	*Mc*	*Umc*	*Mc*	*Umc*	*Mc*	*Umc*	*Mc*
Phenolic acids																
Gallic acid	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Protocatechuic acid	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4-hydroxybenzoic acid	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
P-coumaric acid	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Ferulic acid	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Sinapic acid	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Flavanols																
Catechin	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
(+)-Catechin 3-*O*-glucose	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Epicatechin	√	nd	√	nd	√	√	√	√	√	√	√	√	√	√	√	√
Procyanidin dimers	nd	nd	nd	nd	√	√	√	√	√	√	√	√	√	√	√	√
Procyanidin trimer	nd	nd	nd	nd	√	nd	nd	nd	nd	nd	nd	nd	√	√	√	√
Prodelphinidin dimers	nd	nd	nd	nd	√	√	nd	nd	nd	nd	nd	nd	√	√	nd	nd
(-)-Epicatechin-(2a-7)(4a-8)-epicatechin 3-*O*-galactoside	nd	nd	nd	nd	√	√	nd	nd	nd	nd	nd	nd	√	√	nd	nd

Umc: Unmicronised sample, **Mc**: Micronised sample √: Compound was detected in a sample and **nd:** Compound was not detected

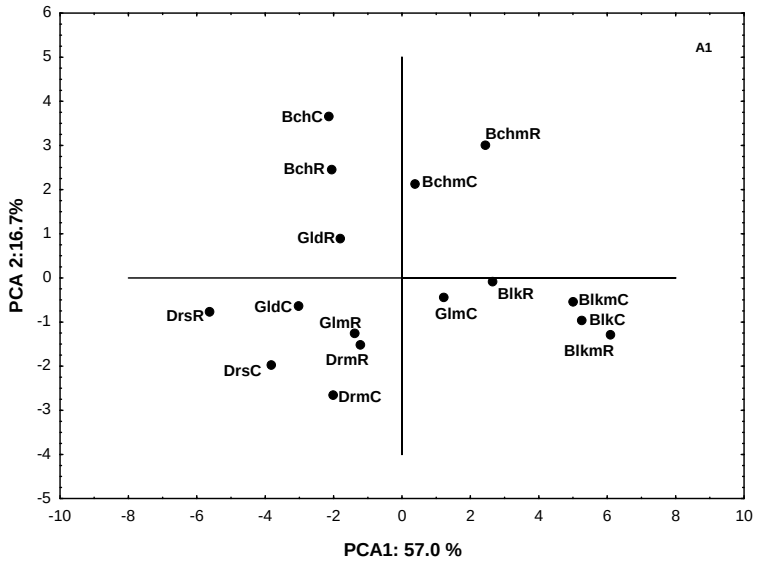
Table 4.2.2 Presence of phenolic compounds in all uncooked and cooked cowpea samples analysed (with or without micronisation) continued

Compounds identified	Blackeye				Bechuana white				Glenda				Dr Saunders			
	Raw		Cooked		Raw		Cooked		Raw		Cooked		Raw		Cooked	
	Umc	*Mc*	*Umc*	*Mc*	*Umc*	*Mc*	*Umc*	*Mc*	*Umc*	*Mc*	*Umc*	*Mc*	*Umc*	*Mc*	*Umc*	*Mc*
Flavonols																
Quercetin	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Quercetin-3- *O* - glucoside	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Quercetin-3-*O*-dihexoside	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Quercetin-3-feruloyl diglucoside	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	√	nd	√
Quercetin-3-diacetoyl diglucoside	nd	nd	nd	nd	nd	nd	nd	nd	√	√	√	√	√	√	√	√
Quercetin-3-succinoyl glucoside	nd	nd	nd	nd	nd	nd	nd	nd	√	√	√	√	√	√	√	√
Quercetin malonyl glucoside	√	nd	√	nd	√	nd	√	nd	nd	nd	nd	nd	nd	nd	nd	nd
Myricetin	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Myricetin-3-*O*-glucoside	nd	nd	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Myricetin-3-*O*-diglucoside	nd	nd	√	√	nd	nd	nd	nd	√	√	√	√	√	√	√	√
Dihydromyricetin 3-O-rhamnoside	nd	nd	nd	nd	√	√	√	√	√	√	√	√	√	√	√	√
Kaempferol	nd	√	nd	nd	nd	√	nd	nd	√	√	nd	nd	√	√	nd	nd
Kaempferol-3-*O*-diglucoside	nd	nd	nd	nd	nd	nd	nd	nd	√	nd	nd	nd	√	nd	nd	nd
Rutin	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Umc: Unmicronised sample, **Mc**: Micronised sample √: Compound was detected in a sample and **nd:** Compound was not detected

Quantification of all 27 phenolic compounds in cowpea samples analysed was limited by lack of standards for each compound identified. Using the peak area of each compound from the UPLC chromatograms, a multivariate data analysis model (PCA) was employed to summarise the variation in phenolic compounds of cowpeas. Figure 4.2.2 (A1&A2) shows the projection of scores of cowpeas and Figure 4.2.2 (B1&B2) illustrates loading projections of peak areas (representing concentration) of phenolic compounds identified.

The first two principal components described 73.7 % of the total variation of phenolic compounds in cowpea samples and showed separation based on cowpea types. On the top of Figure 4.2.2: A1 are samples of Bechuana white, centre (Glenda), bottom/left (Dr Saunders) and bottom/right (Blackeye). The first principal component (PC1) explained 57 % of the total variation where samples on the left side of the plot are differentiated from those on the right side. It was observed that most phenolic compounds were concentrated on the left side of the plot (Figure 4.2.2: B1) which indicates that samples on the left had higher concentrations of these compounds than those on the right side of the plot (Figure 4.2.2: A1). On the lower/left side of the plot the more pigmented Glenda and Dr Saunders cowpeas were differentiated from less pigmented Blackeye on the lower/right side of the plot (Figure 4.2.2.A1). Most phenolic compounds were more concentrated in Glenda and Dr Saunders than Blackeye on the left side. This was expected as cowpea seeds with pigmented seed coats have been reported to have higher levels of phenolic compounds than those that are less pigmented (Nzaramba *et al*., 2005). A study by Ojwang *et al.* (2012) also found that average flavonol content was highest in the red phenotype and lowest in the white phenotype. On the upper/left side of the plot unmicronised samples of Bechuana white cowpeas were separated from the micronised samples (Figure 4.2.2: A1). PC 1 showed that phenolic compounds were more concentrated in unmicronised samples (raw/cooked) of Bechuana white compared with micronised samples. Micronised Bechuana white cowpeas (uncooked and cooked) had lower levels of phenolic compounds compared to other cowpea types.



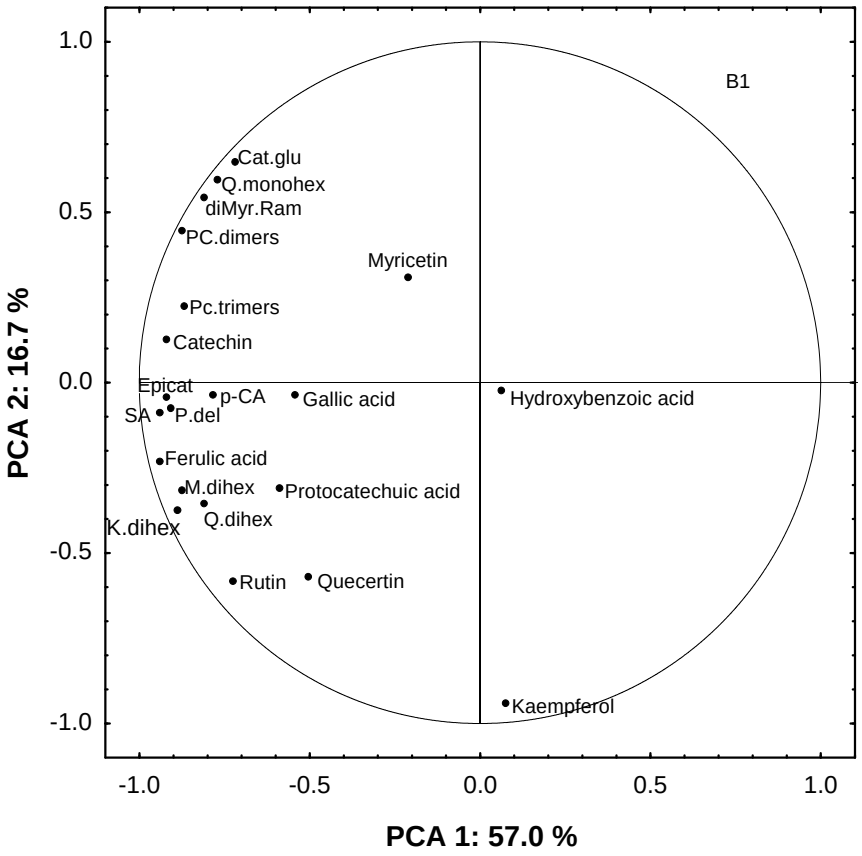


Figure 4.2.2 First and second principal component analysis of cowpea samples scores (A1) and (B1) loadings projections of peak areas (representing concentration) of phenolic compounds identified

BlkR & BlkC: Blackeye Unmicronised (Raw and Cooked), BlkmR & BlkmC: Blackeye micronised (Raw and Cooked), BchR & BchC: Bechuana white Unmicronised (Raw and Cooked), BchmR & BchmC: Bechuana white micronised (Raw and Cooked), GldR & GldC: Glenda Unmicronised (Raw and Cooked), GlmR & GlmC: Glenda micronised (Raw and Cooked), DrsR & DrsC: Dr Saunders unmicronised (Raw and Cooked) and DrmR & DrmC: Dr Saunders micronise (Raw and Cooked). FA: Ferulic acid Cat.gluc: (+)-Catechin 3-O-glucose, Epicat: Epicatechin, PC.dimers: Procyanidin dimer B1, B2, B3 & B4, PC.trimer: Procyanidin trimer, P.del: Prodelphinidin dimers (1, 2, 3), Q.dihex: Quercetin-3-O-dihexoside, Q.Monohex: Quercetin-3-0 glucosides, M.dihex: Myricetin-3-O-diglucoside, K.dihex: Kaempferol-3-O-diglucoside and diMyr.ram: Dihydromyricetin 3-O-rhamnoside.

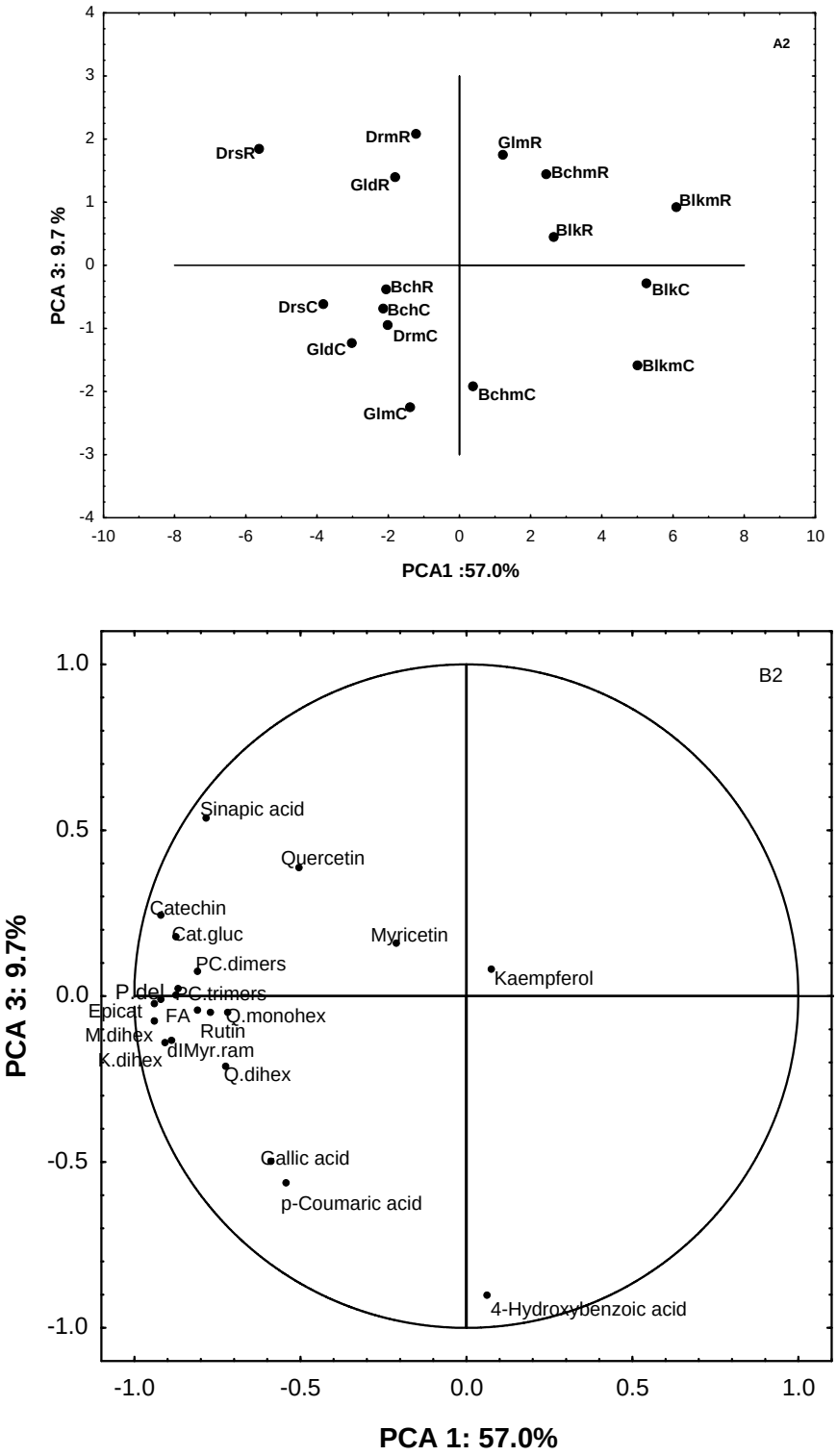


Figure 4.2.2 First and third principal component analysis of cowpea samples scores (A2) and (B2) loadings projections of peak areas (representing concentration) of phenolic compounds identified (continued)

BlkR & BlkC: Blackeye Unmicronised (Raw and Cooked), BlkmR & BlkmC: Blackeye micronised (Raw and Cooked), BchR & BchC: Bechuana white Unmicronised (Raw and Cooked), BchmR & BchmC: Bechuana white micronised (Raw and Cooked), GldR & GldC: Glenda Unmicronised (Raw and Cooked), GlmR & GlmC: Glenda micronised (Raw and Cooked), DrsR & DrsC: Dr Saunders unmicronised (Raw and Cooked) and DrmR & DrmC: Dr Saunders micronise (Raw and Cooked). FA: Ferulic acid Cat.gluc: (+)-Catechin 3-O-glucose, Epicat: Epicatechin, PC.dimers: Procyanidin dimer B1, B2, B3 & B4, PC.trimer: Procyanidin trimer, P.del: Prodelphinidin dimers (1, 2, 3), Q.dihex: Quercetin-3-O-dihexoside, Q.Monohex: Quercetin-3-0 glucosides, M.dihex: Myricetin-3-O-diglucoside, K.dihex: Kaempferol-3-O-diglucoside and diMyr.ram: Dihydromyricetin 3-O-rhamnoside.

The second principal component (PC2) added 16.7 % to the explanation of variation among samples and mostly separated Bechuana white samples (upper side of the plot) from all other samples (bottom side of the plot). Mono-glucosides of quercetin, myricetin and catechin as well as oligomeric derivative of procyanidin were more concentrated in Bechuana white (Figure 4.2.2: B1), whereas phenolic acids, aglycone, diglycosides of quercetin, myricetin and kaempferol were more concentrated in Dr Saunders. Ojwang *et al.* (2012) reported that diglycosides of quercetin were generally the most abundant flavonols in most cowpea varieties, whereas monoglycosides were detected in red cowpea varieties. The same authors found that myricetin glucosides were detected in the black, red, and green cowpea varieties, with much higher levels in the red than the black and green phenotypes. The findings from the present study indicate that cowpea type affected the type and amount of phenolic compound present. More pigmented cowpea types had higher concentrations of phenolic compounds compared to less or non-pigmented cowpeas.

The third principal component (Figure 4.2.2: A2 &B2) explained an additional 9.7 % making the total of 83.4 % of the variation explained by the principal component analysis. The third principal component differentiated raw samples (upper side of the plot) of all cowpeas from the cooked samples (lower side of the plot). PC3 shows that flavonoids such as myricetin, quercetin, catechin and kaempferol were more concentrated in raw samples than cooked samples. On the other hand phenolic acids such as 4-hydroxybenzoic acid, p-coumaric acid and gallic acid were more concentrated in cooked cowpea samples. This may be because phenolic acids such as *p*-coumaric acid are linked to various cell wall components such as arabinoxylans (Hartley *et al.,* 1990). Breakdown of cell structures as a result of cooking may release these compounds thus increasing their extractability in cooked samples. However, micronisation and subsiquent cooking may possibly result in more disruption of cell structures than micronisation or cooking alone. Thus micronised/cooked cowpeas may have higher levels these phenolics acids than cowpeas that were only cooked or micronised.

Table 4.2.3 shows levels of nine phenolic compounds quantified in the cowpea samples and a summary of the effects of micronisation of pre-conditioned cowpeas on these compounds in raw and cooked samples.

Table 4.2.3 Effects of micronisation of preconditioned cowpeas on phenolic composition of uncooked and cooked cowpeas (Db)

Samples	Protocatechuic acid	Gallic acid	Ferulic acid	Hydroxy benzoic acid	*p*-Coumaric acid	Sinapic acid	Catechin	Quercetin	Rutin	TPc
Blackeye										
Unmicronised (Uncked)	2.1(0.1)^{c}	trace	32.2(1.4)^{g}	35.7 (0.5)^{b}	12.8(0.2)^{ab}	trace	83.9(0.4)^{b}	9.2(0.6)^{b}	2.8(0.1)^{b}	**178.5**
Micronised (Uncked)	2.2(0.1)^{c}	trace	26.5 (1.8)^{e}	29.8(1.0)^{a}	26.0(0.2)^{e}	trace	52.9(2.5)^{a}	12.2(0.9)^{bc}	2.4(0.4)^{b}	**152.0**
Unmicronised (Cooked)	10.9(0.5)^{f}	trace	28.5 (0.4)^{f}	63.6 (0.9)^{e}	20.4(0.5)^{c}	trace	54.5(1.8)^{a}	13.9(1.7)^{c}	2.4(0.3)^{b}	**174.2**
Micronised (Cooked)	8.8(0.4)^{e}	trace	49.0(3.7)^{i}	62.1(2.2)^{e}	37.9(0.6)^{g}	trace	51.9(2.0)^{a}	11.5(0.2)^{bc}	2.8(0.3)^{b}	**224.0**
Bechuana white										
Unmicronised (Uncked)	1.7(0.1)^{b}	trace	26.2(1.5)^{e}	72.6(0.8)^{e}	30.2(0.3)^{f}	3.2(0.1)^{b}	459.9(1.2)^{i}	7.3(0.8)^{a}	1.2(0.2)^{a}	**602.3**
Micronised (Uncked)	0.6(0.0)^{a}	trace	20.1(0.5)^{d}	33.0(1.4)^{ab}	30.8(0.7)^{f}	trace	290.3(12.7)^{d}	30.9(1.9)^{e}	1.3(0.2)^{a}	**407.0**
Unmicronised (Cooked)	6.1 (0.1)^{c}	trace	14.3(1.6)^{b}	64.7 (1.8)^{e}	17.4(0.7)^{b}	trace	319.8(3.5)^{e}	10.9(0.3)^{bc}	1.2(0.1)^{a}	**434.4**
Micronised (Cooked)	7.3 (0.1)^{d}	trace	16.5(0.2)^{c}	52.3(1.9)^{c}	9.9(0.8)^{a}	trace	257(1.0)^{c}	17.5(1.3)^{d}	1.0(0.1)^{a}	**361.5**
Glenda										
Unmicronised (Uncked)	14.7(1.2)^{g}	20.3(0.1)^{c}	8.0(0.6)^{a}	50.7(1.4)^{c}	37.8(0.7)^{g}	trace	424.3(4.7)^{h}	149.7(1.7)^{i}	2.6(0.4)^{b}	**708.1**
Micronised (Uncked)	22.4(1.4)^{i}	18.0 (0.8)^{b}	23.5(0.2)^{de}	66.8(2.3)^{ef}	45.2(1.1)^{h}	trace	348.7(7.9)^{f}	138.1(4.7)^{h}	2.3(0.3)^{b}	**665.0**
Unmicronised (Cooked)	69.4(2.5)^{m}	23.3(2.5)^{c}	7.9 (0.4)^{a}	59.0(0.4)^{de}	24.1(0.3)^{d}	trace	368.7(12.1)^{g}	97.3(1.6)^{f}	2.2(0.1)^{b}	**651.9**
Micronised (Cooked)	30.8 (2.8)^{kl}	20.8(0.7)^{c}	19.8(0.5)^{d}	55.9(2.5)^{d}	27.3(1.2)^{e}	trace	314.5(10.6)^{e}	101.6(2.3)^{f}	1.2(0.0)^{a}	**571.9**
Dr Saunders										
Unmicronised (Uncked)	28.7(0.9)^{k}	trace	11.8 (0.2)^{b}	55.5(2.4)^{d}	25.5(2.5)^{e}	3.7(0.3)^{c}	464.4(5.8)^{i}	107.9(0.5)^{g}	4.2(0.2)^{d}	**701.7**
Micronised (Uncked)	18.5 (1.5)^{h}	trace	46.0 (0.7)^{h}	61.2(1.1)^{e}	64.9(3.2)^{i}	1.2(0.1)^{a}	423.9(7.1)^{h}	195.6(14.9)^{j}	4.7(0.8)^{d}	**816.0**
Unmicronised (Cooked)	33.6 (3.2)^{l}	15.7(1.3)^{a}	29.3(1.7)^{f}	51.9(2.0)^{cd}	48.9(1.9)^{h}	trace	457.7(8.4)^{i}	133.7(8.6)^{h}	4.3(0.3)^{d}	**775.1**
Micronised (Cooked)	24.6(1.4)^{j}	19.1(0.2)^{b}	54.0 (2.8)^{j}	70.0(2.7)^{f}	90.1(1.8)^{j}	trace	462.1(9.8)^{i}	126.4(1.8)^{h}	3.2(0.5)^{c}	**849.5**
Effects										
Samples*	*	*	*	*	*	*	*	*	*	
Cooking*	*	*	*	*	no effect	*	*	*	no effect	
Micronisation*	*	no effect	*	*	*	*	*	*	no effect	
Samples*Cooking	*	*	*	*	*	*	*	*	no effect	
Samples*Micronisation	*	*	*	*	*	*	*	*	no effect	
Cooking*Micronisation	*	no effect	*	*	no effect	*	*	*	no effect	
Sample*Cook*Micron	*	no effect	*	*	*	*	*	*	no effect	

$^{A-J}$ = mean values within a column with different letters differ significantly ($p<0.05$), Standard deviations are given in parentheses. *: Significant trace: concentrattions lower than 0.0001 **µg /g TPc**: Total quantified phenolics (phenolic acids + flavonoids); db: dry basis Phenolic compounds expressed as micro gram per gram (µg /g)

Methanolic extracts from the more pigmented cowpea types Dr Saunders (red), Glenda (dark brown) and Bechuana white (light brown) cowpeas contained higher levels of total quantified phenolics (TPCq)than Blackeye (cream) (Table 4.2.3). Madhujith and Shahidi (2005) and Rocha-Guzman *et al*. (2007) found that legumes with pigmented seed coats possess higher levels of total phenolics and flavonoids than those with lighter coloured seed coats.

Ferulic acid, 4-hydroxybenzoic acid and *p*-coumaric acid and were the most abundant phenolic acids in Blackeye and Bechuana white cowpea samples. Gallic acid, *p*-coumaric acid and 4-hydroxybenzoic acid were the most abundant phenolic acids in Glenda. Protocatechuic acid, *p*-coumaric acid and 4-hydroxybenzoic acid were the most abundant phenolic acids in Dr Saunders (Table 4.2.3). Cai *et al*. (2003) identified protocatechuic acid, *4*-hydroxybenzoic acid, *p*-coumaric acid and ferulic acid as the main phenolic acids in cowpeas. Catechin (83.9- 464.4 µg/g) and quercetin (9.2-149 µg/g) were the most abundant flavonoids in all cowpea types analysed. Quercetin has been previously reported in cowpeas by Ojwang *et al.* (2012). Duenas *et al.* (2005) found that legumes e.g. lentils and peas contained up to 92 and 162 mg/100 g catechin respectively.

Total quantified phenolic concentrations were lower in micronised samples of Bechuana white and Glenda than in the unmicronised samples. On the other hand total quantified phenolic concentrations were higher in micronised samples of Blackeye (cooked) and Dr Saunders (uncooked and cooked) than in their unmicronised counterparts. Micronised/cooked and micronised/unckooked samples of Dr Saunders and Glenda had significantly higher ferulic acid and *p*-coumaric acid concentrations compared with the unmicronised. The same trend was observed for *p*-coumaric acid concentration in Blackeye samples and 4-hydroxybenzoic acid concentration in all micronised samples of Dr Saunders as well as in micronised/ uncooked samples of Glenda. On the other hand, the measurable concentrations of catechin and 4-hydroxybenzoic contents in all micronised samples of Bechuana white were significantly lower than in unmicronised samples.

Table 4.2.4 Mean values (all cowpea types) showing the overall effects of micronisation and cooking on phenolic composition (µg /g sample) of cowpeas

	Unmicronised cowpeas	Micronised cowpeas
Phenolic acids (PA)		
Protocatechuic acid		
Uncooked cowpeas	47.2	43.7
Cooked cowpeas	120.0	71.5
Gallic acid		
Uncooked cowpeas	20.3	18.0
Cooked cowpeas	39.0	39.9
Ferulic acid		
Uncooked cowpeas	78.2	116.1
Cooked cowpeas	80.0	139.3
4-Hydroxy benzoic acid		
Uncooked cowpeas	214.5	190.8
Cooked cowpeas	239.2	240.3
***p*-Coumaric acid**		
Uncooked cowpeas	106.3	166.9
Cooked cowpeas	110.8	138.8
Sinapic acid		
Uncooked cowpeas	6.9	1.2
Cooked cowpeas	ND	ND
Flavonoids (FLV)		
Catechin		
Uncooked cowpeas	1432.5	1115.5
Cooked cowpeas	1200.4	1085.4
Quercetin		
Uncooked cowpeas	273.2	376.8
Cooked cowpeas	255.8	256.9
Rutin		
Uncooked cowpeas	10.8	10.7
Cooked cowpeas	10.2	8.2
Total phenolics TPC		
Uncooked cowpeas	2190.6	2030.0
Cooked cowpeas	2035.6	1961.9

Protocatechuic, ferulic and sinapic acid concentrations were also lower in micronised/uncooked samples of Bechuana white than in unmicronised samples. Quercetin was not affected by micronisation in all samples of Blackeye and micronised/uncooked Glenda and Dr Saunders samples. Rutin was also not affected by micronisation in all micronised Blackeye and Bechuana white samples.

In the present study three main patterns in which cowpea phenolic compounds were affected by micronisation were observed: Firstly, cowpea types behaved differently with regard to how their phenolic concentrations were affected by micronisation (Table 4.2.3). For example, for Dr Saunders measurable phenolic acid concentrations were predominantly higher in micronised samples than in unmicronised samples. On the contrary, with Bechuana white most phenolic acid concentrations were predominantly lower in micronised samples than in unmicronised samples. In chapter 4.1 Bechuana white seeds were excessively split upon micronisation and subsequent cooking compared with other cowpea types. Splitting of cowpea seeds during micronisation may have provided exposure to leaching out of the phenolic compounds in more split Bechuana white seeds than in Dr Saunders seeds that remained intact during cooking. Fasina *et al*. (2001) reported that there were higher leaching losses of solids (10-11 g/100 g) from infra-red treated (±140ºC) pinto beans after a 24 h soaking period than from raw, untreated pinto beans (1-5 g/100 g) after a similar soaking period. Fasina *et al*. (2001) suggested that cracks caused by infrared-heating may have allowed easier diffusion of water into the seed and could have facilitated the migration and leaching out of water soluble seed components.

The observed splitting of Bechuana white cowpea seeds after micronisation could be likened to cracking of pinto bean seeds after infra-red treatment reported by Fasina *et al*. (2001). It could be hypothesized that phenolic compounds may leach out with the solids. The leached out phenolic compounds stay within the system because there is no discarding of the cooking water. However, they may be more exposed to oxidative degradation during cooking compared to the intact seeds without any splitting. A study by Makris and Rossister, (2000) found that oxidative conditions [boiling with a phosphate buffer solution (p.H 8) into which air had been bubbled at 97 ºC] resulted in reduced concentration by 98 % and 45 % of quercetin and rutin respectively. In future, studies may be undertaken to study whether splitting of cowpea during micronisation and cooking may play a role in the oxidative degradation of phenolic compounds.

Secondly, phenolic acids were mostly high in micronised samples or remained unchanged by micronisation and cooking in cowpea samples. This may be primarily due to the location of these compounds in cowpea seeds and the mechanism by which micronisation affects structural and physico-chemical properties of cowpea seeds. Phenolic acids such as *p*-coumaric acid and ferulic acid are linked to various cell wall components such as arabinoxylans (Hartley *et al*., 1990). The reduction in cooking due to micronisation is partly attributed to its ability to disintegrate the middle lamella between cotyledon parenchyma cells that results in quicker softening of cooked cowpeas (Mwangwela, 2006). The middle lamella holds individual parenchyma cells, hence giving a fixed structure to the seed cotyledon.

Disruption of the cell wall structure due to micronisation may release phenolic acids from the cell wall increasing their extractability. This may explain the observed high measured phenolic acid concentrations e.g. ferulic acid and *p*-coumaric acid in both micronised (uncooked and cooked) cowpeas. Flavonoids on the other hand were reduced or remained unchanged as a result of micronisation for most samples. Heat generated within cowpea seeds during infrared heat may result in complexion of seed phenols with its macromolecules such as proteins reducing their extractability. Awika *et al*., (2003) working with sorghum, suggested that a reduction in higher molecular phenolic compounds due to thermo processing e.g. “baking” may be attributed to complexation of higher molecular polyphenols with other macromolecules such as protein.

Thirdly, cooking of micronised cowpeas resulted further in high levels of some phenolic compounds (mostly phenolic acids) in micronised samples. Cooking of micronised cowpeas may cause further breakdown of cell structures releasing bound phenolic compounds thus increasing their extractability in micronised cooked samples. Findings from this study provided a basis for understanding how micronisation affected cowpea phenolic compounds. However further information is required in particular regarding the impact of these changes in phenolic compounds on their radical scavenging capacities and potential health benefits.

4.2.5 CONCLUSION

Protocatechuic acid, *p*-coumaric acid, ferulic acid and 4-hydroxybenzoic acids are the most abundant phenolic acids in the cowpeas tested. Catechin, quercetin and myricetin, their mono-and diglycosides and procyanidin dimmers are the main flavonoids identified cowpeas analysed. Cowpea types with pigmented seed coats contain high levels of phenolics and will have more potential to exert health benefits than the less pigmented cowpeas. Cowpea type influences the type and amount of phenolic compounds in the seed and the manner in which phenolic compounds are affected by micronisation and cooking; this may influence seed selection for consumption. Though micronisation results in less extractability of some phenolic compounds, micronised samples retain some phenolic compounds and therefore may still offer potential health benefits to consumers.

4.3 Effects of micronisation of pre-conditioned cowpeas on total phenolic content, total flavonoids and in vitro free radical scavenging properties of uncooked and cooked cowpeas

ABSTRACT

Micronisation, a short-time/high temperature infrared heat treatment applied to moisture-conditioned cowpeas has been found to reduce cowpea cooking times. This part of the study determined the effects of micronisation of pre-conditioned cowpeas on total phenolic and flavonoid contents as well as *in vitro* radical scavenging properties of uncooked and cooked cowpeas. Four cowpea types namely Blackeye (cream), Bechuana white (light brown), Glenda (dark brown) and Dr Saunders (red) were used in the study.

Extracts from more pigmented cowpeas (Dr Saunders, Glenda and Bechuana white) had significantly higher levels of total phenolics (TPC), total flavonoids and radical scavenging properties than Blackeye (less pigmented). For all cowpea types, measurable total phenolic concentrations of extracts from micronised samples (uncooked and cooked) were significantly lower than that of unmicronised samples. Trolox equivalent antioxidant capacity values of micronised samples (uncooked) were lower than that of unmicronised samples for all cowpea types. Reduction in TPC and TEAC values may be possibly due to the heat induced complexion of cowpea macromolecules such as protein with phenolic compounds reducing their extractability. In contrast, micronisation had no significant effect on radical scavenging properties {TEAC and Oxygen radical absorbance capacity (ORAC)} of micronised/cooked Dr Saunders, Glenda and Bechuana white (TEAC). Micronisation and subsequent cooking may have generated heat-induced antioxidants such as Maillard reaction products contributing to radical scavenging properties in micronised (cooked) samples.

4.3.1 INTRODUCTION

Cowpeas are an important source of energy and protein in developing countries of Africa, Latin America and Asia (Phillips *et al*., 2003). They provide an alternative source of protein where meat and meat products are limited or expensive. The dry grain of cowpea is the principle product used for human consumption. (Taiwo *et al.*, 1997b). In most developing countries cowpeas are widely consumed in different forms, one of which is in boiled whole form (Taiwo, 1998).

Utilisation and consumption of cowpeas in part, is limited by their long cooking times (up to 2 h and more) that results in longer time for food preparation and increased high energy consumption (Mwangwela, 2006). Micronisation, an infrared heat treatment applied to pre-conditioned cowpeas has been reported to reduce the cooking time by up to 50 % in cowpeas (Mwangwela *et al.,* 2006). Like other hydrothermal processing technologies, micronisation affects cowpea phenolic compounds. In the previous work (Chapter 4.2), it was observed that cowpea types behaved differently with regard to how their phenolic concentrations were affected by micronisation. Findings from the previous chapter also showed high levels of most phenolic acids in micronised cowpea samples compared with unmicronised samples, whereas flavonoids showed an opposite trend. The reduction in cooking time following micronisation is partly attributed to ability of the micronisation process to disintegrate the middle lamella between cotyledon parenchyma cells (Mwangwela, 2006) and disrupt the cell wall structure which may result in release of phenolic acids from the cell wall, thereby increasing their extractability. Cooking of micronised cowpeas may cause further breakdown of cell structures releasing more bound phenolic compounds thus increasing their extractability in micronised (cooked) samples.

Phenolic compounds are primarily of interest with regards to human health because of their radical scavenging properties. The changes in cowpea phenolics due to micronisation and subsequent cooking may affect their radical scavenging properties. This part of the study aimed at determining effects of micronisation of pre-conditioned cowpeas on total phenolic content, total flavonoids and *in vitro* free radical scavenging properties of uncooked and cooked cowpeas.

4.3.2 MATERIALS AND METHODS

4.3.2.1 Preparation of cowpea samples

Cowpeas samples used in this chapter are described in the previous chapters. The cowpea samples were pre-conditioned, micronised and stored as described in the previous section 4.1.2.2 and cooked samples were prepared as described in section 4.1.2.5.

4.3.2.2 Preparation of cowpea extracts

Extracts from cowpea samples (milled to pass through a 500 µm mesh) were prepared using acidified methanol (1% conc. HCl in methanol). Each sample (0.3 g) was extracted with 30 ml solvent in three phases as follows: 10 ml solvent was added to 0.3 g of the sample in a conical flask and completely covered with aluminium foil. The sample was stirred for 2 h, transferred to a 40 ml plastic centrifuge tube, centrifuged at 3500 rpm for 10 min (25ºC) and decanted, keeping the supernatant. The sample residue was rinsed again with 10 ml of the solvent stirred for 20 min centrifuged again as above and decanted, keeping the supernatant. This step was repeated as in the second time. The supernatants were combined and stored in a glass bottle covered with aluminium foil and kept in the cold room at 4 °C until analysed.

4.3.2.3 Determination of total phenolic content (TPC) of cowpeas

The total phenolic content of cowpea extracts was determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965) as described by Waterman & Mole (1994). Cowpea methanolic extract (0.5 ml) or 0.5 ml catechin solution in acidified methanol (0.0 – 1.0 mg/ml) (used as a standard) was added to a 50 ml volumetric flask containing 10 ml distilled water. Folin-Ciocalteu's phenol reagent (2.5 ml) was added and after 2 min, 7.5 ml of 20% (w/v) sodium carbonate solution was added. The content was mixed and made up to volume with deionised water. The volumetric flask was then stoppered and mixed thoroughly by inverting several times and allowed to stand for 2 h from the addition of sodium carbonate. The absorbance was measured at 760 nm using a T80+ UV/Visible Spectrophotometer. Results were expressed as catechin equivalents (CE, mg catechin equivalents/100 mg sample).

4.3.2.4 Determination of total flavonoid content (TFC) of cowpeas

The total flavonoid content was determined using the aluminium chloride assay according to Amaral, Mira, Nogueira, Pereira da Silva and Floréncio (2009). A 30 µl volume of cowpea extract or catechin solution in acidified methanol (0.0 – 0.8 mg/ml) was added to the wells of a 96 well plate; then 30 µl of 2.5% sodium nitrite, 20 µl of 2.5% aluminium chloride solutions and then 100 µl of 2% sodium hydroxide solution were sequentially added. The samples were mixed well and absorbance was measured at 450 nm on a 96-well plate reader Bio Tek ELx 800 (Biotek Instruments Inc, Winooski, VT, USA). Total flavonoid content was expressed as mg catechin equivalents (CE)/100 mg).

4.3.2.5 Trolox Equivalent antioxidant capacity (TEAC) assay

The free radical scavenging activity of the cowpea phenolic extracts was determined using a method described by Awika *et al.* (2003). The ABTS radical cation ($ABTS^{\cdot+}$) was prepared by mixing equal volumes of 8 mM ABTS and 3 mM potassium persulphate ($K_2S_2O_8$) prepared with deionised water. The solution was allowed to react for at least 12-16 h in the dark at room temperature before use. $ABTS^{\cdot+}$ solution (2.5 ml) was added to 72.5 ml phosphate buffer solution (pH 7.4) to prepare a working solution. The working solution (2.9 ml) was added to cowpea methanolic extracts (0.1 ml) or Trolox standard the water soluble vitamin E analogue (concentration ranging from 100 to 1000µM**)** (0.1 ml) in a test tube and mixed. The test tubes were allowed to stand for 30 min. The absorbance of the standards and samples were measured at 734 nm using a UV/Visible Spectrometer. The results were expressed as µM Trolox equivalent/100 mg sample, on dry matter basis.

4.3.2.6 Oxygen radical absorbance capacity (ORAC)

The ORAC assay was carried out on a FLUOstar OPTIMA plate reader (BMG Labtechnologies, Offenburg, Germany). Procedures were based on a modified method of Ou, Huang, Hampsch-Woodill, Flanagan and Deemer (2002). This assay measures the ability of an antioxidant to quench free radicals by hydrogen donation. AAPH (2, 2'-azobis (2-amidinopropane) hydrochloride) was used as a peroxyl radical generator, Trolox as a standard, fluorescein as a fluorescent probe and phosphate buffer solution (PBS) was used as blank. Cowpea extracts were diluted 100-fold with PBS. Fluorescein working solution (0.139 M) and AAPH (0.11 µM) mixture (200 µl) was added to 10 µl of cowpea extracts or

Trolox serial dilutions. The prepared microplates were placed into the plate reader and incubated at 37˚C. The fluorescence was measured every 5 min for 4 hours. The assay protocol had the following basic parameters: a position delay of 0.5 s, a measurement start time of 0.0 s, 10 flashes per cycle, 300 s cycle time, 485 nm for the excitation filter and 520 nm for the emission filter. The ORAC values of the samples were calculated by integrating the net area under the decay curves (AUC), using Origin software Version 6.0 (Microcal, TM). The results were expressed as µmol TE per 100 mg sample.

4.3.3 STATISTICAL ANALYSES

The experiments were repeated at least twice and analyses were performed in duplicates. MANOVA including cowpea type, micronisation and cooking and their interactive effects as independent variables on total phenolic, total flavonoids and radical scavenging activities was performed. Means for all analyses were compared at $P<0.05$ using Fisher's least significant difference (LSD) test.

4.3.4 RESULTS AND DISCUSSION

Table 4.3.1 shows the total phenolic content and total flavonoid content of all cowpea samples analysed and manova data showing sample and treatment effects are presented. The TPC in leguminous seeds is one of the main parameters indicating the potential antioxidant capacity of seeds (Nwokolo and Ilechukwu, 1996). In the present study, TPC of cowpeas ranged from 3 to 23 mg catechin eq/g. Cai *et al*. (2003) reported TPC values of 0.3 to 3.8 mg protocatechuic acid eq/g in 17 cowpea varieties, while unpublished data by Awika showed a range 2.5 to 13 mg Gallic acid Eq/g in ten cowpea varieties. Variations in reported values for total phenolic content of cowpeas are due to factors such as the assay method used (e.g. type of extraction solvent and standard used for quantification) and type of cowpea sample e.g. variety (Nwokolo and Ilechukwu, 1996).

Table 4.3.1 Effects of micronisation of preconditioned cowpeas on the total phenolic and total flavonoid contents (mg CE/g) of uncooked and cooked samples of different cowpeas types (dry basis)

Cowpea samples	Total phenolics (mg CE/g)	Total flavonoids (mg CE/g)
Blackeye		
Unmicronised (uncooked)	6.0(0.4)^{c}	1.4(0.2)^{b}
Micronised (uncooked)	3.0(0.4)^{a}	1.6(0.3)^{b}
Unmicronised (cooked)	6.3(0.8)^{c}	0.9(0.0)^{a}
Micronised (cooked)	4.0(0.1)^{b}	0.8(0.1)^{a}
Bechuana white		
Unmicronised (uncooked)	19.8(1.2)^{jk}	3.6(0.3)^{e}
Micronised (uncooked)	10.8(0.6)^{f}	3.1(0.2)^{d}
Unmicronised (cooked)	14.0(0.3)^{h}	2.5(0.1)^{c}
Micronised (cooked)	8.3(0.4)^{d}	2.1(0.1)^{b}
Glenda		
Unmicronised (uncooked)	23.0(1.3)^{k}	3.5(0.6)^{e}
Micronised (uncooked)	13.0(0.1)^{g}	3.4(0.5)^{de}
Unmicronised (cooked)	13.3(0.1)^{gh}	2.8 (0.2)^{cd}
Micronised (cooked)	9.1(0.3)^{e}	2.6(0.3)^{c}
Dr Saunders		
Unmicronised (uncooked)	18.7(1.2)^{jk}	3.7(0.1)^{e}
Micronised (uncooked)	15.2(0.5)^{i}	3.7(0.4)^{e}
Unmicronised (cooked)	17.7(0.4)^{j}	3.3(0.3)^{d}
Micronised (cooked)	11.2(0.1)^{f}	3.1(0.3)^{d}
Effects		
Sample	*	*
Cooking	*	*
Micronisation	*	no effect
Samples*Cooking	*	no effect
Samples*Micronisation	*	no effect
Cooking*Micronisation	*	no effect
Samples*Cooking*Micronisation	*	no effect

$^{A-k}$ = mean values within a column with different letters differ significantly ($p<0.05$), Standard deviations are given in parentheses, * Significant and results are expressed as milligram catechin equivalent per gram mg sample

As expected, more pigmented cowpeas types e.g. Glenda Dr Saunders and Bechuana white had significantly higher TPC and TFC than Blackeye (less pigmented). Madhujith and Shahidi (2005) and Rocha-Guzman *et al.* (2007) found that pigmented legumes with dark seed coats possess higher levels of total phenolics and flavonoids than less or non-pigmented legumes with light seed coats. Ojwang *et al.* (2012) also found that the average flavonol content was highest in the red phenotype (970 µg/g) followed by light brown cowpea lines (796 µg/g) and white varieties (270 µg /g) respectively.

Table 4.3.2 Mean values (all cowpea types) showing the effects of micronisation and cooking on total phenolic and total flavonoid contents (mg CE/g) of all cowpeas samples

	Unmicronised	Micronised
Total phenolic content		
Uncooked cowpeas	16.9	10.5
Cooked cowpeas	12.8	8.2
Total flavonoids content		
Uncooked cowpeas	3.1	3.0
Cooked cowpeas	2.4	2.2

Comparing overall mean values of all cowpea types for all treatments, micronisation and cooking significantly reduced total phenolic contents (Table 4.3.2). Heat generated within the cowpea seeds during micronisation may result in degradation of phenolic compounds due to oxidation and/or formation of heat induced insoluble protein-phenol complexes reducing phenolic compound extractability (Siddhuraju and Becker, 2007; Awika *et al.* 2003). Cooking significantly reduced total phenolic and total flavonoid contents of all cowpea types (Table 4.3.2). Although for both uncooked and cooked samples, total flavonoid contents of micronised cowpeas were relatively lower than that of the unmicronised samples, for most cowpea micronisation had no significant effect on total flavonoid contents.

Phenolic compounds are of interest to human health because of their radical scavenging properties. In the present study, two assays ORAC and TEAC were used for comparison to analyse the radical scavenging properties of cowpea samples. The two assays utilise different antioxidant reaction mechanisms. ORAC reactions involve hydrogen atom transfer mechanism, while TEAC involves single electron transfer mechanism (Huang *et al*., 2005). The radical scavenging properties of all cowpea samples analysed are given in Table 4.3.3. All cowpea samples tested exhibited radical scavenging properties. As expected, the more pigmented cowpeas (Bechuana white, Glenda and Dr Saunders) exhibited higher radical scavenging properties than less pigmented cowpeas (Blackeye) for both assays.

Table 4.3.4 shows that generally, mcronised cowpeas (uncooked and cooked) have lower levels of TEAC and ORAC values than unmcronised cowpeas (uncooked and cooked). Low total phenolic concentrations, possibly due to heat induced complexion of seed components e.g. protein-phenol (Awika et al. (2003), may have resulted in their reduced measured ability to scavenge radicals. Howerver, for individual cowpea types e.g. Dr Saunders and Glenda ORAC and TEAC values of the micronised (cooked) samples remained unchanged (Table 4.3.3).

Generally, there was a significant positive correlation ($r = 0.86$, $P < 0.05$) between the total phenolic content and total flavonoid content and radical scavenging properties of cowpeas. This means that high phenolic content led to high radical scavenging properties. However there was a difference in the manner in which micronisation and subsequent cooking affected total phenolic content and antioxidant capacity. For micronised (uncooked) cowpea samples, the radical scavenging properties (TEAC values) were reduced by micronisation in all cowpea types. However TEAC and ORAC values of micronised (cooked sample) for most cowpea types remained unchanged. Further disruption of cowpea cell wall structures during cooking of micronised samples may have released more bound phenolic compounds thus increasing their radical scavenging properties. These results also may suggest that phenolic compounds from uncooked seeds may not be the only compounds contributing compounds to antioxidant activities of cooked/micronised cowpeas. But also heat induced compounds e.g. the formation of Maillard products that are exhibit antioxidant activities (Michalska, Amigo-Benaventb, Zielinski and Dolores del Castillo, 2008).

Table 4.3.3 Effects of micronisation of preconditioned cowpeas on antioxidant capacity of raw and cooked cowpeas using ORAC and TEAC assays (Dry basis)

Samples	Antioxidant capacity (TEAC) (µMTE/g)	Antioxidant capacity (ORAC) (µMTE/g)
Blackeye		
Unmicronised (uncooked)	43.2 (1.2)^{c}	123.7(6.0)^{d}
Micronised (uncooked)	36.8(0.9)^{b}	105.2(1.2)^{c}
Unmicronised (cooked)	37.0 (1.1)^{b}	98.2(3.1)^{b}
Micronised (cooked)	23.5 (0.4)^{a}	85.5(1.8)^{a}
Bechuana white		
Unmicronised (uncooked)	148.0(6.6)^{h}	233.1(4.5)^{j}
Micronised (uncooked)	107.3 (4.8)^{e}	153.2(7.9)^{g}
Unmicronised (cooked)	96.6 (0.4)^{d}	144.5(5.7)^{g}
Micronised (cooked)	95.8(0.4)^{d}	95.8(1.4)^{b}
Glenda		
Unmicronised (uncooked)	157.7 (8.1)^{hi}	235.5(1.1$^{)jj}$
Micronised (uncooked)	116.1 (2.7)^{f}	169.9(7.6)^{h}
Unmicronised (cooked)	97.7 (0.4)^{d}	134.9(4.8)^{f}
Micronised (cooked)	98.4(0.4)^{d}	129.6(1.1)^{f}
Dr Saunders		
Unmicronised (uncooked)	161.1 (1.6)^{i}	221.8(8.8)^{ij}
Micronised (uncooked)	132.5 (1.4)^{g}	219.9 (4.9)^{i}
Unmicronised (cooked)	97.7(0.2)^{d}	128.6(2.3)^{e}
Micronised (cooked)	95.9 (0.1)^{d}	133.1(4.0)^{ef}
Effects		
Sample	*	*
Cooking	*	*
Micronisation	*	*
Samples*Cooking	*	*
Samples*Micronisation	no effect	*
Cooking*Micronisation	*	no effect
Samples*Cooking*Micronisation	*	no effect

$^{A-L}$ = mean values within a column with different letters differ significantly ($p<0.05$), Standard deviations are given in parentheses, * Significant and results are expressed as milligram catechin equivalent per 100 mg sample

Table 4.3.4 Mean values of all cowpea types showing the effects of micronisation and cooking on antioxidant capacity (µMTE/g) of raw and cooked cowpeas (derived from Table 4.3.3)

	Unmicronised	Micronised
TEAC values		
Uncooked cowpeas	127.5	98.2
Cooked cowpeas	82.24	78.4
ORAC values		
Uncooked cowpeas	203.5	162.1
Cooked cowpeas	126.6	111.0

The two research chapter 4.2 and 4.3 provided information on the effect of micronisation on cowpea phenolics and radical scavenging properties. However more information on how micronisation affects the ability of cowpeas to exert health promoting properties is required. In the next chapter the effects of micronisation on cowpea protective effects against Low Density Lipoprotein (LDL) oxidation, oxidative DNA damage and red blood cell haemolysis will be reported.

4.3.5 CONCLUSION

Total phenolic contents, total flavonoids contents and radical scavenging are affected by cowpea types and may influence the application of cowpeas for consumption as a source of antioxidants. The micronized samples show lower total phenolic concentrations compared with unmicronised samples of all cowpea types. However, radical scavenging properties of micronised samples especially cooked samples do not differ from the unmicronised samples for most cowpea types studied. This indicates that micronised samples retain their ability to scavenge highly reactive free radicals and therefore may offer some potential health benefits to consumers.

4.4 Effects of micronisation on cowpea protective effects against Low Density Lipoprotein (LDL) oxidation, oxidative DNA damage and red blood cell haemolysis

ABSTRACT

Cowpeas (*Vigna unguiculata* L. Walp) are legumes recognised as a good source of proteins in developing countries. Consumption of legumes has been associated with lower risks of developing chronic diseases. One of the factors limiting cowpea utilisation is their long cooking times, which results in increased energy consumption. Micronisation of pre-conditioned cowpeas reduces their cooking time. Micronisation may affect cowpea bioactive components and their resultant health promoting properties. In this study, extracts from uncooked (unmicronised) and cooked (unmicronised and micronised) samples of cowpeas were evaluated for their protective effects against *in vitro* copper-induced LDL oxidation, AAPH- induced red blood cell haemolysis and oxidative DNA damage.

In all cowpea types, extracts from uncooked cowpeas inhibited copper-induced LDL oxidation in a dose dependent manner. At the highest sample concentration (10 mg/ml), Dr Saunders exhibited the highest ability for inhibition of LDL oxidation, followed by Bechuana white, Glenda and Blackeye, respectively. Extracts from all samples analysed exhibited protective effects against AAPH-induced red blood cell haemolysis. Extracts from Bechuana white offered the highest protection against red blood cell haemolysis. Extracts from all samples analysed offered protection against AAPH-induced DNA damage in a range of 54 to 77%. Extracts from more pigmented Glenda, Dr Saunders and Bechuana white offered) higher protection against AAPH-induced DNA damage than extracts from Blackeye (less pigmented). Micronisation of pre-conditioned cowpeas and subsequent cooking reduced their ability to inhibit LDL oxidation but did not affect their protective effects against AAPH-induced RBC haemolysis and oxidative DNA damage. Results from this study show that extracts from all cowpea samples protects DNA, LDL and RBC from oxidative damage and have a potential to reduce oxidative stress implicated in the development of chronic diseases. This is because cowpea phenolic compounds possess the ability to reduce oxidative damage associated with development of these diseases.

4.4.1 INTRODUCTION

Legumes play an important role in the traditional diets of many regions throughout the world. Consumption of legumes has been linked to reduced risk of diabetes and obesity and have an inhibitory role in the reduction of coronary heart diseases (Bazzano *et al*., 2001). Reactive oxygen species (ROS) generated in biological systems through endogenous metabolic systems and exogenous sources such as pollution (Tedesco *et al*., 2000) are capable of oxidising cellular proteins, nucleic acids and lipids (Heim *et al*., 2002). ROS contribute to cellular aging, mutagenesis, carcinogenesis and coronary heart diseases possibly through destabilisation of membranes, deoxyribonucleic acid (DNA) damage and oxidation of low-density lipoprotein (LDL) (Heim *et al*., 2002).

Red blood cells are considered as prime targets for free radical attack owing to the presence of both high membrane concentration of polyunsaturated fatty acids and the O_2 transport associated with redox active haemoglobin molecules, which are potent promoters of reactive oxygen species (Girish, Vasudevaraju and Prasada Rao, 2012). DNA damage induced by free radicals is also a common event in all living cells and oxidative modification of low-density lipoprotein (LDL) cholesterol is a major contributing factor in the pathogenesis of atherosclerosis (Madhujith and Shahidi, 2005). Polyphenolic constituents of various legume seeds have been reported to contain potential medicinal properties, including antioxidant activities (Cardador-Martinez *et al*., 2002; Mazur *et al*., 1998). Findings from the previous work (Chapter 4.2) showed that protocatechuic acid, p-coumaric acid, ferulic acid and 4-hydroxybenzoic acids were the most abundant phenolic acids in cowpea samples analysed. Furthermore, catechin, quercetin and myricetin, their mono-and diglycosides and procyanidin dimers were the main flavonoids present in cowpeas. It was also observed that phenolic compounds contributed to radical scavenging properties of cowpeas (Chapter 4.3). Jacob and Burri (1996) hypothesised that antioxidants suppressed the formation of free radicals, quench the existing radicals, and reduce the availability of oxygen in biological systems to prevent the oxidative damage of DNA, proteins and lipids in the human body.

Epidemiological studies have found a significant inverse relationship of per capita consumption of food rich in dietary antioxidants with coronary heart disease mortality (Zhou, Laux and Yu, 2004; Adom, Sorrells and Liu, 2003). Even though cowpea seeds are increasingly consumed as human food, their potential health benefits remain largely unexplored.

Cowpeas are commonly consumed as cooked whole seeds; this presents a challenge as long cooking times are required to obtain a softer palatable texture (Akinyele, Onigbinde, Hussain and Omololu, 1986). Micronisation, an infrared heat treatment has been reported to reduce the cooking time by up to 50 % in cowpeas (Chapter 4.1). Findings from the previous chapter (4.2) found that micronisation affected the extractabilty of cowpea phenolic compounds. The changes in cowpea phenolics and radical scavenging properties due to micronisation may affect their protective effects against LDL oxidation, oxidative DNA damage and red blood cell haemolysis. This chapter aims at studying the effects of micronisation on protective effects of cowpeas against copper-induced LDL oxidation, AAPH -induced oxidative DNA damage and red blood cell haemolysis.

4.4.2 MATERIALS AND METHODS

4.4.2.1 Materials

Cowpea types used in this study were selected as described in section 4.1.2.1. Supercoiled plasmid vector pBR322 DNA (1 mg/ml), agarose (D1 LE) and bromophenol blue were purchased from Whitehead Scientific (Pty) Ltd (Johannesburg, South Africa). Copper sulphate, thiobarbituric acid, sodium hydroxide and trochloroacetic acid were purchased from Merck (Pty) Ltd South Africa (Johannesburg, South Africa). Ethidium bromide, 2, 2'-Azobis (2-methyl-propionamidine) dihydrochloride(AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboylic acid (Trolox) and human low density lipoprotein (LDL) were supplied by Sigma-Aldrich (Pty) Ltd (Johannesburg, South Africa).

4.4.2.2 Micronisation of cowpeas and preparation of cowpea extracts

Cowpea samples were pre-conditioned, micronised and stored as described in section 4.1.2.2 and cooked samples were prepared as described in section 4.1.2.5. Cowpea extracts were prepared as described in section 4.2.2.3. The extracts were stored in a glass bottle covered with aluminium foil and kept in the cold room (4 ºC) until analysed. In this part of the study, the following samples were compared to determine the effects of micrnonisation of cowpeas on protection against LDL oxidation, oxidative DNA damage and RBC haemolysis: Unmicronised (uncooked) samples (control); unmicronised (cooked) samples and micronised (cooked) samples. This is was because cowpeas can only exert health benefit to their consumers in cooked form.

4.4.2.3 Copper-catalysed human LDL oxidation

The ability of methanolic extracts from the cowpeas to inhibit oxidation of low density lipoprotein (LDL) was determined by monitoring formation of thiobarbituric acid reactive substances (TBARS) (Xu *et al*., 2007a) with modification. Cowpea extracts from all cowpea samples were dissolved in 1% HCl conc. in methanol at concentrations 1, 5 and 10 mg/ml into Eppendorf safety lock tubes. Three hundred milligrams of cowpeas (milled to pass through a 500 µm mesh) were extracted with 30 ml of acidified methanol to obtain a solution with 10 mg sample/ml acidified methanol concentration. This was then diluted 2 times to obtain a 5 mg/ml concentration and 10 times to obtain 1mg/ml concentration respectively. Two microlitres of LDL solution (5 mg/ ml), 168 µl of 0.02 M phosphate-buffered saline solution (pH 7.4) and 10 µl sample extract were placed into a 1 ml Eppendorf tube and oxidation was initiated by adding 20 µl of 500 µM copper sulphate solution. Positive controls were prepared by adding 2 µl LDL, 178 µl PBS and 20 µl copper sulphate solution. Negative controls were prepared by adding 2 µl LDL solution and 198 µl PBS. Tubes were sealed and incubated in a water bath at 37 ºC for 3 h. After incubation, the contents were left to cool down for 10 min at 4 ºC. Two hundred microliters of 10 mM EDTA solution, 200 µl of 20% (w/v) trichloroacetic acid solution and 200 µl of 0.67% (w/v) thiobarbituric acid solution in 0.2 M NaOH were added. The tubes were sealed, heated at 80 ºC for 30 min in a water bath, and after cooling they were centrifuged at 1800 x g for 10 min. The supernatant was transferred into a cuvette, the absorbance was measured at 532 nm using a using a UV/Visible Spectrometer.

4.4.2.4 Red blood cell haemolysis assay

Cowpea methanolic extracts from from all cowpea samples were tested for their ability to protect human red blood cell from haemolysis. The red blood cell haemolysis assay was performed according to Tang and Liu (2008). In this assay haemolysis of red blood cell membrane was induced with AAPH (2, 2'-azobis (2-amidinopropane) hydrochloride). AAPH generates peroxyl radicals which attack red blood cell membranes, inducing oxidation of lipids and proteins, which triggers disruptions in the membrane leading to haemolysis (Miki *et al*., 1987). Antioxidants that scavenge peroxyl radicals would reduce the rate of red blood cell haemolysis.

Human blood was obtained from student volunteers from the University of Pretoria (Department of Anatomy). Blood was obtained via vein-puncture into tubes, isolated by centrifugation at 4000 rpm for 10 min using model Z300 centrifuge (Hermle Labortechnik GmbH, Wehingen, Germany) and washed with isotonic phosphate buffer saline PBS, pH 7.4 to remove plasma and platelets. A ten percentage red blood cell solution was made up in isotonic-PBS (pH 7.4) and kept at 4˚C until used for analysis. Preliminary toxicity tests showed that high methanol concentrations in samples damaged the red blood cells. Cowpea extracts were then diluted 100 times in isotonic- PBS (pH 7.4) to obtain lower concentrations that did not cause damage to the cells. For the assay, 100 µl of 10 % RBC in isotonic- PBS (pH 7.4), 40 µl of cowpea extract or trolox standards and 40 µl AAPH solution were added into 1.5 ml safe lock eppendorf tubes. For a negative control 100 µl of 10% RBC in isotonic- PBS (pH 7.4), and 80 µl of isotonic- PBS (pH 7.4) were added into an Eppendorf tube. Two positive controls were prepared by adding 100 µl of 10% RBC in isotonic- PBS (pH 7.4) and 40 µl of AAPH solution into a tube and another tube containing 100 µl of 10% RBC in isotonic- PBS (pH 7.4) and 40 µl of 1 % triton to ensure 100 % red blood cell haemolysis was prepared. Tubes were sealed, mixed by vortexing and incubated at 37 °C for 8 h. After incubation the tubes were mixed by vortexing and centrifuged at 2750 rpm (1184 x g) for 3 min. Supernatant (50 µl) was transferred into a 96 well plate and absorbance measured at 405 nm on a Bio Tek ELx 800 plate reader (Biotek Instruments Inc, Winooski, USA). Results were expressed as percentage inhibition of red blood cell haemolysis.

4.4.2.5 pBR 322 plasmid DNA damage assay

The assay determines the capacity of cowpea extracts to protect the DNA against oxidative damage. Oxidative stress results in conversion of supercoiled plasmid DNA to relaxed circular and linear forms, which can be separated by agarose gel electrophoresis because of their differences in electrophoretic mobility (Aronovitch *et al*., 2007). The protective effects of cowpea methanolic extracts from from all cowpea samples on oxidative DNA damage induced by AAPH were evaluated by the method as described by Aronovitch *et al*., (2007) with modification. To prepare DNA working solution, the supercoiled plasmid pBR322 DNA (1 mg/ml, Whitehead Scientific (Pty) Ltd) was diluted 20 times with double distilled sterile water. Treatment samples were prepared by adding 1.25 µl DNA working solution, 1.25 µl sample extract or Trolox solution and 1.25 µl of 1.5µm AAPH solution into Eppendorf tubes. Positive controls were prepared by adding 1.25 µl DNA working solution, 1.25 µl tris-acetate buffer (pH 8) and 1.25 µl of 3µm AAPH into eppendorf tubes and negative controls by adding 1.25 µl DNA working solution and 2.5 µl tris-acetate buffer solution. Tubes were vortexed and then incubated for 90 min at 37 °C. After incubation, samples were mixed with equal amount (3.75 µl) of the loading buffer (40 % sucrose and 0.025 % bromophenol). Six microlitres of sample mixture was loaded into a 1 % agarose gel containing 40 mM tris-acetate buffer pH 8 and ethidium bromide in an electrophoresis apparatus (Owl Scientific Inc, Worburn, USA). The gel was run at 60 V, 30 mA for 3 h using electrophoresis power supply unit model EPS 301 (Amersham Pharmacia Biotech, Uppsala, Sweden). DNA bands were visualized and photographed on a UV transilluminator (Vilber Laurmat, Marne la Vallee, France). Band intensities were measured using Image Tool for Windows software version 3.00 (The University of Texas Health Center, San Antonio, USA).

4.4.3 STATISTICAL ANALYSES

The experiments were repeated at least twice and analyses were performed in duplicate. Results were expressed as means and standard deviations (SD) were given in parentheses or expressed as error bars on graphs. The data obtained was analysed by one-way ANOVA. Differences between treatments were evaluated at the 95% significance level using the least significant difference test. The statistical analyses were performed using Statistica Version 10 (Statsoft, Tulsa, USA).

4.4.4 RESULTS AND DISCUSSION

4.4.4.1 Protective effects of cowpea extracts against *in vitro* copper-induced LDL oxidation

In this assay, thiobarbituric reactive substances (TBARS), the late stage products of LDL oxidation, were measured at the end of the incubation period (Xu *et al.*, 2007). Figure 4.4.1 shows that incubation of human LDL with copper sulphate solution (positive control) caused oxidation of LDL and produced maximum TBARS. Addition of 100 µm Trolox reduced production of TBARS by 65 % compared to the positive control. The reduction in TBARS production is an indicator that LDL oxidation was inhibited in presence of Trolox. In all cowpea types, extracts from uncooked cowpeas inhibited copper-induced LDL oxidation in a dose dependent manner (Figure 4.4.1). Comparing sample extracts (10 mg/ml) with the positive control, Dr Saunders reduced production of TBARS by 61 %, followed by Bechuana white (56 %), Glenda (52 %) and Blackeye (34 %), respectively (Figure 4.4.1). These results are an indication that extracts from all cowpea types tested inhibited LDL oxidation.

Although no literature has been reported on protective effect of cowpea extracts against LDL oxidation, extracts from cereal and other legume grains were reported to inhibit lipid peroxidation (Djordjevic, Šiler-Marinkovic and Dimitrijevic-Brankovic, 2011). These authors found that extracts from rye inhibited lipid peroxidation by 58%, followed by wheat and barley (55 and 51%, respectively. Djordjevic *et al*. (2011) also reported that extracts from lentils inhibited lipid peroxidation by 54 %, followed by soybeans (49 %), mung beans (47 %) and red kidney beans (39%). Madhujith and Shahidi (2005) compared the LDL-oxidation inhibition by white kidney, red pinto, Swedish brown, and black kidney common beans and stated that the differences in anti-LDL oxidation activities were due to their phenolic composition. Results in the present study shows that extracts from more pigmented cowpea types, namely Dr Saunders, Glenda and Bechuana white, inihibited LDL oxidation more than Blackeye (less pigmented) (Figure 4.4.1). This is in agreement with the results reported in Chapter 4.3 which showed that extracts from Dr Saunders, Glenda and Bechuana white had higher total phonolic content and radical scavenging capacities than Blackeye. A study by Xu *et al.* (2007) observed that black beans, lentils, black soybeans, red kidney beans, and pinto beans exhibited a higher TBARS inhibitory activity than yellow and green peas, chickpea, and yellow soybeans.

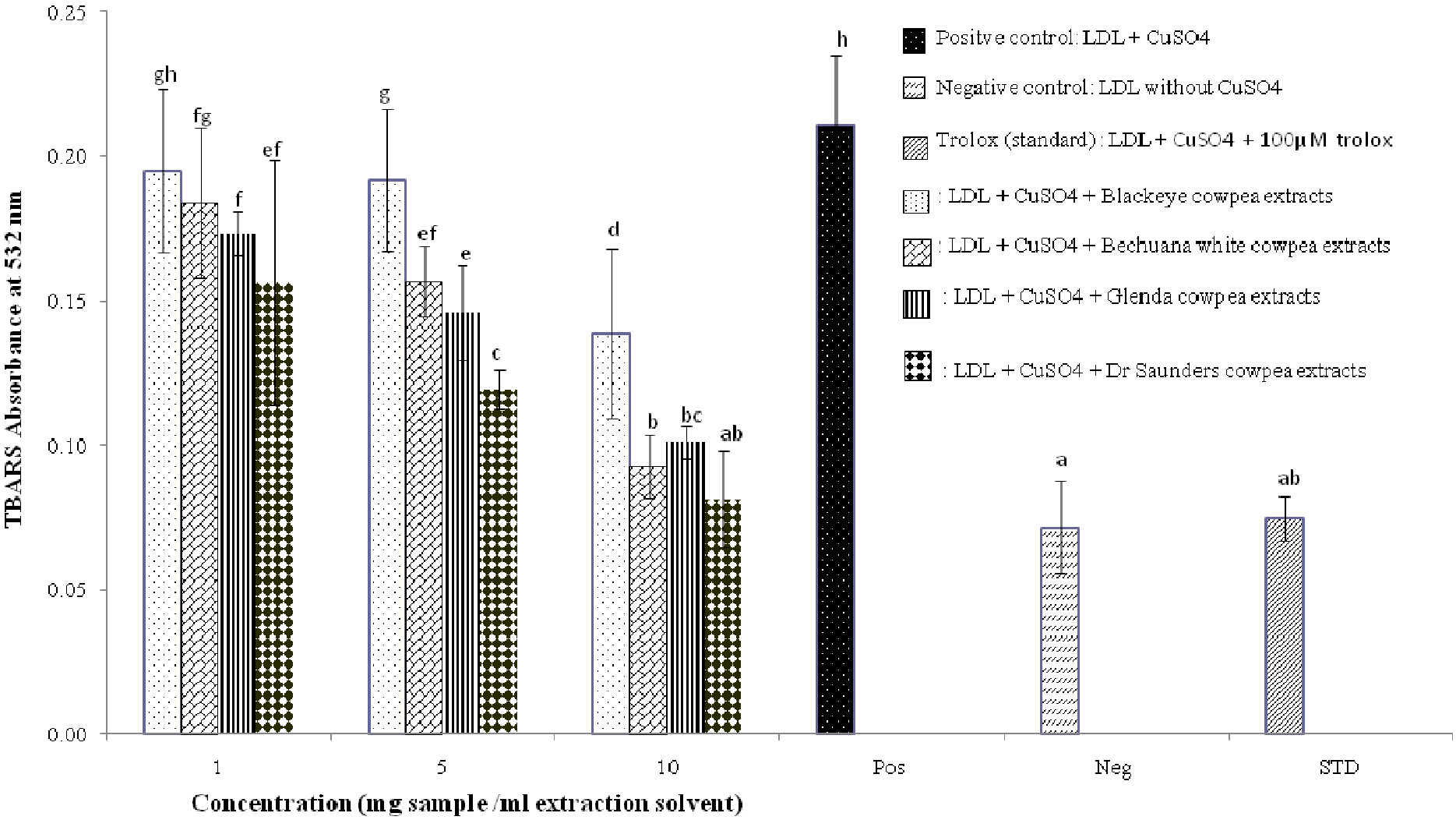


Figure 4.4.1: Protective effect of extracts from uncooked cowpeas against copper-induced LDL oxidation

[a-g:] Graph bars with different letters differ significantly ($P<0.05$)

Comparing unmicronised (cooked) and micronised (cooked) cowpeas, micronisation significantly reduced the antioxidant capacities of all cowpea types against LDL oxidation (Table 4.4.1). Antioxidants such phenolic compounds are known to offer protective effects against LDL oxidation (Gallegos-Infante, Rocha-Guzman, Gonzalez-Laredo and Pulido-Alonso, 2010). Results reported in Chapter 3.3 showed that extracts from micronised samples had significantly lower levels of total measurable phenolics compared with the unmicronised samples. This may influence the ability of extracts from micronised cowpea samples to inhibit LDL oxidation. Larrauri, Rupérez and Saura-calixto (1997) reported that drying temperatures of 100 and 140 °C resulted in significant reduction in total phenolic content resulting in a decrease of up to 50% in antioxidant activity in red grape pomace peels. Siddhuraju and Becker (2007) reported a reduction in total extractable phenolics and radical scavenging activities of cowpeas autoclaved at 120°C for 20 min.

Table 4.4.1: Effects of micronisation of preconditioned cowpeas (followed by cooking) on protective effect of their extracts (10 mg/ml) against copper-induced LDL oxidation

Sample	Antioxidant activity against LDL oxidation (µm TE/100 mg)
Blackeye	
Unmicronised (uncooked)	0.7 (0.1)^{d}
Unmicronised (cooked)	0.5 (0.0)^{c}
Micronised (cooked)	0.1 (0.0)^{a}
Bechuana white	
Unmicronised (uncooked)	1.3 (0.1)^{f}
Unmicronised (cooked)	0.9 (0.0)^{e}
Micronised (cooked)	0.2 (0.0)^{ab}
Glenda	
Unmicronised (uncooked)	1.3 (0.0)^{f}
Unmicronised (cooked)	0.9 (0.1)^{e}
Micronised (cooked)	0.3 (0.1)^{ab}
Dr Saunders	
Unmicronised (uncooked)	1.5 (0.0)^{g}
Unmicronised (cooked)	1.2 (0.2)^{e}
Micronised (cooked)	0.4 (0.0)^{b}

$^{a-g}$ = mean values within a column with different letters differ significantly ($P<0.05$), Standard deviations are given in parentheses and results are expressed as micro molar trolox equivalent (TE) per 100 mg of the sample

4.4.4.2 Protective effects of cowpea extracts against *in vitro* AAPH-induced erythrocyte haemolysis

Erythrocytes have been used as a model to investigate oxidative damage in biomembranes due to their high susceptibility to peroxidation (Zhu *et al.,* 2002). Figure 4.4.2 indicates that extracts from all cowpea samples tested protected red blood cells against AAPH-induced haemolysis. Carvalho, Ferreira, Mendes, Silva, Jose, Pereira, Jerónimo and Silva (2010) reported that walnut seed, green husk and leaf methanolic extracts protected the erythrocyte membrane from hemolysis induced by AAPH. It is known that polyphenols enhance red blood cell resistance to oxidative stress both in vitro and in vivo (Youdim, Shukitt-Hale, MacKinnon, Kalt and Joseph, 2000). Antioxidant properties of polyphenols are attributed mainly to their free radical scavenging and metal-chelating properties (Van Acker *et al*., 1996). In an erythrocyte cell model, these polyphenols may quench peroxyl radicals before they attack the lipid molecules of the erythrocyte membrane, thereby breaking the free-radical chain reaction and ultimately oxidative hemolysis (Carvalho *et al*., 2010).

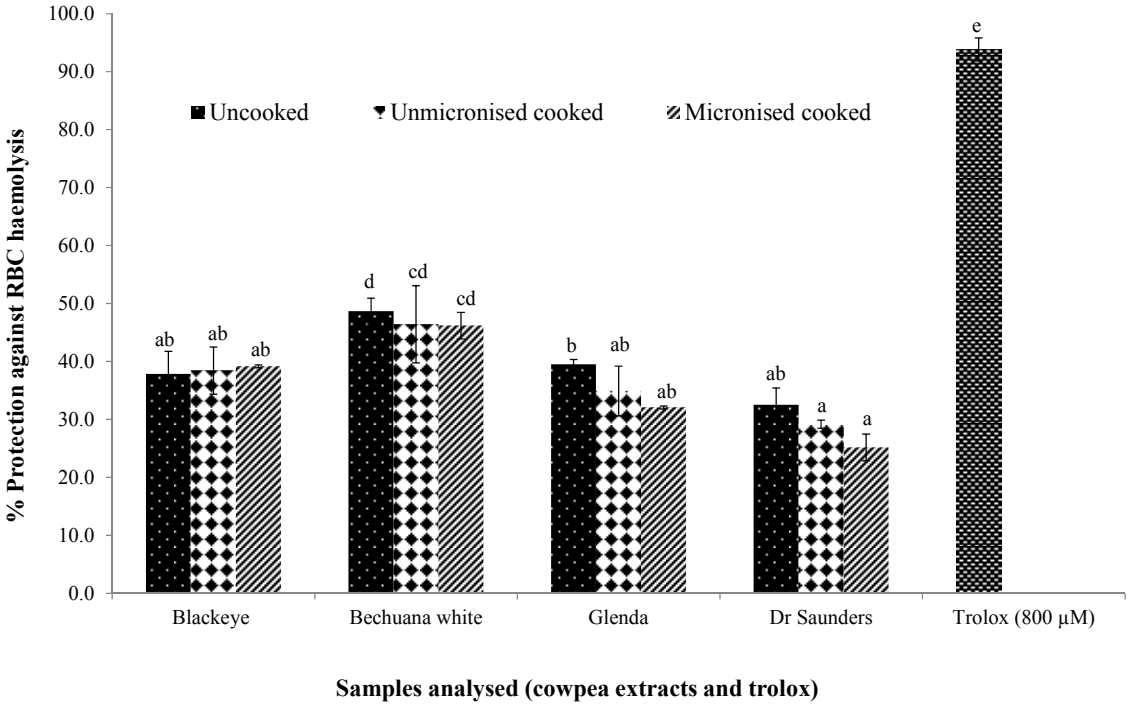


Figure 4.4.2 Effects of micronisation of preconditioned cowpeas (followed by cooking) on their protective effects of cowpea extracts against AAPH-induced red blood cell haemolysis

[a-d] = Graph bars with different letters differ significantly (P<0.05)

Among uncooked samples, extracts from Bechuana white offered the highest protection against AAPH-induced red blood cell haemolysis (Figure 4.4.2). There was no significant difference between Blackeye, Glenda and Dr Saunders extracts protection against AAPH-induced red blood cell haemolysis (Figure 4.4.2). As shown in Chapter 4.3, Glenda and Dr Saunders had levels of total phenolic three times higher than Blackeye. These results suggests that protection of RBC membrane by cowpeas may not necessarily dependent on their total phenolic content and antioxidant capacities, but rather phenolic types present in the extracts, their structure and their interaction with the cell membrane. LC-MS results in Chapter 4. 2 showed that mono-glucosides of quercetin, myricetin and catechin as well as oligomeric derivative of procyanidin were more concentrated in Bechuana white compared to other cowpea types. Tabart, Kevers, Pincemail, Defraigne and Dommesa (2009) reported differences in flavonoid's ability to protect red blood cells from haemolysis. Chen, Chan, Ho, Fung and Wang (1996) reported that quercetin had higher inhibitory effects against RBC haemolysis compared to other flavonoids such as myricetin, and kaempferol. The authors suggested that this could be due multiple factors such as their hydrophobicity/hydrophilicity, total number and location of the hydroxyl groups of an aromatic ring of a flavonoid and the interaction between the flavonoids with the phospholips, haemoglobin, iron and other components of the red blood cells. Micronisation and cooking did not have a significant effect on protective effects of all cowpea sample extracts against AAPH-induced red blood cell haemolysis (Figure 4.4.2). It is important to note that although there was increase or decrease in some phenolics upon micronisation, the radical scavenging properties of micronised /cooked samples for most cowpea types remained unchanged after micronisation (Chapter 4.3). This may explain why the ability of cooked cowpeas (micronised and unmicronised) to inhibit AAPH-induced red blood cell haemolysis did not differ.

4.4.4.3 Protective effects of cowpea extracts against *in vitro* AAPH-induced oxidative DNA damage

Oxidative stress results in conversion of supercoiled plasmid DNA to relaxed circular and linear forms. These three forms can be separated by agarose gel electrophoresis because of their differences in electrophoretic mobility (Aronovitch *et al.*, 2007). Figure 4.4.3 shows the electrophoretic pattern of plasmid DNA after AAPH-induced DNA oxidative damage in presence of extracts from uncooked (unmicronised) and cooked (unmicronised and micronised) cowpeas.

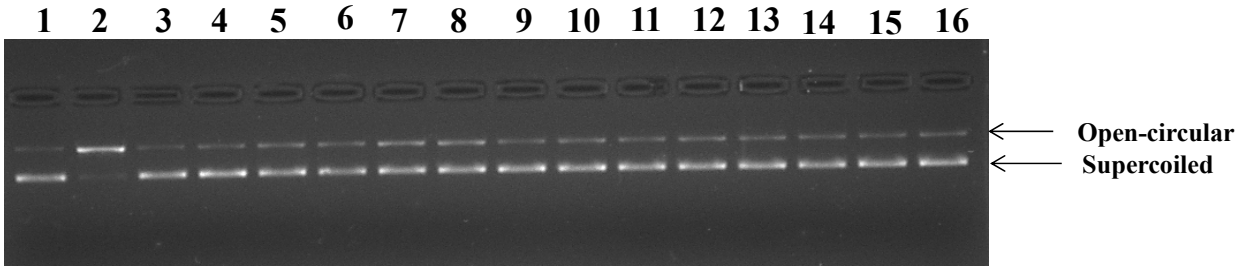


Figure 4.4.3 Effects of cowpea phenolic extracts on AAPH-induced oxidative supercoiled pBR 322 plasmid DNA damage

Lane 1: pBR 322 plasmid DNA; Lane 2: pBR 322 plasmid DNA+ AAPH; Lane 3: pBR 322 plasmid DNA+ AAPH+ 10 µmol trolox; Lane 4: pBR 322 plasmid DNA+ AAPH+ 100 µmol trolox; Lane 5: pBR 322 plasmid DNA+ AAPH+ extracts from Blackeye (uncooked), Lane 6: pBR 322 plasmid DNA+ AAPH+ extracts from Blackeye (unmicronised, cooked), Lane 7: pBR 322 plasmid DNA+ AAPH+ extracts from Blackeye (micronized, cooked), Lane 8: pBR 322 plasmid DNA+ AAPH+ extracts from Bechuana white (un cooked), Lane 9: pBR 322 plasmid DNA+ AAPH+ extracts from Bechuana white (unmicronised, cooked), Lane 10: pBR 322 plasmid DNA+ AAPH+ extracts from Bechuana white (micronised, cooked), Lane 11: pBR 322 plasmid DNA+ AAPH+ extracts from Glenda (uncooked), Lane 12: pBR 322 plasmid DNA+ AAPH+ extracts from Glenda (unmicronised, cooked), Lane 13: pBR 322 plasmid DNA+ AAPH+ extracts from Glenda (micronised, cooked), Lane 14: pBR 322 plasmid DNA+ AAPH+ extracts from Dr Saunders (uncooked), Lane 15: pBR 322 plasmid DNA+ AAPH+ extracts from Dr Saunders (unmicronised, cooked), Lane 15: pBR 322 plasmid DNA+ AAPH+ extracts from Dr Saunders (micronised, cooked).

Only two bands were present in the agarose gel, the upper band represents the open-circular DNA (supercoiled DNA converted to open-circular DNA) and the lower band represents the supercoiled DNA in its original form. Absence of the lower band (supercoiled DNA) in lane 2 is an indication that AAPH caused extensive damage. In the presence of extracts from all cowpeas (Lane 5 -16), the supercoiled DNA original form is retained compared with lane 2 (positive control) where there was extensive oxidative DNA damage with almost no supercoiled DNA left (lower band). This is an indication of the protective effects of cowpea extracts against oxidative DNA damage. Figure 4.4.4 shows the quantitative percentage protection of cowpea extracts from uncooked (unmicronised) and cooked (unmicronised and micronised) against AAPH-induced oxidative DNA damage. Addition of 100 µm Trolox protected the DNA from oxidative damage by 92 % compared with plasmid DNA without AAPH (no damage).

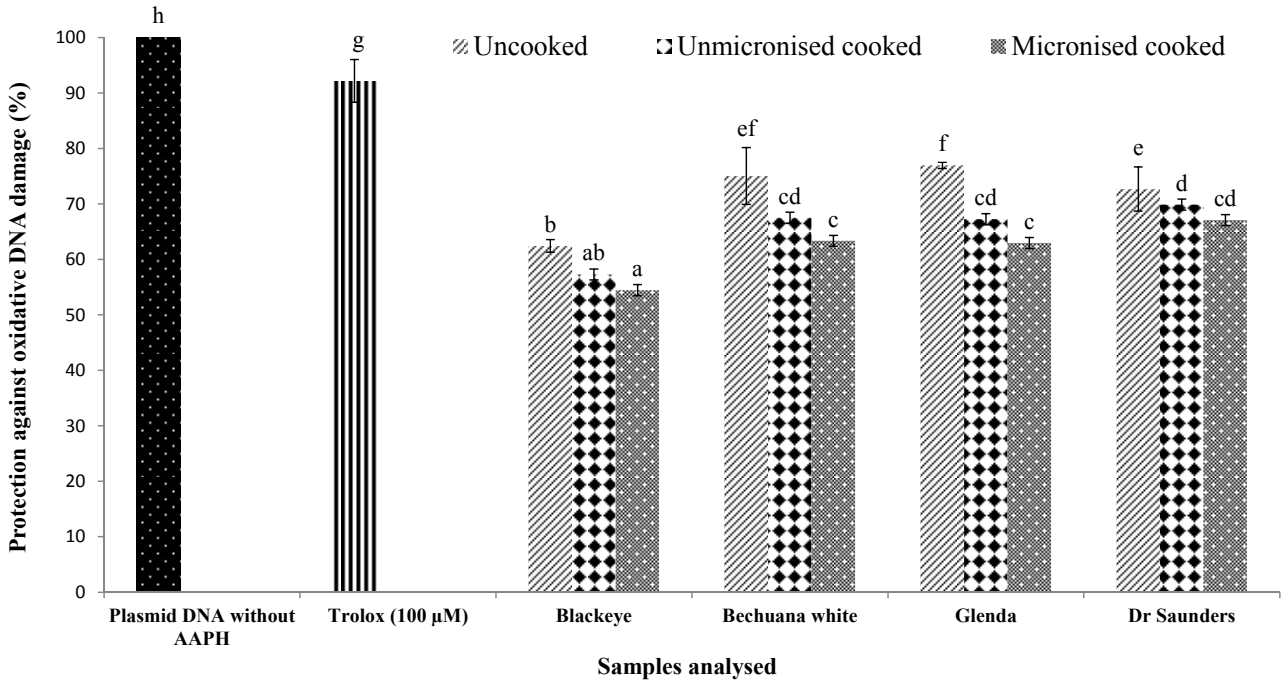


Figure 4.4.4 Effects of micronisation of pre-conditioned cowpeas (followed by cooking) on protective effects of their extracts against AAPH-induced oxidative supercoiled pBR 322 plasmid DNA damage

[a-d] = Graph bars with different letters differ significantly ($P<0.05$)

Extracts from all cowpea samples analysed protected the DNA oxidative damage by 54 to 77 % compared with plasmid DNA without AAPH. Extracts from more pigmented cowpea samples namely Glenda, Dr Saunders and Bechuana white cowpeas offered higher protection against AAPH-induced oxidative DNA damage than less pigmented Blackeye cowpeas. This is in agreement with results reported for total phenolics, total flavonoids and radical scavenging properties previously in Chapter 4.2 and 4.3. This may indicate that the ability of cowpea extracts to protect the DNA from oxidative damage could be attributed to the concentration of antioxidants in the sample. The preventive capability of cowpea extracts may be due to free radical scavenging activity of their constituent polyphenols. Madhujitha, Amarowicz and Shahidi, (2004) reported that common bean extracts were able to effectively retain supercoiled DNA against AAPH-induced damage. Madhujitha *et al*. (2004) also reported that beans with coloured seed coats (red, brown, and black) exhibited stronger protection against *in vitro* AAPH radical-induced DNA damage compared with white beans.

Figure 4.4.4 shows that micronisation of pre-conditioned cowpeas (followed by cooking) did not have a significant effect on protective effects of cowpea extracts against AAPH-induced oxidative DNA damage. As mentioned earlier, the radical scavenging properties of cooked samples for most cowpea types remained unchanged after micronisation (Chapter 4.3). This may explain why there was no change in antioxidant capacities of cowpea extracts against AAPH-induced oxidative DNA damage after micronisation.

4.4.4.4 Correlation coefficients between total phenolics, total flavonoids, radical scavenging properties of cowpea extracts and their *in vitro* protective effects against oxidative damage

Table 4.4.2 shows correlation coefficients (*r*) between total phenolics, total flavonoids, radical scavenging properties (data obtained from Chapter 4.3) and protection of biomolecules e.g. DNA, LDL and RBC from oxidative damage of cowpea samples. There was a significant positive correlation between total phenolics, radical scavenging properties (TEAC & ORAC) and protection of cowpea extracts against AAPH-induced DNA damage (Table 4.4.2). These results suggests that the protective effects of cowpea extracts against oxidative DNA damage may be attributed to total concentration and antioxidant activity of phenolics in cowpeas.

Table 4.4.2: Correlation coefficients (r) between total phenolic content and protection of biomolecules e.g. DNA, LDL and RBCs from oxidative damage

Assay	Correlation coefficient (r)
Total phenolics vs % protection against DNA damage	0.93 (*)
Total phenolics vs LDL oxidation (TBARS production)	-0.74 (*)
Total phenolics vs Protection RBCs haemolysis	0.04 (ns)
TEAC/ABTS vs % protection against DNA damage	0.93 (*)
TEAC/ABTS vs LDL oxidation (TBARS production)	-0.70 (*)
TEAC/ABTS vs Protection RBCs haemolysis	0.04 (ns)
ORAC vs % protection against DNA damage	0.90 (*)
ORAC vs LDL oxidation (TBARS production)	-0.61 (*)
ORAC vs Protection RBCs haemolysis	0.14 (ns)

*Significant at P<0.05 and ns (not significant)

It has been hypothesised that phenolic antioxidants suppress the formation of free radicals, quench the existing radicals, and reduce the availability of oxygen in biological system to prevent the oxidative damage of DNA (Jacob and Burri, 1996).

A significant negative correlation was observed between total phenolics, radical scavenging properties (TEAC and ORAC) and the production of TBARS, the final products of LDL oxidation (Table 4.4.2). This means that samples with high total phenolic contents and antioxidant activity also exhibited the highest protective effective effects against LDL oxidation by reducing the production of TBARS. LDL oxidation is a lipid peroxidation reaction which is characterized by three phases; initiation (lag phase), propagation and decomposition. Antioxidants such as phenolic compounds may lower the rate of initiation by scavenging lipid peroxyl radicals, this result in a prolonged lag phase during incubation (Esterbauer, Gebicki, Puhl and Jürgens, 1992; Abuja, Murkovic and Pfannhauser, 1998). Xu *et al.* (2007) reported a positive correlations between TBARS inhibitory activity and the total phenolic content ($r = 0.79$) of nine common food legumes.

No significant correlation was observed between total phenolics, radical scavenging properties and protection of RBC against oxidative damage. This means that there was no direct dependency of quantitative protection of RBC against oxidative damage on total phenolic concentration of the sample. Some studies have found a positive correlation between total phenolic concentration of the sample and quantitative protection of RBC against oxidative damage, while other studies did not find any correlation. This may be probably because multiple factors such as phenolic compound hydrophobicity/hydrophilicity, total number and location of the hydroxyl groups of an aromatic ring of phenolic compound and the interaction their with components of the red blood cells plays a significant role in their protection against RBC haemolysis (Chen *et al.*,1996). Positive correlate ions were found between total phenolic content in the mushroom extracts and their ability to protect the RBCs from haemolysis (Cheung, Cheung and Ooi, 2003). On the contrary, Lim, Cheung, Ooi and Ang (2002) found no correlation between the total phenolic content and the protective effects of the brown sea weed against AAPH induced erythrocytes haemolysis.

4.4.5 CONCLUSIONS

Extracts from all cowpea types used in the study offer protection against copper-induced LDL oxidation, AAPH-induced oxidative DNA damage and red blood cell haemolysis. Results from this study shows that extracts from cowpea types with dark coloured seed coats offer higher protection against oxidative DNA damage and LDL oxidation than those from light coloured seed coats. Oxidative damage of DNA, lipids and proteins in the human body is normally considered to be one of the causes of some chronic diseases. Cowpeas can be considered an important source of natural antioxidants, hence, a food with potential health benefits. Micronised and cooked cowpea samples have reduced potential to inhibit oxidation of LDL, but their protection against oxidative DNA damage and RBCs is not affected by micronisation. Micronised cowpea samples retain some bioactivity and therefore may offer potential health benefits to cowpea consumers. In future, in vivo studies involving animal and human trials may be undertaken to understand bioavailability of cowpea bioactive components and the potential to exert health benefits.

5. GENERAL DISCUSION

This chapter is divided into two sections. The first section critically discusses the methodologies used in the research in terms of their principles, advantages and disadvantages. The second section discusses the main findings of the research with emphasis on the effects of micronisation on sensory properties of cooked cowpeas, phenolic compounds and bioactive properties of cowpeas.

5.1 METHODOLOGIES

Cowpea plays an important role in the traditional diets in many regions of the world, especially Asia, Africa and South America, which include most of the world's developing countries (Phillips *et al*., 2005). One of the major forms in which cowpea is utilised is as cooked whole seeds (Demooy and Demooy, 1990). Cooking of cowpeas is achieved by boiling the grains in excess water until they acquire the desired softness (Olapade, Okafor, Ozumba and Olatunji, 2002). Cowpeas have been reported to cook for up to 2 hours and even longer (Akiyele *et al*., 1986; Olapade *et al*., 2002). This results in increased energy consumption and long food prepation times. The changing socio-economic conditions and demand brings pressure to bear on the food industry to develop convenience foods which are easy to prepare, have acceptable sensory properties, nutritious and wholesome in nature with long shelf life. To achieve these goals, various processing operations are employed. Micronisation, an infrared heat treatment applied to pre-conditioned cowpeas has been reported to effectively reduce the cooking time (Mwangwela, 2006).

The micronisation process uses infrared energy with wavelengths between 1.0 and 3.4 microns to heat water in biological materials by inducing molecular vibrations at a frequency of 8.8 x 107 to 1.7 x 108 MHz (Cenkowski and Sosulski, 1998). A tabletop microniser was used and infrared energy was applied on stationary cowpea seeds. Cowpea seeds were micronised for 6 min to a final surface temperature of 153 °C. Although the cowpeas were micronised from one direction only, the micronisation treatment was still effective since the cowpeas used were small and medium seeded and small quantities were micronised at a time in this study. This however may be a problem if large seeded cowpeas and larger quantities are to be used. Vibrating troughs or belts may be used in pilot scale equipment to facilitate uniform exposure of the samples to the infrared radiation (Cenkowski, Hong, Scanlon and Arntfield, 2003). Such vibrating troughs or belts may therefore be useful during

micronisation processing of large quantities of cowpeas to ensure uniformly micronised seed especially when working with large seeded cowpeas.

Reduction in cooking time is achieved when infrared heating is combined with moisture pre-conditioning of cowpeas. Tempering cowpeas to 41 % moisture facilitates partial starch gelatinisation and protein denaturation during micronisation (Mwangwela, 2006). The reduction in legume cooking time due to micronisation has been attributed to its ability to disintegrate the middle lamella between cotyledon parenchyma cells, denaturation of protein and pre-gelatinisation of starch within the cotyledon parenchyma cells that result in quicker softening of cooked cowpeas (Mwangwela, 2006; Arntfield *et al*., 2001).

The sensory quality was evaluated using descriptive sensory evaluation. Einstein (1991) defines Descriptive Sensory Evaluation as "the identification, description and quantification of sensory attributes of a food material or product using human subjects who have been specifically trained for this purpose". As such, the success of the descriptive sensory analysis depends primarily on the collective ability of the descriptive sensory panel to reliably and precisely grade the sensory attributes of the product. Because they are human subjects they can be affected by different forms of setbacks e.g. emotional, physiological differences social and physical conditions. This is confirmed by the observation in some sample attributes mostly aromas and flavours where there were significant panellist effects (results not shown). This is attributed to factors such as the degree of understanding or perception of certain attributes, definitions and individual scaling behaviour. To minimise such errors of judgment, panellists had access to reference samples throughout training and during the evaluation period. Training sessions helped the panel to become familiar with the product, agree on the attribute to be used in the evaluation sessions and also use a linear scale for quantification. Panel check and feedback calibration models in Compusense Five ® release 5.0 were used during training to evaluate the collective ability of the descriptive sensory panel to reliably and precisely grade the sensory attributes of cowpea samples.

The second and third phase of the study focused on evaluating the effects of micronisation followed by cooking on cowpea phenolic compounds and bioactive properties. Acidified methanol (1% conc. HCl in methanol) was used for the extraction of phenolics from the cowpea samples. The type of solvent used for extraction plays an important role in ensuring

maximum extraction of phenolics (Naczk and Shahidi, 2004). Due to the chemical nature of food phenolics, no completely satisfactory solvent extraction procedure is suitable for extraction of all phenolics in foods. Solubility of phenolics is dependent on the type of solvent (polarity), degree of polymerisation of phenolics, interaction of phenolics with other food constituents and formation of insoluble complexes (Waterman and Mole, 1994; Naczk and Shahidi, 2004). Polar solvents are suitable for extracting polar phenolic compounds, while non-polar solvents are suitable for extracting non-polar phenolic compounds. Acidified methanol has been recommended for the extraction of phenolics in beans (Madhujith and Shahidi, 2005).

The total phenolic content of cowpea samples was determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965). This method was chosen because it is a simple and reproducible assay which is widely used for studying total phenolic antioxidants (Macdonald, Wood and Garg, 2006). It is based on the reducing power of phenolic hydroxyl groups (Sun, Ricardo da Silva and Spranger, 1998). Phenolic compounds react with the Folin-Ciocalteu reagent under basic conditions, through the dissociation of a proton from the phenolic hydroxyl group which leads to the formation of a phenolate anion (Macdonald *et al.*, 2006). Folin-Ciocalteu method however is not specific and detects all phenolic hydroxyl groups in extracts. Folin-Ciocalteu reagent can also be reduced by non-phenolic compounds e.g. extractable proteins (Naczk and Shahidi, 2004). Therefore the total phenolic content of the extracts may be overestimated if the sample contains significant amount of proteins composed of phenolic amino acid such as tyrosine with reducing propeties. It is therefore important that this assay be combined with other methods e.g. using LC/MS analysis that are more specific to determining phenolic compounds in the sample.

The aluminium chloride assay was used to estimate total flavonoid content of cowpea samples. In this assay, aluminium ions react with the C-4 keto group and either the C3 or C5 hydroxyl group of flavones and flavonols to form an acid stable complex and also with the dihydroxy groups in the A- and B-ring of flavonoids to form an acid labile complex (Chang *et al.*, 2002). This assay however is specific for flavones and flavonols because some flavonoid compounds, such as the flavanones react with aluminium chloride to form complexes that have an absorption λ_{max}, that is different from that of flavone and flavonol complexes (Chang *et al.*, 2002). Total flavonoid content should be the sum of all flavonoids

present in the sample. Therefore, this method could be combined with the 2, 4-dinitrophenyllhydrazine method specific to flavanones to better represent the total flavonoid content (Chang *et al*., 2002). Both the Folin-Ciocalteu and aluminium chloride assay methods gives good estimates of total phenolic and total flavonoid content respectively but these methods do not give quantitative amounts of each phenolic compound present in the samples analysed.

Each plant has a unique phenolic composition profile, which makes identification and quantification of these compounds difficult. An Ultra Performance Liquid Chromatography (UPLC), coupled with diode array detector and a mass spectrometer (UPLC / PDA /MS) system was used in the study. Using the UPLC system, complete separation of phenolic compounds in cowpeas could be done within 26 min. By contrast, typical HPLC analysis of phenolic compounds (phenolic acids and flavonoids) takes 60 min (Wu and Prior, 2005). This method provides UV and MS spectra for each peak which makes identification of each peak possible sometimes without the need for a reference compound and by comparing with literature data (He, Lian and Lin, 1997). Electrospray ionization (ESI) (for mass spectrometry) was chosen because it is a gentle ionization technique at atmospheric pressure. It generates mainly the deprotonated molecules or pseudomolecular ion $[M-H]^-$ in negative mode (Gioacchini, Roda, Galletti, Bocchini, Manetta and Baraldini, 1996) for rapid determination of molecular mass of the compounds (Soong and Barlow, 2005). Negative mode was chosen because deprotonation of phenolic compounds is easier as these compounds are weakly acidic (Friedrich, Eberhardt and Galensa, 2000). Positive ion mode was not used because it is reported to generate higher background signal noise (Sun and Miller, 2003). Mass spectrometry detection has a great advantage of being able to distinguish between compounds that co-elute (Shui, Leong and Wong, 2005) and/or have overlapping chromatographic peaks (Bocchi, Careri, Groppi, Mangia, Manini and Mori, 1996).

Antioxidant capacities of cowpea samples were determined using the TEAC and ORAC assays. The TEAC assay is based on the neutralisation of radical cations formed by a single-electron oxidation of a synthetic ABTS chromophore to a strong absorbing $ABTS^{\bullet+}$. The antioxidants reduce the radicals depending on the antioxidant activity, concentration of the antioxidants and the duration of the reaction (Re, Pellengrin, Proteggente, Pannala, Yang and Rice-evans, 1999).

This method is preferred for its simplicity, speed of analysis and it can be used over a wide pH range (Lemaska, Szymusiak, Tyrakowska, Zielinski, Soffer and Rietjens, 2001). ABTS assay is also a good method for evaluating both lipophilic and hydrophilic antioxidants because the radical is soluble in water and organic solvents (Rivero-Pérez, Muñiz, and González-Sanjosé, 2007; Alvarez-Suarez, Tulipani, Romandini, Vidal and Battino, 2009). The limitation of the assay is that the $ABTS^{\bullet+}$ reagent is unstable and reacts with any hydroxylated aromatics (Roginsky and Lissi, 2005). The ORAC assay measures the capacity of an antioxidant to quench peroxyl radicals (Cao and Prior, 1999). In this method fluorescein is used as a probe which is attacked by peroxyl radicals ($ROO^{\bullet}$) generated by 2, 2'-azobis (2-methyl-propionamidine) dihydrochloride (AAPH) resulting in loss of fluorescence which is recorded at specific time intervals as the reaction goes to completion. In the presence of an antioxidant, $ROO^{\bullet}$ removes a hydrogen atom from the antioxidant to form peroxide and a stable antioxidant radical, resulting in a delay or inhibition of the reaction between $ROO^{\bullet}$ and flourescein (Ou *et al*., 2001). Unlike ABTS, ORAC directly measures the antioxidant scavenging activity of an antioxidant against the biologically relevant peroxyl radical induced by thermal decomposition of AAPH at 37 °C (Ou *et at*., 2001; Madhujith and Shahidi, 2009). It is the only assay that combines both inhibition time and degree of inhibition into a single value (Madhujith and Shahidi, 2009), the ORAC value. The ORAC assay is a standardised method therefore results can be easily compared across laboratories. However, the equipment used in the assay is expensive and its availability to some laboratories is limited.

To evaluate the potential health promoting properties of antioxidants in the sample, it is important to study their antioxidant activity in relevant biomolecules, cellular system or animal models. The first level of testing is using simple biological systems that represent targets of oxidative damage. In this study the cowpea extracts were evaluated for their ability to protect LDL against copper-induced oxidative damage, AAPH-induced red blood cell haemolysis and oxidative DNA damage.

Copper-induced human LDL oxidation was used as an *in vitro* model to determine the protective effect of cowpea extracts against LDL oxidation. The method is based on the induction of LDL oxidation with Cu^{2+} which leads to lipid peroxidation and decomposition of lipid peroxides to aldehydes and other compounds (Esterbauer et al.,1992). The decomposition products or thiobarbituric reactive substances (TBARS) are then measured

with the thiobarbituric acid assay (TBA assay). The TBA assay is the most frequently used method to assess the resistance of LDL to lipid oxidation (Schnitzer, Pinchuk, Bor, Fainaru, Samuni and Lichtenberg, 1998). The challenge with this assay is that use of plasma or serum may result in variable results depending on the nutritional status of the donor. This method requires isolation of LDL by preparative ultracentrifugation or use of expensive commercially available isolated LDL whose cost may be a limiting factor. The method has also been criticised for low specificity and for the fact that heating of polyunsaturated fatty acids in hot acid, as applied in the TBA assay is very harsh and may result in autoxidation so TBARS may be formed during the assay itself. This was avoided by the addition of EDTA to chelate copper which catalyses this reaction (Esterbauer *et al.*, 1992).

The red blood cell haemolysis assay is a convenient test model to determine the ability of cowpea sample extracts to protect the red blood cell membrane from haemolysis. In this assay decomposition of AAPH produces peroxyl radicals which attack the susceptible polyunsaturated fatty acids within the red blood cell membrane to induce lipid peroxidation (Deng, Chen, Zhou, Yang and Lui, 2006). Phenolic antioxidants in the sample scavenge the radicals before they damage the red blood cell membranes, thus preventing haemolysis (Blasa, Candiracci, Accorsi, Piacentini and Piatti, 2007). The advantage of this method is its simplicity and the fact that from a small amount of blood sample many samples can be tested simultaneously. Furthermore, it is a biologically relevant method, because red blood cells play an important role in antioxidant protection of blood. The disadvantage of this assay is poor repeatability when using different batches of blood because biologically originated substrates may contain different levels of endogenous chain breaking antioxidants such as vitamin E which may interfere with the assay (Roginsky and Lissi, 2005). To overcome this problem the same batch and source of blood was used for all analyses.

AAPH-induced breakage of supercoiled plasmid DNA was used as an experimental model to evaluate the protective effect of cowpea sample extracts against oxidative DNA damage. Peroxyl radicals generated by thermal decomposition of AAPH induce single and double strand breaks on supercoiled plasmid DNA resulting in relaxed circular DNA (Aronovitch *et al.*, 2007). Using agarose gel electrophoresis, the three forms (linear, circular and supercoiled) of DNA are separated due to their differences in electrophoretic mobility (Aronovitch *et al.*, 2007). The advantage of this method is that it allows large amounts of

DNA to be processed in one run. Although DNA migration assay is a sensitive biomarker of DNA damage, the method quantifying the relative DNA damage has low sensitivity and accuracy. Other limitations of this method include: (a) occurrence of other DNA lesions introduced into the plasmid DNA samples through the use of ethidium bromide dye for staining (Elliot, Astley, Southon and Archer, 2000) and (b) weaker binding ability of ethidium bromide to supercoiled plasmid DNA in comparison to the circular or linear forms of DNA (Milligan, Arnold and Ward, 1992). This method could be improved by employing an anion exchange/HPLC method to measure the extent of DNA damage (Elliot *et al*., 2000).

5.2 RESEARCH FINDINGS

5.2.1 Effects of micronisation of pre-conditioned cowpeas on cooking time and sensory properties of cooked cowpeas

Figure 5.1 summarises suggested changes in cowpea seeds during micronisation that affected cowpea cooking time and sensory characteristics of cooked cowpeas. As described earlier (Chapter 4.1) micronisation of pre-conditioned cowpeas reduced the cooking time of all cowpeas analysed. Researchers have suggested that reduction in legume cooking times may be attributed to several changes in their physicochemical properties during micronisation. The reduction in cooking due to the micronisation process was attributed to its ability to disintegrate the middle lamella between cotyledon parenchyma cells that result in quicker softening of cooked cowpeas (Mwangwela, 2006; Arntfield *et al.*, 2001). Micronisation leads to cracking and fissures as well as excessive splitting of the cowpeas cowpeas seed coat (Mwangwela, 2006). Fractures and splitting of seed coat and cotyledons improves water uptake, leading to a softer texture. It has also been reported that micronisation pre-gelatinises starch which can reduce the time and energy required to complete starch gelatinisation during cooking. Arntfield *et al.* (1997) also reported that micronising of lentils increased protein solubility resulting in increased softening of lentils. Production of micronised cowpeas with reduced cooking time presents an opportunity for utilisation of cowpeas as an alternative product for dry grains on shop shelves especially for urban consumers whose busy life style makes convenience an important factor in food choices.

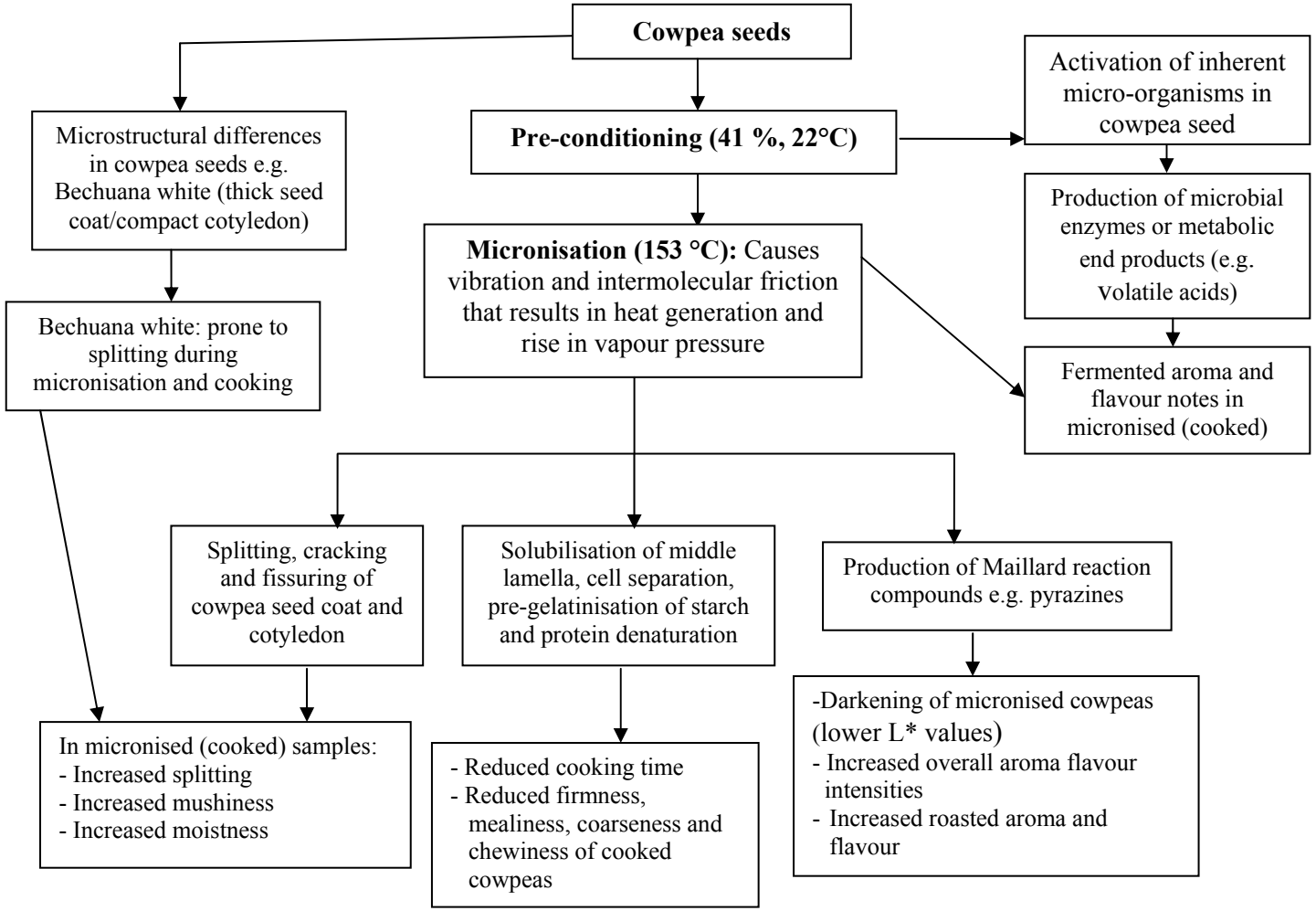


Figure 5.1 Diagram showing the suggested changes in cowpea seeds during micronisation (followed by cooking) that affects their cooking and sensory characterisics

This study showed that micronisation of pre-conditioned cowpeas affected sensory characteristics as described in Chapter 4.1. Micronisation of pre-conditioned cowpeas increased splitting in all cowpea types. During micronisation there is an inter-molecular friction among seed molecules. This results in heat generation and vaporisation of water molecules that increases the volume and pressure within the seed (Fasina *et al.,* 2001). The increase in pressure results in visible cracks on the seed coat before cooking thus increasing splitting in micronised (cooked) cowpeas (Mwangwela, 2006). Increased cowpea splitting upon micronisation was previously reported by Mwangwela (2006). Micronised cowpeas were mushier than the unmicronised samples. Micronisation results in degradation of cell wall (cellulose and pectic substance) and cotyledon components, e.g. starch (Arntfield *et al.,* 2001; Mwangwela, 2006). These changes result in a more porous cowpea cell wall that facilitates leaching of the seed components into the cooking water. This might explain a thick broth referred to as 'mushy" in micronised cooked cowpeas. Micronisation also resulted in significant reduction in firmness, mealiness and coarseness in micronised cooked cowpeas (Chapter 4.1). An indicator that micronised cooked cowpeas was softer than the unmicronised cooked samples. As stated earlier, micronisation causes the disintegration of the middle lamella, denaturation of protein and pre-gelatinisation of starch that result in more softening of cooked/micronised cowpeas (Figure 5.1).

Micronisation of pre-conditioned cowpeas led to browning of cowpea seeds as indicated by the lower L-values measured by a colour meter. Browning of cowpea seeds upon micronisation was previous reported by Mwangwela (2006) and Phadi (2004). Maillard reaction is a non-enzymatic reaction that takes place on heating and is related to dark colour development in food products (Martins *et al.,* 2001). The chemical mechanisms involved in the initial stages of the Maillard reaction have been studied in detail and involve the condensation of the carbonyl group of the reducing sugar with the amino compound to give a glycosylamine (Martins *et al.,* 2001). Colour formation is attributed to the final stage of the reaction, where condensation between carbonyls (especially aldehydes) and amines occurs to give high molecular mass, coloured products known as melanoidins (Michalska *et al*., 2008).

Maillard reaction is also responsible for the roasted aroma and flavour notes perceived by the descriptive sensory panel in micronised cooked cowpeas. The final stage of the reaction is of great importance for flavour formation when carbonyl compounds react with each other, as well as with amino compounds and amino acid degradation products, such as hydrogen sulphide and ammonia. It is these interactions that lead to the formation of flavour compounds, including important heterocyclics, such as pyrazines, pyrroles, furans, oxazoles, thiazoles and thiophenes (Mottram, 1991). Use of raw cowpea flour in baked products such as cookies and bread has been associated with a raw legume flavour (McWatters, Ouedraogo, Resurreccion, Hung and Phillips, 2003). Incorporating flour from micronised cowpeas with more toasted/roasted flavour notes may reduce the raw legume flavour note and introduced toasted/roasted flavour notes common to baked products.

Fermented aroma and flavour notes were perceived in micronised cooked cowpea samples of some cowpea types by a descriptive sensory panel. Pre-conditioning cowpeas to 41 % moisture for 18 h at ambient temperature may have resulted into activity of microorganisms inherently present in the seeds, leading to production of microbial enzymes or metabolic end products with fermented flavours e.g. volatile acids. Beal, Niven, Brooks and Gill (2005) found that incubating barley or wheat grains at 30 °C for 24 h resulted in production of acetic and butyric acids. Fermented flavour compounds produced at the pre-conditioning stage may have been carried over even after infrared heating of cowpeas. Since these flavours were perceived in micronised samples of some cowpea types and not in the others, further investigations may be undertaken. For example, evaluating cowpea type differences in sugar composition as sugars may serve as substrate for microbial activity.

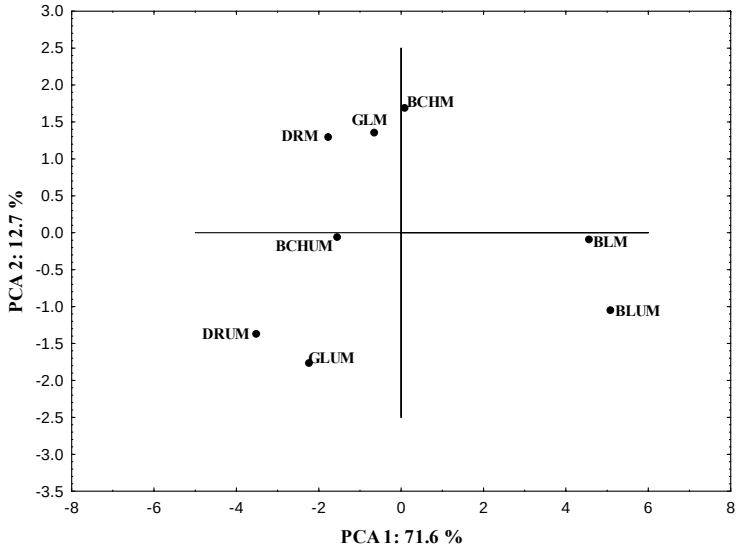
Results from descriptive sensory analysis shows that micronisation of pre-conditioned cowpeas affects the appearance, aroma, flavour and textural properties of micronised cooked cowpeas. A recently study (unpublished) by Mothupi (2012) established that generally, micronised cowpeas were equally acceptable to the target consumers as the conventionally cooked cowpea grains. It is important to note that consumer panellists in her study were mostly students and were not regular consumers of cowpeas. Therefore it is important that extensive consumer acceptability studies be conducted and it would be interesting to investigate the consumer's response to micronised cowpeas in regions e.g. West Africa where cowpea is produced and consumed the most.

The second phase of the study characterised phenolic compounds in cowpeas. The literature has described phenolic compounds ranging from simple phenols to highly polymerised compounds that are associated with both bitterness and astringency in plant foods (Drewnowski and Gomez-Carneros, 2000; Lesschaeve and Noble, 2005). Simple phenols like phenolic acids are associated with sensory characteristics such as bitterness and astringency (Peleg and Noble, 1995). Higher-molecular-weight polymers such as tannins are more likely to be astringent (Nobel, 1994). Legumes such as beans were perceived to be bitter (Mkanda *et al.,* 2007) and astringent (Koehler *et al*., 1887). A study by Kobue-Lekalake, Taylor and De Kock (2007) found that water extracts from different sorghum cultivars were perceived to have different degrees of bitterness and astrigency. Water extracts from sorghums cultivars with the highest levels of total phenolics and condensed tannins were the most bitter and astringent (Kobue-Lekalake, Taylor and De Kock, 2007).

Figure 5.2 shows PCAs indicating variations between cowpea samples in terms of sweetness, bitterness and astringency in relation to their phenolic composition. PCA 1 explained 71.7 % of variations between samples on the right side of the plot from those on the left. On the left all samples (micronised and unmicronised) of Dr Saunders, Glenda and Bechuana white which had higher phenolic contents were perceived as more bitter and astringent compared to Blackeye samples (lower phenolic contents) on the right of the plot that were perceived as sweet. Figure 5.2 shows that micronized Dr Saunders and Glenda cowpeas had relatively higher levels of procyanidin oligomers and these were perceived as realtively more astringent.

However, it is important to note that although cowpea samples were characterised with phenolic compounds such as phenolic acids, flavonols, flavanols and oligomers of procyanidins and prodelphindins (Chapter 4.2), descriptive sensory analysis results (Chapter 4.1) showed that bitterness and astringency were not “strongly” perceived by the panellists in all cowpea samples. The top scores for both bitterness and astrigency were less than 3 on a 10 line scale used by the panellists thus may not be objectionable to cowpea consumers. Concentration and chemical structure of phenolic compounds has been reported to contribute to flavour perception of both bitterness and astringency (Lesschaeve and Noble, 2005). For example, variations in proanthocyanidin composition, such as polymer size, extent of

galloylation, and formation of derivatives, affect both bitterness and astringency (Lesschaeve and Noble, 2005). Astringency increases and bitterness decreases with the degree of polymerisation. Futhermore, in a food matrix that consists of other food components bitterness and astringency perception may be masked (Lesschaeve and Noble, 2005).



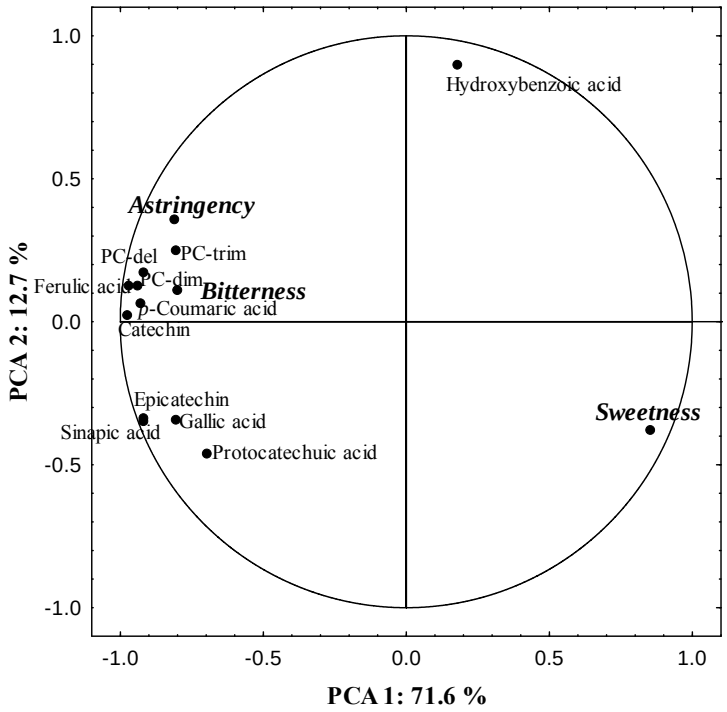


Figure 5.2 First and second principal component analysis of cowpea samples scores and loadings projections of peak areas (representing concentration) of phenolic compounds and flavour characteristics (sweetness, bitterness and astringency)

BLUM=Blackeye unmicronised, BLM=Blackeye micronised, BCHUM = Bechuana white unmicronised, BCHM = Bechuana white micronised, GLUM=Glenda unmicronised, GLM=Glenda micronised, DRUM=Dr Saunders unmicronised, and DRM= Dr Saunders micronised, PC-dim: Procyanidin dimer, PC-trim: Procyanidin trimer, PC-del: Prodelphinidin dimers

5.2.2 Phenolic compounds, *in vitro* radical scavenging and bioactive properties of cowpeas

This section discusses the findings on cowpea phenolic compound profiles and bioactive properties of cowpeas. Results from the study indicated that extracts from samples of cowpeas with more pigmented seed coats had higher levels of total phenolics (determined by both Folin-Ciocalteu and UPLC-MS methods) and total flavonoids than those with less pigmented seed coats. This is probably because phenolic compounds are not distributed uniformly in plants. At the tissue level, the outer layers of plants or plant-based foods contain higher levels of phenolic than those located in the inner parts (Naczka and Shahidi, 2004). Phenolic compounds are mainly concentrated in the seed coat (Aparicio-Fernandez, Yousef, Loarca-Pina, de Mejia, and Lila, 2005) and are responsible for the seed coat colour (Dueñas *et al*., 2006). Nzaramba *et al.* (2005) found that cowpea seeds with dark-coloured seed coat (e.g. black, purple or dark red) contain high levels of phenolic compounds that resulted in higher antioxidant activities. These results were expected and selection of the samples used in the study had been based on the supposition that cowpeas with darker shades of colour would have high phenolic concentration, resulting in higher radical scavenging.

When discussing phenolics in plants foods, flavonoids are the predominant class described because they account for approximately two-thirds of the dietary phenols (Scalbert and Williamson, 2000). However, phenolic acids account for almost all of the remaining third, and there is an increasing awareness and interest in the antioxidant behaviour and potential health benefits associated with phenolic acids (Scalbert and Williamson, 2000). In the present study, phenolic acids identified in cowpea extracts were gallic acid, protocatechuic acid, ferulic acid sinapic acid, 4-hydroxybenzoic acid and *p*-coumaric acid. All these phenolic acids have been previously reported in cowpeas (Cai *et al*. 2003; Sosulski and Dabrowski, 1984).

Total flavonoid conents in the present study ranged from 140 mg/ 100g (Blackeye) to 370 mg/100 g (Dr Saunders) in cowpeas analysed. Total dietary intake of flavonoids has been estimated to vary from 100 to 1000 mg/day (Aherne and O'Brien, 2002). Consumption of 50 g (¼ of a cup) of Dr Saunders cowpeas per day may contribute to 106 mg of total dietary flavonoid intake. Fourteen flavonols consisting of quercetin, myricetin, kaempferol and glycosides of quercetin, myricetin and kaempferol were identified in cowpeas tested (Figure

5.3). All flavonols identified in cowpea samples were previously reported in cowpeas by Wang *et al*. (2008) and Ojwang *et al*. (2012). Flavonol aglycones, such as quercetin are hydrophilic and can passively diffuse across biological membranes (Beattie, Crozier and Duthie, 2005). Flavonol glycosides, in contrast, are more water-soluble molecules which greatly limits their rate of diffusion through cell membranes (Beattie *et al*., 2005). Thus, if the glycosides, which occur in the major flavonoid components of cowpeas are to be absorbed into the circulatory system some form of transport system is likely to be involved. It has been proposed that flavonol glucosides, such as quercetin-3-glucoside, can be absorbed intact into the small intestine using the sodium-dependent glucose transporter (Hollman, de Vries, van Leeuwen Mengelers and Katan, 1995). Colon bacteria are able to hydrolyse flavonoid glycosides and they can still be bioavailable (Hollman and Katan, 1999). Absorption of some flavonoid glycosides e.g. quercetin-3-o-rhamanosylglucoside without microbial hydrolysis have been reported (Hollman and Katan, 1999)..

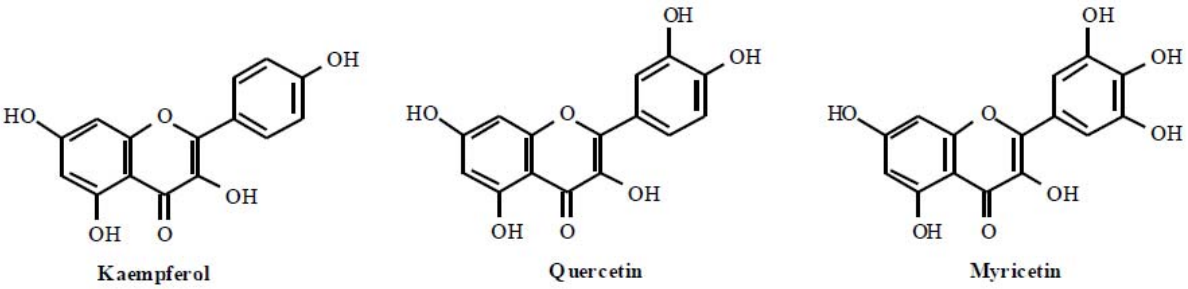


Figure 5.3 Structures of flavonol aglycones identified in cowpeas (Beattie *et al*., 2005).

Cowpea anthocyanins such as delphinidin-3-O-glucoside, cyanidin-3-O-glucoside and petunidin-3-O-glucoside were predominatly reported in cowpeas by Ojwang *et al. (*2012). However, no published data is available on flavan-3- ols such as prodelphinidin dimers, procyanidin dimers and trimers idententifed in cowpeas in the present study (Figure 5.4).

Proanthocyanidins in other legumes are mainly procyanidins (Dueñas *et al.*, 2006). Prodelphinidin and procyanidin dimers and trimers have been previously reported as dominant phenolic compounds in lentils (Amarowicz *et al.*, 2010) and adzuki bean extracts (Amarowicz, and Pegg, 2008; Ariga and Hamano, 1990).

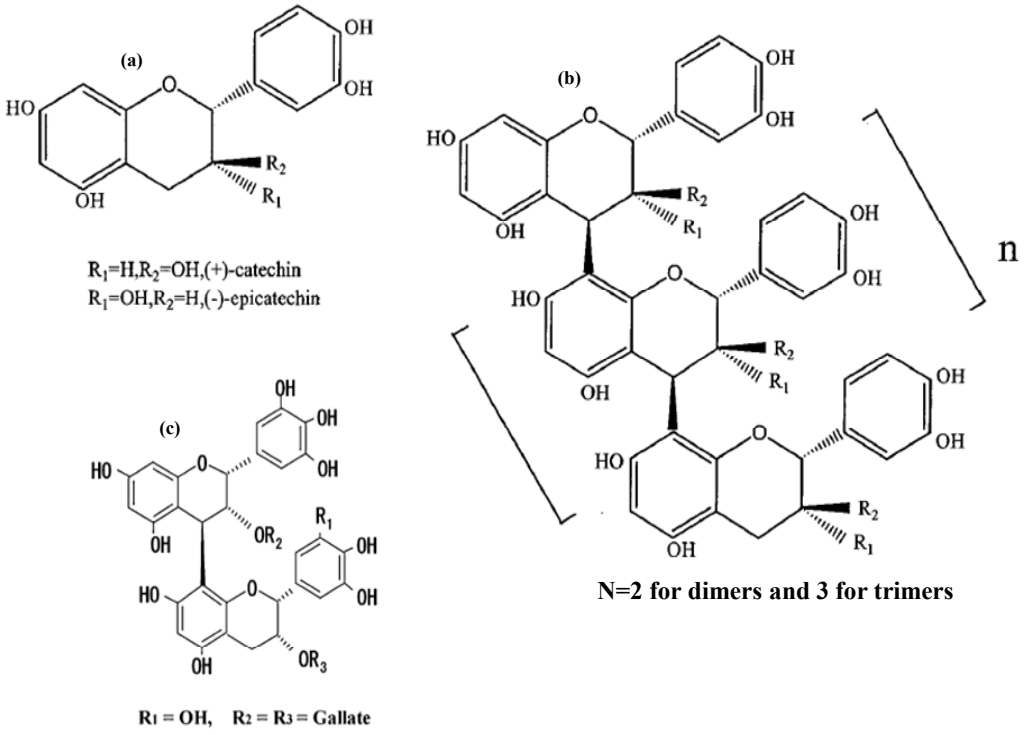


Figure 5.4 Chemical structures of flavan-3-ols identified in cowpea samples (a) (+)-Catechin and (+)-Epicatechin, (b) Procyanidin dimers and trimers and (c) Prodelphidins (Zhu *et al.*, 2002)

A recent study by Awika (unpublished) found tannins in cowpeas. In their study catechins and (epi) afzelechins were the major flavan-3-ol units that made up the tannin polymers of cowpeas. Monomeric flavan-3-ol was the largest group (up to 69 %) and oligomers (degree of polymerisation in a range of 2-4 accounted to almost 20 % of total cowpea tannins. This indicates that cowpea tannins are less polymerised compared to other grains such as marama beans and sorghum reported to have highly galloylated prodelphinidins and proanthocyanidins respectively (Shelembe, Cromarty, Bester, Minnaar, and Duodu, 2012). Phenolic compound differerences in molecular size, polarity, and solubility, have been reported to affect their bioavailability and their physiological and nutrional effect (Bravo, 1998). For example, molecular size, polarity and solubility may affect the distribution of

phenolic compounds in different macromolecules, subcellular organelles, cells, organs, and tissues (Bravo, 1998). Studies have shown that low molecular weight phenolics including proanthocyanidins oligomers were more bioavailable than high molecular phenolics e.g. condensed tannins (Bravo, 1998). Non extractable polyphenols (higher molecular and highly polymerised phenolics) were extensively recovered in faeces, confirming their resistance to intestinal digestion and/ or absorption. Conversely, extractable polyphenols (low molecular and less polymerised phenolics) were excreted only in minor amounts, suggesting that digestion and or absorption of these polyphenolic compounds occurs in the gut (Bravo, Saura-Calixto and Gofii, 1992; Bravo, Mafias and Saura-Calixto, 1993; Bravo, Abia, Eastwood and Saura-Calixto, 1994). Therefore, cowpea tannins may be soluble and easily absorbed thus offering more physiological beneficial effects compared to those present in other grain such as sorghum.

Phenolic compounds are of interest to human health primarily because of their antioxidant activity. The chemical structure of phenolic compounds gives them the ability to act as free radical scavengers and metal chelators (Dueñas *et al.*, 2005). The type of compound, the degree of methoxylation and the number of hydroxyl groups are some of the parameters that determine the antioxidant activity. The contribution of phenolic acids as antioxidants is dependent on the numbers and positions of the hydroxyl groups in relation to the carboxyl functional group (Rice-Evans *et al.*, 1996). Flavonoids generally have higher antioxidant activity than phenolic acids (Fukomoto and Mazza, 2000). This is attributed to the complexity of the flavonoid molecule compared to phenolic acids. Some of the structural features and nature of substitutions on rings B and C which determine the antioxidant activity of flavonoids include the degree of hydroxylation and the positions of the –OH groups in the B ring (Pietta, 2000). Results from this study indicated that extracts from all cowpea samples used exhibited radical scavenging properties. Extracts from cowpea seeds with more pigmented seed coats exhibited higher radical scavenging properties than those with less pigmented seed coats. Madhujith *et al.*(2004) also demonstrated that coloured beans possessed superior antioxidant activity compared to white beans.

Phenolic compounds are considered to be natural antioxidants and represent an important group of bioactive compounds in foods which may prevent the development of many diseases, including coronary heart diseases and cancers (Kahkonen, Hopia, Vuorela, Rauha,

Pihlaja, Kujala and Heinonen, 1999). The body has defence mechanisms to prevent free radical damage and to repair damage (Campos-Vega *et al*., 2010), but when the defence is not sufficient, disease may develop. The uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, and degenerative processes associated with aging (Tedesco *et al*., 2000). Phenolic compounds can have complementary and overlapping mechanisms of action, including modulation of detoxifying enzymes, stimulation of the immune system, regulation of lipid and hormone metabolism, antioxidant, antimutagen, and antiangiogenic effects, reduction of tumor initiation, and promotion and induction of apoptosis (Campos-Vega *et al.,* 2010). Figure 5.5 shows a proposed schematic diagram illustrating the process involved in health promotion due to cowpea antioxidants.

Coronary heart disease and chronic degenerative diseases (e.g. cancer) are responsible for many deaths in North America and rest of the world. In sub-Saharan Africa, families suffer from malnution mostly among children children, while diet related chronic diseases have become a common phenomenon in urban African populations. Akinboboye, Idris, Akinboboye and Akinkugbe (2003) reported that the prevalence of coronary artery diseases and cardiovascular diseases was steadily increasing in Sub Saharan Africa. This is in part due to changes in lifestyle mostly influenced by the western lifestyle. Studies have found a link between childhood malnutrion to repressed immunity leading to increased risks of chronic degenerative disease e.g cancer in adulthood (Sawaya, Martins, Hoffman and Roberts, 2003). Potential mechanisms for the link between malnution and chronic degenerative disease include longterm effects of childhood undernutrition on energy expenditure, fat oxidation, regulation of food intake and susceptibility to the effects of high-fat diets (Sawaya *et al*., 2003). There is evidence that suggests that consumption of plant foods can reduce rates of mortality associated with CHD in the early, middle, and late stages of CHD (Rao and Al-Weshahy, 2008). Oxidation of the circulating LDL creates oxidised LDL, which is thought to play a key role in the pathogenesis of atherosclerosis, the underlying disorder leading to heart attack and ischemic stroke (Chu, Sun, Wu and Liu, 2002).

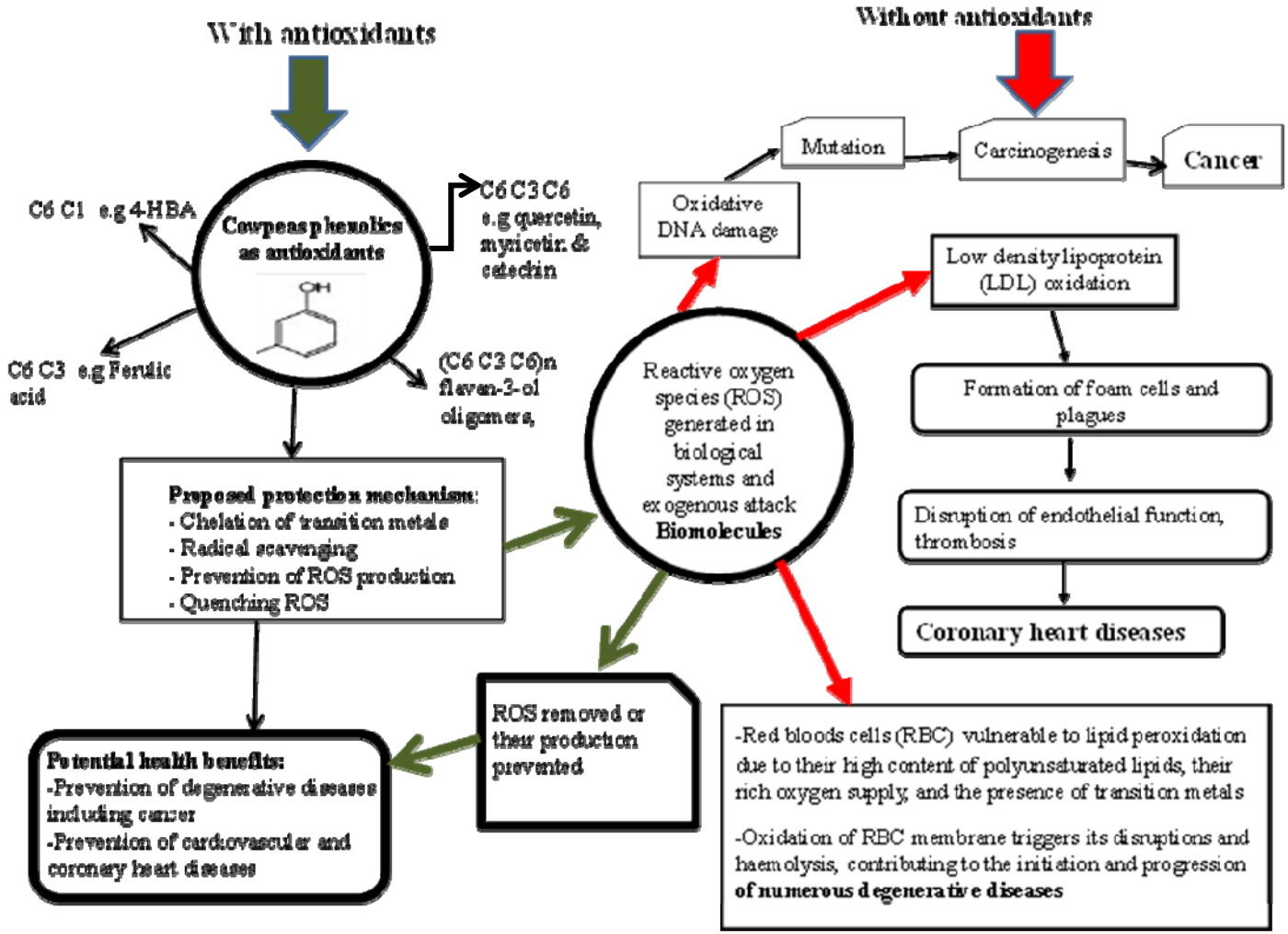


Figure 5.5 Proposed schematic diagram illustrating the process involved in antioxidant and health promoting properties of cowpeas

The findings of this study indicated that the presence of extracts from cowpeas inhibited copper-induced human LDL oxidation as described in research Chapter 4.3. As with radical scavenging properties, extracts from cowpeas with more pigmented seed coats exhibited stronger protection against LDL oxidation compared with those with less pigmented seed coats. Extracts from pigmented cowpeas exhibited stronger protection because of their high levels of phenolic levels and radical scavenging properties. The protective effect may be through scavenging of hydroxyl radicals, peroxyl radicals and chelation of transition metals (Abuja *et al.*, 1998).

Reactive oxygen species (ROS) have been implicated in the mechanism of the damage to red blood cells (RBC) in b-thalassemia, sickle cell anaemia, and other haemoglobinopathies (Tedesco *et al*., 2000). Human red blood cells, because they are oxygen carriers with high polyunsaturated fatty acid content on their membranes and high cellular concentration of haemoglobin are particularly exposed to oxidative damage (Tedesco *et al*., 2000). The haemoglobin released from erythrocytes is potentially dangerous because in reacting with hydrogen peroxide (H_2O_2) it is converted into oxidized forms: methaemoglobin and ferrylhaemoglobin, which are powerful promoters of oxidative processes (Tedesco *et al*., 2000). The free haemoglobin exposed to H_2O_2 causes haeme degradation with the release of iron ions catalytically active in initiating free radical reaction and lipid peroxidation (Tedesco *et al*., 2000). Many defence mechanisms have developed in living organisms to limit the levels of ROS and the damage they inflict. Included among them are endogenous enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Ames, Shigenaga and Hagen, 1993). In addition to these endogenous mechanisms, much attention has been paid to the antioxidant role of some dietary compounds such as polyphenols (Tedesco *et al*., 2000).

Results from this study showed that extracts from all cowpea types protected against AAPH-induced red blood cells haemolysis. Antioxidant properties of polyphenols are attributed mainly to their free radical scavenging and metal-chelating properties (Van Acker *et al.,* 1996). In an erythrocyte cell model, these polyphenols may quench peroxyl radicals before they attack the lipid molecules of the erythrocyte membrane, thereby breaking the free-radical chain reaction and ultimately oxidative haemolysis (Carvalho *et al*., 2010). The protective effect of phenolic compounds can also be due to their ability to bind to the plasma membrane (Blasa *et al*., 2007) and their ability to penetrate lipid bilayers (Lopez-Revuelta,

Sanchez-Gallego, Hernandez- Hernandez, Sanchez-Yague, and Llanillo, 2006). Dai, Miao, Zhou, Yang, and Liu (2006) previously reported that flavonols and their glycosides are effective antioxidants protecting human red blood cells from free radical induced oxidative haemolysis.

In the present study it was also observed that Bechuana white, a light brown cowpea type, protected RBC from haemolysis more than other cowpea samples. The UPLC/PDA/MS analysis showed that this sample was characterised by higher levels of flavonoids such as catechin, catechin 3-O-glucoside, procyanidin dimers, procyanidin dimers and dihydromyricetin 3-O-rhamnoside compared with the other samples. Chen *et al*. (1996) reported that there was variation in the inhibitory effects of flavonoids such quercetin, myricetin, and kaempferol against RBC haemolysis flavonoids such quercetin, myricetin, and kaempferol. Chen *et al.* (1996) suggested that variation in the inhibitory effects of flavonoids could be due to multiple factors such as their hydrophobicity/hydrophilicity, total number and location of the hydroxyl groups of an aromatic ring of a flavonoid and the interaction between the flavonoids with the phospholipids, haemoglobin, iron and other components of the red blood cells. In the future it would be worth investigating the mechanism in which cowpea phenolic compounds protects RBC from haemolysis. Studies may be undertaken to demonstrate if cowpea phenolics protected the RBC through: cell membranes permeability via a passive diffusion mechanism or/and if cowpea phenolics bind to the RBC membrane. Dimeric or oligomeric flavan-3-ol such as procyanidins and prodelphinidins have increased the number of hydroxyl groups in the polymer structure and therefore may interact to a greater degree with RBC membrane protein, through hydrogen bonding and hydrophobic interactions. Phenolic acids (low molecular weight) on the hand may diffuse into the RBC membrane resulting in increased the intracellular antioxidant activities of RBC.

Extracts from cowpeas protected supercoiled plasmid DNA (pBR 322) against AAPH induced oxidative damage as described in research Chapter 4.3. This an indication of the potential of the extracts to protect against free radical oxidative DNA damage which may lead to mutations and altered gene expression that may lead to the development of cancer (Laparra,Vélez Barberá, Farré, and Montoro, 2008). Reactive oxygen species can damage DNA, and division of cells with unrepaired or misrepaired damage leads to mutations (Nijveldt, Van Nood, Van Hoorn, Boelens, Van Norren, and Van Leeuwen, 2001). Reactive

oxygen species can interfere directly with cell signaling and growth (Nijveldt, *et al*., 2001). The cellular damage caused by reactive oxygen species can induce mitosis, increasing the risk that damaged DNA will lead to mutations, and can increase the exposure of DNA to mutagens (Nijveldt, *et al*., 2001). The preventive capability of cowpea extracts against oxidative DNA damage may be due to free radical scavenging activity of their constituent polyphenols.

The ability of cowpea extracts to inhibit *in vitro* LDL oxidation, RBC haemolysis and DNA damage indicates that cowpeas have a potential to inhibit oxidative stress, which is implicated in the development of various chronic diseases (Table 5.1). Cowpeas may therefore be incorporated in food systems not only for their nutritional values but also their potential health benefits. As stated earlier, methanolic extracts were used for analysis and do not represent the form in which cowpeas are ingested. Furthermore, these health promoting benefits depends on absorption and metabolism of cowpea phenolic compounds. Further bioavailability studies of cowpea phenolic compounds may be undertaken to determine their absorption and metabolism.

Table 5.1 A summary of potential health benefits of consuming cowpeas (per serving 50 g, cooked cowpea samples) based on the present research findings

Bioactive properties	**Antioxidant activity (50 g (¼ cup) of cowpeas)**	**Potential health benefit**
Inhibition of LDL oxidation	**(µM TE/100 g cowpeas)**	
Blackeye	350.0	Reduced risk of coronary heart diseases
Bechuana white	650.0	
Glenda	650.0	
Dr Saunders	750.0	
Inhibition of RBC haemolysis	**(µM TE/100 g cowpeas)**	
Blackeye	49.5	Reduced risk of degenerative diseases including cancer
Bechuana white	75.5	
Glenda	58.0	
Dr Saunders	52.0	
Inhibition of DNA damage	**Percentage inhibition (%)**	
Blackeye	30.0	Reduced risk of degenerative diseases including cancer
Bechuana white	37.5	
Glenda	38.0	
Dr Saunders	36.0	

µM TE: Antioxidant activity expressed as micro molar Trolox equivalent

5.2.3 Effects of micronisation of pre-conditioned cowpeas on their phenolic compounds, in vitro radical scavenging and bioactive properties

Table 5.2 summarises the effects of cooking as well as micronisation of pre-conditioned cowpeas followed by cooking on their phenolic composition, radical scavenging properties and protective effects against LDL oxidation, DNA damage and RBC haemolysis. Findings from this study indicate that for all cowpea types, total phenolics contents of uncooked and cooked cowpeas were reduced by micronisation process. Figure 5.6 summarises some of the reasons for changes in cowpea phenolic compounds and bioactive properties as a result of micronisation and subsequent cooking. Generally, two main patterns in which micronisation of pre-conditioned cowpeas and subsequent cooking affected their phenolic composition and the resultant bioactive properties were observed:

(1) Cowpea types behaved differently in the manner in which they were affected by micronisation. Firstly, cowpea types behaved differently with regard to how their phenolic concentrations were affected by micronisation (Table 4.2.3). For example, for Dr Saunders measurable phenolic acid concentrations were predominantly higher in micronised samples than in unmicronised samples. On the contrary, with Bechuana white most phenolic acid concentrations were predominantly lower in micronised samples than in unmicronised samples. Penicela (2010) found that cowpea cotyledon compactness and seed coats thickness affected their cooking quality. Differences in thickness of cowpea seed coat or cotyledon compactness may affect the manner in which micronisation causes fissures or splitting of cowpea seeds which may predispose cowpea phenolics to leaching out upon cooking and thus exposed to oxidative degradation. Penicela (2010) reported that cowpea samples e.g. Bechuana white with compact cotyledons and thick seed coats were more split during cooking compared to the porous/thin cowpeas. Mwangwela (2006) found that Bechuana white seeds were more split upon micronisation compared to other cowpeas used in their studies. Excessive splitting due to micronisation and cooking of Bechuana white may have facilitated leaching out of their phenolic components.

Table 5.2 A summary showing overall effects of micronisation of pre-conditioned cowpeas followed by cooking on their phenolic compouns and bioactive properties (comparing mean values of all cowpea types)

ANALYSES	Effect of cooking	% effect	Effect of micronisation	% effect	Effect of micronisation and subsequent cooking	% effect
Phenolic acids:						
Protocatechuic acid	Higher*	154.0	Higher	7.3	Higher	40.0
Ferulic acid	No change		Higher	48.5	Higher	74.0
4-Hydroxybenzoic acid	Higher	11.5	Lower	11.0	No change	
p-Coumaric acid	No change		Higher	57.0	Higher	25.3
Flavonoids:						
Catechin	Lower	16.2	Lower	22.9	Lower	10.0
Quercetin	Lower	7.5	Higher	37.9	No change	
Rutin	Lower	6.6	No change		Lower	19.6
Total flavonoids	Lower	22.6	No change		No change	
Total phenolics	Lower	24.0	Lower	38.7	Lower	36.0
Antioxidant activity:						
ABTS/TEAC	Lower	35.5	Lower	23.0	No change	
ORAC	Lower	38.0	Lower	20.0	Lower	12.0
Protective effects against:						
LDL oxidation	Lower	27.0	Not determined	Not determined	Lower	71.4
DNA damage	No change		Not determined	Not determined	No change	
RBC haemolysis	No change		Not determined	Not determined	No change	

*This means cooked cowpea had higher protocatechuic acid content than uncooked.

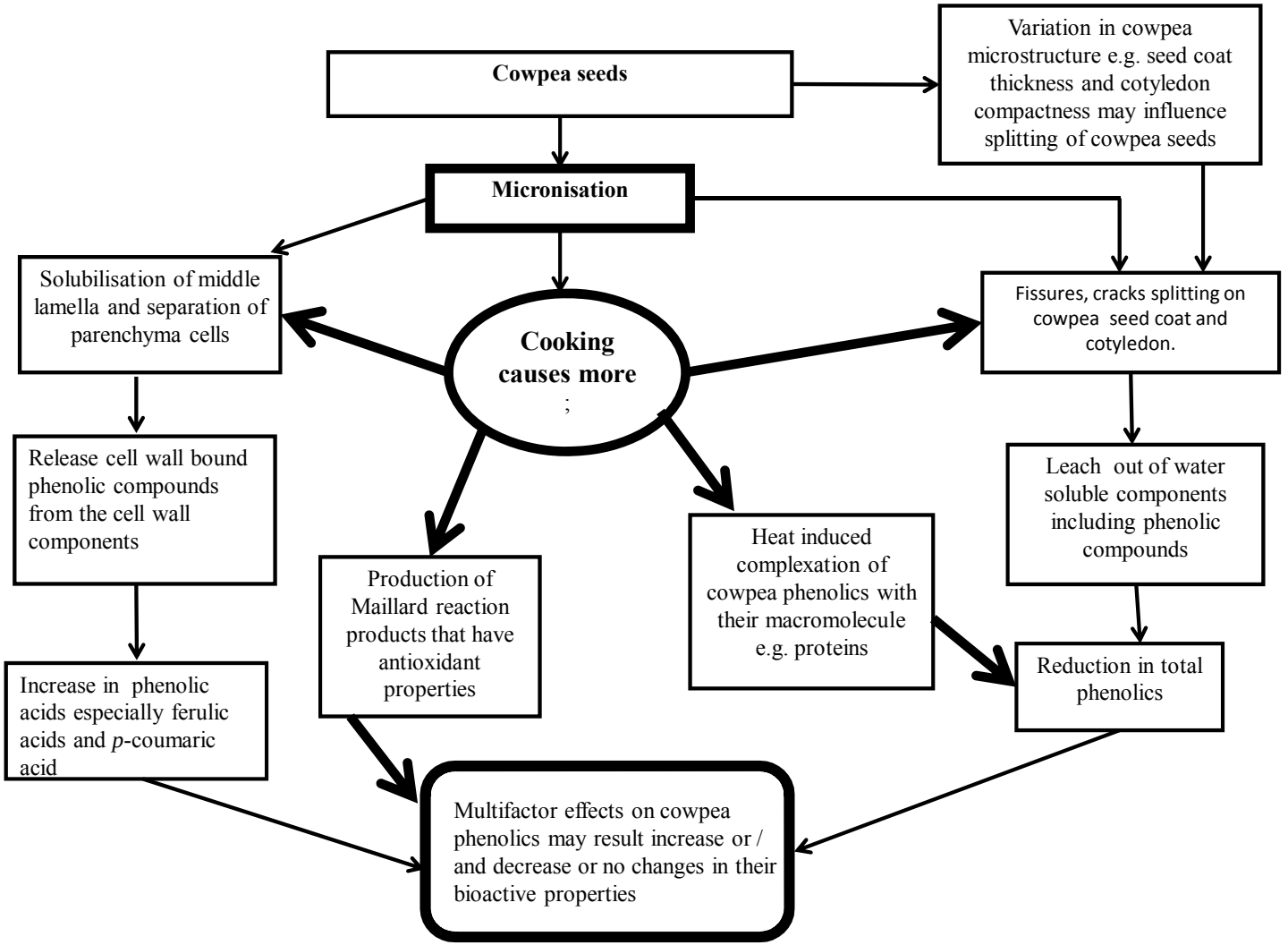


Figure 5.6 Proposed reasons for changes in cowpea phenolic compounds and bioactive properties upon micronisation and cooking

(2) Generally phenolic acids were mostly increased or unchanged by micronisation and cooking while flavonoids were decreased or remained unchanged (Table 5.2). Some phenolic compounds especially phenolic acids are found in cell walls, while others are present within the plant cell vacuoles (Bengoechea, Sancho, Bartolome, Estrella, Gomez-Cordoves and Hernandez, 1997). Cell wall phenolics are linked to various cell wall components and contribute to the mechanical strength of cell walls as well as playing a regulatory role in plant growth and morphogenesis in the cell in response to stress and pathogens (Naczk and Shahidi, 2004). Ferulic and *p*-coumaric acids are the major phenolic acids that occur in bound form within cell walls. These compounds may be esterified to pectins and arabinoxylans or cross-linked to cell wall polysaccharides in the form of dimers such as dehydroferulates and truxillic acid (Naczk and Shahidi, 2004). Mwangwela (2006) reported that micronisation resulted in physical, chemical and structural changes in cowpea seeds. Mwangwela (2006) found that micronisation process resulted in disintegration of middle lamella, cracks/fissured seed coats as well as separation of cowpea parenchyma cell. This indicates that the integrity of the cell wall structure was interrupted, and possibly resulting in release of phenolics linked to the cell wall component. Therefore higher levels of phenolic acids such as ferulic and *p*-coumaric acids observed in micronised (uncooked) and micronised (cooked) cowpea samples may be attributed to these changes.

Mwangwela (2006) also observed that micronisation resulted in mildly crosslinked cytoplasmic matrix (low temperatures) and highly crosslinked cytoplasmic matrix especially (high temperatures). These crosslinks formed may have involved the interaction of cowpea phenolic compounds with macromolecules such as proteins. Interactions of plant phenolics with proteins may lead to the formation of insoluble complexes (Shahidi and Naczk, 2004). Flavonoids due to their more complex structure e.g. more hydroxyl groups compared to phenolic acids may interact to a greater degree with cowpea macro molecules e.g. proteins. Polyphenol–protein interactions are affected by the size, length, and flexibility of polyphenol molecules and the number and stereospecificity of binding sites on both the polyphenol and protein molecules (de Freitas and Mateus, 2001; Hagerman, Rice, and Ritchard, 1998; Shahidi and Naczk, 2004). The formation of phenolic-protein complexes may have caused a reduction in extractability of flavonoids. It is important to note that complexes e.g. protein-phenol complexes may have a detrimental effect on the in vivo bioavailability of both phenolics and proteins (Lowry, McSweeney and Palmer, 1996).

6. CONCLUSIONS AND RECOMMENDATIONS

Reduction in cooking time of cowpeas as a result of micronisation implies that less energy and time will be required for preparation of cooked cowpeas. Such advantages could improve utilisation of cowpeas. Production of micronised cowpeas with reduced cooking time presents an opportunity for utilisation of cowpeas as an alternative product for dry grains on shop shelves especially for urban consumers whose busy life style makes convenience an important factor in food choices.

Micronisation affects the sensory characteristics of cooked cowpeas, e.g. increasing mushiness and seed splitting. Some cowpea types are more prone to splitting and mushiness than others, and thus should rather be used in food applications like pastes or soups where intact cooked seeds are not necessary. Micronisation also results in darker cowpeas with roasted flavour notes on cooking. Incorporating flour from micronised cowpeas with more toasted/roasted flavour notes will reduce the raw legume flavour note normally perceived in baked products composited with raw legume flours. On the other hand introduction of uncharacteristic aroma/flavour notes in cooked cowpeas that may be unfamiliar to cowpea consumers could elicit negative consumer reaction to micronised cowpeas. Extensive consumer acceptability studies are required to determine how to effectively market micronised cowpeas with shorter cooking times but varying sensory properties to target consumers.

Protocatechuic acid, *p*-coumaric acid, ferulic acid and 4-hydroxybenzoic acids are the most abundant phenolic acids in cowpea samples. Catechin, catechin-3-*O* -glucoside, myricetin, rutin, and quercetin and its mono and diglycosides are the main flavonoids present in cowpeas. The type and content of phenolic compound depends on cowpea types. More of the flavonols are identified in red and dark brown compared to light brown and cream cowpeas. The red cowpea type contained all the dimers and oligomeric flavan-3-ol species identified in this study. Generally, more pigmented cowpea types have the highest level of phenolic compounds than the less pigmented cowpeas. Cowpea seed coat pigmentation can be used as selection criteria by breeders, nutritionists, food scientists and consumers for health application.

Extracts from all cowpea samples tested exhibit strong radical scavenging properties and protection against *in vitro* LDL oxidation, RBC haemolysis and DNA damage. Phenolic compounds in cowpeas contribute to these protective effects because cowpeas with high phenolic contents exert strong protection against oxidative damage of the biological molecules. The ability of cowpea extracts to inhibit *in vitro* oxidative damage of the biological molecules (LDL, RBC and DNA) indicates that cowpeas have a potential to inhibit oxidative stress, which is implicated in the development of various chronic diseases. The findings from this study should form the basis to promote consumption of cowpeas not only for their nutritional values but also their potential health benefits.

Phenolic acids e.g. ferulic acid and p-coumaric acid are rendered more extractable by micronisation and cooking. These compounds are mostly linked to the cell wall materials and disruption of cell wall structure due to micronisation increases their extractability. In contrast, flavonoids become less extractable or remain unchanged by micronisation and cooking. Flavonoids due to their more complex structure compared to phenolic acids may interact with cowpea macromolecules e.g. proteins during micronisation and subsequent cooking. This will result in the formation of insoluble protein-phenol complexes, thus reducing their extractability. The multifactorial effects of micronisation and subsequent cooking on cowpea phenolics result in changes in their bioactive properties. Micronised cowpea samples have reduced potential to inhibit oxidation of LDL, but their protection against oxidative DNA damage and RBC is not affected by micronisation. Micronised cowpea samples retain some bioactivity and therefore can offer potential health benefits to cowpea consumers.

Cowpea type influences the manner in which phenolic compounds are affected by micronisation and subsequent cooking, and can direct seed selection for health applications. Cowpea types that are prone to splitting have more phenolic losses than those that remain intact during micronisation and subsequent cookig. This is because splitting results in leaching out of water soluble seed components including phenolic compounds. Cowpea types that are less prone to splitting can be recommended for micronisation if the final product is intended for health applications. However, further research is needed to test this hypothesis.

Results from this study provide a basis for understanding cowpea phenolic compounds and their potential to promote health. Furthermore, this study improved our understanding of how micronisation affects phenolic compounds. However, it is important to note that in the present study all the analyses were done *in vitro*. There is a need for conducting *in vivo* studies to understand bioavailability of cowpea phenolics and their effect on human health. Animal and clinical studies may be undertaken to confirm the claim that cowpea is a potential source of antioxidants and may be consumed to exert health benefits to their consumers. Furthermore more information on how processing technologies such as micronisation may affect the absorption and metabolism of cowpea phenolic compounds is required.

7. REFERENCES

ABBOTT, J., 1999. Quality measurement of fruits and vegetables. *Postharvest Biology and Technology* **15**, 207-225.

ABUJA, P. M., MURKOVIC, M. & PFANNHAUSER, W., 1998. Antioxidant and prooxidant activities of elderberry (*Sambucus nigra*) extract in low-density lipoprotein oxidation. *Journal of Agricultural and Food Chemistry***, 46,** 4091-4096.

ADOM, K.K., SORRELLS, M.E. & LIU, R.H., 2003. Phytochemical profiles and antioxidant activity of wheat varieties, *Journal of Agricultural and Food Chemistry* **51** 7825–7834.

AFOAKWA, E.O., YENYI, S.E. & SAKYI-DAWSON, E., 2006. Response surface methodology for optimizing the pre-processing conditions during canning of a newly developed and promising cowpea (*Vigna unguiculata*) variety. *Journal of Food Engineering* **73**, 346-357.

AGURS-COLLINS, T., SMOOT, D., AFFUL, J., MAKAMBI, K. & ADAMS-CAMPBELL, L., 2006. Legume intake and reduced colorectal adenoma risk in African-Americans. *Journal of the National Black Nurses Association* **17**, 6–12.

AHERNE, A.S. & OBRIEN, N.M., 2002. Dietary flavonols: Chemistry, food content and metabolism. *Nutrition* **18**, 75–81.

AKINBOBOYE, O., IDRIS, O, AKINBOBOYE, O. & AKINKUGBE, O., 2003. Trends in coronary artery disease and associated risk factors in sub-Saharan Africans. *Journal of Human Hypertension* **17**, 381–387

AKINYELE, I.O., ONIGBINDE, A.O., HUSSAIN, M.A. & OMOLOLU, A., 1986. Physicochemical characteristics of 18 cultivars of Nigerian cowpeas (*V. unguiculata*) and their cooking properties. *Journal of Food Science* **51**, 1483-1485.

ALASALVAR, C., GRIGOR, J.M., ZHANG, D., QUANTICK, P.C. & SHAHIDI, F., 2001. Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different coloured carrot varieties. *Journal of Agricultural and Food Chemistry* **49**, 1410–1416.

ALVAREZ-SUAREZ, J. M., TULIPANI, S., ROMANDINI, S., VIDAL, A. & BATTINO, M., 2009. Methodological aspects about determination of phenolic compounds and *in vitro* evaluation of antioxidant capacity in the honey: A review. *Current Analytical Chemistry* **5,** 293-302.

AMARAL, S., MIRA, L., NOGUEIRA, J. M. F., PEREIRA DA SILVA, A., & FLORÊNCIO, M. H., 2009. Plant extracts with anti-inflammatory properties – A new approach for characterization of their bioactive compounds and establishment of structure–antioxidant activity relationships. *Bioorganic and Medicinal Chemistry* **17**, 1876–1883.

AMAROWICZ, R. & PEGG, R. B., 2008. Legumes as a source of natural antioxidants. *European Journal of Lipid Science and Technology*, **110**, 865–878.

AMAROWICZ, R., TROSZYŃSKA, A., BARYŁKO-PIKIELNA, N. & SHAHIDI, F., 2004. Polyphenolics extracts from legume seeds: Correlations between total antioxidant activity, total phenolics content, tannins content and astringency. Journal of Food Lipids 11, 278–286.

AMES, B.N., SHIGENAGA, M.K. & HAGEN, T.M., 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proceedings of National Academy of Science USA 90, 7915–7922.

ANDERSON, J. W., SMITH, B. M. & WASHNOCK, C. S., 1999. Cardiovascular and renal benefits of dry bean and soybean intake. *American Journal of Clinical Nutrition* **70**, 464S–474S.

ANDREASEN, M. F., CHRISTINSEN, L. P., MEYER, A. S. & HANSEN. A., 2003 Content of phenolic acids and ferulic acid dehydrodimers in 17 rye (*Secale cereale* L.) varieties. *Journal of Agricultural and Food Chemistry* ***48***, 2837-2842.

ANDREASEN, M.F., LANDBO, A., CHRISTENSEN, L.P., HANSEN, A. & MEYER, A.S., 2001. Antioxidant effect of phenolic rye (*Secale cereale L.*) extracts, monomeric hydroxycinnamates, and ferulic acid dehydrodimers on human low density lipoproteins. *Journal of Agricultural and Food Chemistry* **49**, 4090–4096.

ANG, E.C. & BOATRIGHT, W.L., 2003. Olfactory perception of major odorants found in the headspace of aqueous soy protein isolate slurries. *Journal of Food Science* **68**, 388–393.

ANYANGO, J.O., DE KOCK, H.L. & TAYLOR, J.R.N., 2011. Evaluation of the functional quality of cowpea-fortified traditional African sorghum foods using instrumental and descriptive sensory analysis. *LWT - Food Science and Technology* **44**, 2126–2133.

APARICIO-FERNANDEZ, X., YOUSEF, G. G., LOARCA-PINA, G., DE MEJIA, E. & LILA, M. A., 2005. Characterization of polyphenolics in the seed coat of black Jamapa bean *(Phaseolus vulgaris L.). Journal of Agricultural and Food Chemistry* **53**, 4615–4622.

AREMU, C.Y., 1991. Selected physico-chemical properties of five varieties of cowpea. *Food Chemistry* **41**, 123-128.

ARIGA, T. & HAMANO, M., 1990. Radical scavenging action and its mode in procyanidins B-1 and B-3 from azuki beans to peroxyl radicals. *Agricultural and Biological Chemistry* **54**, 2499–2504.

ARNTFIELD, S.D., SCANLON, M.G., MALCOLMSON, L.J., WATTS, B., RYLAND, D. & SAVOIE, V. 1997. Effect of tempering and end moisture content on the quality of micronized lentils. *Food Research International* **30**, 371-380.

ARNTFIELD, S.D., SCANLON, M.G., MALCOLMSON, L.J., WATTS, B.M., CENKOWSKI, S., RYLAND, D. & SAVOIE, V., 2001. Reduction in lentil cooking time using micronization: Comparison of 2 micronisation temperatures. *Journal of Food Science* **66**, 500-505.

ARNTFIELD, S.D., ZHANG, M.Z., NYACHOTI, C.M., GUENTER, W. & CENKOWSKI, S., 2004. Processing conditions for micronization of peas (*Pisum Sativu*) and *in-vitro* evaluation of the product. *Technical Sciences* **7**, 29-38.

ARONOVITCH, Y., GODINGER, D., ISRAELI, A., KRISHNA, M. C., SAMUNI, A. & GOLDSTEIN, S., 2007. Dual activity of nitroxides as pro- and antioxidants: Catalysis of copper-mediated DNA breakage and H_2O_2 dismutation. *Free Radical Biology and Medicine,* **42**, 1317-1325.

ASTADI, I. R., ASTUTI, M., SANTOSO, U. & NUGRAHENI, P. S., 2009. In vitro antioxidant activity of anthocyanins of black soybean seed coat in human low density lipoprotein (LDL). *Food Chemistry* **112**, 1 659-663.

AVILA, M. A., VELASCO, J. A., CANSADO, J. & NOTARIO, V., 1994. Quercetin mediates the down-regulation of mutant p53 in the human breast cancer cell line MDAMB468. *Cancer Research* **54**, 2424–2428.

AWIKA J.M., ROONEY, L.W., WU, X., PRIOR, R.L. & CISNEROS-ZEVALLOS, L., 2003. Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *Journal of Agricultural and Food Chemistry* **51**, 6657-6662.

AWIKA, J.M., DYKES, L., GU, L., ROONEY, L.W. &PRIOR, R.L., 2003. Processing of sorghum (Sorghum bicolor) and sorghum products alters procyanidin oligomer and polymer distribution and content. *Journal of Agricultural and Food Chemistry* **51**, 5516–5521.

BALAMAZE, J., MUYONGA, J.M., KYAMUGANGIRE, W.M., KIKAFUNDA, J.K., NAKIMBUGWE, D. & UGEN, M., 2008. Influence of variety, growth location and storage conditions on development of hard-to-cook defect in common bean (*Phaseolus vulgaris* L.). *African Journal of Food Agriculture and Nutritional Development* ***8***, 333–348.

BARROGA, F. C., LAURENA, A. C. & MENDOZA E. M. T., 1985. Polyphenols in mung (*Vigna radiata* (L.) Wiczek): Determination and removal. *Journal of Agricultural and Food Chemistry* **33**, 1006–1009.

BAZZANO, L., HE, J., OGDEN, L. G., LORIA, C., VUPPUTURI, S., MYERS, L. & WHELTON, P. K., 2001. Legume consumption and risk of coronary heart disease in US men and women. *Archives of International Medicine* **161**, 2573-2578.

BEAL, J.D., NIVEN, S.J., BROOKS, P.H. & GILL, B.P., 2005. Variation in short chain fatty acid and ethanol concentration resulting from the natural fermentation of wheat and barley for inclusion in liquid diets for pigs. *Journal of the Science of Food and Agriculture* **85**, 433–440.

BEATTIE, J., CROZIER, A. & DUTHIE, G.G., 2005. Potential health benefits of berries. *Current Nutrition and Food Science* **1,** 71-86

BELLIDO, G.G., ARNTFIELD, S.D., SCANLON, M.G. & CENKOWSKI, S., 2003. The effect of micronisation operational conditions on the physiochemical properties of navy beans (*Phaseolus Vulgaris* L.). *Journal of Food Science* **68**, 1731-1735.

BEMILLER, J.N. & WHISTLER, R.L. 1996. *Carbohydrates*. In O.R. Fennema (Ed) Food chemistry, New York: Marcel Dekker, pp 191-195.

BENGOECHEA, M.L., SANCHO, A.I., BARTOLOME, B., ESTRELLA, I., GOMEZ-CORDOVES, C. & HERNANDEZ, T., 1997. Phenolic composition of industrially manufactured purées and concentrates from peach and apple fruits. *Journal of Agricultural and Food Chemistry* **45**, 4071–4075.

BENINGER, C. W. & HOSFIELD, G. L., 2003. Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from *Phaseolus vulgaris* L. seed coat color genotypes. *Journal of Agricultural and Food Chemistry* ***51*****,** 7879-7883.

BIRD, T. A., SCHULE, H. D., DELANEY, P. B., SIMS, J. E., THOMA, B. & DOWER, S. K., 1992. Evidence that MAP (mitogen-activated protein) kinase activation may be a necessary but not sufficient signal for a restricted subset of responses in IL-1- treated epidermoid cells. *Cytokine* **4**, 429–440.

BLASA, M., CANDIRACCI, M., ACCORSI, A., PIACENTINI, M. P. & PIATTI, E., 2007. Honey flavonoids as protection agents against oxidative damage to human red blood cells. *Food Chemistry* **104**, 1635–1640.

BLOCK, G., PATTERSON, B. & SUBAR, A., 1992. Fruit, vegetables, and cancer prevention: a review of epidemiological evidence. *Nutrition and Cancer* **18**, 1–29.

BOCCHI, C., CARERI, M., GROPPI, F., MANGIA, A., MANINI, P. & MORI, G., 1996. Comparative investigation of UV, electrochemical and particle beam mass spectrometric detection for high-perfomance liquid chromatographic determination of benzoic and cinnamic acids and of their corresponding phenolic acids. *Journal of Chromatography A,* ***753***, 157-170.

BOMSER, J., MADHAVI, D. L., SINGLETETARY, K. & SMITH, M. A. L., 1996. In vitro anticancer activity of fruit extracts from Vaccinium species. *Planta Medica* **41**, 212–216.

BRAVO L., 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews* **56**, 317–33.

BRAVO, L., ABIA, R., EASTWOOD, M.A. & SAURA-CALIXTO, F., 1994**.** Degradation of polyphenols (catechin and tannic acid) in the rat intestinal tract: effect on colonic fermentation and faecal output. *British Journal of Nutrition* **71**, 933-46.

BRAVO, L., MAFIAS, E. & SAURA-CALIXTO F., 1993. Dietary nonextractable condensed tannins as indigestible compounds: effects on faecal weight, and protein and fat excretion. *Journal of the Science of Food and Agriculture* **63**, 63-68.

BRAVO, L., SAURA-CALIXTO. F. & GOFII, L**.,** 1992**.** Effects of dietary fibre and tannins from apple pulp on the composition of faeces in rats. *British Journal of Nutrition* **67**, 463-73.

BROWN, D.F., SENN, V.J., DOLLEAR, F.G. & GOLDBLATT, L.A., 1973. Concentrations of some aliphatic aldehydes and ketones found in raw and roasted Spanish and runner peanuts. *Journal of the American Oil Chemist Society* **50**, 16-20.

BRYNGELSSON, S., DIMBERG, L.H. & KAMAL-ELDIN, A., 2002. Effects of commercial processing on levels of antioxidants in oats (*Avena sativa* L.). *Journal of Agricultural and Food Chemistry* **50**, 1890–1896.

BUNEA, A., ANDJELKOVIC, M., SOCACIU, C., BOBIS, O., NEACSU, M., VERHE, R. & VAN CAMP, J., 2008. Total and individual carotenoids and phenolic acids contents in fresh, refrigerated and processed spinach (*Spinacia oleracea* L.). *Food Chemistry* **108**, 649–656.

CAI, R., HETTIARACHCHY, N.S. & JALALUDDIN, M., 2003. High-performance liquid chromatography determination of phenolic constituents in 17 varieties of cowpeas. *Journal of Agricultural and Food Chemistry* **51**, 1623-1627.

CAMPOS-VEGA, R., LOARCA-PINA, G. & OOMAH, B.D., 2010. Minor components of pulses and their potential impact on human health. *Food Research International* **43**, 461–482.

CAO, G.H. & PRIOR, R. L., 1999. Measurement of oxygen radical absorbance capacity in biological samples: oxidants and antioxidants. *Methods in Enzymology* **299**, 50-62.

CAO, G., SOFIC, E. & PRIOR, R. L., 1997. Antioxidant and prooxidant behavior of flavonoids: Structure-Activity Relationships. *Free Radical Biology and Medicine* ***22***, 749-760.

CAO, G., ALESSIO, H.M. & CULTER, R.G., 1993. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine* **14**, 303-311.

CARDADOR-MARTINEZ, A., LOARCA-PINA, G. & OOMAH, B. D., 2002. Antioxidant activity in common beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry* **50**, 6975-6980.

CARRÃO-PANIZZI, M.C. & KITAMURA K., 1995. Isoflavone content in Brazilian soybean cultivars. *Breeding Science* **45**, 295–300.

CARVALHO, M., FERREIRA, J.P., MENDES, V.S., SILVA, R., JOSÉ A. PEREIRA, J.A., JERÓNIMO, C. & SILVA, B. M., 2010. Human cancer cell antiproliferative and antioxidant activities of walnut *Juglans regia L. Food and Chemical Toxicology* **48**, 441–447.

CENKOWSKI, S., AMES, N. & MUIR, W.E., 2006. Infrared processing of oat groats in a laboratory-scale microniser. *Canadian Biosystems Engineering* **48**, 3.17–3.25.

CENKOWSKI, S., HONG, J.T., SCANLON, M.G. & ARNTFIELD, S.D. 2003. Development of a mathematical model for high-intensity infrared processing (Micronization) of peas. *Transactions of the ASAE* **46**, 705-713.

CENKOWSKI, S. & SOSULSKI, F.W. 1997. Physical and cooking properties of micronized lentils. *Journal of Food Process Engineering* **20**, 249-264.

CHANG, C., YANG, M., WEN, H. & CHERN, J., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis* **10**, 178-182.

CHANG, R.L., HUANG, M. T., WOOD, A. W., WONG, C. Q., NEWMARK, H. L. & YAGI, H., 1985. Effect of ellagic acid and hydroxylated flavonoids on the tumorigenicity of benzo[a]pyrene and (6)-7 beta, 8 alphadihydroxy-9 alpha, 10 alpha epoxy- 7,8,9,10-tetrahydrobenzo[a]pyrene on mouse skin and in the newborn mouse. *Carcinogenesis* **6**, 1127–1133.

CHAU. K.J & CHOU.S.K., 2005. A comparative study between intermittent microwave and infrared drying of bioproducts. *International Journal of Food Science and Technology* **40,** 23–39.

CHEN, Z.Y., CHAFUNG, P.T., HO, K.Y., FUNG, K.P. & WANG, J., 1996. Antioxidant activity of natural flavonoids is governed by number and location their aromatic hydroxyl groups. *Chemistry and Physics of Lipids* **79**, 157-163.

CHENG, J., DAI, F., ZHOU, B., YANG, L. & LIU Z., 2007. Antioxidant activity of hydroxycinnamic acid derivatives in human low density lipoprotein: Mechanism and structure-activity relationship. *Food Chemistry* **104**, 132–139.

CHEUNG, L. M., CHEUNG, P.C.K. & OOI, V.E.C., 2003. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry* **81**, 249-255.

CHOI, J. A., KIM, J. Y., LEE, J. Y., KANG, C. M., KWON, H. J., YOO, Y. D., KIM,T.W., LEE, Y.S. & LEE, S.J., 2001. Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. *International Journal of Oncology* **9**, 837–844.

CHU, Y.F., SUN, J., WU, X. & LIU, R.H., 2002. Antioxidant and antiproliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry* **50,** 6910–6916.

CHUNG, T. W., MOON, S. K., CHANG, Y. C., KO, J. H., LEE, Y. C. & CHO., G., 2004. Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: Complete regression of hepatoma growth and metastasis by dual mechanism. *The FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* **18**, 1670–1681.

CHUNG, W.K. & HWANG, I.K., 1996. Quality characteristics of vegetable soybeans at different harvesting time. *Korean Journal of Crop Science* **41**, 103–108.

CZOCHANSKA, Z., FOO, L.Y. & PORTER, L.J., 1979. Compositional changes in lower molecular weight flavans during grape maturation. *Phytochemistry* **18**, 1819 –22.

DAI, F., MIAO, Q., ZHOU, B., YANG, L. & LIU, Z. L., 2006. Protective effects of flavonols and their glycosides against free radical-induced oxidative hemolysis of red blood cells. *Life Sciences* 78, 2488–2493.

DAVIS, D.W., OELKE, E.A., OPLINGER, E.S., DOLL, J.D., HANSON, C.V. & PUTNAM, D.H., 1991. *Cowpea: Alternative Field Crops Manual*. www.hort.purdue.edu/newcrop/afcm/cowpea.html; Last accessed in May 2010.

DE FREITAS, V. & MATEUS, N., 2001. Structural features of procyanidin interactions with salivary proteins. *Journal of Agricultural and Food Chemistry* **49**, 940–945.

DEMOOY, B.G. & DEMOOY, C.J. 1990. Evaluation of cooking time and quality of seven diverse cowpea (*Vigna unguiculata* (L) Walp) varieties. *International Journal of Food Science and Technology* **25**, 209-212.

DENG, S.L., CHEN, W, F., ZHOU, B., YANG, L. & LUI, Z.L., 2006. Protective effects of curmmin and its analogues against free radical-induced oxidative haemolysis of human red blood cells. *Food Chemistry* **98**, 112-119.

DEWANTO, V., WU, X., ADOM, K. K. & LIU, R. H., 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry* **50**, 3010–3014.

DÍAZ-BATALLA, L., WIDHOLM, J. M., FAHEY, G. C., CASTAÑO-TOSTADO, E. & PAREDES-LÓPEZ O., 2006. Chemical components with health implications in wild and cultivated Mexican common bean seeds (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry*. **54**, 2045–2052.

DJORDJEVIC, T.M., SILER-MARINKOVIC, S.S. & DIMITRIJEVIC-BRANKOVIC, S.I., 2011. Antioxiodant activity and total phenolic content in some cereals and legumes. *International Journal of Food Properties* **14**, 175–184.

DREWNOWSKI, A. & GOMEZ-CARNEROS, C., 2000. Bitter taste, phytonutrients, and the consumer. *American Journal of Clinical Nutrition* **72**, 1424–1435.

DUEÑAS, M., HERNÁNDEZ, T. & ESTRELLA, I., 2006. Assessment of in vitro antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. *Food Chemistry* **98**, 95–103.

DUEÑAS, M., FERNÁNDEZ, D., HERNÁNDEZ, T., ESTRELLA, I. & MUNOZ, R., 2005. Bioactive phenolic compounds of cowpeas (*Vigna sinensis* L). Modifications by fermentation with natural microflora and with *Lactobacillus plantarum* ATCC 14977. *Journal of the Science of Food and Agriculture* **85**, 297 304.

DURKEE, A. B. & THIVIERGE, P. A., 1977. Ferulic acid and other phenolics in oat seeds. *Journal of Food Science* ***42***, 551-552.

EHLERS, J.D. & HALL, A.E. 1997. Cowpea (*Vigna unguiculata* L. *Walp). Field Crops Engineering* **33**, 337-346.

EINSTEIN, M.A., 1991. Descriptive techniques and their hybridization. In: *Sensory Science Theory and Applications in Foods*. Lawless, H.T. & Klein, B.P. (Eds) Marcel Dekker: New York. pp 317-338.

ELLIOT, R.M., ASTLEY, S.B., SOUTHON, S. & ARCHER, D.B., 2000. Measurement of cellular repair activities for oxidative DNA damage. *Free Radical Biology and Medicine* **28**, 1438-1446.

ESTERBAUER, H., GEBICKI, J., PUHL, H. & JÜRGENS, G., 1992. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology and Medicine, **13***, 341-390.

FASINA, O., TYLER, R.T, PICKARD, M.D. & ZHENG, G.H., 1999. Infrared heating of hull-less and pearled barley. *Journal of Food Processing and Preservation* **23**, 135-151.

FASINA, O., TYLER, B., PICKARD, M., ZHENG, G. & WANG, N., 2001. Effect of infrared heating on the properties of legume seeds. *International Journal of Food Science and Technology* **36**, 79-90.

FEREGRINO-PEREZ, A. A., BERUMEN, L. C., GARCIA-ALCOCER, G., GUEVARA-GONZALEZ, R. G., RAMOS-GOMEZ, M., REYNOSO-CAMACHO, R., 2008. Composition and chemopreventive effect of polysaccharides from common beans *(Phaseolus vulgaris L.)* on azoxymethane-induced colon cancer. *Journal of Agricultural and Food Chemistry* **56**, 8737–8744.

FLIGHT, I. & CLIFTON, P., 2006. Cereal grains and legumes in the prevention of coronary heart disease and stroke: a review of the literature. *European Journal of Clinical Nutrition* **60**, 1145–1159

FOOD AND AGRICULTURE ORGANIZATION, 2006. Double burden of malnutrition: Fighting hunger and obesity. http://www.fao.org/ag/magazine/0602sp1.htm accessed 29 June, 2010.

FRIEDRICH, W., EBERHARDT, A. & GALENSA, R., 2000. Investigation of proanthocyanidins by HPLC with electrospray ionization mass spectrometry. *European Food Research and Technology* 56-64.

FUKUMOTO, L.R. & MAZZA, G., 2000. Assessing antioxidant and prooxidant activities of phenolic compounds. *Journal of Agricultural and Food Chemistry* **48,** 3597-3604.

FUNG, T., HU, F. B., FUCHS, C., GIOVANNUCCI, E., HUNTER, D. J., STAMPHER, M. J., COLDITZ, G.A. & WILLETT, W.C., 2003. Major dietary patterns and the risk of colorectal cancer in women. *Archives of Internal Medicine* **163**, 309–314.

GACULA, M. C., 1997. *Descriptive Sensory Analysis in practice*. Trumbull, CT: Food and Nutrition Press.

GALLEGOS-INFANTE, J.A., ROCHA-GUZMAN, N.E., GONZALEZ-LAREDO, R.F. & PULIDO-ALONSO. J., 2010. Effect of processing on the antioxidant properties of extracts from Mexican barley (*Hordeum vulgare*) cultivar. *Food Chemistry* **119**, 903–906.

GIOACCHINI, A.M., RODA, A., GALLETTI, G. C., BOCCHINI, P., MANETTA, C. & BARALDINI, M., 1996. High-performance liquid chromatographic-electrospray mass spectrometric analysis of phenolic acids and aldehydes. *Journal of Chromatography A* **730**, 31-37.

GIRISH, T.K., VASUDEVARAJU, P. & PRASADA RAO, U.J.S., 2012. Protection of DNA and erythrocytes from free radical induced oxidative damage by black gram (Vigna mungo L.) husk extract. *Food and Chemical Toxicology* **50**, 1690–1696.

GRACE, S, C., SALGO, M.G. & PRYOR, W.A., 1998. Scavenging of peroxynitrite by a phenolic/peroxidase system prevents oxidative damage to DNA. *FEBS Letters* **426**, 24–28

GRANITO, M., BRITTO, Y. & TORRES, A., 2007. Chemical composition, antioxidant capacity and functionality of raw and processed *Phaseolus lunatus*. *Journal of the Science of Food and Agriculture* **87**, 2801–2809.

GREENWOOD, D., CADE, J., DRAPER, A., BARRETT, J. H., CALVERT, C. & GREENHALGH, A., 2000. Seven unique food consumption patterns identified among women in the UK women's cohort study. *European Journal of Clinical Nutrition* **54**, 314–320.

GRINBERG, L.N., NEWMARK, H., KITROSSKY, N., RAHAMIM, E., CHEVOIN, M. & RACHMILEWITZ, E.A., 1997. Protective effects of tea polyphenols against oxidative damage to red blood cells. *Biochemical Pharmacology* **54**,973-978

HA, T.J., LEE, M. H., JEONG, Y. N., LEE, J. H., HAN, S.I., PARK, C.H., PAE, S.B., HWANG, C.D., BAEK, I.Y. & PARK, K.Y., 2010. Anthocyanins in cowpeas *(Vigna unguiculata (L.) Walp. Food Science and Biotechnology* **19**, 821−826.

HAGERMAN, A. E., RIEDL, K. M., JONES, G. A., SOVIK, K. N., RITCHARD, N. T., V., 2000. Effect of antioxidant protection by p-coumaric acid on oxidation low-density lipoprotein cholesterol. *American Journal of Physiology: Cell Physiology* **279**, C954–C960.

HAGERMAN, A. E., RIEDL, K. M., JONES, G. A., SOVIK, K. N., RITCHARD, N. T., HARTFELD, P. W. & RIECHEL, T. L., 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry* **46**, 1887-1892.

HARTZFELD, P. W., & RIECHEL, T. L., 1998. High Molecular Weight Plant Polyphenolics (Tannins) as Biological Antioxidants. *Journal of Agricultural and Food Chemistry* **46**, 1887-1892.

HARBORNE, J.B., 1989. *Methods in Plant Biochemistry*, Academic Press Limited, London pp 1-552.

HARBORNE, J. B., 1998. *Phytochemical Methods*. 3rd ed. London: Chapman and Hall. pp.40-90.

HARBORNE, J.B., BAXTER, H. & MOSS, G.P., 1999. *Phytochemical Dictionary* Taylor & Francis, Philadelphia, PA, pp. 1–976.

HARTLEY, R. D., MORRISON, W. H., HIMMELSBACH, D. S. & BORNEMAN, N. S. 1990. Cross-linking of cell wall phenolics to arabinoxylans in graminaceous plants. *Phytochemistry* **29**, 3701–3709.

HATANO, T., MIYATAKE, H., NATSUME, M., OSAKABE, N., TAKIZAWA, T., ITO, H. & YOSHIDA. T., 2002. Proanthocyanidin glycosides and related polyphenols from cacao liquor and their antioxidant effects. *Phytochemistry* **59**, 749-758.

HAVEMAN-NIES, A., TUCKER, K., DE GROOT, L., WILSON, P. W. F. & VAN STAVEREN, W. A., 2001. Evaluation of dietary quality in relationship to nutritional and lifestyle factors in elderly people in the US Framingham Heart Study and the European SENECA study. *European Journal of Clinical Nutrition* **55**, 870–880.

HE, X., LIAN, L. & LIN, L., 1997. Analysis of lignan constituents from *Schisandra chinensis* by liquid chromatography-electrospray mass spectrometry. *Journal of Chromatography A,* **757**, 81-87.

HEDGES, H.J & LISTER, C. E., 2006. *The Nutritional Attributes of Allium Species*. New Zealand Institute for Crop & Food Research Report No. 1814

HEIM, K.E., TAGLIAFERRO., A.R & BOBILY, A., 2002. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationship. *Journal of Nutritional Biochemistry* **13**, 572-584

HENSHAW, F.O., McWATTERS, K.H., OGUNTUNDE, A.O. & PHILLIPS, R.D., 1996. Pasting properties of cowpea flour: Effects of soaking and decortication method. *Journal of Agricultural and Food Chemistry* **44**, 1864-1870.

HERRMANN, K., 1989**.** Occurrence and content of hydroxycinnamic acid and hydroxybenzoic acid compounds in foods. *Critical Reviews in Food Science and Nutrition* **28**, 315-345.

HETHERINGTON, M.M., BELL, A. & ROLLS, B.J., 2000. Effect of repeat consumption on pleasantness, preference and intake. *British Food Journal* **102,** 507-521.

HETHERINGTON, M.M., PIRIE, L.M. & NABBI, S., 2002. Stimulus satiation: effects of repeated exposure to foods on pleasantness and intake. *Appetite* **38**, 19-28.

HOLLMAN, P. C. H. & KATAN, M. B., 1999. Dietary Flavonoids: Intake, health effects and bioavailability. *Food and Chemical Toxicology* **48**, 1773-1998.

HOLLMAN, P.C.H., DE VRIES, J.H.M., VAN LEEUWEN, P.A.M., MENGELERS, M.J.B. & KATAN, M.B., 1995. Absorption of dietary quercetin glycosides and quercetin in heathy ileostomy volunteers. *American Journal of Clinical Nutrition* **62**, 1276-82.

HOLT, R. R., LAZARUS, S. A., SULLARDS, M. C., ZHU, Q. Y., SCHRAMM, D. D., HAMMERSTONE, J. F., FRAGA, C. G., SCHMITZ, H. H. & KEEN, C. L., 2002. Procayanidin dimer B2 [Epicatechin-(4β-8)-epicatechin] in human plasma after the consumption of a flavanol-rich cocoa. *American Journal of Clinical Nutrition* ***76,*** 798-804.

HORAX, R., HETTIARACHCHY, N.S., CHEN, P. & JALALUDDIN, M. 2004. Preparation and characterization of protein isolate from cowpea (*Vigna unguiculata* L. Walp). *Journal of Food Science* **69**, 114-118.

HOU, D.X., MASUZAKI, S., HASHIMOTO , F., UTO, T., TANIGAWA , S., FUJII, M. & SAKATA, Y., 2007. Green tea proanthocyanidins inhibit cyclooxygenase-2 expression in LPS-activated mouse macrophages: Molecular mechanisms and structure–activity relationship. *Archives of Biochemistry and Biophysics* **460**, 67–74.

HUANG, D., OU, B. & PRIOR, R, R. L., 2005. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry* **53**, 1841-1856.

HUNG, Y.C., McWATTERS, K.H., PHILLIPS, R.D. & CHINNAN, M.S., 1990. Effects of pre-decortication drying treatment on the microstructure of cowpea products. *Journal of Food Science* **55**, 774-807.

HUNTER, E. A. & MCEWAN, J. A., 1998. Evaluation of an international ring trial for sensory profiling of hard cheese. *Food Quality and Preference* **9**, 343–354.

IKEDA, I., IMASATO, Y., SASAKI, E., NAKAYAMA, M., NAGAO, H., TAKEO, T., YAYABE, F. & SUGANO, M., 1992. Tea catechins decrease micellar solubility and intestinal absorption of cholesterol in rats. *Biochimica et Biophysica Acta* **1127**, 141–146.

INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE (IITA), 2012). Press release *www.iita.org/cowpea*. Accessed 10/11/2012

ISSANCHOU, S. & LESSCHAEVE, I., 1995. Screening individual ability to perform descriptive analysis of food products: Basic statements and application to a camembert cheese descriptive panel. *Journal of Sensory Studies* **10**, 349–368.

JACOB, R.A. & BURRI, B.J., 1996. Oxidative damage and defense. *American Journal of Clinical Nutrition* **63**, S985–S990.

JACOBS, D.R., MEYER, K.A., KUSHI, L.H. & FOLSOM, A.R., 1998. Whole grain intake may reduce risk of coronary heart disease death in postmenopausal women: The Iowa Women's Health Study, *American Journal Clinical Nutrition* **68,** 248–257.

JANG, Y., LEE, J. H., KIM, O. Y., PARK, H. Y., & LEE, S. Y., 2001. Consumption of whole grain and legume powder reduces insulin demand, lipid peroxidation, and plasma homocysteine concentrations in patients with coronary artery disease: Randomized controlled clinical trial. *Arteriosclerosis, Thrombosis, and Vascular Biology* **21**, 2065–2071.

JAYATHILAKAN, K. & SHARMA, G. K., 2006. Role of sugar–amino acid interaction products (MRPs) as antioxidants in a methyl linoleate model system. *Food Chemistry* **95**, 620–626.

KAHKONEN, M.P., HOPIA, A.L., VUORELA, H.J., RAUHA, J.P., PIHLAJA, K., KUJALA, T.S. & HEINONEN, M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry* **47**, 3954-3962.

KANSKI, J., AKSENOVA, M., STOYANOVA, A. & BUTTERFIELD, D.A., 2002. Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure–activity studies. *Journal of Nutrition and Biochemistry* **13**, 273–281.

KAWABATA, K., YAMAMOTO, T., HARA, A., SHIMIZU, M., YAMADA, Y., MATSUNAGA, K., TANAKA, T. & MORI, H., 2000. Modifying effects of ferulic acid on azoxymethane-induced colon carcinogenesis in F344 rats. *Cancer Letters* **157**, 15–21.

KHATUN, M., EGUCHI, S., YAMAGUCHI, T., TAKAMURA, H. & MATOBA, T., 2006. Effect of the thermal treatment on radical-scavenging activity of some spices. *Food Science and Technology Research* **12**, 178–185.

KHOKHAR, S., & APENTEN, R.K.O., 2003. Iron binding characteristics of phenolic compounds: some tentative structure–activity relations. *Food Chemistry* **81,** 133–140.

KIKUZAKI, H., HISAMOTO, M., HIROSE, K., AKIYAMA, K. & TANIGUCHI, H., 2002. Antioxidant properties of ferulic acid and its related compounds. *Journal of Agricultural and Food Chemistry* **50**, 2161–2168.

KINSELLA, J. E., FRANKEL, E. N., GERMAN, J. B. & KANNER, J., 1993. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technology* **47**, 85-89.

KOBUE-LEKALAKE, R., TAYLOR, J. R. N. & DE KOCK, H. L., 2007. Effects of phenolics in sorghum grain on its bitterness, astringency and other sensory properties. *Journal of the Science of Food and Agriculture* ***87***, 1940-1948.

KOEHLER, H.H., CHANG, C., SCHEIER, G. & BURKE, D. W., 1987. Nutrient composition, protein quality, and sensory properties of thirty-six cultivars of dry beans (Phaseolus vulgaris L.). *Journal of Food Science* **52**, 1335-1340.

KOREN, E., KOHEN, R. & GINSBURG, I., 2010. Polyphenols enhance total oxidant-scavenging capacities of human blood by binding to red blood cells. *Experimental Biology and Medicine* **235**, 689–699.

LAPARRA, J. M., VELEZ, D., BARBERA, R., FARRE, R. & MONTORO, R., 2008. As_2O_3-induced oxidative stress and cycle progression in human epithelial cell line (Caco-2). *Toxicology in Vitro* **22**, 444-449.

LARRAURI, J. A., RUPEREZ, P. & SAURA-CALIXTO, F., 1997. Effect of drying temperature on the stability of polyphenols and the antioxidant activity of red grape pomace peels. *Journal of Agricultural and Food Chemistry* **45**, 1390-1393.

LAWLESS, H. T., & HEYMANN, H. (1998). *Sensory Evaluation of Food: Principles and Practices*. New York: Chapman and Hall.

LEE, S. C., KIM, J. H., JEONG, S. M., KIM, D. R., HA, J. U. & NAM, K. C., 2003. Effect of far-infrared radiation on the antioxidant activity of rice hulls. *Journal of Agricultural and Food Chemistry* **51**, 4400–4403.

LEE, S. C., JEONG, S. M., KIM, S. Y., PARK, H. R., NAM, K. C. & AHN, D. U., 2006. Effect of far-infrared radiation and heat treatment on the antioxidant activity of water extracts from peanut hulls. *Food Chemistry* **94**, 489-493.

LEMASKA,K., SZYMUSIAK H., TYRAKOWSKA,B., ZIELINSKI,R., SOFFER, A.E. & RIETJENS, I.M., 2001. The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxyflavones. *Free Radical Biology and Medicine* **37**, 869-881.

LERER-METZGER, M., RIZKALLA, S. & LUO, J., 1996. Effects of long-term low glycemic index starchy food on plasma glucose and lipid concentrations and adipose tissue cellularity in normal and diabetic rats. *British Journal of Nutrition* **75**, 723–732.

LESSCHAEVE, I., & NOBLE, A., 2005. Polyphenols: Factors influencing their sensory properties and their effects on foods and beverage preferences. *American Journal of Clinical Nutrition* **81**, 330s-335s.

LÉVY, C.M. & KÖSTER, E.P., 1999. The relevance of initial hedonic judgements in the prediction of subtle food choices. *Food Quality and Preference* **10**, 185-200.

LIM, S. N., CHEUNG, P. C. K. , OOI, V. E. C. & ANG, P. O., 2002. Evaluation of antioxidative activity of extracts from a brown seaweed, *Sargassum siliquastrum. Journal of Agricultural and Food Chemistry* **50**, 3862-3866.

LIU, K., HUNG, Y.C. & PHILLIPS, R.D., 1993a. Mechanism of Hard-to-Cook defect in cowpeas: Verification via microstructure examination. *Food Structure* **12**, 51-58.

LIU, K., PHILLIPS, R.D & McWATTERS, K.H., 1993b. Mechanism of pectin changes during soaking and heating as related to hard-to-cook defect in cowpeas. *Journal of Agricultural and Food Chemistry* **41***, 1476-1480.*

LIYANA-PATHIRANA, C. M. & SHAHIDI, F., 2005. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *Journal of Agricultural and Food Chemistry* **53**, 2433-2440.

LONG-ZE, L., HARNLY, J.M., PASTOR-CORRALES, M.S. & LUTHRIA, D.L., 2008. The polyphenolic profiles of common bean (*Phaseolus vulgaris* L.). *Food Chemistry* **107**, 399–410.

LOPES, G.K.B., SCHULMAN, H.M. & HERMES-LIMA, M., 1999. Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. *Biochimica et Biophysica Acta* **1472**, 142-152.

LOPEZ-REVUELTA, A., SANCHEZ-GALLEGO, J. I., HERNANDEZ-HERNANDEZ, A., SANCHEZ-YAGUE, J., & LLANILLO, M., 2006. Membrane cholesterol contents influence the protective effects of quercetin and rutin in erythrocytes damaged by oxidative stress. *Chemico-Biological Interactions* **161**, 79–91.

LOWRY, J.B., MCSWEENEY, C.S. & PALMER, B., 1996. Changing perceptions of the effect of plant phenolics on nutrient supply in the ruminant. *Australian Journal of Agricultural Research* **47** 829–842.

LUTHRIA, D.L. & PASTOR-CORRALES, M.A., 2006. Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris L.*) varieties. *Journal of Food Composition and Analysis* **19**, 205–211.

MACDONALD, L. K., WOOD, L. G. & GARG, M. L., 2006. Methodology for the determination of biological antioxidant capacity *in vitro*: a review. *Journal of the Science of Food and Agriculture* **86,** 2046-2056.

MADHUJITH, T. & SHAHIDI, F., 2005. Antioxidant potential of pea beans (*Phaseolus vulgaris* L.). *Journal of Food Science* **90,** 85 -90.

MADHUJITH, T. & SHAHIDI, F., 2009. Antioxidant potential of barley as affected by alkaline hydrolysis and release of insoluble-bound phenolics. *Food Chemistry* **117**, 615-620.

MADHUJITH, T., AMAROWICZ, R. & SHAHIDI, F., 2004. Antioxidants in beans and their effects on inhibition of radical-induced DNA damage. *Journal of the American Oil Chemists' Society* **81**,691-96.

MANACH, C., SCALBERT, A., MORAND, C., RÉMÉSY, C. & JIMÉNEZ, L., 2004. Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition* **79**,727 – 747.

MANGIAPANE, H., THOMSON, J., SALTER, A., BROWN, S., BELL, G. D. & WHITE, D. A., 1992. The inhibition of the oxidation of low density lipoprotein by (1) - catechin, a naturally occurring flavonoid. *Biochemical Pharmacology* **43**, 445–450.

MARTINS S.I.F.S., JONGEN W.M.F. & VAN BOEKEL M.A., 2001. A review of Maillard reaction in food and implication of kinetic modelling. *Trends in Food Science and Technology* **11**, 364-373.

MARUYAMA, C., ARAKI, R., KAWAMURA, M., KONDO, N., KIGAWA, M., KAWAI, Y., 2008. Azuki bean juice lowers serum triglyceride concentrations in healthy young women. *Journal of Clinical Biochemistry and Nutrition* **43**, 19–25.

MAZUR, W. M., DUKE, J. A., WAHALA, K., RASKU, S. & ADLERCREUTZ, H., 1998. Isoflavonoids and lignans in legumes: nutritional and health aspects in humans. *Journal of Nutritional Biochemistry* **9**, 193–200.

MAZZA, G. & MINIATI, E., 1993. *Anthocyanins in Fruits, Vegetables and Grains*; CRC Press: Boca Raton, FL.

McWATTERS, K., OUEDRAOGO, J.B., RESURRECCION, V.A., HUNG, Y-C. & PHILLIPS, R.D., 2003. Physical and sensory characteristics of sugar cookies containing mixtures of wheat, fonio (*Digitaria exilis*) and cowpea (*Vigna unguiculata*) flours. *International Journal of Food Science and Technology* **38**, 403-410.

MEILGAARD, M. C., CIVILLE, G. V. & CARR, B. T., 1991. *Sensory Evaluation Techniques* (2nd ed.). Boca Raton, FL: CRC Press.

MEILGAARD, M.C., DALGLEISH, C.E. & CLAPPERTON, J.F., 1979. Beer flavour terminology. *Journal of the Institute of Brewing* **85**, 38-42.

MERKEN, H.M. & BEECHER, G.R., 2000. Measurement of food flavonoids by high-performance liquid chromatography: a review. *Journal of Agricultural and Food Chemistry* **48**, 577–599.

MEYER, A.S., DONOVAN, J.L., PEARSON, D.A., WATERHOUSE, A. L. & FRANKEL, E. N., 1998. Fruit hydroxycinnamic acids inhibit human low-density lipoprotein oxidation *in vitro*. *Journal of Agricultural and Food Chemistry* **46**, 1783-1787.

MICHALSKA, A., AMIGO-BENAVENT, B.M., ZIELINSKI, H. & DOLORES DEL CASTILLO, M., 2008. Effect of bread making on formation of Maillard reaction products contributing to the overall antioxidant activity of rye bread. *Journal of Cereal Science* **48**, 123–132.

MIKI, M., TAMAI, H., MINO, M., YAMAMOTO, Y. & NIKI, E., 1987. Free radical chain oxidation of erythrocytes by molecular oxygen and its inhibition by alpha-tocopherol. *Archives of Biochemistry and Biophysics*. ***258***, 373-380.

MILLIGAN, J.R., ARNOLD, A.D. & WARD, J.F., 1992. The effect of superhelical density on the yield of single-strand breaks in γ- irradiated plasmid DNA. *Radiation Research* **132**, 69-93.

MKANDA, A.V., MINNAAR, A. & DE KOCK, H.L., 2007. Relating consumer preferences to sensory and physicochemical properties of dry beans (*Phaseolus vulgaris*). *Journal of the Science of Food and Agriculture* **87**, 2868–2879.

MOTHUPI, O., 2012. Micronised cowpeas: Are they acceptable to the consumers? *BSc Honours Project Report*, University of Pretoria. Pretoria, South Africa

MUNOZ, A. M. & CIVILLE, G. V., 1998. Universal, product and attribute specific scaling and the development of common lexicons in descriptive analysis. *Journal of Sensory Studies* **13**, 57–75.

MURRAY, J. M. & DELAHUNTY, C. M., 2000. Selection of standards to reference terms in a Cheddar cheese flavour language. *Journal of Sensory Studies* **15**, 179–199.

MURRAY, J. M., 1999. Determination of consumer preference for cheese using descriptive sensory analysis. *PhD thesis*, National University of Ireland. Cork, Ireland

MURRAY, J.M., DELAHUNTY, C.M. & BAXTER, I.A., 2001. Descriptive sensory analysis: past, present and future. *Food Research International* **34**, 461–471.

MWANGWELA, A.M., 2006. Physicochemical characteristics of conditioned and micronised cowpeas and functional properties of the resultant flours. *PhD (Food Science) Thesis,* University of Pretoria. Pretoria, South Africa.

MWANGWELA, A.M., WANISKA, R.D. & MINNAAR, A., 2006**.** Hydrothermal treatments of two cowpea (*Vigna unguiculata* L. Walp) varieties: Effect of micronisation on physico-chemical and structural characteristics. *Journal of the Science of Food and Agriculture* **86**, 35-45.

MWANGWELA, A.M., WANISKA, R.D., MCDONOUGH, C. & MINNAAR, A., 2007. Cowpea cooking characteristics as affected by micronisation temperature: a study of the physicochemical and functional properties of starch. *Journal of the Science of Food and Agriculture* **87**, 399–410.

NACZK, M. & SHAHIDI, F., 2004. Extraction and analysis of phenolics in food. *Journal of Chromatography A* **1054**, 95-111.

NARDINI, M., D'AQUINO, M., TOMASSI, G., GENTILI, V., DI-FELICE, M., & SCACCINI, C., 1995. Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. *Free Radical Biology and Medicine* **19**, 541–552.

NEVEU, V., PEREZ-JIMÉNEZ, J., VOS, F., CRESPY, V., DU CHAFFAUT, L., MENNEN, L., KNOX, C., EISNER, R., CRUZ, J., WISHART, D. & SCALBERT A. 2010. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database, doi: 10.1093/database/bap024 (Version 1.5.2, available at http://www.phenol-explorer.eu). Last accessed on 23 October, 2012.

NIELSEN, R. G. & ZANNONI, M., 1998. Progress in developing an international protocol for sensory profiling of hard cheese. International Journal of Dairy Technology 31, 57–64.

NIJVELDT, R.J., VAN NOOD, E., VAN HOORN, D.E., BOELENS, P.G., VAN NORREN, K. & VAN LEEUWEN, P.A., 2001. Flavonoids: a review of probable mechanisms of action and potential applications. *American Journal of Clinical Nutrition* **74**, 418-25.

NIWA, T., DOI, U., KATO, Y. & OSAWA, T., 1999. Inhibitory mechanism of sinapic acid against peroxynitrite-mediated tyrosine nitration of protein in vitro. *FEBS Letters* **459**, 43–46.

NIWA, Y., KANOH, T., KASANA, T. & NEIGISHI, M., 1988. Activation of antioxidant activity in natural medicinal products by heating, brewing and lyophilisation. A new drug delivery system. *Drugs under Experimental and Clinical Research* ***14***, 361-372.

NJOKU, O. H. & OKEMADU, P.C., 1989. Biochemical changes during the natural fermentation of the African Oil Bean *(Pentaclethra macrophylla)* for the production of ugba. *Journal of the Science of Food and Agriculture* **49,** 457-465.

NOBLE, A. C., ARNOLD, R. A., BUECHSENSTEIN, J., LEACH, E. J., SCHMIDT, J. O. & STERN, P. M., 1987. Modification of a standardized system of wine flavor terminology. *American Journal of Enology and Viticulture* **38**, 143–146.

NOBLE, A. C., ARNOLD, R. A., MASUDA, B. M., PECORE, S. D., SCHMIDT, J. O. & STERN, P. M., 1984. Progress towards a standardized system of wine terminology. *American Journal of Enology and Viticulture* **35**, 107–109.

NOBLE, A.C., 1994. Bitterness in wine. *Physiological Behaviour* **56**, 1251–5.

NWOKOLO, E. & ILECHUKWU, S.N., 1996. Cowpea (*Vigna unguiculata* (L.) Walp. In Nwokolo, E. & Smart, J., (Eds.) *Food and Feed from Legumes and Oilseeds.*Chapman & Hall: London, pp 229-242.

NZARAMBA, M.N, HALE, A.L, SCHEURING, D.C. & MILLER, J.C., 2005. Inheritance of antioxidant activity and its association with seed-coat color in cowpea. *Journal of the American Society of Horticultural Sciences* **130**, 386-391.

OGIWARA, T., SATOH, K., KADOMA, Y., MURAKAMI, Y., UNTEN, S., ATSUMI, T., SAKAGAMI, H. & FUJISAWA, S., 2002. Radical scavenging activity and cytotoxicity of ferulic acid. *Anticancer Research* **22**, 2711–2717.

OJWANG, L.O., DYKES, L. & AWIKA, J.M., 2012. Ultra performance liquid chromatography−tandem quadrupole mass spectrometry profiling of anthocyanins and flavonols in cowpea (Vigna unguiculata) of varying genotypes. *Journal of Agricultural and Food Chemistry* **60**, 3735−3744.

OKAKA, J.C. & POTTER, N.N., 1979. Physico-chemical and functional properties of cowpea powders processed to reduce beany flavour. *Journal of Food Science* **44**, 1235–1240.

OLAPADE, A., OKAFOR, G.I., OZUMBA, A.U. & OLATUNJI, O., 2002. Characterization of common Nigerian cowpea (*Vigna unguiculata L. Walp*) varieties. *Journal of Food Engineering* **55**, 101-105.

OU, B., HAMPSCH-WOODILL, M. & PRIOR, R. L., 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry* **49,** 4619-4626.

OU, B., HUANG, D., HAMPSCH-WOODILL, M., FLANAGAN, J. & DEEMER, E. K. 2002. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assay: a comparative study. *Journal of Agricultural and Food Chemistry* **50**, 3122-3128.

PELEG, H. & NOBLE, A.C., 1995. Perceptual properties of benzoic acid derivatives. *Chemical Senses* **20**, 393– 400.

PELEG, H., GACON, K., SCHLICH, P. & NOBLE, A. C., 1999. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *Journal of the Science of Food and Agriculture* **79**, 1123–1128.

PENICELA, L., 2010. The influence of seed coat and cotyledon structure on cooking characteristics of cowpeas. *MSc (Agric) Food Science and Technology Dissertation*, University of Pretoria. Pretoria, South Africa

PETRIDOU, E., NTOUVELIS, E., DESSYPRIS, N., TERZIDIS, A., & TRICHOPOULOS, D., 2005. Maternal diet and acute lymphoblastic leukemia in young children. *Epidemiology Biomarkers and Prevention* **14**, 1935–1939.

PHADI, M. M. 2004. Physico–chemical effects of micronization on cowpeas (*Vigna unguiculata* L. Walp). *MInst Agrar Dessertation*, University of Pretoria.

PHILLIPS, R.D, MCWATTERS, K.H., CHINNAN, M.S., HUNG, Y.C., BEUCHAT, L.R., SEFA-DEDEH, S., SAKYI-DAWSON, E., NGODDY, P., NNANYELUGO, D., ENWERE, J., KOMEY, N.S., LIU, K., MENSA-WILMOT, Y., NNANNA, I.A., OKEKE, C., PRINYAWIWATKUL, W. & SAALIA, F.K., 2003. Utilization of cowpeas for human food. *Field Crops Research* **82**, 193-213

PHILLIPS, R.D. & MCWATTERS, K.H., 1991. Contribution of cowpeas to nutrition and health. *Food Technology* **45**, 127-130.

PIETTA, P. G., 2000. Flavonoids as antioxidants. *Journal of Natural Products* **63**, 1035-1042.

PIGGOTT, J. R. & CANAWAY, P. R., 1981. Finding the word for it: methods and uses of descriptive sensory analysis. In *Flavour* '81 (pp. 202–243). Berlin: Walter de Gruyter & Co, Berlin.

PITTAWAY, J. K., AHUJA, K. D. K., CEHUN, M., CHRONOPOULOS, A., ROBERTSON, I. K., NESTEL, P.J., 2006. Dietary supplementation with chickpeas for at least 5 weeks results in small but significant significant reductions in serum total and low density lipoprotein cholesterols in adult woman and men. *Annals of Nutrition and Metabolism* **50**, 512–518.

PLINER, P., 1982. The effect of mere exposure on liking for edible substances. *Appetite* **3**, 283-290.

PORCHEROT, C. & ISSANCHOU, S., 1998. Dynamics of liking for flavoured crackers: test for predictive value of a boredom test. *Food Quality Preferences* **9**, 21-29.

PROCTOR, J.R. & WATTS, B.M. 1987. Development of a modified Mattson Bean Cooker procedure based on sensory panel cookability evaluation. *Canadian Institute of Food Science and Technology Journal* **20**, 9-14.

RAINEY, B. A., 1986. Importance of reference standards in training panellists. *Journal of Sensory Studies* **1**, 149–154.

RAKIC, S., PETROVIC, S., KUKIC, J., JADRANIN, M., TESEVIC, V., POVRENOVIC, D. & SILER-MARINKOVIC, S., 2007. Influence of thermal treatment on phenolic compounds and antioxidant properties of aok acorns from Serbia. *Food Chemistry* **104**, 830–834.

RANDHIR, R., KWON, Y.I. & SHETTY, K., 2008. Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. *Innovative Food Science Emerging Technology* **9**, 355–364.

RANDHIR, R., LIN, Y.T., & SHETTY, K. 2004. Stimulation of phenolics, antioxidant and antimicrobial activity in dark-germinated mungbean sprouts in response to peptide and phytochemical elicitors. *Processing and Biochemistry* **39**, 637–646.

RANILLA, L. G., GENOVESE M. I. & LAJOLO, F.M. 2007. Polyphenols and antioxidant capacity of seed coat and cotyledon from Brazilian and Peruvian bean cultivars (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry* **55**, 90-98.

RAO, V. & AMIR AL-WESHAHY, A., 2008. Plant-based diets and control of lipids and coronary heart disease risk. Current Medicine Group LLC ISSN. *Current Atherosclerosis Reports* **10,** 478–485.

RE, R., PELLENGRIN, N., PROTEGGENTE, A., PANNALA., YANG, M. & RICE-EVANS, C., 1999. Antioxidant activity applying an improved ABTS radical cation discolouration assay. *Free Radical Biology and Medicine* **26**, 1231-1237.

REDDY, N. R., PIERSON, M. D., SATHE, S. K., & SALUNKHE, D. K., 1985. Drybean tannins: a review of nutritional implications. *Journal of the American Oil Chemists' Society* **62**, 541–549.

REINERS, J.J., CLIFT, R. AND MATHIEU, P., 1999. Suppression of cell cycle progression by flavonoids; dependence on the aryl hydrocarbon receptor. *Carcinogenesis* **20**, 1561–1566.

RICE-EVANS, C.A., MILLER, N. J. & PAGANGA, G., 1996. Structure and antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine* **20**, 933 – 956.

RIVERO-PÉREZ, M. D., MUÑIZ, P. & GONZÁLEZ-SANJOSÉ, M. L., 2007. Antioxidant profile of red wines evaluated by total antioxidant capacity, scavenger activity, and biomarkers of oxidative stress methodologies. *Journal of Agricultural and Food Chemistry* **55**, 5476-5483.

ROBARDS, K., PRENZLER, P.D., TUCKER, G., SWATSITANG, P. & GLOVER, W., 1999. Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry* **66**, 401–436

ROCHA-GUZMAN, N.E., GONZALES-LAREDO, R.F., IBARRA-PEREZ, F.J., NAVA-BERUMEN, C.A., & GALLEGOS-INFANTE, J. 2007. Effect of pressure cooking on the antioxidant activity of extracts from three common bean (*Phaseolus vulgaris* L.) cultivars. *Food Chemistry* **100**, 31-35.

ROGACHEV, I. & AHARONI. A., 2012. UPLC-MS-based metabolite analysis in tomato. In Plant Metabolomics : Methods and Protocols. *Methods in Molecular Biology*, **860**, 129-144.

ROGINSKY, V. & LISSI, E. A., 2005. Review of methods to determine chain-breaking antioxidant activity in food. *Food Chemistry* **92**, 235-254.

SACCHETTI, G., PINNAVALA, G.G., GUIDOLINE, E. & ROSA, D.M., 2004. Effect of extrusion temperature and feed composition on the functional, physical and sensory properties of chestnut and rice flour based snack-like products. *Food Research International* **37**, 527–534.

SADEGHI, A.A., FATTAH, A.N.A. & CHAMANI, M., 2010. The Effects of micronisation on ruminal starch degradation of corn grain. *World Applied Sciences Journal* **16,** 240-243.

SAIJA, A., TOMAINO, A., TROMBETTA, D., PASQUALE, A., UCCELLA, N., BARBUZZI, T., PAOLINO, D. & BONINA, F., 2000. In vitro and in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents. *International Journal of Pharmaceuticals* **199**, 39–47.

SANZI CALVO, M. & ATTIENZA DEL REY, J., 1999. Sensory analysis of beans (*Phaseolus vulgaris*). *Biotechnology, Agronomy, Society and Environment* **3**, 201–204.

SAWAYA, A.L., MARTINS, P., HOFFMAN, D. & ROBERTS, S. B., 2003. The link between childhood undernutrition and risk of chronic diseases in adulthood: A case study of Brazil**.** *Journal of Human Hypertension* **17,** 381–387

SCALBERT, A. & WILLIAMSON G., 2000. Dietary intake and bioavailability of polyphenols. *Journal of Nutrition* **130**, 2073S–85S.

SCALBERT, A., MANACH, C., MORAND & RÉMÉSY., 2005. Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition* **45**, 287-306.

SCALBERT, A., MORAND. C., MANACH, C. & REMESY C., 2002. Absorption and metabolism of polyphenols in the gut and impact on health. *Biomedicine and Pharmacotherapy* **56,** 276-282.

SCANLON, M.G., MALCOLMSON, L.J., ARNTFIELD, S.D., WATTS, B., RYLAND, D. & PROKOPOWICH, D.J., 1998. Micronization pre-treatments for reducing the cooking time of lentils. *Journal of Science of Food and Agriculture* **76**, 23-30.

SCHNITZER, E., PINCHUK, I., BOR, A., FAINARU, M., SAMUNI, A. M. & LICHTENBERG, D., 1998. Lipid oxidation in unfractionated serum and plasma. *Chemistry and Physics of Lipids* ***92***, 151-170.

SCOTT, D., 1975. Oxidoreductases. In *Enzymes in Food Processing* (G. Reed, ed.) pp. 219–254. Academic Press. New York.

SEERAM, N. P. & NAIR, M. G., 2002. Inhibition of lipid peroxidation and structure-activity-related studies of the dietary constituents anthocyanins, anthocyanidins, and catechins. *Journal of Agricultural and Food Chemistry* **50**, 5308-5312.

SEFA-DEDEH, S. & STANLEY, D.W. 1979. Textural implications of the microstructure of legumes. *Food Technology* **10**, 77-83.

SEFA-DEDEH. S., STANELY, D.W. & VOISEY, P.W., 1978. Effects of soaking time and cooking conditions on texture and microstructure of cowpeas (*V. unguiculata*). *Journal of Food Science* **43**, 1832–1838.

SENSOY, I., ROSEN, R.T., HO,C. & KARWE.M.V., 2006. Effect of processing on buckwheat phenolics and antioxidant activity**.** *Food Chemistry* **99,** 388–393.

SHAHIDI, F. & NACZK, M., 1995. *Food Phenolics: Sources, Chemistry, Effects and Applications*; Technomic Publishing: Lancaster, PA, 1 pp 1-20.

SHAHIDI, F. & NACZK, M., 2004. *Phenolics in Food and Nutraceuticals*. London: CRC Press LLC. pp 1-20, 95-98, 403-427.

SHAHIDI, F., CHAVAN, U. D., NACZK, M. & AMAROWICZ, R., 2001. Nutrient distribution and phenolic antioxidants in air-classified fractions of Beach pea (Lathyrus maritimus L.). *Journal of Agricultural and Food Chemistry* **49**, 926–933.

SHARMA, G. K., 2009. Micronzation. Defence research and development organization, Ministry of defence, Government of India (DRDO) *Science Spectrum* pp169-171.

SHELEMBE, J.S., CROMARTY, D., BESTER, M.J., MINNAAR, A. & DUODU, K.G., 2011 Characterisation of phenolic acids, flavonoids, proanthocyanidins and antioxidant activity of water extracts from seed coats of marama bean (*Tylosema esculentum*) – an underutilised food legume. *International Journal of Food Science and Technology* **47**, 648-655.

SHIGA, T.M., CORDENUNSI, B.R. & LAJOLO, F.M., 2009. Effect of cooking on non-starch polysaccharides of hard-to-cook beans. *Carbohydrate Polymers* **76**, 100–109.

SHUI, G., LEONG, L. P. & WONG, S. P., 2005. Rapid screening and characterisation of antioxidants of *Cosmos caudatus* using liquid chromatography coupled with mass spectrometry. *Journal of Chromatography B* **827**, 127-138.

SIDDHURAJU, P. & BECKER, K., 2007. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata L. Walp*) seed extracts. *Food Chemistry* **101**, 10–19.

SIDDHURAJU, P., & BECKER, K., 2001. Effect of various domestic processing methods on antinutrients and in vitro protein and starch digestibility of two indigenous varieties of Indian tribal pulse, *Mucuna pruriens var. utilis*. *Journal of Agricultural and Food Chemistry* **49**, 3058–3067.

SINGLETON, V. L. & ROSSI, J., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* **16**, 144-158.

SOONG, Y. Y. & BARLOW, P. J., 2005. Isolation and structure elucidation of phenolic compounds from longan (*Dimocarpus longun* Lour.) seed by high-performance liquid chromatography-electronspray ionization mass spectometry. *Journal of Chromatography A* **1085**, 270-277.

SOSULSKI, F. W., & DABROWSKI, K. J. 1984. Composition of free and hydrolyzable phenolic acids in the flours and hulls of ten legume species. *Journal of Agricultural and Food Chemistry* **32**,131–133

SOSULSKI, F., KRYGER, K. & HOGGE, L., 1982. Free, esterified and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. *Journal of Agricultural and Food Chemistry* **30**, 337–340.

STEINBERG, D., 1997. Low density lipoprotein oxidation and its pathobiological significance. *Journal of Biological Chemistry* **272,** 20963–20966.

SUGIHARA, N., ARAKAWA, T., OHNISHI, M & FURUNO, K., 1999. Anti- and pro-oxidative effects of flavonoids on metal-induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with α-linolenic acid. *Free Radical Biology and Medicine* **27**, 1313-1323.

SULMONT, C., LESSCHAEVE, I., SAUVAGEOT, F. & ISSANCHOU, I., 1999. Comparative training procedures to learn odour descriptors: effects on profiling performance. *Journal of Sensory Studies*, **14**, 467–490.

SUN, W. & MILLER, J. M., 2003. Tandem mass spectrometry of the B-type procyanidins in wine and B-type dehydrodicatechins in an autoxidation mixture of (+)-catechin and (-)-epicatechin. *Journal of Mass Spectrometry* ***38***, 438-446.

SUN, B., DA SILVA, J.R.M. & SPRANGER, I., 1998. Critical factors of vanillin assay for catechins and proanthocyanidins. *Journal of Agricutural and Food Chemistry* **46**, 4267-4274.

SUZUKI, A., KAGAWA, D., FUJII, A., OCHIAI, R., TOKIMITSU, I. & SAITO, I., 2002. Short- and long-term effects of ferulic acid on blood pressure in spontaneously hypertensive rats. *American Journal of Hypertension* **15**, 351–357.

TABART, J., KEVERS, C., PINCEMAIL, J., DEFRAIGNE, J.O. & DOMMES, J.2009. Comparative antioxidant capacities of phenolic compounds measured by various tests. *Food Chemistry* **113**, 1226–1233

TAIWO, K.A., 1998. The potential of cowpea as human food in Nigeria. *Technovation* **18**, 469-481.

TAIWO, K.A., AKANBI, C.T. & AJIBOLA, O.O., 1997a. Establishing processing conditions for canning cowpea seeds in tomato sauce**.** *International Journal of Food Science and Technology* **32**, 313-324.

TAIWO, K.A., AKANBI, C.T. & AJIBOLA, O.O., 1997b. Effect of soaking and cooking time on the cooking properties of two cowpea varieties. *Journal of Food Research* **53**, 187 – 204.

TANAKA, T., KOJIMA, T., KAWAMORI, T., WANG, A., SUZUI, M., OKAMOTO, K. & MORI, H., 1993. Inhibition of 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis by the naturally occurring plant phenolics caffeic, ellagic, chlorogenic and ferulic acids. *Carcinogenesis* **14,** 1321–1325.

TANG, Y. & LIU, Z., 2008. Chemical kinetic behavior of chlorogenic acid in protecting erythrocyte and DNA against radical induced oxidation. *Journal of Agricultural and Food Chemistry* ***56,*** 11025-11029.

TAO, M. H., XU, W. H., ZHENG, W., GAO, Y. T., RUAN, Z. X., CHENG, J. R., 2005. A case–control study in Shanghai of fruit and vegetable intake and endometrial cancer. *British Journal of Cancer* **92**, 2059–2064.

TEDESCO, I., RUSSO, G. L., NAZZARO, F., RUSSO, M. & PALUMBO, R. 2001. Antioxidant effect of red wine anthocyanins in normal and catalase-inactive human erythrocytes. *Journal of Nutritional Biochemistry* **12,** 505-511.

TEDESCO, I., RUSSO, M., RUSSO, P., IACOMINO, G., RUSSO, G. L., CARRATURO, A., FARUOLO, C., MOIO. L., & PALUMBO, R., 2000. Antioxidant effect of red wine polyphenols on red blood cells. *Journal of Nutritional Biochemistry* **11,** 114-119.

THOMPSON, L.U., 1994. Antioxidants and hormone-mediated health benefits of whole grains. *Critical Reviews in Food Science and Nutrition.* **34**, 473 – 497.

THORNGATE, J.H. & NOBLE, A. C., 1995. Sensory evaluation of bitterness and astringency of 3*R* (+)-epicatechin and 3*S* (-)-catechin. *Journal of Science of Food and Agriculture* **53**, 1–5.

TROSZYNSKA, A., AMAROWICZ, R., LAMPARSKI, G., WOLEJSZO, A. & BARYLKO-PIKIELNA, N., 2005. Investigation of astringency of extracts obtained from selected tannin-rich legume seeds. *Food Quality and Preferences* **17**, 31-35.

TSUDA, T., HORIO, F. & OSAWA, T. 2003. Dietary cyanidin 3-O-β-D-glucoside rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *Journal of Nutrition* **133**, 2125–2130.

TSUDA, T., MIZUNO, K., OHSHIMA, K., KAWAKISHI, S. & OSAWA, T., 1995. Supercritical carbon dioxide extraction of antioxidative components from tamarind (*Tamarindus indica* L.) seed coat. *Journal of Agricutural and Food Chemistry* **43**, 2803-2806.

TURKMEN, N., SARI, F. & VELIOGLU, Y. S., 2005. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chemistry* **93**, 713-718.

VAN ACKER, S.A.B.E., VAN DEN BERG, D. J., TROMP, M.N.J.L., GRIFFOEN, D.H., VAN BENNEKOM, W.P., VAN DER VIJGH, W.J.F. & BAST, A., 1996. Structural aspects of antioxidant activity of flavonoids. *Free Radical Biology and Medicine* **20**, 331–342.

VARA-UBOL, S., CHAMBERS, E. IV and CHAMBERS, D.H. 2004. Sensory characteristics of chemical compounds potentially associated with beany aroma in foods. *Journal of Sensory Studies* **19**, 15–26.

VELIE, E. M., SCHAIRER, C., FLOOD, A., HE, J.-P., KHATTREE, R. & SCHATZKIN, A., 2005. Empirically derived dietary patterns and risk of postmenopausal breast cancer in a large prospective cohort study. *American Journal of Clinical Nutrition* **82**, 1308–1319.

VENN, B. J. & MANN, J. I., 2004. Cereal grains, legumes and diabetes. *European Journal of Clinical Nutrition* **58**, 1443–1461.

VERSTRAETEN, S. V., KEEN, C. L., SCHMITZ, H. H., FRAGA, C. G. & OTEIZA, P. I. 2003. Flavan-3-ols and procyanidins protect liposomes against lipid oxidation and disruption of the bilayer. *Free Radical Biology and Medicine*, **34**, 84-92.

VIDAL, S., CHEYNIER, V., WATERS, E. & NOBLE, A. C., 2003. *Effect of tannin composition and wine carbohydrates on astringency and bitterness.* In: Meiselman H, ed. 5th Pangborn Sensory Science Symposium. Boston, MA: Elsevier.

VINSON, J.A., DABBAGH, Y.A., SERRY, M.M. & JANG, J., 1995. Plant flavonoids, especially tea flavones, are powerful antioxidants using an in vitro oxidation model for heart disease. *Journal of Agricultural and Food Chemistry* **43,** 2800-2802.

WANG, M.L., GILLASPIE, A. G., MORRIS, J.B., PITTMAN, R.N., DAVIS, J. & PEDERSON. 2008. Flavonoid content in different legume germplasm seeds quantified by HPLC. *Plant Genetic Resources: Characterisation and Utilisation* **6**, 62-69.

WANG, J. & MAZZA, G. 2002. Effects of anthocyanins and other phenolic compounds on the production of tumor necrosis factor R in LPS/ IFN-γ-activated RAW 264.7 macrophages. *Journal of Agricultural and Food Chemistry* **50**, 4183–4189.

WANG, M.L., GILLASPIE, A. G., MORRIS, J.B., PITTMAN, R.N., DAVIS, J. & PEDERSON., 2008. Flavonoid content in different legume germplasm seeds quantified by HPLC. *Plant Genetic Resources: Characterisation and Utilisation* **6**, 62-69.

WATERMAN, P.G. & MOLE, S. 1994. *Analysis of Phenolic Plant Metabolites.* London: Blackwell Scientific Publications. pp.1-20.

WILLCOX, J. K., ASH, S. & CATIGNANI, G., 2004. Antioxidants and prevention of chronic disease. *Critical Reviews in Food Science and Nutrition* **44,** 275-295.

WRAY, S.L. & CENKOWSKI. S., 2002. Nutritional changes of yellow peas during infrared processing. *Transactions of the ASAE* **45,** 1023-1027.

WU, X. & PRIOR, R. L., 2005. Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in Common Foods in the United States: Fruits and Berries. *Journal of Agricultural and Food Chemistry* **53**, 2589−2599.

XING, N., CHEN, Y., MITCHELL, S. H. & YOUNG, Y. F. C., 2001. Quercetin inhibits the expression and function of the androgen receptor in LNCaP prostate cancer cells. *Carcinogenesis* **22**, 409–414.

XU, B.J. & CHANG S. K. C., 2008. Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. *Food Chemistry* **110**, 1–13.

XU, B. J. & CHANG S. K. C., 2007. Comparative studies on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of Food Science* **72**, S159–S166.

XU, B. J., YUAN, S. H. & CHANG, S. K., 2007. Comparative studies on the antioxidant activities of nine common food legumes against copper-induced human low-density lipoprotein oxidation *in vitro*. *Journal of Food Science* **72**, S522-S527.

YAN, J., CHO, J., KIM, H., KIM, K., JUNG, J., HUH, S., SUH, H., KIM, Y. & SONG, D., 2001. Protection against b-amyloid peptide toxicity in vivo with long-term administration of ferulic acid. *British Journal of Pharmacology* **133**, 89–96.

YOUDIM, K., SHUKITT-HALE, B., MACKINNON, S., KALT, W. & JOSEPH, J., 2000. Polyphenolics enhance red blood cell resistance to oxidative stress: in vitro and in vivo. *Biochimica et Biophysica Acta (BBA) General subjects.* **1523**, 117–122.

YOUDIM, K. A., MCDONALD, J., KALT, W. & JOSEPH, J. A. 2002. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *Journal of Nutritional Biochemistry* **13**, 282–288.

YU, H., HONG, J. & WU, D., 1999. Effect of sodium ferulate on proliferation of rabbit aortic smooth muscle cells induced by oxidized LDL. *Zhongguo Zhong Yao Za Zhi* **24**, 365–366.

YU, L., HALEY, S., PERRET, J. & HARRIS, M., 2002. Antioxidant properties of hard winter wheat extracts. *Food Chemistry* **78,** 457–461.

YUAN, X., WANG, J. & YAO, H., 2005. Antioxidant activity of feruloylated oligosaccharides from wheat bran. *Food Chemistry* **90**, 759–764.

ZANG, L., COSMA, G., GARDNER, H., SHI, X., CASTRANOVA, V., VALLYATHAN,
ZHENG, G.H., FASINA, O., SOSULSKI, F.W. & TYLER, R.T., 1998. Nitrogen solubility of cereals and legumes subjected to micronization. *Journal of Agricultural and Food Chemistry* **46**, 4150-4157.

ZHENG, G.H., FASINA, O., SOSULSKI, F.W. & TYLER, R.T. 1998. Nitrogen solubility of cereals and legumes subjected to micronization. *Journal of Agricultural and Food Chemistry* **46**, 4150-4157.

ZHOU, K., LAUX, J.J. & YU, L., 2004. Comparison of Swiss red wheat grain and fractions for their antioxidant properties, *Journal of Agricultural and Food Chemistry* **52** 1118–1123.

ZHU, Q. Y., HOLT, R. R., LAZARUS, S. A., OROZCO, T. J. & KEEN, C.L., 2002. Inhibitory effects of cocoa flavanols and procyanidin oligomers on free radical-induced erythrocyte hemolysis. *Experimental Biology and Medicine*, **227**, 321-329.

8. APPENDIX

Publications and presentations from this work

Scientific Paper:

Kayitesi, E., Duodu, K. G., Minnaar, A. and De Kock, H. L. Effect of micronisation of pre-conditioned cowpeas on cooking time and sensory properties of cooked cowpeas. ***Journal of the Science of Food and Agriculture* 93,** 838-845.

Conference posters:

Poster presentation at Global Pulse Researchers Meeting Feb, 2012, Kigali, Rwanda "Transforming Grain-Legume Systems to Enhance Nutrition and Livelihoods" **Poster**: E. Kayitesi, H.L. de Kock, K.G. Duodu and A. Minnaar. Effects of micronisation on cowpea phenolic compounds and potential health promoting properties

Technical reports:

Technical report on phytochemical quality of cowpea types – formed part of progress report for USAID funded Dry Grain Pulse CRSP programme.