Nutritional value of South African quality protein maize before and after storage

By

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DECLARATION

“I declare that the dissertation hereby handed in for the qualification Magister Scientiae Agriculturae at the University of the Free State, is my own independent work and that I have not previously submitted the same work for a qualification at/in another University/faculty”.

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Lekgolwa T. Phalafala

DEDICATION

It is very fitting that this work be dedicated to my late father Ngoako Phalafala. He was a truly great parent and it has been my personal privilege to have received the benefits of his wisdom. I would also like to dedicate this work to my mother Morongoa Phalafala. She took good care of me when I was a teenager.

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ABBREVIATIONS AND ACRONYMS

^oC	Degree celcius
%	Percent
µL	Micro litre(s)
µm	Micro metre(s)
AAS	Atomic absorption spectroscopy
ANOVA	Analysis of variance
ARC	Agricultural Research Council
Ca	Calcium
CIMMYT	International Maize and Wheat Improvement Center
CV	Coefficient of variation
DMSO	Dimethyl sulphoxide
DPn	Degree of polymerization by number
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
Fe	Iron
g	Gram(s)
GC	Gas chromatography
GOPOD	Glucose oxidase-peroxidase
h	Hour(s)
ha	Hectare(s)
Hg/ha	Hectogram/hectare
HNO_3	Nitric acid
HPLC	High performance liquid chromatography
K	Potassium
kg	Kilogram(s)
kg/ha	Kilogram/hectare
LSD	Least Significant Difference
Mg	Magnesium
mg	Milligram(s)
mg/kg	Milligram/kilogram
mg/ml	Milligram/millilitre
min	Minute(s)

ml	Millilitre(s)
Mn	Manganese
MOPS	3-Morpholinopropanesulfonic acid
Na	Sodium
NaOH	Sodium hydroxide
P	Phosphorus
PCA	Principal component analysis
QPM	Quality protein maize
RP-HPLC	Reversed phase-high performance liquid chromatography
SADC	Southern African Development Community
SE-HPLC	Size exclusion-high performance liquid chromatography
UN	United Nations
Zn	Zinc

CHAPTER 1

GENERAL INTRODUCTION

Maize (*Zea mays* L.) is one of the most important sources of proteins, minerals, carbohydrates and fats for humans and animals. The nutritional quality of maize is very important and is mainly determined by the genotype and the growing conditions as well as the postharvest technology. The protein produced by normal maize is of low biological quality for human and animal consumption, especially because of a low content of essential amino acids such as lysine and tryptophan. Quality protein maize (QPM) is nutritionally improved maize that possesses twice the quantity of lysine and tryptophan compared with non-QPM. QPM cultivars have been developed by various companies in the world, to offer a nutritional solution to people who solely depend on maize as their source of protein.

From a nutritional quality preservation point of view, little is known about the response of QPM nutritional parameters when subjected to different storage conditions as practiced by small-scale farmers on their farms, as well as commercial storage conditions. In South Africa, poor storage conditions often prevail which affect seed composition and quality deterioration is likely to occur (Hell *et al*., 2000).

The conservation of genetic resources in germplasm banks and breeder collections assumes genetic stability during storage (Revilla, 2006). Wongdechsarekul and Kongkiattikajorn (2010) reported that maize grains can be stored for long periods without microbial spoilage, but biochemical changes always take place during ageing. During storage, functional and nutritional characteristics of the maize grain are altered (Reed, 1992). When grains are placed into storage they are exposed to a broad range of complex ecological factors that work against the store manager’s objective of maintaining grain quality (Lopes *et al*., 2008). Seed deterioration during storage involves progressive impairment of performance and function of seeds. Seed quality deterioration is an inevitable and irreversible process which cannot be prevented but it can be slowed under specific conditions (Doijode, 2001). Unfavourable conditions are one of the factors that are responsible for the decline in nutritional status and germination potential of maize seeds in storage. Adequate and effective storage of maize grains is therefore a major research thrust for enhanced maize nutritional value (Olakojo and Akinlosutu, 2004).

Maize quality is of great significance for producers, processors and consumers of maize, because it affects the nutritional and commercial value of grains. The most important factors that determine the quality of a seed are appearance, milling quality, cooking and processing quality and nutritional quality (Koutroubas *et al*., 2004). According to Thanapornpoonpong and Vearasilp (1999), seed quality deterioration is a serious problem for the seed industry, because commercial kernels must meet the requirements of the quality standards. Kernel quality is a multiple criterion characteristic that consists of some vital kernel attributes: chemical composition and germination vigour, size, crop and varietal purity, moisture content and weight of the kernel (Šimić *et al*., 2005; 2007). The condition of the kernel in storage is mostly determined by the initially stored kernel quality and the storage conditions (Šimić *et al*., 2005). During storage, kernel quality can remain at the original state or deteriorate to a point that may make the kernel undesirable for planting purposes (Anfinrud, 1997; Al-Yahya, 2001; Guberac *et al*., 2003; Šimić *et al*., 2004). Cereal grains can be stored for long periods without microbial spoilage, however, biochemical changes do occur during storage (McDonough *et al*., 2004).

The condition or quality of stored maize is usually determined by its moisture content and storage temperature (Bern and Herum, 2003). Moisture is important economically because too much moisture facilitates growth of fungi and the removal of moisture requires energy, therefore increasing cost (Paulsen *et al*., 2003). High temperatures and excessive moisture in the stored products or high humidity under ambient storage conditions permit the proliferation of insects and moulds, which cause large losses of qualitative and nutritional properties (Iconomou *et al*., 2006). The safe moisture level for storage depends on grain condition and cultivar, the storage environment, and climate (Bern and Herum, 2003).

Frequent changes in temperature, relative humidity and extended storage time also cause significant nutrient losses. The possible causes of seed quality deterioration during storage are not only the high temperatures and relative humidity, but also the natural ageing of the seeds (Shah *et al*., 2002). Storage losses always prevail irrespective of the type and efficacy of the storage method being used. However, the losses vary according to the type of storage. Natural seed ageing results in the reduction of quality faster than when the seeds are exposed to laboratory conditions (Gutiérrez *et al*., 1993). Hsu and Sung (1997) and Bailly *et al*. (1998) indicated that spontaneous natural ageing results in increased lipid oxidation, reduced level and activities of antioxidants and several enzymes involved in scavenging free radicals and peroxides. The kernels that deteriorate quickly under accelerated natural ageing usually demonstrate noticeable decline in vigour (McDonald, 1999). According to Fujikura and Karssen (1995), the negative effects of natural ageing on seed viability and quality are linked with the nucleic acid and protein level. Liklatchev *et al*. (1984) pointed out that accelerated ageing leads to the same biochemical changes that occur in natural ageing.

Maize kernels need adequate storage conditions to be commercially acceptable for planting purposes (Murdolelono and Hosang, 2009). Seed storage, critical for germplasm preservation, is important to farmers, breeders, and industries interested in seed processing and commercial trade (Mohamed-Yasseen *et al*., 1994). Storage of maize grain after harvest is very important for future use by farmers and consumers. Most farmers have problems in preserving their maize seeds for a long period of time especially after the harvest season.

Storage of surplus seed is a type of subsistence strategy allowing farmers to avoid the risk of seed scarcity by smoothing out fluctuations in both the availability of seed resources and variations in yield of agricultural crops. Storage of seeds serves as a means to extend the season, to delay marketing until prices go up, to provide a reserve for more uniform retail distribution, or to decrease the frequency of purchase by the consumer or food service establishment (Prussia and Shewfelt, 1993). Storing the seeds using sustainable methods is very important. According to Tsugio (2001), retaining the viability of seeds during the long periods of storage is the first priority of genebanks. Therefore, optimal storage conditions are very critical when it comes to the improvement of the shelf life of maize genotypes (Bern and Herum, 2003).

Storage is usually in an artificial environment where quality and nutritive changes take place because of the interactions between physical, chemical and biological factors (Chulze, 2010). Maize seed has to be stored from harvesting to the next planting season with minimal quality losses and safe maize seed storage has become an important issue in

South Africa. As QPM is bred for enhanced nutritional value, the question arises as to how the composition of QPM is influenced by different storage conditions. Thus, the objectives of this study were to determine the nutritional composition of South African open-pollinated and hybrid QPM cultivars, compare them with normal maize hybrids and to measure the effect of different storage conditions on the nutritional characteristics.

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CHAPTER 2

LITERATURE REVIEW

2.1 AN OVERVIEW OF MAIZE PRODUCTION

Maize is one of the principal locally produced field crops and the most important source of proteins and carbohydrates in the Southern African Development Community (SADC) region for animal and human consumption (Department of Agriculture, Forestry and Fisheries, 2011). According to Fox and Manley (2009), the world production of maize annually exceeds 700 million tonnes and it reached around 790 million tonnes in 2008. The recent maize statistics estimated that the world production of maize exceeded 950 million tonnes for the 2012/2013 season with the United States being the major producer accounting for 40% of the total production. China is the second-major producer of maize with 195 million tonnes. The third-largest producer is Brazil with over 67 million tonnes. Mexico and Argentina are some of the top maize producers in the world. The European Union is responsible for just 65.5 million tonnes with Italy being the leading producer (Farmer’s weekly, 2012).

The total maize production on the African continent was estimated at about 43.4 million tonnes in 2007 (FAOSTAT, 2008), but the production increased to over 49 million tonnes in between 2007 and 2009 (Forum for Agricultural Research in Africa, 2009). The overall maize production in Sub-Saharan Africa is estimated at approximately 34.4 million tonnes annually (Aquino *et al*., 2001). The production of maize varies from country to country in Africa. On average, South Africa is the leading maize producer on the African continent and produced more than 11 million tonnes in 2013 on 2.7 million ha (Department of Agriculture, Forestry and Fisheries, 2013), but the total production in 2009 exceeded 12 million tonnes (Mariote, 2007; FAOSTAT, 2009). The West Africa is responsible for approximately 11 million tonnes with Nigeria as the major producer (Forum for Agricultural Research in Africa, 2009). The East Africa is responsible for 7.8 million tonnes with Tanzania as the largest producer (Mariote, 2007; Forum for Agricultural Research in Africa, 2009). The North Africa and Central Africa are responsible for 5.0 million tonnes and 969 000 tonnes, respectively. The Southern Africa is responsible for approximately 14.6 million tonnes with South Africa being the leading producer (Forum for Agricultural Research in Africa, 2009).

South Africa is self-sufficient when it comes to maize production. Maize production accounts for about 40% of the entire area cultivated in South Africa (Mqadi, 2005). About 12.6 million tonnes of maize grain is produced in South Africa annually on approximately 2.9 million ha of land. In 2008, about 13.2 million tonnes of maize was produced on 3.3 million ha of land (Department of Agriculture, Forestry and Fisheries, 2011). According to a published report by the Department of Agriculture, Forestry and Fisheries (2013), the reduction of land for maize cultivation in South Africa has negatively impacted maize production. Of the South African maize which was produced between 1995 and 1997, 48% was for human consumption and 39% for animal feed (Pingali and Pandey, 2001).

Maize in South Africa is produced countrywide, but it is mainly produced in the Free State, North West and Mpumalanga provinces (Department of Agriculture, Forestry and Fisheries, 2011). The Free State is the leading maize producer in the country in 2013 with 4.9 million tonnes produced on a 1.2 ha of land (Department of Agriculture, Forestry and Fisheries, 2013). It produces 40% of all the commercial maize in South Africa, of which 67% is white maize and 33% yellow maize (Anon, 2006). Mpumalanga is the second-largest producer in 2013 with 2.6 million tonnes on 470 000 ha of land. It produces 21% of the total commercial maize, of which 49% is white maize and 51% yellow maize (Anon, 2006). The third-largest producer in the country in 2013 is the North West province with total maize production of 2.0 million on 740 000 ha. Of the 2.0 million tonnes produced in the North West province in 2013, 1.6 million tonnes were white maize and 472 500 tonnes yellow maize on 565 000 ha and 175 000 ha, respectively (Department of Agriculture, Forestry and Fisheries, 2013).

The Northern Cape province is the fourth-largest producer in 2013 in the country with 637 300 tonnes produced on 53 200 ha of land (Department of Agriculture, Forestry and Fisheries, 2013). Of the 637 300 tonnes produced in the Northern Cape in 2013, 25 300 tonnes were white maize and 612 000 tonnes yellow maize on 2 200 ha and 51 000 ha, respectively. The fifth-largest producer in the country in 2013 is the Gauteng province with total maize production of 568 800 tonnes on 117 500 ha. Of the 568 800 tonnes produced in the Gauteng in 2013, 377 400 tonnes were white maize produced on 74 000 ha and 191 400 tonnes yellow maize produced on 43 500 ha.

The total maize production in the KwaZulu-Natal province in 2013 is 546 500 tonnes on 95 000 ha of land and is the sixth-largest producer. Of the 546 500 tonnes produced in the KwaZulu-Natal in 2013, 258 500 tonnes were white maize produced on 47 000 ha and 288 000 tonnes yellow maize produced on 48 000 ha. The Limpopo province is the seventh-largest producer in the country in 2013 with 282 500 tonnes produced on 53 500 ha of land. Of the 282 500 tonnes produced in Limpopo in 2013, 165 000 tonnes were white maize and 117 500 tonnes yellow maize on 30 000 ha and 23 500 ha, respectively (Department of Agriculture, Forestry and Fisheries, 2013).

The eighth-largest producer in the country in 2013 is the Eastern Cape province with total maize production of 102 500 tonnes on 18 700 ha. Of the 102 500 tonnes produced in the Eastern Cape in 2013, 18 500 tonnes were white maize produced on 3 700 ha and 84 000 tonnes yellow maize produced on 15 000 ha. The Western Cape province is the ninth and the smallest producer in 2013 in the country with 33 000 tonnes produced on 3 300 ha of land. Of the 33 000 tonnes produced in the Western Cape in 2013, 3 000 tonnes were white maize and 30 000 tonnes yellow maize on 300 ha and 3000 ha, respectively (Department of Agriculture, Forestry and Fisheries, 2013).

Maize in Sub-Saharan Africa and South Africa in particular, is largely grown under dryland conditions (Mataruka, 1985). However, supplementary irrigation is applied by commercial farmers in some cases where the crop needs early growth support and when the mid-season drought severely affects the entire development of the crop (Sibiya, 2009). The Free State produces 34% of maize under dryland conditions followed by the North West (32%), Mpumalanga (24%) and KwaZulu-Natal (3%).

On average, the largest maize consumers worldwide are the United States at 282.5 million tonnes; China at 201 million tonnes; Europe at 69.5 million tonnes; and Brazil at 56 million tonnes (Farmer’s weekly, 2012). In addition, 16 countries with the highest maize grain consumption are located in Sub-Saharan Africa, with the average per capita consumption of over 60 kg per annum (FAOSTAT, 2008). Sub-Saharan Africa does not produce sufficient maize to meet its needs and must therefore import approximately 3.0 million tonnes of maize annually (Pingali and Pandey, 2001; FAOSTAT, 2008). It has therefore become very important to develop high-yielding and early maturity cultivars in order to meet growing demand for maize while reducing the production costs (Maredia *et* *al*., 2000; Rohrbach *et al*., 2003) as the demand for maize in the developing world is likely to double by the year 2050 (Forum for Agricultural Research in Africa, 2009). Early-maturing cultivars are seen as a viable option to keep up with the pace of population growth.

2.2 ECONOMIC IMPORTANCE

Maize is the fundamental economic food crop in Africa, Asia, Latin America, and in some other parts of the world (Li *et al*., 2001). The importance of maize around the world is reflected by the wide variety of its uses and the role it plays as a staple food crop, especially in the lives of poor people. With the increasing population and the high levels of poverty in Sub-Saharan Africa and Central America, the demand for maize as a food continues to grow (Ortiz, 1998). According to Chetty (2004), the agronomic importance of maize as a food crop throughout the world is an undeniable fact, motivating investigations into efficient maize production. It is used for human consumption, in animal in feed, starch industry and oil production (Vasal, 2000; Amin *et al*., 2007). It plays a significant role in human and animal nourishment in a number of developed and developing countries worldwide (Séne *et al*., 2000; Prasanna *et al*., 2001; Azevedo *et al*., 2003; Bantte and Prasanna, 2003; Hussain *et al*., 2006). It serves as a source for raw material for industrial use (Miracle, 1966; Crow and Kermicle, 2002). Maize is largely used as an energy plant species, but specialized versions for protein, fat and starch are also widespread (Turi *et al*., 2007). Normal maize hybrids have many important uses in foods, medicine, beverages, ethanol, and industrial applications, amounting to an average annual utilisation of about 23% of the annual market of the grain worldwide (Watson, 2003).

2.3 ECONOMIC LOSSES DUE TO SEED STORAGE

The loss of maize seed due to various factors can be measured in economic terms. Maize storage losses largely occur in the form of kernel weight loss, as a result of deterioration processes of the grain but it is difficult to estimate the actual storage losses. The loss of value of stored maize kernels often hampers maize production in South Africa. The problem of low seed quality as a result of inappropriate storage conditions affects farmers, consumers as well as animals. The seeds that have lost quality are rejected by the buyers, hence the grower loses cash. The consumer, on the other hand, does not get value for his money. Maize kernels, just like the seeds of many other crop species, lose quality after short periods of storage which in turn results in losses (Mohamed-Yasseen *et al*., 1994). Maize storage losses affect the quantity and quality of maize available for sale post-harvest and hence its price (Kohl and Uhl, 1998; Armah and Asante, 2006). Knowing the ideal conditions for storage of maize is important if storage losses are to be mitigated (Cephas, 2008). Losses during storage vary according to region, environmental conditions, storage temperature, method of storage and storage time.

Losses in developed countries are far less because of the good storage facilities and the ability to maintain it (Bern *et al*., 2003). The quantitative losses are difficult to estimate because they vary from region to region and even from year to year (Méndez-Albores *et al*., 2003). In Mexico, quantitative losses due to lack of an efficient post-harvest system have been estimated at around 10% of the maize used for food and feed, without taking into consideration the quality losses (Méndez-Albores *et al*., 2003). Gwinner *et al*. (1991) and Louwaars *et al*. (1994) indicated that poor seed storage conditions cause up to 10% loss in maize kernel quality, particularly in the tropics mostly through loss of viability. Losses in maize during storage are common and occur to a larger extent in the developing countries than in the developed countries. According to Basappa *et al*. (2007), storage losses are found to be high and almost similar with the losses at the harvesting stage.

Despite maize being one of the most important cereal crops produced in South Africa, little is known about the economic losses due to storage. Although maize loss due to storage is a global concern, South Africa has not published data covering all regions that produce maize. Nevertheless, limited quality losses have been reported in maize traditionally stored under unfavourable conditions along the coastline of KwaZulu-Natal. A survey of storage facilities available in northern KwaZulu-Natal revealed that none of the ware-houses or silos used was properly functioning (Thamaga-Chitja *et al*., 2004). In South Africa, rural maize storage methods such as the traditional silo (*ingolobane*) and storing maize on the roof revealed 85.7% and 50.0% of households reporting losses respectively after a 12 month storage period. The study further showed 34.8% and 66.7% maize losses after a 24 month storage period in metal tanks and sacks, respectively (Thamaga-Chitja *et al*., 2004).

2.4 OVERVIEW OF MAIZE AND QUALITY PROTEIN MAIZE

2.4.1 Maize

Maize is one of the most important cereal crops that forms part of many diets worldwide and regularly produces protein comparable with that of sorghum, but very low compared with that of soybean (*Glycine max*) (Creech and Alexander, 1978). The protein quantity produced by maize is of low biological quality for humans and animals, especially because of a shortage of essential amino acids such as lysine and tryptophan (Creech and Alexander, 1978; Gibbon *et al.*, 2003; Milan-Carrillo *et al*., 2004; Badu-Apraku *et al*., 2006). According to Paulis *et al.* (1992), Gupta (1994) and Li and Gill (2004), the nutritional quality of maize protein is low because of the unbalanced proportion of zein, which is extremely deficient in lysine and tryptophan.

The nutritional quality of maize seeds depends on the essential amino acid composition of storage proteins (Landry and Delhaye, 2007). The amino acid composition of the endosperm proteins consists of high levels of proline and glutamine while the quantity of the essential amino acid lysine in particular, is restrictive for the efficient metabolism of the maize grain (Ingversen, 1983). The major storage proteins, zeins, which account for 50%-70% of the endosperm proteins in maize kernels, have a shortage of lysine and tryptophan, but are rich in glutamine and hydrophobic amino acids (Azevedo *et al*., 2003; Huang *et al.*, 2006). The rising demand for maize as a source of protein resulted in a search for maize varieties that have a balanced nutritional quality.

2.4.2 Quality protein maize

QPM describes maize genotypes with double the amount of the limiting amino acids lysine and tryptophan compared with maize landraces, and has been bred to help mitigate human undernourishment in areas where protein deficiency is a serious problem and where maize is the main protein source in the diet, such as in different parts of Sub-Saharan Africa (Krivanek *et al.*, 2007; Nurit *et al.*, 2009). According to Vasal (2001), QPM refers to maize homozygous for the *o2* allele, with increased lysine and tryptophan content but without undesirable characteristics of a soft endosperm. QPM kernels look like the normal maize kernels but they can be detected through laboratory tests (Villegas *et al*., 1992) and, more recently, through the use of a light table (Vivek *et al*., 2008). QPM is a viable option as an animal feed, since it can help mitigate the requirement of supplementary protein from other sources in balanced feeds (Vivek *et al*., 2008). QPM holds better nutritional and biological importance and is fundamentally interchangeable with ordinary maize in farming and seed phenotype (Prasanna *et al*., 2001).

Numerous nutritional quality characteristics are important in breeding programmes of maize genotypes (Montes *et al*., 2008). Traditional breeding methods have achieved the release of numerous QPM hybrids, both in Africa and Latin America (Danson *et al*., 2006). According to Vivek *et al*. (2008), numerous national maize research programmes in developing countries mainly South Africa, Brazil, China, Ghana and more recently, India, have made a significant contribution towards the development of QPM. Several national research programmes in countries such as South Africa, Ghana, Brazil and China have released numerous QPM hybrids (Vasal, 1999). Numerous African, Latin American and Asian countries, particularly China, are focused on more QPM hybrid releases where hopes and optimism for QPM promotion and dissemination are remarkably high (Vasal, 1999). Bhatnagar *et al*. (2004) proposed that improvement and adoption of QPM would enhance the nutritional status of food and feed maize products. The improved open-pollinated QPM varieties are generally better suited to resource-poor farmers' needs than the hybrids because of lower costs and distributional problems (Vivek *et al*., 2008).

QPM research started soon after the discovery of mutants, such as the opaque-2 and floury-2 genes that are responsible for the high protein yield in two maize landraces from the Andean highlands of South America by a doctoral student, Lynn Bates, working with prof. Edwin Mertz at Purdue University, USA (Ortega and Bates, 1983; Prasanna *et al*., 2001; Vivek *et al*., 2008). QPM breeding started with the purpose of enhancing the nutritional status of maize grain protein (Nurit *et al*., 2009). The International Maize and Wheat Improvement Center (CIMMYT) started a QPM hybrid programme in response to the growing demand for maize with high protein quality in developing countries (Bjarnason and Vasal, 1992). The insertion of the opaque-2 gene into the normal maize alters the amino acid profile and composition of protein, particularly in the endosperm portion, resulting in the increase of non-zein proteins (albumins, globulins and glutelin) and two-fold increase in the levels of lysine and tryptophan contents and the reduction of the zein fraction in comparison with normal maize hybrids (Mertz *et al*., 1964; Lopes *et al*., 1995; Gaziola *et al*., 1999; Prasanna *et al*., 2001; Darrigues *et al*., 2006; Vivek *et al*., 2008).

According to Geetha *et al.* (1991), opaque-2 mutants had reduced levels of α-zeins in maize endosperm resulting in small unexpanded protein bodies. Sofi *et al.* (2009) reported that other mutations such as floury-2 resulted in irregular shaped protein bodies. According to Danson *et al.* (2006), opaque-2 mutation increases the lysine content in maize endosperm by reducing the content of zein, while genetic modifiers alter the soft texture of the opaque seeds into hard endosperm. The alteration of the zeins brought protein fractions to a balance thereby doubling lysine and tryptophan contents (Vivek *et al.*, 2008). Maize seeds that contain the homozygous *o2* mutant have higher levels of lysine and tryptophan due to the suppression or reduction of the synthesis of the lysine-deficient zein fraction (Mertz *et al.*, 1964; Habben *et al.*, 1993). Sofi *et al.* (2009) indicated that the elevated lysine content in opaque-2 mutants is due to higher levels of an elongation factor of protein synthesis. According to Bantte and Prasanna (2003), unfavourable pleiotropic effects imposed severe constraints on successful exploitation of these mutants. The insertion of the maize mutant opaque-2 gene into maize landraces (Mertz *et al.*, 1964; Nelson, 1969) yielded high lysine and tryptophan maize with soft, chalky endosperm with increased susceptibility to insect pests and reduced yields (Lambert *et al.*, 1969; Sreeramulu and Baumann, 1970; Wessel-Beaver and Lambert, 1982; Villegas *et al.*, 1992; Moro *et al.*, 1995; Lin *et al.*, 1997; Toro *et al.*, 2003; Scott *et al.*, 2004; Danson *et al.*, 2006; Ngaboyisonga *et al.*, 2008). Lopes *et al.* (1995) also stated that the maize mutation opaque-2 causes a reduction in storage protein synthesis and the formation of a soft, starchy endosperm in the kernel. Not only did the opaque-2 gene have negative effects, it also caused agronomic problems related to processing which prevented its acceptance by many people including maize breeders who are accustomed to harder grains throughout the world, particularly the developing world (Glover and Mertz, 1987; Lauderdale, 2002; Krivanek *et al.*, 2007). The soft endosperm of opaque-2 maize varieties initially resulted in 25% yield loss due to the lower density of the opaque grains, as well as elevated vulnerability to fungal ear rots and storage pests (Vasal, 2000).

Prasanna *et al.* (2001) and Vivek *et al.* (2008) reported that interdisciplinary and intensive research efforts by CIMMYT research team initially led by Dr. Surinder Vasal, a breeder, and Dr. Evangelina Villegas, a cereal chemist, led to amelioration of the negative characteristics of the opaque phenotype. This newly developed maize variety without negative effects was termed QPM (Vasal *et al.*, 1980; Gevers and Lake, 1992; Nelson, 2001). QPM has a number of characteristics that differentiate it from high lysine soft opaque-2 maize genotypes (Vasal, 1999). The undesirable characteristics were suppressed through the systematic introgression of genetic modifiers introduced by CIMMYT and the University of Illinois, USA (Wessel-Beaver and Lambert, 1982) into opaque-2 germplasm, which converted the soft and floury mutant endosperm to hard and vitreous endosperm while preserving higher lysine content (Hohls *et al*., 1996; Vasal, 2002). According to Sofi *et al*. (2009), the modifier genes do not have any effect of their own as such but interact to improve kernel hardness and appearance and increased kernel weight and density.

The development of QPM germplasm with hard endosperm involved several phases including the development of QPM donor stocks and QPM gene pools (Martinez *et al*., 1996). The hard endosperm relates to its chemical composition (Pratt *et al*., 1995). The protein content and the zein protein have been linked with seed hardness (Pratt *et al*., 1995; Eyherabide *et al*., 1996; Robutti *et al*., 1997; Landry *et al*., 2004). When it comes to processing, safe food processing technologies used for preservation of quality characteristics of food materials such as maize are very important issues (Baysal *et al*., 2010). Seed proteins, particularly in hard endosperm QPM cultivars are important in food processing (Shewry *et al*., 2002). The hardness of maize endosperm determines the extent of quality and yield of maize by-products during dry milling (O’Kennedy, 2011). Hard endosperm maize provides good storage and milling characteristics (Holding and Larkins, 2006). Dorsey-Redding *et al*. (1991) indicated that kernel hardness contributes to grain test weight. Maize cultivars with high percentages of hard endosperm are attractive when it comes to dry milling (Hill *et al*., 1991).

Current QPM research around the world focuses on its adaptability to various growing conditions (Krivanek *et al*., 2007). In Sub-Saharan Africa, 17 countries are growing QPM genotypes on around 200 000 ha with Ghana accounting for approximately 70 000 ha (Sofi *et al*., 2009). Research towards the development of high-yielding QPM varieties is currently underway in many parts of the world (Sofi *et al*., 2009) and it is predicted that by 2020, about 30% of maize area will be under QPM genotypes (Gill, 2008). The improvement and adoption of QPM would enhance the nutritional status of food and feed maize products (Bhatnagar *et al*., 2004).

2.5 CHEMICAL COMPOSITION OF THE MAIZE KERNEL

Cereals such as maize differ greatly in their grain composition, with both genetics and environment influencing the kind and level of chemical compounds (Bullock *et al*., 1989; Awika and Rooney, 2004). The chemical composition of maize is influenced by a number of factors such as soil, climatic conditions and storage (Ereifej and Haddad, 2001). Several seed scientists have evaluated the chemical composition of the seed and discovered that on average the seed normally contains minerals, carbohydrate (63%), proteins (19%) and fat (6.5%) (Oluyemi *et al*., 1976).

2.5.1 Minerals

Maize has a substantial amount of phosphorus (P) with a fair quantity of manganese (Mn), magnesium (Mg) and iron (Fe) and a small amount of zinc (Zn), potassium (K), calcium (Ca), and sodium (Na). Each of these mineral elements plays a significant role in the growth and development of maize (Battal *et al*., 2003). Bajaj *et al*. (1994) stated that approximately 80% of the minerals are concentrated in the grain germ. The endosperm of maize contains 250 g/kg minerals and the remaining 750 g/kg is concentrated in the embryo (Department of Agriculture, 2003). However, cereals including maize also contain these mineral fractions in starch, but they have been found to be low compared with minerals found in potato starch (Svihus *et al*., 2005). These minerals have been reported to negatively interact with starch in the amorphous region of the granules through interference with swelling and subsequent digestion (Blennow *et al*., 2000).

Ca is involved in cell division and plays a main role in the protection of membrane reliability (Fageria *et al*., 1991). Cereal grains possess negligible amounts of Ca (Hambidge *et al*., 2005). Maize, like other cereal crops, is extremely low in Ca (Loy and Wright, 2003), with a content of about 0.2 g/kg (Blair, 2007). Pfahler and Linskens (1974) reported the average Ca content to be 0.39 g/kg in three single-cross hybrids and two maize inbred lines. A Ca content of 0.7 g/kg was reported in both normal maize and QPM hybrids (Zhai and Zhang, 2007). In addition, Ca contents of four improved white dent maize genotypes IWD MSB-W, TZSR-W, W-composite and TZB grown in Nigeria were found to be 0.44 g/kg, 0.50 g/kg, 0.62 g/kg and 0.76 g/kg, respectively (Iken *et al*., 2002). However, total Ca content obtained from high-moisture maize dried at 80^{o}C, 90^{o}C and 100^{o}C was 0.05 g/kg, 0.04 g/kg and 0.03 g/kg, respectively (Bhuiyan *et al*., 2010).

Mg is a constituent of chlorophyll and a cofactor for various enzymatic reactions (Fageria *et al*., 1991). Shobha *et al*. (2010) found the Mg content to vary between 1.31 g/kg and 1.46 g/kg in tested maize genotypes. High-moisture maize dried at 80^{o}C, 90^{o}C and 100^{o}C had an Mg content of 1.4 g/kg, 1.3 g/kg and 1.4 g/kg, respectively (Bhuiyan *et al*., 2010). However, Mg contents of four improved white dent maize genotypes IWD MSB-W, TZSR-W, W-composite and TZB grown in Nigeria were found to be 0.0012 g/kg, 0.0013 g/kg, 0.0011 mg/kg and 0.0011 g/kg, respectively (Iken *et al*., 2002).

Na is one of the most important mineral elements found in maize. The Na content in maize kernels is usually low (Blair, 2007). Pfahler and Linskens (1974) observed the average Na content to be 0.22 g/kg in three single-cross hybrids and two maize inbred lines. The total Na content obtained from high-moisture maize dried at 80^{o}C, 90^{o}C and 100^{o}C was 7.3 g/kg, 7.1 g/kg and 7.0 g/kg, respectively (Bhuiyan *et al*., 2010).

K plays several roles in plant metabolism and to perform these roles effectively, it should interact positively with other essential nutrients (Fageria, 2009). It functions as a cofactor or activator for many enzymes of carbohydrate and protein metabolism (Fageria *et al*., 1991). Maize grain contains nearly a balanced chemical compounds, except some, such as K, which appears to be low (Blair, 2007). Pfahler and Linskens (1974) found the average K content to be 6.2 g/kg in three single-cross hybrids and two maize inbred lines. High-moisture maize dried at 80^{o}C, 90^{o}C and 100^{o}C had a K content of 3.3 g/kg, 3.1 g/kg and 3.2 g/kg, respectively (Bhuiyan *et al*., 2010). However, K contents of four improved white dent maize genotypes IWD MSB-W, TZSR-W, W-composite and TZB grown in Nigeria were found to be 0.0045 g/kg, 0.0048 g/kg, 0.0045 g/kg and 0.0039 g/kg, respectively (Iken *et al*., 2002).

Fe boosts many plant enzymes that are responsible for redox reactions of photosynthesis and respiration (Sajedi *et al*., 2009). Maximizing the concentrations of Fe in staple food crops through breeding has been proposed as one strategy to reduce the undesirable effects of extensive mineral deficiencies in people (Menkir, 2008). Fe associates itself in one way or another with the important functions of plant metabolism (Mathan and Amberger, 1977). Fe deficiency symptoms first emerge on the younger tissues or leaves (Fageria, 2009). Iken *et al*. (2002) reported Fe contents of 0.29 g/kg, 0.26 g/kg, 0.36 g/kg and 0.26 g/kg in for improved white dent maize genotypes IWD MSB-W, TZSR-W, W-composite and TZB grown in Nigeria, respectively. High-moisture maize dried at 80°C, 90°C and 100°C had a Fe content of 27.9 g/kg, 25.2 g/kg and 18.7 g/kg, respectively (Bhuiyan *et al.*, 2010).

Zn plays a vital role in protein and carbohydrate synthesis and it is involved in the regulation of saccharide, nucleic acid and lipid metabolism (Sajedi *et al.*, 2009). Marginal Zn shortages and suboptimal Zn concentration have been recognized in many population groups in both developing and developed countries (Hemalatha *et al.*, 2009). According to Menkir (2008), increasing the concentrations of Zn in crops including maize is one important strategy to fight mineral deficiencies in rural communities. The total Zn content obtained from high-moisture maize dried at 80°C, 90°C and 100°C was 15.4 g/kg, 14.0 g/kg and 14.9 g/kg, respectively (Bhuiyan *et al.*, 2010). However, Zn contents of four improved white dent maize genotypes IWD MSB-W, TZSR-W, W-composite and TZB grown in Nigeria were found to be 0.03 g/kg, 0.02 g/kg, 0.02 g/kg and 0.05, respectively (Iken *et al.*, 2002).

Mn is considered an activator of various kinds of enzymatic reactions and is involved in photosynthesis (Sajedi *et al.*, 2009). It is a constituent of the enzymes arginase and phosphotransferase (Fageria *et al.*, 1991). The influence of Mn on Fe nutrition in plants regulates the change in the oxidation state of Fe (Kuo and Mikkelsen, 1981). Numerous maize genotypes show a wide variation in terms of the Mn content. The Mn contents of four improved white dent maize genotypes IWD MSB-W, TZSR-W, W-composite and TZB grown in Nigeria were found to be 0.06 g/kg, 0.40 g/kg, 0.43 g/kg and 0.023, respectively (Iken *et al.*, 2002). High-moisture maize dried at 80°C, 90°C and 100°C had a Mn content of 8.7 g/kg, 5.1 g/kg and 5.6 g/kg, respectively (Bhuiyan *et al.*, 2010).

P is one of the most important mineral elements in maize and is necessary for plant development and reproduction. Unavailability of P can decrease seed size, seed numbers, and viability (Fageria, 2009). Blair (2007) stated that maize contains a high level of P (2.5 g/kg-3.0 g/kg). Debet and Gidley (2006) reported that the P content in normal maize was 0.19 g/kg and 0.02 g/kg in waxy maize. Shobha *et al.* (2010) found the P content to vary between 3.11 g/kg and 3.49 g/kg in tested maize genotypes. Zhai and Zhang (2007) reported Ca contents of 2.1 g/kg and 2.2 g/kg in normal maize and QPM genotypes, respectively. High-moisture maize dried at 80°C, 90°C and 100°C had a P content of 2.6 g/kg, 2.5 g/kg and 2.6 g/kg, respectively (Bhuiyan *et al.*, 2010). However, P contents of four improved white dent maize genotypes IWD MSB-W, TZSR-W, W-composite and TZB grown in Nigeria were found to be 0.0012 g/kg, 0.0016 g/kg, 0.0021 g/kg and 0.0024, respectively (Iken *et al*., 2002).

2.5.2 Fat

Fat is the most valuable constituent in maize and is a highly heritable trait (Johnson and May, 2003; White and Weber, 2003). Fats are divided into two major groups, acyl lipids and non-saponifiable lipids. The acyl lipids contain fatty acid esters that are hydrolysed by alkali. The non-saponifiable lipids, particularly hydrocarbons, sterols, carotenoids, and tocopherols, are those extracted by organic solvents after saponification (White and Weber, 2003). Morrison (1981) classified lipids experimentally into three major categories namely: non-starch lipids, starch surface lipids and internal starch lipids. Maize fat is considered to be the finest vegetable oil because of its flavour, colour, stability, and clarity at refrigerator temperatures (Orthoefer *et al*., 2003; Watson, 2003; Loy and Wright, 2003). The maize kernel contains about 3-5% fat, with most of the fat being concentrated in the germ (Bajaj *et al*., 1994; Orthoefer *et al*., 2003). The endosperm contains approximately 20% of the fat while the embryo contains about 80% (Department of Agriculture, 2003). Orthoefer *et al*. (2003) reported that 85% of the fat in maize is concentrated in the germ and the rest is distributed among the endosperm (15%), bran (1.3%) and tip cap (0.7%).

The fat content of different maize genotypes shows wide variation (Casa *et al*., 2010). Yellow maize in South Africa has a lower fat content (3.5%) than white maize (3.9%) (Anon, 2006). Graham (1993) reported more than 15% fat in high-fat QPM grown in Mexico. Jugenheimer (1961) found that two maize hybrids, Illinois 6021 (6.39%) and Illinois 6052 (6.27%) grown in Illinois, United States, yielded 30% more fat than hybrid, U.S. 13 (4.77%). Hasjim *et al*. (2009) observed between 4.2% and 5.1% fat in four QPM lines. Debet and Gidley (2006) found 0.8% and 0.2% fat content in normal maize and waxy maize, respectively. Chanvrier *et al*. (2007) observed 0.2% and 0.1% fat contents in normal and waxy maize genotypes, respectively. Loy and Wright (2003) and Blair (2007) stated that maize fat has a high proportion of unsaturated fatty acids and is an excellent source of linoleic acid. The fatty acid composition of maize does not significantly differ from that of other cereals. Léder (2004) stated that fatty acid composition of sorghum

(*Sorghum bicolor* [L.] Moench) is similar to that of maize fat, with high concentrations of linoleic (49%), oleic (31%) and palmitic acids (14%).

Maize starch frequently contains lipids, but the content and composition differ from cultivar to cultivar (Morrison, 1988; Morrison *et al*., 1993a). The two most important groups of starch bound lipids which are available in small quantities are: free fatty acids and lysophospholipids (Morrison, 1978a). These starch bound lipids are considered to be complexed with amylose in granules and require hot aqueous alcohol for extraction (Kitahara *et al*., 1994). The interaction between starch and lipids is of vital importance to manufacturers and consumers when it comes to food processing (Blazek, 2008). Lipids and phospholipids are known to form stable complexes with long chains of starch, with both amylose and long branched chains of amylopectin, which result in the restricted swelling of granules (Medcalf *et al*., 1968; Batres and White, 1986; Morrison *et al*., 1993a; 1993b). The presence of naturally occurring chemical compounds such as lipids can affect the functional properties of starch granules (Leach, 1965). Lipids binding to granular starches in maize influence many important properties of starch-containing foods by changing the granule swelling, solubilisation, and crystallization of starch polymers (Morrison 1978b; Morrison and Milligan, 1982; Hahn and Hood, 1987).

2.5.3 Enzymes

Enzymes are proteins that catalyse many chemical reactions and are the most important functional proteins together with the membrane proteins, non-regulatory proteins and proteins of organelles (O’Kennedy, 2011). Enzymes such as lipase are responsible for the breakdown of fats in the maize kernel. Lipoxygenase is an enzyme that catalyses oxidation of unsaturated fatty acids with oxygen to yield peroxides of the fatty acids. Peroxidase is an enzyme responsible for the oxidation processes in different substrates using hydrogen peroxide and spontaneously produces water and free radicals (Balešević-Tubić, 2005). Amylase is one of the most important enzymes in plants found mainly in the endosperm of maize seeds and plays very important roles during germination (Subbarao *et al*., 1998). In addition, amylase is important in sprouting vigour and during seed germination and is secreted from the cells of the aleurone layer and scutellum to mobilize starch (Subbarao *et al*., 1998; Egwim and Oloyeide, 2006; Coulibaly and Chen, 2011). Amylase is important in food processing quality and causes nutritional, functional and compositional changes in grain during malting and brewing (Gupta *et al*., 2010). The α-amylase is a common type of enzyme that randomly cleaves α-1,4 linkages and is able to hydrolyse intact starch granules with the production of soluble starch fragments in germinating pea (*Pisum sativum* L.) and rice (*Oryza sativa* L.) seeds (Murata *et al*., 1968; Juliano and Varner, 1969; MacGregor, 1983; Synowiecki, 2007). The ß-amylase enzyme promotes rapid hydrolysis of outer chains by breaking off sugar units without hydrolysing the α-1,6 linkages.

2.5.4 Starch

Starch is one of the most plentiful biotic resources produced in nature by higher plants as a carbohydrate energy reserve and consists primarily of branched glucans with an architecture that allows the formation of a semi-crystalline insoluble granule (MacGregor, 1983; Gidley and Bociek, 1988; Blennow *et al*., 2001; Desai, 2004; Zeeman *et al*., 2007). Lagarrigue *et al*. (2007) defined starch as the most widely used thickener in the food industry. Starch is a major component of maize and is extracted mainly through wet-milling. According to Mason and D’Croz-Mason (2002), starch extraction by wet-milling is the second largest end-use of maize grain following livestock feed. Traditionally, high amylose starch from maize has been mainly used as an ingredient in gum candies and as an adhesive for corrugated cardboard (Wu *et al*., 2009).

Starch, the primary component of most plant storage organs, a component in many essential food products with a long tradition in drug formulation, has recently emerged as a candidate for new uses as a biodegradable polymer in microspheres within the pharmaceutical industry and its use largely depends on its functional properties (Elfstrand *et al*., 2004; Maache-Rizzoug *et al*., 2008). Zobel (1988) indicated that typical natural maize starch granules range in degree of crystallinity from about 15% to 45%. The understanding of starch phase transitions is very important in the food processing operations and the physical properties of food products since short chain lengths exhibit A-type crystallinity and intermediate chain length is related to the C-type crystallinity (Hizukuri *et al*., 1983; Hizukuri 1985; Maache-Rezzoug *et al*., 2008). In addition, starch granules have a semi-crystalline nature with C polymorphs which are a mixture of A and B polymorphs, with intermediate behaviour and properties and the differences could be related to the A and B polymorphs in the starches (Sarco and Wu, 1978; Blanshard, 1987; Gernat *et al*., 1990; Davydova *et al*., 1995).

The molecular structure of maize starch plays an important role in its nutritional and technological functionality (Creek *et al.*, 2007). The nutritional value of maize starch is dependent on its primary composition and physical structure and subsequent processing (Buckow *et al.*, 2009). Structural and physicochemical characteristics of starches are associated with their botanical sources (Peroni *et al.*, 2006). Starch structural characteristics such as amylose content, branch chain length distribution of amylopectin (Jane *et al.*, 1999) and phospholipids and lipid contents (Tester and Morrison, 1990; Lim *et al.*, 1994) affect their functional properties. Maize starches that contain more amylopectin molecules exhibit higher gelatinisation temperature and enthalpy changes (Kasemsuwan *et al.*, 1995).

Starch structure can be modified in different ways prior to utilisation in industrial applications of which enzymatic and chemical modifications are the most common (Elfstrand *et al.*, 2004). Starches utilised in the food industry are modified chemically by cross-linking, substitution and acid hydrolysis to alter their functionality (Jayakody, 2009). Creek *et al.* (2007) pointed out that structural features such as degree of branching and molecular weight depend on the type of maize and may alter the determination of amylose content.

Creech and Alexander (1978) stated that maize produces the highest content of starch among the cereal crops. Most maize averages about 58%-75% starch yield in laboratory-scale wet milling tests (Mertz, 1970; Eckoff *et al.*, 1996; Paulsen *et al.*, 2003; Rodriguez-Nogales *et al.*, 2006; Bewley *et al.*, 2006; Sofi *et al.*, 2009). Starch concentration and composition in the maize seed are quantitative characteristics influenced by many genes (Wilson *et al.*, 2004). The starch content in yellow maize (73.3%) was reported to be higher than in white maize (72.9%) (Anon, 2006). The starch content of five QPM lines was reported to be 73.0%, 66.9%, 71.7%, 74.1% and 72.4% (Hasjim *et al.*, 2009). A large portion of starch is concentrated in the endosperm. Watson (2003) reported that starch accounts for 88% of the weight of mature endosperm. According to Freeman (1973), starch tends to be lower in maize with high fat content.

Starch is the main storage component in mature maize seed and comprises amylose and amylopectin (Séne *et al.*, 1997; Edwards *et al.*, 2002; Hernández-Uribe *et al.*, 2004; Shang-Jing *et al.*, 2006; Liu *et al.*, 2009b). The amylose/amylopectin ratio in starch granules has a distinct impact on the physicochemical properties of starches (Corcuera *et al.*, 2007; Buckow *et al.*, 2009). Burešová *et al.* (2010) reported that the nutritional quality of starch is dependent largely on amylose/amylopectin ratio. The relative ratio of amylose and amylopectin varies to a great extent within plant species (Synowiecki, 2007; Stawski, 2008). In most common types of cereal endosperm starches, the relative weight percentages of amylose and amylopectin range between 72% and 82% amylopectin, and 18% and 28% amylose (Buléon *et al.*, 1998).

Amylose

The amylose content is an important factor that determines the digestibility of starch (Liu *et al.*, 2009a). Amylose is a linear glucan with α(1→4) glycosidic linkages and limited branching that produces few non-reducing end-groups. In normal maize genotypes, amylose is organised in a helical conformation, tightly associated with lipids (Séne *et al.*, 1997). The amylose fraction is essentially linear, although there is now proof that amylose is not absolutely linear (Curá *et al.*, 1995). According to Wilson *et al.* (2004), maize is composed of approximately 21% amylose. In normal maize, amylose makes up 25% to 30% of the starch. Chanvrier *et al.* (2007) observed 30.8% and 80.0% amylose in normal and high-amylose maize starches, respectively. The maize sugary-2 mutants produce starch with 40% amylose (Bewley *et al.*, 2006). However, there are certain maize varieties known to have very high amylose content. Polaske *et al.* (2005) found 53.3%, 63.9% and 69.9% in three high-amylose varieties which had high-amylose modifying genes. However, high temperature during grain-filling of cereals including maize was found to contribute to high amylose content (Tester *et al.*, 1991; 1995). Chen *et al.* (2006) reported 50% and 80% in two high-amylose maize lines, respectively. A study of comparison of methods for colometric amylose determination in cereal grains done by Mahmood *et al.* (2007) revealed that the standard consisted of normal maize starch with 27% amylose and high-amylose maize starch with 85% amylose.

Maize genotypes with high amylose content vary considerably in size, shape, and relative crystallinity and have a B-type crystal structure in contrast to A-type crystals in normal maize genotypes (Cheetham and Tao, 1998; Chen *et al.*, 2006; Liu *et al.*, 2009a). Certain mutant plants, commonly called waxy because of the waxy appearance of the seed endosperm, have much lower amylose content, or even lack the amylose component completely (Huang, 2006). Buckow *et al.* (2009) reported waxy maize genotype with less than 1% amylose while Polaske *et al*. (2005) reported that waxy maize and normal maize genotypes produced 1.3% and 25.5% amylose, respectively. Herrero-Martinez *et al*. (2004) and Debet and Gidley (2006) observed 0% amylose content in waxy maize.

Amylopectin

Amylopectin is one of the largest chemical compounds found in starch and has a molecular weight that is approximately 1000 times that of amylose due to its many branches that contain 30 glucose units each (Manners, 1989; Buckow *et al*., 2009). It is a more branched molecule of very high molecular mass ranging from 10^6 to 10^8 and comprises hundreds of short α(1→4) glucan chains which are interlinked by α(1→6) branch points. The α(1→4) chains of amylopectin consist of A chains that carry no additional chains as opposed to B chains which bear A chains or other B chains. C chains carry other chains as branches but contain the sole reducing terminal residue. Normal maize is composed of amylopectin ranging from 70%-75%. Wilson *et al*. (2004) reported that maize contains about 79% amylopectin. High-moisture maize dried at 80^{o}C, 90^{o}C and 100^{o}C had an amylopectin content of 388 g/kg, 380 g/kg and 370 g/kg, respectively (Bhuiyan *et al*., 2010). The chain length distribution of amylopectin in maize influences the pasting properties of starch (Jane *et al*., 1999; Wang *et al*., 1993; Franco *et al*., 2002). The exact nature of amylopectin formation is not clearly understood, and so are the enzymes and genes involved in the starch biosynthesis (Fisher *et al*., 1996; Huang and Wang, 1998; Beckles *et al*., 2001).

2.5.5 Protein

Protein is the second largest chemical component in maize after starch. It is an important quality parameter in maize and plays a significant role in food products (Arief, 2008). Protein in maize is of particular importance for the health of persons whose major staple food is maize (Koutroubas *et al*., 2004). Li and Vasal (2004) reported that maize contributes about 42 million tonnes of protein a year worldwide, which corresponds to roughly 15% of the world annual production of food-crop protein. Protein content and composition are important when it comes to the nutrition they provide and the impact on quality of targeted end-uses of cereals (Shewry and Halford, 2002). The nutritional quality of maize is mainly determined by the protein content of grain and amino acids. Payne (1983) stated that breeding for protein quantity in cereals has the drawback that as protein level accumulates the overall nutritional value goes down. This is because increasing protein up to 15% increases the proportion of zein protein to metabolic and structural proteins in the grain (Payne, 1983). According to Rooney *et al*. (2004), the protein content of maize is inversely proportional to starch content and grain yields. Maize with high protein content has increased zein content and decreased amino acid content (Rooney *et al*., 2004).

The chemical composition of protein in maize is different from other cereal crops. Maize seed normally contains approximately 9-11% protein (Bajaj *et al*., 1994; Sofi *et al*., 2009; Fox and Manley, 2009). However, the endosperm averages 8-9% protein (Lawton and Wilson, 2003). According to Rodriguez-Nogales *et al*. (2006), the endosperm of the mature kernel contains 6.5% of the protein and the germ the remaining 2.5%. The protein content of maize seed can vary significantly depending on the type, variety, agronomic, and other environmental factors (Lawton and Wilson, 2003). Landry *et al*. (2005) stated that the protein content of a maize grain is dependent upon the genotype and the soil status with regard to the nitrogen levels in which the crop is grown. The average protein content in white maize was found to be 9.3% and in yellow maize 9.5% (Anon, 2006). Vasal (1999) observed CIMMYT QPM genotypes to have between 8.9% and 9.9% protein. The actual protein content in the maize seed is of importance from a nutritional point of view. Gibbon and Larkins (2005) reported protein varying between 10.1% and 13.5% in some maize genotypes. Hasjim *et al*. (2009) observed between 9.6% and 13.3% protein in four QPM lines. Jovanović *et al*. (2005) found maize genotypes to be comprised of 12.4% of protein content. Protein contents found in QPM inbreds CML 176, CML 177, CML 178, CML 181, CML 473, CML 490, CML 490, CML 491, CML 492, CML 493 were 7.34%, 7.57%, 8.23%, 7.34%, 7.34%, 8.03%, 7.14%, 8.13% and 6.79%, respectively (Bello *et al*., 2012). Protein contents of 7.21%, 7.10%, 7.34%, 7.23% and 6.89% were found in open-pollinated QPM varieties Obatanpa, EV8766-SR, EV8363-SR, Pool 18-SR and Pool 19-SR, respectively (Bello *et al*., 2012). In addition, protein contents found in pure QPM hybrids Mama-ba, CIDA-ba Dada-ba, ART98-SW6-OB, ART98-SW4-OB, TZPB-OB, ILEI-OB were 8.14%, 7.57%, 8.23%, 6.34%, 8.34%, 8.33%, 8.12% and 6.02%, respectively (Bello *et al*., 2012).

Maize protein is classified into the following groups on the basis of differences in their solubility: zeins (highly alcohol soluble), albumins (water soluble), globulins (salt soluble) and glutelins (dilute alkali soluble) (Esen, 1986; 1987; Singh, 2005; Momany *et* *al*., 2006). The proportion of different protein fractions of normal maize endosperms, on average, are zeins (60%), albumins (3%), globulins (3%) and glutelin (34%) (Schnieder, 1955; Vasal, 1999; Sofi *et al*., 2009). According to Lasztity (1984), proteins can be divided into two major groups based on their functionality: cytoplasmic and storage proteins. The cytoplasmic proteins are composed of mainly globulins and albumins, and are located in the aleurone layer and embryo. Storage proteins are located in the endosperm and are generally soluble in alcohol (prolamins) and dilute alkali solutions (glutelins). These storage proteins can be divided into two types of proteins: low and high molecular weights. The low molecular weight proteins contain one polypeptide chain with only intramolecular disulfide bonds and high molecular weight proteins contain numerous polypeptide chains which are cross-linked via intermolecular disulfide bonds (Lasztity, 1984; Shewry and Tatham, 1990).

Zein

The zein of maize is defined as the protein extracted from maize with aqueous alcohols (Parris and Dickey, 2001; Rodriguez-Nogales *et al*., 2006). The endosperm of maize possesses a cluster of four structurally distinct alcohol-soluble proteins called zeins, which are encoded by definite classes of structural genes that belong to a large gene family clustered in several genomic regions (Prasanna *et al*., 2001). The maturing endosperm of maize is composed of zeins, the main alcohol-soluble storage proteins within protein bodies (Vitale *et al*., 1982; Wilson, 1987; Lending and Larkins, 1989; Boston *et al*., 1991; Prasanna *et al*., 2001; Rodriguez-Nogales *et al*., 2006). Zein is a major constituent of storage protein in maize (Lawton and Wilson, 2003) and is limited to a small area in the protein bodies (Larkins *et al*., 1979). Larkins and Hurkman (1978) reported that zeins are synthesized in the rough endoplasmic reticulum and are deposited in the kernel as relatively large, insoluble protein bodies that make up about 10% to 15% of the endosperm by volume. Zein plays a vital role in industrial polymers due to its commercial application (Shukla and Cheryan, 2001). It is a hydrophobic protein generally processed to yield films and coatings (Parris and Dickey, 2001; Lawton, 2002). Zein that is produced commercially is made up of α-zeins (Wilson, 1988). According to Zhu *et al*. (2007), two types of zein are presently being produced for industrial purposes, namely: white and yellow zein. The zeins of cereals were shown to be important when it comes to the nutrition they offer and the impact they have on the quality of targeted end-uses of cereals (Shewry and Tatham, 1990; Shewry and Halford, 2002).

Storage proteins contain approximately 50% of the total protein in mature cereal grains and have significant impacts on nutritional quality for people and animals (Bajaj *et al*., 1994; Shewry and Halford, 2002). Zein constitutes about 44%-79% of the endosperm protein, depending on the maize variety and the separation method used (Larkins, 1981; Hoseney *et al*., 1993; Hamaker *et al*., 1995; Bjarnason and Vasal, 1996; Landry *et al*., 2000; Hunter *et al*., 2002). According to Larkins *et al*. (1993) and Singh (2005), the zeins are the main determinants of the amino acid composition in maize seeds because of their abundance. Numerous nomenclatures have been proposed to differentiate the different types of zeins (Landry and Moureaux, 1970; Paulis and Wall, 1977; Wilson, 1991). Previously zein was divided into two classes: α-zein and ß-zein (McKinney, 1958; Pomes, 1971). Now it is divided into four major classes, namely: gamma (γ)-zein, delta (δ)-zein, ß-zein and α-zein (Esen, 1987; Kirihara *et al*., 1988; Coleman and Larkins, 1999; Woo *et al*., 2001) which constitute about 50-70% of the maize endosperm. These four zein classes are all rich in glutamine, leucine and proline (Sofi *et al*., 2009). Although the precise mechanisms of how these storage proteins interact are unclear (Holding and Larkins, 2006), but research is currently at an advanced stage of understanding the interactions of some proteins (Coleman *et al*., 1996; Hinchliffe and Kemp, 2002; Kim *et al*., 2002; Coleman *et al*., 2004; Randall *et al*., 2004).

The zeins vary in their quantity, molecular weight and solubility (Lawton, 2002; O'Kennedy, 2011). Zeins have usually been fractioned based on solubility in alcoholic or aqueous solvents and their high content of proline and amide nitrogen (Osborne, 1924; Wilson *et al*., 1981; Esen, 1986). Zein solubilised from whole maize seed without using a reducing agent will consist of mixtures of aggregates and monomers (Tsai, 1980). Lawton (2002) pointed out that zein that is extracted without using reducing agents is known as native zein.

The major prolamin fraction of storage proteins is called the α-zeins (Esen, 1986) which accounts for approximately 70% of the total (Thompson and Larkins, 1989). The α-zeins are a complex group of closely related polypeptides of M_r 19 and 22 kD, which are soluble in aqueous alcohols and are rich in cysteine (Larkins *et al*., 1984; Wallace *et al*., 1990; Sofi *et al*., 2009) and are the most abundant proteins in the maize endosperm (Villegas *et al*., 1980; Lending *et al*., 1988; Prasanna *et al*., 2001; Gibbon and Larkins, 2005). The γ-zeins are the next most abundant class of storage proteins which are very soluble in both aqueous and alcoholic solvents and account for 20% of the total zeins present in maize endosperm (Wallace *et al*., 1990; Lawton, 2002). The γ-zeins are rich in methionine (Sofi *et al*., 2009) and have M_r 16 and 27 kDa (Larkins *et al*., 1984). The ß- and δ-zeins are also rich in methionine (Shewry and Halford, 2002). The M_r 14 kD ß-zeins and M_r 10 kD δ-zeins are a minor group of storage proteins and are soluble in aqueous alcohols containing a reducing agent (Wallace *et al*., 1990).

Zein possesses small amounts of essential amino acids, particularly lysine and tryptophan (Rooney and Serna-Saldivar, 2003). Misra *et al*. (1972) and Lin *et al*. (1997) reported that zeins contain only 1.0 g/kg lysine. The zein portion in normal maize usually possesses a higher proportion of leucine (18.7%), phenylalanine (5.2%), isoleucine (3.8%), valine (3.6%) and tyrosine (3.5%), but lesser amounts of other essential amino acids such as threonine (3%), histidine and cysteine (1%), methionine (0.9%), lysine (0.1%) and is fundamentally devoid of tryptophan as it is not present in the α-zeins of maize seed (Gibbon and Larkins, 2005; Sofi *et al*., 2009; Huang *et al*., 2006). The amino acids such as lysine, threonine and metheonine in maize are synthesized from asparatic acid (Shewry, 2007). Misra *et al*. (1975) observed zein content ranging from 49.3% to 77.5% in three maize hybrids. Di Fonzo *et al*. (1979) found zein content of 65.5% in various maize landraces. Azevedo *et al*. (2003) observed zein content varying between 41.5% and 71.9% in four maize genotypes.

Albumins

Albumins are protein fractions that are very soluble in water solutions and their molecular weights range from 10 to 18 KDa range (González-Pérez and Arellano, 2009). They belong to a widely distributed family of seed proteins (González-Pérez and Arellano, 2009). Normal maize generally has lower albumin content (3.2%) than opaque-2 maize (13.2%). According to Bjarnason and Vasal (1992), the albumin content of normal endosperm maize is lower than the albumin content found in soft endosperm maize. A study by Konopka *et al*. (2007) suggested that the amount of albumin present in the seed is associated with kernel size.

Globulins

Globulins are traditionally classified as proteins that are soluble in salt solutions. They have been extensively characterised and their molecular weights are 150-190 KDa (González-Pérez and Arellano, 2009). Globulins, particularly α-globulins, are largely sequestered at the peripheral matrix surrounding the glutelins (Krishnan *et al*., 1992) and are deposited into the protein storage vacuoles (Krishnan *et al*., 1986; Li *et al*., 1993). Globulins contribute towards the total protein content present in the maize kernel. They are, however, not available in large quantities. Previous research by Konopka *et al*. (2007) suggested that the amount of globulin present in seed is associated with kernel size. Normal maize (0.15%) normally has lower globulin content than QPM (0.39%) (Vivek *et al*., 2008).

Glutelins

Glutelins are hydrophobic proteins that are soluble in dilute alkali (González-Pérez and Arellano, 2009). Glutelins are stored in the inner region of the protein storage vacuoles as crystalloids and they are the second major protein fraction in maize after zein proteins (Krishnan *et al*., 1992). Glutelin was found to be 17% in both normal endosperm maize and soft endosperm maize (Bjarnason and Vasal, 1992). Misra *et al*. (1972) and Lin *et al*. (1997) indicated that glutelins have high lysine content (2%). Opaque-2 maize (50%) has considerably higher glutelin content than normal maize (35.1%). Konopka *et al*. (2007) suggested that the glutelin content was affected by the type of cultivar and the kernel size, and showed to decrease with a decrease in kernel size.

2.5.6 Amino acids

Amino acids are the building blocks of protein. The two most important amino acids found in maize are lysine and tryptophan. Amino acid composition differs greatly between species and genotypes (González-Pérez and Arellano, 2009). The low concentration of these amino acids is the main cause of poor nutritional quality in maize.

Lysine

Lysine is one of the primary amino acids found in maize. Nutritional deficiencies in animals whose diets rely heavily on maize are often caused by a shortage of lysine (Yu *et al*., 2004). Lysine content in normal maize is very low (Zhai and Zhang, 2007). However, it was found to be almost double in QPM. Zhai and Zhang (2007) reported lysine contents of 0.27% and 0.41% in normal maize and QPM genotypes, respectively. Moro *et al*. (1996) found the lysine content ranging from 1.6-2.6% and 2.7-4.5% in normal maize and homozygous recessive (*o2o2*) maize, respectively. Vasal (1999) indicated that in QPM hybrids the lysine content varies between 3.8% and 4.5%, while Hunter *et al*. (2002) found it to vary between 1.5% and 3.8% in normal maize genotypes. Lysine contents found in QPM inbreds CML 176, CML 177, CML 178, CML 181, CML 473, CML 490, CML 490, CML 491, CML 492, CML 493 were 3.18%, 3.16%, 3.27%, 3.01%, 3.08%, 3.29%, 3.29%, 3.15% and 3.26%. Lysine contents of 3.35%, 3.49%, 3.47%, 3.56% and 3.48% were found in open-pollinated QPM varieties Obatanpa, EV8766-SR, EV8363-SR, Pool 18-SR and Pool 19-SR, respectively. In addition, lysine contents found in pure QPM hybrids Mama-ba, CIDA-ba Dada-ba, ART98-SW6-OB, ART98-SW4-OB, TZPB-OB, ILEI-OB were 3.66%, 3.59%, 3.59%, 3.68%, 3.83%, 3.59%, 3.67% and 3.36%, respectively (Bello *et al*., 2012). Landry *et al*. (2000) observed a lysine content of 3.14%, 1.49% and 3.14% in tested normal genotypes. Misra *et al*. (1975) observed the lysine content to vary between 1.6% and 3.5% in normal maize hybrids. Azevedo *et al*. (2003) reported the lysine content ranging from 1.87% to 3.78% in normal maize genotypes. Lysine content in maize endosperm protein is considered to be one of the most important traits for determining the nutritional quality of food and feed (Yang *et al*., 2005). Efforts are made to increase the lysine content and total protein levels simultaneously (Yu *et al*., 2004).

Tryptophan

Tryptophan is one of the essential amino acids found in maize and is available in small quantities. Vivek *et al*. (2008) observed the tryptophan content to be 0.4% and 0.9% in normal maize and opaque-2 maize, respectively. Zhai and Zhang (2007) reported tryptophan content of 0.08% and 0.12% in normal maize and QPM genotypes, respectively. Vasal (1999) reported that tryptophan in protein varies between 0.90% and 1.10%. Tryptophan content of white and yellow QPM hybrids varies between 1% and 1.10%. However, in the white and yellow normal maize hybrids it varies between 0.55% and 0.60%. Tryptophan contents found in QPM inbreds CML 176, CML 177, CML 178, CML 181, CML 473, CML 490, CML 490, CML 491, CML 492, CML 493 were 0.60%, 0.79%, 0.63%, 0.71%, 0.63%, 0.52%, 0.56%, 0.67% and 0.46%, respectively (Bello *et al*., 2012). Tryptophan contents of 0.67%, 0.66%, 0.75%, 0.70% and 0.62% were found in open-pollinated QPM varieties Obatanpa, EV8766-SR, EV8363-SR, Pool 18-SR and Pool

19-SR, respectively (Bello *et al*., 2012). In addition, tryptophan contents found in pure QPM hybrids Mama-ba, CIDA-ba Dada-ba, ART98-SW6-OB, ART98-SW4-OB, TZPB-OB, ILEI-OB were 0.70%, 0.71%, 0.71%, 0.78%, 0.82%, 0.72%, 0.67% and 0.64%, respectively (Bello *et al*., 2012).

2.6 EFFECT OF STORAGE CONDITIONS ON CHEMICAL COMPOSITION AND QUALITY OF THE MAIZE KERNEL

Storage conditions have an influence on chemical composition and quality of a maize kernel and biochemical changes occur to various extents depending on the temperature and storage time (Rehman *et al*., 2002). The nutritional content of maize seeds can be maintained by decreasing storage temperature and relative humidity. The two factors in question, if not properly controlled, can cause a reduction in quality and chemical composition of the maize kernel. Seed deterioration is a complex phenomenon, but it is believed to be initiated differently under different storage conditions depending on the transition temperature of seed cytoplasm (Murthy *et al*., 2003). NRC (1988) reported that storage has the same effect on QPM and normal maize because of the endosperm hardness that is virtually the same. Rehman *et al*. (2002) reported that the nutritional quality of QPM and normal maize grains was adversely affected as a result of storage at high temperature. A noticeable symptom of seed deterioration is membrane degradation which results in solute leakage, enzyme activity, respiration and hormonal changes (Woodstock and Grabe, 1967; Roos, 1980; Copeland and McDonald, 1985). This is due to changes in phospholipids (Tatipata, 2009). Although biochemical changes in seeds have been observed, the possible causes are not yet clear (Anderson, 1970; Chauhan, 2011).

2.6.1 Minerals

Minerals are one of the important nutrient sources produced by cereals, particularly maize. However, many of the minerals may be lost when exposed to the unfavourable storage conditions. Different storage conditions such as high temperatures and relative humidity are some of the factors that may cause the level of minerals in maize to decline. However, a reduction in mineral composition was observed by Fagbohun *et al*. (2011), but it is believed that the decrease was facilitated by the mycoflora which were also placed with the seeds in the same container. Membrane deterioration as a result of change in phospholipids is the primary cause of deterioration (Copeland and McDonald, 1985;

Tatipata, 2009). Although the process of deterioration is not clear, it is believed that the changes occur as a result of inactive enzymes which lead to lipid peroxidation (Wilson and McDonald, 1986; Vertucci and Leopold, 1986).

Storage conditions may have a negative impact on Ca level. Ca content of sundried soybean seeds was reduced from 2.2 g/kg to 2.1 g/kg during 20 weeks of storage, but it is believed that the cultured mycoflora could have played a role in the decrease of Ca content (Fagbohun and Lawal, 2011). The Ca composition usually remains stable under very low temperature and relative humidity. Fagbohun *et al*. (2011) reported biochemical changes in the Ca content ranging from 5.9 g/kg to 2.4 g/kg in shelled melon seeds during 20 weeks of storage, but it is believed that the cultured mycoflora is the cause of this change.

Mg content, like Ca, is stable under low temperature and relative humidity during storage. It is only under extreme high temperature that the chemical composition changes. Sundried soybean seeds had Mg content dropping from 1.589 g/kg to 1.585 g/kg during seed storage, but the mycoflora which were placed in the same storage with the soybean seeds could have caused the changes (Fagbohun and Lawal, 2011). In contrast, Mg content was reduced from 59.1g/kg to 57.5 g/kg in shelled melon seeds during 20 weeks of storage, but it is believed that the cultured mycoflora is the reason behind this change (Fagbohun *et al*., 2011).

The chemical composition level of Na in maize kernel remains stable during storage, especially under suitable storage conditions. Fagbohun *et al*. (2011) observed biochemical changes in the Na content ranging from 27.1 g/kg to 24.7 g/kg in shelled melon seeds during 20 weeks of storage, but it is believed that the mycoflora played a role in the drop of Na content since they were placed in the same container with the seeds during storage. Similarly, Na content of sundried soybean seeds was reduced from 0.28 g/kg to 0.27 g/kg during 20 weeks of storage, but it is believed that the cultured mycoflora could have played a role in the decrease of Na content (Fagbohun and Lawal, 2011).

Changes in lipids destabilise the chemical composition level of K during storage (Vertucci, 1992). High fat content results in rapid deterioration of seed which in turn affects the chemical composition of K (Thomison, 2002). Fagbohun and Lawal (2011) found biochemical changes in K content ranging from 0.46 g/kg to 0.61 mg/kg in sundried soybean seeds during 20 weeks of storage, but this increase could be due to the inoculated mycoflora which were placed in the same storage with the soybean seeds. During 20 weeks of storage, K content had increased from 51.5 g/kg to 52.5 g/kg in shelled melon seeds, but it is believed that the mycoflora that were placed in the same container with the seeds could have played a role in the increase of K content (Fagbohun *et al*., 2011). However, K content of winter squash seeds stored in kraft paper, polyethylene bags, glass containers and aluminium foil for five years had increased under different storage conditions, and the increase was associated with low seed germination when the seeds were tested in the field for germination (Doijode, 2000).

Storage conditions may affect the chemical composition level of Fe. High fat content in maize kernels causes changes in Fe levels, especially when the kernels are exposed to unfavourable storage conditions (Thomison, 2002). Fe content of sundried soybean seeds during 20 weeks of storage was reduced from 0.072 g/kg to 0.065 g/kg, but it is believed that the cultured mycoflora could have played a role in the decrease of Fe content (Fagbohun and Lawal, 2011). Fagbohun *et al*. (2011) found biochemical changes in Fe content of shelled melon seeds ranging from 11.1 g/kg to 6.3 g/kg during 20 weeks of storage, due to the mycoflora that were placed in one container with seeds.

Changes in lipids destabilise the chemical composition level of Zn during storage (Vertucci, 1992). High fat content results in the rapid deterioration of the seed which in turn affects Zn content (Thomison, 2002). Fagbohun *et al*. (2011) found biochemical changes in Zn content of shelled melon seeds ranging from 7.5 g/kg to 6.3 g/kg during 20 weeks of storage, due to the mycoflora that were placed in the same container with seeds. Zn content of sundried soybean seeds was reduced from 0.06 g/kg to 0.08 g/kg during 20 weeks of storage, but it is believed that the cultured mycoflora could have played a role in the decrease of Zn content (Fagbohun and Lawal, 2011).

The amount of Mn decreases when seed is exposed to unfavourable storage conditions. This may be due to the fat content that changes during storage in the seeds (Vertucci, 1992) which in turn results in rapid deterioration of the seeds (Thomison, 2002). During 20 weeks of storage, Mn content of sundried soybean seeds was reduced from 0.014 g/kg to 0.013 g/kg, but it is believed that the cultured mycoflora could have played a role in the decrease of Mn content (Fagbohun and Lawal, 2011). Fagbohun and Faleye (2012) found that the Mn content of okra seeds dropped form 17.5 g/kg to 7.5 g/kg during seed storage, but the mycoflora which were placed in the same storage with the okra seeds could have caused the changes.

Changes in lipids destabilise the chemical composition level of P during storage (Vertucci, 1992). Fat content may result in rapid deterioration of seed which in turn affects P content (Thomison, 2002). Sundried soybean seeds had P content dropping from 5.9 g/kg to 5.6 g/kg during 20 weeks of storage, but it is believed that the mycoflora that were placed in one container with seeds could have played a role in the decrease of P content (Fagbohun and Lawal, 2011).

2.6.2 Fat

Maize seeds with high fat content are very susceptible to harsh storage conditions (Kausar *et al*., 2009). According to Braccini *et al*. (2000), under natural conditions, seed storage may cause reduction in fat content of soybean seeds. However, the fat content of brown rice remained stable during storage for 12 months at 5°C but decreased significantly during storage at 35°C (Shin *et al*., 1986). Arulnandhy and Senanayake (1991) observed significant decrease in fat content in deteriorated soybean seeds. The fat content in sundried soybean seeds dropped form 19.15% to 18.37% during seed storage, but the mycoflora which were placed in the same container with soybean seeds could have caused the changes (Fagbohun and Lawal, 2011). Sawazaki *et al*. (1985) found biochemical changes in fat content of bean seeds during 11 months of storage. The extended storage conditions resulted in decrease in fat content of *Dendrocalamus strictus* seeds (Ravikumar *et al*., 2002). The high fat content in maize kernels causes a rapid deterioration of chemical composition (Thomison, 2002). Sendeko (1975) observed that content of phospholipids in non-viable seeds of maize were reduced during storage. According to Black *et al*. (1969), storage of a wide range of fat-containing agricultural commodities including maize generally results in a drop in fat content. Changes in the fat constituents of seeds are believed to be associated with losses of seed viability during storage (Vertucci, 1992).

Grains undergo changes in fat content during storage as a result of the production of free fatty acids which break down fats (Serna-Saldivar, 2010). According to Balešević-Tubić *et al*. (2005), an increase in the content of free fatty acids and auto-oxidation of lipids during storage are the major causes of the rapid deterioration of maize kernels. The fat content of sunflower seeds were significantly influenced by storage conditions of temperature and relative humidity (Ghasemnezhad and Honermeier, 2009) which caused oil spoilage and increase of free fatty acid (Sisman and Delibas, 2004; Sisman, 2005). According to Sauer (1992), cereal grains including maize grain experiencing intrinsic deterioration during storage have shown to have a lower fat content. The deterioration of phospholipids composing the cell membrane causes successive increase of the permeability coefficient (Sotome *et al*., 2005). The residual lipids originating from the germ of processed maize lead to a rapid increase in fat acidity during storage and a dramatic change in texture (Nago *et al*., 1997). Fat deterioration during storage is a complex process, however, Mod *et al*. (1983) suggested that the oxidation of ferulate esters of hemicellulose contribute to cross-linking and increase strength of cell walls during seed storage. In contrast, Zhou *et al*. (2002), proposed that the release of free phenolic acids alters integrity of the cell wall and at the same time the phenolic acids exert an effect through their antioxidant activity on the formation of free fatty acids that can further aggregate with amylose during seed storage.

Lipoxygenase is thought to be a major contributor to lipid peroxidation and free-radical generation (Doijode, 2001). According to Wilson and McDonald (1986), seed storage exposes fat to constant attack by oxygen, causing hydroperoxides, oxygenated fatty acids and free radicals. The exact mechanism of seed deterioration is not clear. It is assumed that the fat content readily oxidizes, which deteriorates the original state of the kernel in storage (Wilson and McDonald, 1986). Vertucci and Leopold (1986) reported that the oxidative reaction occurs through non-enzymatic auto-oxidation. Nevertheless, fat degradation is a major cause of deterioration, releasing free fatty acids, which initiates oxidative deterioration by providing substrate for lipoxygenase, which primarily attacks membranes (Hailstones and Smith, 1988; Gidrol *et al*., 1989; Doijode, 2001; Sukesh and Chandrashekar, 2011). These deteriorative changes result in the loss of vigour (McDonald, 1976; Copeland and McDonald, 1995). Moisture content and oxygen during storage are the main causes for lipids autoxidation in soybean which result in loss of enzymatic activity and membrane integrity which in turn lead to rapid seed deterioration and declination of quality (Ching, 1973; Wilson and McDonald, 1992; Hartman *et al*., 1994; McDonald, 1999).

2.6.3 Enzymes

Enzymes are very sensitive to changes in storage temperature. They spontaneously decay during storage. Lipase and lipoxygenase are the two principal enzymes involved in degradation of lipids in seeds particularly during storage (Angelo and Ory, 1983). Amylase is responsible for initiating seed germination during malting, but its role is affected during storage (Subbarao *et al*., 1998). During spontaneous natural ageing, many enzymes including amylase and peroxidase become less functional (Dey and Mukherjee, 1986; Puntarulo and Boveris, 1990). According to Chauhan (2011), during the natural ageing of wheat seeds, the enzymes may undergo biochemical changes in content by losing or gaining specific functional groups or by conversion of amino acids within the protein structure.

During accelerated ageing, the activities of various enzymes such as peroxidase, dehydrogenase, and amylase decrease (Ravikumar *et al*., 2002; Goel *et al*., 2003; Kumar *et al*., 2011; Chauhan *et al*., 2011). Damaged grains have a higher concentration of enzymes when determined with free amino nitrogen assay (Serna-Saldivar, 2010). During six month storage period, the activities of both α-amylase and β-amylase were reduced (Desikachar *et al*., 1960; Daliwal *et al*., 1991). Peroxidase and catalase activities were also reduced during storage of rice seeds (Zhou *et al*., 2002). However, the activities of lipoxygenase, proteases and lipases increased during storage of rice seeds (Dhaliwal *et al*., 1991).

2.6.4 Starch

Unfavourable storage conditions cause the starch content to drop over time. However, the starch content in rice seeds was not affected by storage and remained unchanged during six month storage (Zhou *et al*., 2002). Daneke and Decker (1988) found starch to aggregate under ambient humidity conditions. They suggested that the aggregation of starch could have been avoided if the seeds were stored in a perfectly dry environment. According to Ravikumar *et al*. (2002), extended storage conditions result in decrease in starch content of *Dendrocalamus strictus* seeds. Sawazaki *et al*. (1985) found biochemical changes in starch content of bean seeds during 11 months of storage.

Environmental factors such as temperature and harvest date have been suggested to affect the properties of starch during storage (Yusuph *et al*., 2003; Noda *et al*., 2004; Tester *et al*., 2005). Recent studies have shown that there is no intrinsic long-term degradable loss in starch yield due to storage at either ambient conditions or at $4^{o}C$ until after 4 years (Eckhoff, 2004). Wuttisela *et al*. (2008) reported that starch association and crystallization on cooling and storage negatively influence preservation strength, resulting in contraction and the disappearance in kernel moisture. The endosperm of maize contains amylase which is the major enzyme that rapidly initiates the starch degradation process into soluble substrates for the other enzymes to further degrade starch (Beck and Ziegler, 1989). However, the mechanism of starch degradation in the maize endosperm is different from those in other plant tissues.

Amylose

Under normal storage conditions, maize amylose does not change in content. However, unfavourable storage conditions, such as high temperature, may cause some changes. The amylase enzyme is normally the enzyme that initiates the amylose degradation. During storage, the carbonyl groups of reducing sugars form complex intermediate compounds which may cause the activity of amylolytic enzyme leading to a reduction in amylose content (Marshall and Chrastil, 1992; Rehman *et al*., 2002). According to Strelec *et al*. (2010), amylose content of wheat varieties stored at various storage conditions was reduced.

Amylopectin

The chemical composition of amylopectin does not change under normal storage conditions. During storage, the carbonyl groups of reducing sugars associate to form complex intermediate compounds which may facilitate the activity of amylolytic enzyme leading to a reduction in amylopectin content (Marshall and Chrastil, 1992; Rehman *et al*., 2002).

2.6.5 Protein

Protein content, under normal storage conditions does not change. During 11 months of storage, the protein content of bean seeds did not change (Sawazaki *et al*., 1985). Bhullar and Jenner (1985) and Wilhelm *et al*. (1999) reported that maize protein content is less susceptible to heat stress, and generally accumulates under average heat conditions.

Under unfavourable conditions, seed storage may cause reduction in protein content (Braccini *et al*., 2000). Storage significantly influenced the protein content of sunflower seeds (Ghasemnezhad and Honermeier, 2009) due to increase in temperature and relative humidity which caused oil spoilage and increase in free fatty acid (Sisman and Delibas, 2004; Sisman, 2005). Arulnandhy and Senanayake (1991) observed significant decrease in protein content in deteriorated soybean seeds. During 20 weeks of storage, the protein content had increased from 40.94% to 42.33% in sundried soybean seeds, but it is believed that the mycoflora that were placed in the same container with seeds could have played a role in the decrease of protein content (Fagbohun and Lawal, 2011). According to Ravikumar *et al*. (2002), long periods of storage result in the decrease in protein content of *Dendrocalamus strictus* seeds.

Rehman *et al*. (2002) found that protein digestibility in maize decreased by 5.19% and 9.0% at 25^{o}C and 45^{o}C respectively, from an initial 77.0% during six months of storage. During storage, protein content of rice seeds was not affected by storage and remained unchanged during the entire six month storage period (Zhou *et al*., 2002). Although the mechanisms involved in the deterioration of protein during storage is not clear, Moritaka and Yasumatsu (1972) suggested that the formation of disulphide linkages from sulfhydryl groups together with an increase in the strength of micelle binding of starch, inhibits swelling of starch granules. They reported that lipids form free fatty acids, which can agglomerate with amylose and carboxyl compounds and hydroperoxides, which can accelerate protein oxidation and condensation plus accumulation of volatile carbonyl compounds.

Zein

Zein is one of the chemical components in maize that does not change in content under normal storage conditions. Under unfavourable storage conditions, zein content decreases with increase in storage time. During storage, the free amino acid groups of zein may associate to form complex intermediate compounds resulting in the activity of proteolytic enzyme which causes a reduction in zein content (Marshall and Chrastil, 1992; Rehman *et al*., 2002).

Albumins

During storage particularly at high temperature, albumin undergoes some structural changes resulting in proteolysis and amino acid solubility leading to a reduction in albumin content (Rehman *et al*., 2002). Albumin content of rice varieties was reduced during seed storage and the reduction was associated with poor cooking quality when the seeds were tested for cooking quality (Dhaliwal *et al*., 1991). Kapoor *et al.* (2011) observed decrease in content of albumins of five rice seed varieties during storage, and the decrease has been associated with lack of energy (Gidrol *et al*., 1998), protein denaturation and elevated superoxide dismutase activity (Kalpana and Rao, 1995).

Globulins

During storage particularly at high temperature, globulin results in structural changes which may affect the activity of the proteolytic enzymes which in turn results in the reduction of globulin content. Sawazaki *et al.* (1985) found biochemical changes in globulin content of bean seeds during 11 months of storage.

Glutelins

Maize storage conditions may affect the levels of glutelin. During storage particularly at high temperature, glutelin may undergo some structural changes which affect the activity of proteolytic enzymes which in turn results in the reduction of glutelin content. Chrastil (1990) reported that the molecular weight of glutelin increases considerably in rice during storage.

2.6.6 Amino acids

Essential amino acids require optimal storage conditions in order to keep their quality and content intact. Bad storage conditions pose a threat to amino acids during storage. Lysine and tryptophan are sensitive to extreme storage conditions. According to Ravikumar *et al.* (2002), long periods of storage result in increase in total free amino acid content of *Dendrocalamus strictus* seeds. Sukesh and Chandrashekar (2011) found that the total free amino acid content increased over time under sealed condition at 28°C. According to Sukesh and Chandrashekar (2011), the increased amino acid content signifies the breaking of proteins during storage.

Lysine

Lipids in the maize kernel affect the composition of the lysine during storage. At prolonged storage, lipids degrade which leaves the lysine vulnerable to external conditions such as heat and cold stress (Balešević-Tubić *et al*., 2005). During storage, lysine content rapidly deteriorated due to high fat content which formed complex matter with lysine, resulting in a drop in lysine content (Thomison, 2002). During storage, particularly at high temperature, the lysine may undergo some structural changes which result in proteolysis and amino acid solubility leading to a reduction in lysine content (Rehman *et al*., 2002).

Tryptophan

Maize fat causes the tryptophan content to change, more especially during storage. Changes in lipids during storage result in the rapid deterioration of the kernels (Vertucci, 1992; Thomison, 2002). At an extended storage period, fat content degrades which leaves tryptophan exposed to harsh environmental conditions (Vertucci, 1992) resulting in changes in tryptophan content.

2.7 FACTORS INFLUENCING SEED QUALITY DURING STORAGE

A number of factors influence the quality of seeds during storage. The three most important are temperature, relative humidity and moisture content (Chayjan and Esna-Ashari, 2010). During storage, ageing of the kernels at elevated temperature and high moisture conditions considerably influences biochemical metabolisms of lipids in the kernel (Suriyong, 2007). Seed quality largely depends on factors such as storage temperature, relative humidity, and moisture content (Jayas and White, 2003; Khaldun and Haque, 2009; Tatipata, 2009). The quality of maize kernels reaching the consumer depends on the characteristics of the kernels at harvest time, handling of the harvested seeds, storage conditions and processing technology (Kigel, 1999).

2.7.1 Temperature

During storage, high temperature may reduce the lifespan of the grain. Low temperature is very effective in preserving seed quality in that it enhances the period of longevity of seeds (Desai, 2004). Alabadan (2006) observed that temperature is one of the most important factors influencing the quality of grain during storage and also the main factor that regulates the pest insect populations in stored grain. Maize kernels normally must be dried first before storage to reduce moisture level in the seed. Ideal maize kernel drying temperatures will differ depending on the cultivar, harvest moisture, ecological conditions but generally range from 35°C to 45°C (Smith *et al*., 2004). Agrawal *et al*. (1998) also recommended that drying temperatures for maize seed range from 35°C to 46°C. Seed deterioration is more at 5°C than at -18°C and -196°C (Doijode, 2001). Seeds can be preserved at room temperature and remain viable for 12 months. They must be kept in sealed plastic, metal or glass containers in cold chambers at low temperatures to maintain their quality longer during storage (Karaboon *et al*., 2005).

2.7.2 Relative humidity

Relative humidity is the amount of moisture in the air. It has the most influence on seed longevity because it affects seed moisture content (Herbage Seed Unit, 1994). High relative humidity causes the development of microbial activities that lead to seed decay. A suitable relative humidity for a perfect storage depends on the type of kernel, length of storage period and ambient temperature (Herbage Seed Unit, 1994). A relative humidity of 50% is ideal to keep maize kernel quality stable for a period of more than 12 months (Smith *et al*., 2004). Storage of maize grain in a high relative humidity environment should be avoided in order to prevent deterioration of quality. Maize kernels exposed to extreme conditions of relative humidity (100%) during storage are vulnerable to extremely pronounced peroxidative lipid degradation (Balešević-Tubić *et al*., 2005).

2.7.3 Moisture content

Seed moisture is the amount of water in the seed and is critical during storage. Grain moisture content is one of the most important factors that influence storage period of grain and the entire health status of the grain (Volenik *et al*., 2007). Moisture content is the most common significant factor associated with colourisation in maize grains during storage, even at low temperature (Lacey and Magan, 1991). Somado *et al*. (2006) reported that seed moisture content is a key factor that determines storability of germplasm preserved in seed genebanks. Low moisture content enhances the period of longevity of the seeds (Desai, 2004). Doijode (2001) reported that high seed moisture shortens the seed life and rapidly reduces viability during storage. High moisture content favours the development of microorganisms which cause rapid seed deterioration (Desai, 2004).

Seed moisture of less than 16% is ideal for storage of seeds (Doijode, 2001; George, 2009). Seeds exposed to cold storage (0-5^{o}C) need to be sealed in moisture-proof containers to prevent the influence of relative humidity on seed content which in turn may result in seed deterioration (Desai, 2004). Such conditions would be fairly appropriate to keep maize seed quality in storage for a period of one year or more (Smith *et al*., 2004). The moisture content of freshly harvested maize intended for long-term storage using bags must be low, to reduce the incidence of discoloration (Armah and Asante, 2006). Zhai and Zhang (2007) reported moisture content of 12.03% and 12.11% in normal maize and QPM genotypes, respectively to be ideal for storage. Nonetheless, maize can also be stored safely at 14% moisture content for 6-12 months (Dendy, 2001). However, for long term storage of more than a year, maize should be dried to below 13% (Bern *et al*., 2003).

2.8 MAIZE STORAGE

Seed storage enables the maintenance of germplasm (Modi, 2004). According to Maree (2008), the rationale of seed storage is to safeguard the planting stocks from one season to the next. The objective of seed storage is to maintain seed quality for the longest duration possible (Maree, 2008). According to Thamaga-Chitja *et al*. (2004), proper storage plays a vital role in stabilising food supply at the household level by ensuring seasonal food production. Successful seed storage is key to maize producers' seed security and may also enable rural people to make a living through selling and storing excess maize seeds (Wambugu *et al*., 2009).

The efficiency of maize storage is dependent on limited storage losses, duration of storage and storage volume (Thamaga-Chitja *et al*., 2004). Seeds must be stored under optimal conditions to ensure that they maintain quality in accordance with the market demands (Takavarasha and Rukovo, 1989; Santos *et al*., 2010). Maintaining seed quality during storage is necessary as far as food security is concerned. Doijode (2001) and Pascual *et al*. (2006) reported that seed quality can be retained during storage, but it does not improve.

Seed deterioration can be measured by the germination percentage and it is a clear indication of poor viability and vigour (Khaldun and Haque, 2009). The declination of viability with high moisture content is related to the hygroscopic nature of kernels, especially under high temperatures, which in turn is associated with the relative humidity

(Delouche *et al*., 1973). The value of kernels can decline during storage resulting in grain that is not usable for planting the crop in the following year (Zielinski and Mos, 2009).

Cleanliness and good organization of the stored seeds are important for efficient maize seed storage (Smith *et al*., 2004). According to Joao Abba and Lovato (1999), suitable and safe storage conditions are described as those that maintain kernel quality without loss of vigour for a period of three years. Storage longevity, particularly for maize, may vary from six up to 20 months or longer if the seeds are to be carried over (Šimić *et al*., 2005). Dry, cool conditions are best for longer storage (Doijode, 2001). Agrawal *et al*. (1998) reported that under normal storage conditions, maize kernels can be preserved for up to 24 months and retain acceptable levels of germination and vigour, but normally maize seed has to be stored from harvesting to the next planting generally about seven to nine months (Mettananda *et al*., 2001).

2.9 STORAGE TECHNOLOGY

Storage technology is aimed at preserving maize quality without excessive storage losses. A seed is a living organism and therefore it respires, hence it needs to be stored in an environment that is conducive to secure the nutritive value of the seed. Deterioration is a natural phenomenon in living organisms such as maize seeds and it can be reduced through the use of appropriate storage technology (Doijode, 2001). Rising consumer health consciousness and the mounting demand for food with high quality, stimulated researchers to come up with strategies to improve the maintenance of good quality food during storage (Chanvrier *et al*., 2007). Different traditional and commercial storage methods are available for maize grain storage, but it is important to choose a method that is cheap and efficient. A method that successfully keeps the stored grain stable for a long time is usually expensive.

The general problem associated with storage methods is that they do not totally eliminate quality deterioration of the seed and subsequent storage losses (Okereke and Nwosu, 1987). Losses will always be a limiting factor to maize during storage. One major problem associated with storage is a lack of space. Commercial farmers require huge storage facilities such as modern maize silos to store all of their seeds. However, this system of storage does not eradicate all of the problems associated with storage. One method that can be used is cooling. However, lowering the temperature and relative humidity has some consequences, in that, as the temperature and relative humidity drop, the cooling coils freeze up. Lack of knowledge with regard to the various storage systems used by smallholder farmers is a serious concern despite the importance of maize in South Africa. The major storage problems reported by over 85% of the farmers surveyed include uncertain returns from storage as a result of future price unpredictability, lack of working capital to construct cribs and store maize, and physical losses of stored maize (Armah and Asante, 2006).

Attempts have been made to slow down the rate of seed deterioration in storage although some methods proved to be costly (Adebisi *et al*., 2004; Daniel *et al*., 2009). The cooling methods are too expensive to operate and maintain, but are very effective, when it comes to arresting seed deterioration. Daniel *et al*. (2009) reported that low-input storage methods that can reduce the operational costs and technological sophistication associated with cooling will benefit seed storage operations in humid environments if appropriate procedures are used and standardized. Implementing standardized seed storage for commercial seed operations or for genebanks faces major restrictions in Africa (Daniel, 2007). The storage of maize seeds in Africa is a serious problem largely caused by the inadequate facilities and poor methods for seed storage applied by rural farmers (Wambugu *et al*., 2009). Méndez-Albores *et al*. (2003) stated that the efficient maintenance of grain quality is not possible due to the lack of proper post-harvest technology including storage structures.

Maize grain can be stored in a number of ways. However, despite significant advances in seed storage methods, many African and South African communities still heavily rely on conventional storage methods to preserve the maize kernels (Olakojo and Akinlosotu, 2004; Thamaga-Chitja *et al*., 2004). Fantinatti and Usberti (2007) reported that relevant methods that provide excellent environment for seed storage are not many.

2.9.1 Maize silos

Maize silos are usually used for commercial storage, although they are not popular in developing countries (Dendey, 2001). Commercial farmers require commercial storage facilities with huge space such as commercial maize silos in order to store all their harvested grain. They have so far proved to be the most efficient method for storing commercial maize. However, operational costs for the cooling systems to maintain the condition of the stored seeds stable are very expensive. According to Smith *et al*. (2004), seed conditioning facilities usually possess various bulk storage capacities, but this frequently proves to be insufficient when huge amounts of seed must be preserved for a long period of time. Air conditioning does not bring relative humidity to acceptable levels required for successful seed storage because of the cooling coils that freeze up.

2.9.2 Crib storage

Crib storage is very easy to construct and economic and very popular to the farmers, particularly small-scale farmers (Hossain *et al*., 2003; Cephas, 2008). Cribs can be constructed with farm materials, sawn wood or metal. Crib storage for maize is gradually gaining ground, particularly in southern Nigeria (Okereke and Nwosu, 1987). Crib storage in Nigeria is widely used to store maize cobs, particularly in the humid and dry tropics (Cephas, 2008). During crib storage stored maize continues to dry as a result of the natural ventilation which is dependent on the design of the crib (Cephas, 2008). Storing seeds in cribs has some shortcomings in that the method provides little protection against pests (Cephas, 2008). Previous approaches to maize preservation relied on the use of chemicals when storing maize in cribs (Yakubu, 2010). Dales and Golob (1997) reported that maize was stored on farms after harvest by applying permethrin dust at a rate of 50 g of 0.5% dust per 90 kg grain to avoid insect infestation. Application of chemicals to the stored maize does not eradicate all problems associated with storage. The costs of chemicals are too high to afford. In contrast, the chemicals may get rid of the pests, but may not prevent biochemical changes during storage. According to Modi (2004), some smallholder farmers in South Africa rely on the use of smoke as a traditional method of preserving the seed, and the method has yielded positive results when it comes to maintaining seed quality.

2.9.3 Metal tanks and baskets

Subsistence farmers usually store maize in sacks, clay pots, jars, baskets or metal tanks (Thamaga-Chitja *et al*., 2004). A survey conducted by Thamaga-Chitja *et al*. (2004) in northern KwaZulu-Natal, South Africa, showed that subsistence farmers used traditional silos (*inqolobane*), metal tanks and sacks as rural maize storage methods. The technology involves storing the dry maize in sealed containers to prevent the entry of pathogens that may reduce the quality of the grain. Storing seeds in metal containers can limit movement of moisture from an ambient atmosphere into the seed better than other containers like sacks (Huda, 2001). According to Khaldun and Haque (2009), seed kept in metal containers has the lowest moisture content followed by polythene bags, earthen pots, jute bags and bamboo bins. These technologies have proved to be unsuitable as they cannot prevent or delay the natural deterioration of the seed. Woven baskets are constructed from sisal and woven river reeds to provide airtight storage and are mainly used in the Limpopo province to store maize (Thamaga-Chitja *et al*., 2004).

Traditional methods of storage are not suitable when it comes to preserving maize seeds without significant storage losses (Thamaga-Chitja *et al*., 2004). None of the traditional methods can be practiced on a large scale. Storing seeds through the use of smoke can be practiced on a small-scale. On the other hand, storing seeds in pots, jars, metal tanks, and baskets with a view to maintaining quality is a short-term solution to subsistence farmers who produce small quantities of maize on very limited land.

2.9.4 Bags and sacks

Bags made of polyethylene have traditionally been used to store maize seeds. According to Agrawal *et al*. (1998) and Smith *et al*. (2004), maize seeds are typically stored either in bulk or bagged. Maize storage in bags was the most widely used method among many small-scale farmers (Udoh *et al*., 2000). In developing countries, particularly Pakistan, maize grains are preserved in jute bags and earthen pots under unfavourable temperature and moisture conditions (Rehman *et al*., 2002). The silo bag is a hermic storage system constructed from high density polythene that reduces the mass transfer between the intergranular gas phase and the external environment (Santos *et al*., 2010). The hermic environment reduces oxygen build-up due to the respiration of the maize kernels and microorganisms, which then inhibits the development of aerobic fungi and can eradicate insect infestations (Donahaye *et al*., 1991; Sinha, 1995). Polyethylene bags are effective in maintaining high viability at 50^{o}C and could be used for seed storage (Doijode, 1997). However, storing seeds in bags such as polyethylene bags is not good for commercial purposes. Soybeans lasted for ten months using vacuum plastic bags with a relative humidity of 8% (Tatipata, 2009). A research trial done on rice seed storage has shown that poly-sacks are suitable if seed moisture content is high (Abeysiriwardane, 1985). In contrast, a study done on maize storage demonstrated that poly-sacks were effective only under cold room conditions (Mettananda *et al*., 2001). Under room temperature, germination of maize kernels stored in poly-sacks had viability below acceptable levels after six, eight and 12 months of storage (Mettananda *et al*., 2001).

2.9.5 Dry storage

Seed drying involves decreasing moisture content of the seed using drying technique that is not harmful to its viability (Sastry *et al*., 2007). Somado *et al*. (2006) reported that drying seeds with the aim to decrease their moisture content to a level that prolongs shelf life during storage in seed genebanks is very convenient hence increases the regeneration intervals. Considerable research efforts in many countries around the world are focused on dry storage (Somado *et al*., 2006). Stern (1991) indicated that kernels may maintain their viability for several years if stored under dry conditions. The major problem associated with dry storage is that drying temperatures above 43^{o}C are very harmful to the viability of the seeds (Muckle and Stirling, 1971). According to Chulze (2010), it is important to select injury-free seeds for storage and to avoid damage before and during drying, and during storage.

2.10 ANALYTICAL METHODS

Analytical methods are very important when it comes to evaluating the nutritional quality and composition of the grain. A number of methods are used to determine the contents and the chemical composition of maize. Some of the methods are high performance liquid chromatography (HPLC), reversed phase-HPLC, size exclusion-HPLC and atomic absorption spectroscopy. The nutritional content of the grain is dependent on the type of the method used. During amylose/starch and protein analyses, some analytical methods fail to determine the exact content of starch or protein due to the inefficiency to predict starch or protein that has undergone some changes. Gérard *et al*. (2001) and Zhu *et al*. (2008) found different amylose content in the same sample using SE-HPLC, concanavalin A, differential scanning calorimetry and iodine binding capacity methods. This observation says a lot about the reproducibility of some of the analytical methods.

2.10.1 High performance liquid chromatography

HPLC is used to analyse many chemical compounds, including zein proteins and vitamins (Coulibaly and Chen, 2011). It is regarded as a relevant method specifically designed for analysing cereal proteins due to its sensitivity, precision, and specificity (Rodriguez-Nogales, 2006). HPLC is an instrumental form of liquid chromatography that employs stationary phases consisting of small particles, thereby achieving more efficient separations than those used in conventional liquid chromatography (Moreno-Arribas, 2003). Moreover, HPLC methods employ a variety of strategies such as the application of different types of stationary phases, different compositions of the mobile phase and a wide variety of selective detectors, making it more suitable for the determination of peptides and proteins (Rodriguez-Nogales, 2006). HPLC permits separation of various kinds of compounds within a shorter period than conventional chromatography (Batey and Curtin, 1996). It has many advantages over traditional methods of analysis in particular in relation to speed, sensitivity and selectivity (Pozo *et al*., 1990).

2.10.2 Reversed phase-high performance liquid chromatography

Reversed phase-HPLC (RP-HPLC) is a liquid chromatography technique that can be used to analyse zein protein. RP-HPLC has largely been employed to determine the relationship between zein classes and kernel texture (Dombrink-Kurtzman and Bietz, 1993; Dombrink-Kurtzman, 1994; Eyherabide *et al*., 1996; Mestres and Matencio, 1996; Robutti *et al*., 1997). It has been widely used in cereal chemistry for the extraction of cereal proteins and has proven to be a reliable technique for the qualitative and quantitative investigation and isolation of gliadin and glutenin in wheat (Paulis and Bietz, 1986; Wiesser *et al*., 1994). RP-HPLC is superior to most other methods in speed, sensitivity, reproducibility and suitability for quantification. According to Bietz (1990), it offers excellent reproducibility and due to its speed, it can automatically analyse multiple samples within a short time. It resolves proteins primarily on the basis of differences in surface hydrophobicity. Lasztity (1996) reported that RP-HPLC complements other chromatic and electrophoretic methods since it fractionates proteins by surface hydrophobicity.

RP-HPLC provides unique profiles that are mainly characteristic of genotype (Smith and Smith, 1986). According to Wilson (1991), RP-HPLC separates maize alcohol-soluble proteins into four groups: peaks 1, 2, 3 and 4. Peaks 1, 2 and 3 are alcohol soluble glutelins and the multiple peak area 4 contains the major zeins (Paulis and Bietz, 1986). Zein classes have been quantified, using RP-HPLC, and correlated to degree of hardness evaluated using different hardness measurements (Paulis *et al*., 1993; Pratt *et al*., 1995; Mestres and Matencio, 1996; Lee *et al*., 2006).

2.10.3 Size exclusion-high performance liquid chromatography

Size exclusion-HPLC (SE-HPLC) is a liquid chromatographic separation method which permits the analysis of oligomer and polymer range and is normally used for the separation of proteins in terms of their molecular weight (Rodriguez-Nogales *et al*., 2006). SE-HPLC is an important method for revealing quality differences and was developed to analyse zein proteins from other ethanol-based compounds such as zein from xanthophylls (Huebner and Bietz, 1999; Zhu *et al*., 2007). The technique of SE-HPLC is now used widely to estimate the relative amounts, and the apparent molecular weights of amylose and amylopectin in starch (Jackson *et al*., 1988; Blennow *et al*., 2001; Gérard *et al*., 2001; Meyer, 2004; Zhu *et al*., 2008; Cho *et al*., 2009). SE-HPLC coupled with refractive index, laser light-scattering and fluorescent labelling detectors has also been used to determine the molecular weight distribution of whole and debranched starch, and chain length distribution of amylopectin (Lehtonen, 1988; Bradbury and Bello, 1993; Yoo and Jane, 2002a,b; Millan-Testa *et al*., 2005; Charoenkul *et al*., 2006; Leong *et al*., 2007).

2.10.4 Atomic absorption spectroscopy

Atomic absorption spectroscopy (AAS) is an analytical method that has been widely used to analyse mineral elements such as K, Na, Fe, Mn and Zn (Lorenz *et al*., 1974; Walsh, 1977; Abbas *et al*., 2011). It is one of the three techniques for analyses and is the most widely used (Beaty and Kerber, 1993). AAS uses the absorption of light to measure the concentration of gas-phase atoms. The atoms absorb ultraviolet and make transitions to higher electronic energy levels. In many other studies, AAS has been used to determine amylose and P content, and has yielded good results within a short period of time (Pérez, 2000).

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CHAPTER 3

THE MINERAL COMPOSITION OF SOME SOUTH AFRICAN QUALITY PROTEIN MAIZE GENOTYPES

3.1 ABSTRACT

Maize seeds contain varying amounts of minerals which play an important role in nutrition of plants, animals and humans. The objective of this study was to determine the mineral composition of South African open-pollinated quality protein maize (QPM) varieties and QPM hybrids, compared with normal maize hybrids. The genotypes were obtained from the Agricultural Research Council (ARC), Potchefstroom. Analysis of variance (ANOVA) indicated that there were significant differences (p=0.01) among the maize genotypes, and there was large variation for mineral content in the 15 genotypes. QPM genotypes, in general, had higher K, P and Zn content than normal maize hybrids. For the other minerals the type of maize had no influence.

3.2 INTRODUCTION

Maize contains varying amounts of mineral elements. Each of these mineral elements, calcium (Ca), potassium (K), iron (Fe), magnesium (Mg), manganese (Mn) sodium (Na), phosphorus (P) and zinc (Zn) performs a vital role in the growth and development of maize plants (Battal *et al*., 2003). Zn plays an important role in protein and carbohydrate synthesis and takes part in metabolism regulation of saccharides, nucleic acid and lipid metabolism (Sajedi *et al*., 2009). Mg is required for many biological processes in the maize plant like photosynthesis and protein metabolism. Ca is an essential nutrient that plays a vital role in many enzyme-mediated processes. Fe is one of most important trace elements that play a vital role in the energy transfer within the plant and is also involved in vital metabolic functions as an instrinsic component of myoglobin and cytochromes (Connolly and Guerinot, 2002; Hemalatha *et al*., 2007). K strengthens maize stalks and reduces harvest losses (NJAES, 2004). Mn is involved in many plant functions including the activation of numerous enzymes concerned with carbohydrate metabolism and phosphorylation reactions (Severson and Shacklette, 1988).

A thorough knowledge of minerals in maize kernels is important to understand the use of maize for preventing malnutrition. Mineral analysis of maize kernels is also one of the most reliable tools for estimating fertilizer needs of plants (Hussaini *et al*., 2008). Of the mineral elements found in maize, P is generally high and a fair amount of Fe and Zn is readily available. However, many of these nutrients are lost during milling. All of the above nutrients can be easily added to maize flour or maize meal during the milling process, but this has cost implications. Feil *et al.* (2005) pointed out that breeding for higher concentrations of minerals in food crops is one option for improving the health of humans suffering from the consequences of mineral deficiency.

Maize is one of the most important sources of minerals, despite some of the mineral fractions being low. The majority of poor people in developing countries obtain minerals from maize. It plays an important role for people who consume it on a daily basis. Half of the world's population get their minerals from maize. A body that is deficient in minerals is very susceptible to all kinds of diseases that are related to growth. Higher protein quality was observed in quality protein maize (QPM) than in normal maize, but a consensus has not been reached that it is better than traditional maize varieties in all aspects of nutritional quality.

One of the reasons for research on maize quality is to release maize genotypes with satisfactory mineral yield for the maize consumers without any further supplementation from other sources of minerals. The establishment of a wide range of variation in maize genotypes could give many options to breed for high mineral content which should be sufficient to safeguard against kwashiorkor and malnutrition (Nuss and Tanumihardjo, 2011).

Chemical analysis of maize seed for mineral content has been used to reveal the shortage, sufficiency or excessive status of a range of mineral elements in a soil-plant system (Hussaini *et al*., 2008). The importance of nutritional quality of QPM and normal maize makes the analyses for mineral content and other characteristics desirable (Van Bruijnsvoort *et al*., 2001). Despite ongoing research with the aim of improving quality, little is known on whether QPM is superior in nutritional quality to normal maize with special reference to the mineral content. The environment under which these cultivars are produced could possibly have an effect on the levels of the mineral content (Hussaini *et al*., 2008). As a result, it is important to measure the mineral content of different genotypes grown under the same conditions. The aim of this study was to determine the mineral composition of South African open-pollinated QPM varieties and QPM hybrids, and to compare them with normal maize hybrids.

3.3 MATERIALS AND METHODS

3.3.1 Planting location

The trial was planted in Potchefstroom at the Agricultural Research Council (ARC)-Grain Crops Institute (26°74''S; 27°8'E) in November 2008. The ARC-Grain Crops Institute is located in Potchefstroom in the North West Province, South Africa and its mandate crops are diverse cereals grown in summer. Potchefstroom is located at an altitude of 1344 m above sea level with average minimum and maximum temperatures of 9.61^{o}C and 25.48^{o}C, respectively, and with an average annual total rainfall of 618.88 mm.

3.3.2 Plant material

The genotypes used in this study were eight South African open-pollinated QPM varieties (SYN2QYQPM, SYN4QYQPM, SYN11QYQPM, SYN13QYQPM, SYN2QWQPM, SYN5QWQPM, SYN12QWQPM and SYN15QWQPM), one QPM hybrid (QS7608) and six normal maize hybrids (CRW3505, C3505, CB341xI37F2, CB346xI37F2, P6479F2 and CB389xI37F2) obtained from the ARC-Grain Crops Institute, Potchefstroom.

3.3.3 Experimental design and procedures

The trial was planted for one season at a density of about 50 000 plants per hectare on sandy clay loam. The experimental design was randomised complete block design with three replications. The genotypes were grown in two-row plots. The rows were 5 m long with 0.25 m spacing apart, and row width was 75 cm. A compound fertilizer was applied at a rate equivalent to 300 kg per hectare of 3:2:1 (N:P:K) and then top-dressed with limestone ammonium nitrate (LAN) in order to avoid nutritional stress. Standard cultural practices including ploughing, disking, and application of herbicides were done at the site in order to make nutrients easily accessible to the genotypes and to minimise competition for nutrients between the planted genotypes and weeds as well as to reduce damage from insect pests. The trial was planted under rain-fed conditions. After harvesting, the seeds were dried to 12.5% moisture content and shelled with a stationary sheller. After shelling, a light table was used to confirm the status of the QPM genotypes.

3.3.4 Mineral extraction and analyses

The extraction steps of mineral elements (Ca, K, Fe, Mg, Mn, Na, P and Zn) were done according to the dry-ashing method outlined by the AOAC (2000). Maize seed samples were ground to a fine powder using a 1KA analysis grinder, A10 Yellowline (Merck Chemicals Pty Ltd) with a 1 mm sieve. Approximately 2 g of maize flour was then weighed into glazed, high-form porcelain crucibles and ashed in a furnace at 550^{o}C for 3 h. A few drops of nitric acid (HNO_3) (55%) were added to the samples for digestion. The samples were then placed in a hot sand-bath until they were completely dry, after which they were returned to the oven for 1 h at 550^{o}C for further ashing. After cooling, 10 ml of 1:2 HNO_3 was added to the samples for further digestion. The samples were placed back in a hot sand-bath until they became warm. The samples were then transferred to 100 ml volumetric flasks and filled to the mark with distilled water. Ca, K, Fe, Mg, Mn, Na, P and Zn content were measured in triplicate using an Atomic Absorption Spectrophotometer (Spectra AA 300).

3.3.5 Data analyses

Analyses of variance were carried out using Agrobase (2005). Principal Component Analyses (PCA) and the Pearson's correlation coefficient determination for the mineral elements were done using number cruncher statistical system (NCSS) software (NCSS, 2004).

3.4 RESULTS AND DISCUSSION

The mean squares for all measured minerals were highly significant indicating differences among genotypes (Table 3.1). The highest Ca contents were 29.50 mg/kg and 29.25 mg/kg for the open-pollinated QPM genotype SYN2QYQPM and normal maize hybrid C3505, respectively (Table 3.2). The lowest was 16.75 mg/kg and was observed in the open-pollinated QPM genotype SYN5QWQPM.

Table 3.1 Mean squares for measured minerals of maize samples

Soure	Ca	Fe	K	Mg	Mn	Na	P	Zn
Block	0.467	1.309	1280.443	49.072	0.138	0.657	613.750	0.040
Entry	62.446**	178.482**	1614908.425**	13953.764**	2.173**	55.009**	1104630.507**	33.720**

**P=0.01

Table 3.2 Mean values for the concentration of mineral elements in mg/kg (rank in parenthesis)

No.	Genotype	Type	Ca	Fe	K	Mg	Mn	Na	P	Zn
1	CRW3505	Normal hybrid	26.38(8)	48.28(1)	2935.00(9)	756.67(13)	7.50(9)	10.75(13)	2111.00(12)	23.00(13)
2	QS7608	QPM hybrid	27.25(6)	31.52(11)	4749.00(1)	979.00(1)	8.39(3)	15.50(9)	2828.77(6)	34.00(1)
3	C3505	Normal hybrid	29.25(2)	36.86(9)	3073.00(8)	843.30(3)	7.49(8)	18.25(6)	2742.66(8)	22.11(14)
4	CB341xI37F2	Normal hybrid	28.00(5)	26.41(14)	2730.00(10)	910.00(2)	7.25(11)	19.00(4)	1535.10(14)	21.69(15)
5	CB346xI37F2	Normal hybrid	23.67(10)	32.05(10)	2539.27(12)	748.33(15)	7.33(10)	17.50(8)	2046.54(13)	24.16(12)
6	P6479F2	Normal hybrid	28.50(4)	40.25(6)	2120.00(15)	760.00(7)	7.17(13)	13.50(11)	1352.77(15)	24.75(10)
7	CB389xI37F2	Normal hybrid	23.63(9)	46.25(4)	2171.88(14)	757.50(12)	8.17(5)	21.50(2)	2273.63(11)	26.50(9)
8	SYN2QYQPM	Open-pollinated QPM	29.50(1)	36.88(8)	2425.00(13)	765.00(4)	8.17(5)	19.50(3)	2841.25(5)	27.33(6)
9	SYN4QYQPM	Open-pollinated QPM	26.50(7)	46.75(3)	3525.00(5)	762.50(5)	7.17(13)	23.25(1)	2737.34(9)	29.94(2)
10	SYN11QYQPM	Open-pollinated QPM	22.00(11)	46.95(2)	3625.00(4)	761.67(6)	6.83(15)	15.00(10)	2771.00(7)	29.00(4)
11	SYN13QYQPM	Open-pollinated QPM	29.00(3)	26.13(15)	3242.58(6)	758.33(9)	7.58(7)	9.44(15)	2863.63(4)	27.50(7)
12	SYN2QWQPM	Open-pollinated QPM	17.00(14)	29.25(13)	3149.55(7)	760.00(7)	7.25(11)	11.38(12)	2571.00(10)	24.25(11)
13	SYN5QWQPM	Open-pollinated QPM	16.75(15)	31.50(12)	4068.75(2)	757.50(11)	9.33(2)	18.63(5)	3259.87(2)	29.75(3)
14	SYN12QWQPM	Open-pollinated QPM	18.75(13)	43.00(5)	2646.88(11)	755.00(14)	8.33(4)	18.31(7)	3212.88(3)	27.83(5)
15	SYN15QWQPM	Open-pollinated QPM	19.67(12)	39.69(7)	3762.50(3)	758.33(9)	9.83(1)	10.19(14)	3473.25(1)	27.50(7)
	Average QPM		19.66	36.85	3466.03	748.15	8.10	15.69	2951.00	28.57
	Average normal maize		26.57	38.35	2594.86	795.97	7.49	16.75	2010.28	23.70
	LSD (0.05)		1.24	1.98	39.52	12.41	0.52	1.19	34.80	1.34
	CV (%)		3.29	3.17	0.76	0.94	4.03	4.43	0.81	3.03
	Mean		24.39	37.45	3117.63	788.88	7.85	16.11	2574.71	26.62

The open-pollinated QPM genotypes SYN5QWQPM, SYN2QWQPM, SYN12QWQPM and SYN15QWQPM revealed a Ca content of 16.75 mg/kg, 17.00 mg/kg, 18.75 mg/kg and 19.67 mg/kg, respectively. The normal maize genotypes C3505, CB341xI37F2 and P6479F2 showed relatively higher Ca content of 29.25 mg/kg, 28.00 mg/kg and 28.50 mg/kg, respectively than the QPM genotypes (Table 3.2). However, one QPM genotype SYN2QYQPM had the highest Ca content, but normal hybrid C3505 ranked second. Some normal maize genotypes such as CB346xI37F2 and CB389xI37F2 had relatively low Ca content. The average value (26.57 mg/kg) of Ca for the normal maize genotypes was significantly higher than that of the QPM genotypes (22.94 mg/kg). The average mean for both QPM and normal maize genotypes supports earlier reports that maize generally contains low amounts of Ca (Loy and Wright, 2003; Hambidge *et al*., 2005; Blair, 2007). In addition, this average mean for these maize samples under study was lower than average mean of four maize genotypes assessed by Iken *et al*. (2002).

There were significant differences between the maize genotypes for Fe content. The Fe content ranged from 26.13 mg/kg to 48.28 mg/kg. The normal maize genotype CRW3505 showed significantly higher Fe content (48.28 mg/kg) than most other genotypes. The lowest Fe content (26.13 mg/kg) was observed in the open-pollinated QPM genotype SYN13QYQPM. Brkić *et al*. (2003) reported a range of 13.6-30.3 mg/kg for Fe among 28 maize crosses in a study of corn-belt inbred lines. Hemalatha *et al*. (2007) observed a low Fe content of cereals ranging from 1.32 mg/kg in rice to 6.51 mg/kg in sorghum in a study of Zn and Fe contents and their bio-accessibility in cereals and pulses consumed in India.

There was no pattern in terms of Fe values, and values seemed to be related to genotype rather than to the type of maize. That is, some QPM genotypes showed higher Fe content than the normal maize while other normal maize genotypes showed higher Fe content than some QPM genotypes. Generally the average Fe value (38.35 mg/kg) for the normal maize genotypes was similar to that of the QPM genotypes (36.85 mg/kg), however, they were all higher than those reported by Brkić *et al*. (2003) and Hemalatha *et al*. (2007). Nonetheless, the average Fe content for both QPM and normal maize samples was very low compared with average Fe content observed in four maize genotypes by Iken *et al*. (2002).

The QPM hybrid QS7608 and the open-pollinated QPM genotype SYN5QWQPM had the highest K content of 4749 mg/kg and 4068.75 mg/kg, respectively (Table 3.2). The normal maize genotype C3505 had a relatively high K content of 3073 mg/kg compared with other genotypes. The QPM K content was generally higher than that of normal maize. Normal maize ranked in the last four positions for K content. The K content ranged from 2120.00 mg/kg to 4749.99 mg/kg. The lowest K content (2120.00 mg/kg) was observed in normal maize genotype P6479F2. The average K content for both QPM and normal maize samples was very high compared with average K content observed by Iken *et al*. (2002).

There were significant differences among the maize genotypes in terms of K content. Seven QPM genotypes topped the rankings although two QPM genotypes were outperformed by some normal maize genotypes. The average value (3466.03 mg/kg) for the QPM genotypes suggests that the K content of the QPM genotypes was significantly higher than that of the normal maize genotypes (2594.86 mg/kg).

There were significant differences in Mg content among the QPM and normal maize genotypes. The highest Mg content (979.00 mg/kg) was observed in the QPM hybrid QS7608 (979.00 mg/kg) followed by the normal maize genotype CB341xI37F2 with 910.00 mg/kg. The lowest Mg content (748.33 mg/kg) was observed in the normal maize genotype CB346xI37F2. Brkić *et al*. (2003) observed the lowest Mg content of 1019 mg/kg in maize lines. They reported Mg content ranging from 1019 mg/kg to 1466 mg/kg. The average Mg content for both QPM and normal maize samples was very high compared with average Mg content observed in four maize genotypes by Iken *et al*. (2002). In addition, the average value (795.97 mg/kg) for the normal maize genotypes suggests that the Mg content of the normal maize genotypes was somewhat higher than that of the QPM genotypes (784.15 mg/kg). There was no pattern in the rankings for Mg content, and it seems that Mg content was determined by genotype rather than by type of maize.

The open-pollinated QPM genotypes SYN15QWQPM, SYN5QWQPM and SYN12QWQPM and the QPM hybrid QS7608 ranked in the first four positions for Mn content. The open-pollinated QPM genotype SYN11QWQPM ranked last (Table 3.1). The Mn content ranged from 6.83 mg/kg to 9.83 mg/kg. The highest Mn content (9.83

mg/kg) was observed in the open-pollinated QPM genotype SYN15QWQPM. The lowest Mn content (6.83 mg/kg) was seen in the open-pollinated QPM genotype SYN11QYQPM. Similar results have been reported by Brkić *et al*. (2003) where the Mn content ranged from 6.38 mg/kg to 11.01 mg/kg in 28 maize inbred lines. However, the average Mn content for both QPM and normal maize samples was very low compared with average Mn content observed in four maize genotypes by Iken *et al*. (2002). In addition, the average value (7.49 mg/kg) for normal maize genotypes suggests that the Mn content of the normal maize genotypes was significantly lower than that of the QPM genotypes (8.10 mg/kg).

The open-pollinated QPM variety SYN4QYQPM had the highest Na content. The Na content ranged from 9.44 mg/kg to 23.25 mg/kg. The lowest Na content was observed in the open-pollinated QPM genotype SYN13QYQPM. The average Na content for both QPM and normal maize genotypes was high compared with those reported in the literature, hence contradicting earlier reports that maize is very deficient in Na (Blair, 2007). Generally, the average value for the normal maize genotypes (16.75 mg/kg) suggests that the Na content of the normal maize genotypes was somewhat higher than that of the QPM genotypes (15.67 mg/kg).

The QPM genotypes had higher P content than other maize genotypes. The QPM genotypes ranked in the first seven positions regarding P content (Table 3.1). The P content ranged from 1352.77 mg/kg to 3473.25 mg/kg. Of the different P contents observed in both QPM and open-pollinated QPM genotypes, SYN15QWQPM had highest content, and total mean of P suggests that P is generally high and therefore supporting earlier findings that maize generally has a high P content (Blair, 2007). This value was significantly higher than for all the normal hybrids. In addition, Šimić *et al*. (2009) observed a high P content of 3190 in grain of 294 F4 maize lines and two parent lines, compared with an average content under study. The lowest P content was observed in the normal maize genotype P6479F2. However, the average P content for both QPM and normal maize samples was very high compared with average P content observed in four maize genotypes by Iken *et al*. (2002). In addition, the mean for the normal maize genotypes was 2010.28, and 2950.10 for the QPM genotypes, indicating a significant difference.

The Zn content ranged from 21.69 mg/kg to 34 mg/kg. QPM genotypes ranked in the first eight positions for Zn content (Table 3.1). The QPM hybrid QS7608 and open-pollinated QPM varieties SYN4QYQPM, SYN11QYQPM and SYN5QWQPM yielded high Zn content of 34.00 mg/kg, 29.94 mg/kg, 29.00 mg/kg and 29.95 mg/kg, respectively, among the measured QPMs. The Zn content (34.00 mg/kg) observed in the QPM genotype QS7608, was significantly higher than that of all the normal hybrids. The normal maize genotypes CB346xI37F2 and P6479F2 had Zn content of 24.16 mg/kg and 24.75 mg/kg, respectively, compared with other normal maize genotypes. The lowest Zn content (21.69 mg/kg) was observed in the normal maize genotype CB341xI37F2. Brkić *et al*. (2003) reported Zn content which ranged from 16.0 mg/kg to 23.6 mg/kg in 28 maize inbred lines. Hemalatha *et al*. (2007) observed low Zn content of cereals ranging from 10.8 mg/kg in rice genotypes to 22.4 mg/kg in sorghum genotypes in a study of Zn and Fe contents and their bio-accessibility in cereals and pulses consumed in India. The average Zn value (28.57 mg/kg) for the QPM genotypes was significantly higher than that of the normal genotypes (23.70 mg/kg). In addition, the average Zn content for both QPM and normal maize samples nearly matched the average Zn content observed in four maize genotypes by Iken *et al*. (2002).

Generally, the variation observed between the genotypes in terms of the mineral composition could have been caused by the genetic potential of each cultivar (House, 1999; Oikeh *et al*., 2003). That is, some genotypes could have been favoured by their genetic ability to obtain minerals from the soil. Onwueme (1978) and Guchhait *et al*. (2008) reported cocoyam accessions which produced varying mineral composition due to their differences in genetic potential of each cultivar to obtain minerals from the soil.

There were highly significant positive correlations between K and Zn, and P and Mn, meaning (Table 3.3). Highly significant positive correlations between K and Zn mean that maize varieties with high K content also had high P content. Similarly, highly significant positive correlations between P and Mn mean that maize varieties with high P content also had high Mn content. In addition, significant positive correlations were observed between P and Zn, and P and K.

Table 3.3 Pearson's correlation coefficients for the eight mineral elements extracted from the tested 15 maize genotypes

Mineral element	Fe	Zn	Mn	Na	Ca	K	Mg
Zn	0.09						
Mn	-0.08	0.40					
Na	0.21	0.13	-0.06				
Ca	-0.05	-0.19	-0.42	0.10			
K	-0.19	0.68**	0.37	-0.18	-0.24		
Mg	-0.41	0.17	0.00	0.12	0.38	0.43	
P	0.05	0.58*	0.64**	-0.05	-0.46	0.59*	-0.14

*P≤ 0.05,** P≤0.01

The first, second and third principal components (PC1, PC2, and PC3) in the analysis accounted for 74% of the total variability, while PC1 and PC2 alone accounted for 36% and 22% of the variability, respectively (Table 3.4). PC1 variation was mainly caused by P, K, Zn, Mn and Mg. PC2 variation was mainly caused by Mg, Fe, Ca and K. PC3 analysis explained 15.79% of variation, largely caused by Na and Fe. In general eigen vectors of the PC1 had the largest negative values for P, K, Zn and Mn and a large positive value for Ca. The smallest positive values also existed for mineral elements such as Fe and Na. Eigen vectors of the PC2 had the largest positive value for Mg and a large negative value for Fe. Similarly, PC3 had the largest negative value for Na. The smallest negative values were also shown for mineral elements such as Fe, Zn, Ca, and Mg. In general, there was no mineral element that showed dominance in all three PC analyses.

Table 3.4 Principal component analyses depicting eigen values and eigen vectors for the eight mineral elements

Mineral element	Eigen vectors		
	PC1	PC2	PC3
Fe	0.04	-0.47	-0.49
Zn	-0.45	0.11	-0.36
Mn	-0.44	-0.09	0.09
Na	0.06	-0.00	-0.74
Ca	0.32	-0.40	-0.24
K	-0.47	0.31	0.00
Mg	-0.05	0.69	-0.14
P	-0.51	-0.16	-0.03
Eigen value	2.88	1.75	1.26
Individual%	36.01	21.90	15.79
Cumulative%	36.01	57.90	73.70

The first three PC’s, which accounted for 74% of the total variation in kernel mineral concentrations, stratified the genotypes into four groups based on differences in their grain mineral compositions. The grouping of the genotypes and the mineral elements in the same quadrant implies that the genotypes have similar amounts of a particular mineral which appears in the same quadrant (Figs. 3.1 and 3.2).

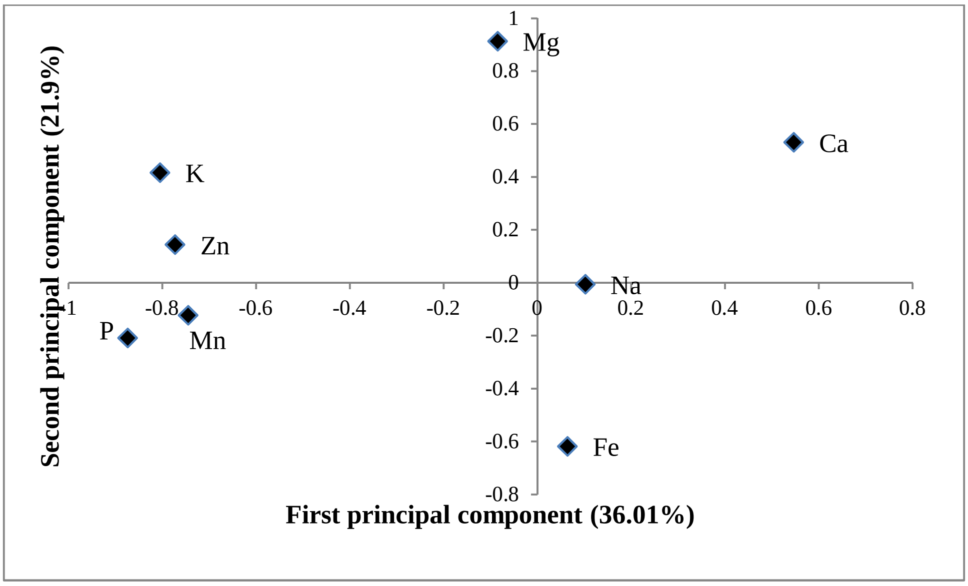

Figure 3.1 Principal component analysis showing variation of mineral elements

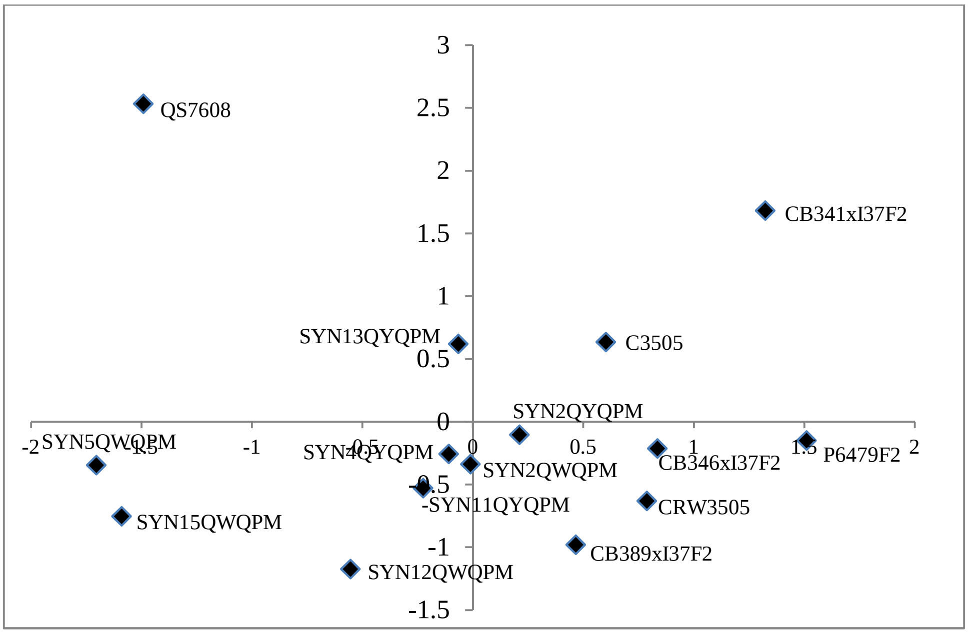

Figure 3.2 Biplot depicting the distribution of 15 maize genotypes for mineral content

The normal maize genotypes CB341xI37F2 and C3505 were grouped together in the first quadrant (Fig. 3.2). In the second quadrant, the QPM hybrid QS7608 and the open-pollinated QPM genotype SYN13QYQPM were grouped together. The open-pollinated QPM genotypes SYN5QWQPM, SYN4QYQPM, SYN2QWQPM, SYN11QYQPM, SYN15QWQPM and SYN2QWQPM were grouped together in the third quadrant. In the fourth quadrant, the normal maize genotypes CB346xI37F2, P6479F2, CWR3505 and CB389xI37F2 and the open-pollinated QPM genotype SYN2QYQPM were grouped together.

The normal maize genotypes CB341xI37F2 and C3505 had similar Ca concentration as they appear in the top-right quadrant (Figs. 3.1 and 3.2). The QPM hybrid QS7608 and the open-pollinated QPM genotype SN13QYQPM had similar K and Zn content as they appear in the top-left quadrant (Figs. 3.1 and 3.2). The open-pollinated QPM genotypes SYN4QYQPM, SYN11QYQPM, SYN2QWQPM, SYN5QWQPM, SYN12QWQPM and SYN15QWQPM had similar amounts of P and Mn as they were both found in the bottom-left quadrant (Figs. 3.1 and 3.2). The normal maize genotypes CRW3505, CB346xI37F2, P6479F2 and CB389xI37F2 and the open-pollinated QPM genotype SYN2QYQPM had similar Fe content as they both appear in the bottom-right quadrant (Figs. 3.1 and 3.2). The normal maize genotypes CB341xI37F2, C3505, CRW3505, CB346xI37F2, P6479F2 and CB389xI37F2 and the open-pollinated QPM genotype

SYN2QYQPM had similar Na content as Na appears in both top-right and bottom-right quadrants where both genotypes are also found (Figs. 3.1 and 3.2). The normal maize genotypes CRW3505, CB341xI37F2, CB346xI37F2, CB389xI37F2 and P6479F2 and the open-pollinated QPM genotypes SYN5QWQPM and SYN15QWQPM and the QPM hybrid QS7608 differed in mineral content since they are distant from each other on the biplot.

3.5 CONCLUSIONS

Generally, the normal maize genotypes were not necessarily lower in mineral content than QPM genotypes or vice-versa. Higher and lower mineral levels were observed in both normal maize and QPM hybrids. For instance, normal maize genotype CRW3505 (48.28 mg/kg) had higher Fe content than all QPM genotypes. In contrast, QPM genotype SYN5QWQPM (16.75 mg/kg) had lower Ca content than all normal maize genotypes. Therefore, it cannot be concluded that QPM genotypes had higher mineral contents than all normal maize genotypes or vice versa.

The results from this study indicate that there were significant differences among the maize genotypes studied. They indicate that genetic potential exists for the concurrent improvement of K and Zn, P and Mn, Zn and P, and K and P without lowering the concentrations of Na, Ca and Mg. They further suggest that increasing the concentrations of minerals in maize through breeding can help minimise the adverse effects of mineral deficiencies in humans particularly South Africans as there are many poor South Africans in rural areas who depend largely on maize as their most important source of minerals. This was one single trial, and this would have to be repeated over locations and seasons to make sound conclusions, but it nevertheless gave a good indication of trends.

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CHAPTER 4

THE STARCH AND AMYLOSE CONTENTS OF SOUTH AFRICAN QUALITY PROTEIN MAIZE AND NORMAL MAIZE HYBRIDS

4.1 ABSTRACT

Starch is the major constituent and principal carbohydrate of maize and it is composed of amylose and amylopectin. The ratio between these two components influences the industrial use of starch. The objective of this study was to determine the starch and amylose content of South African open-pollinated quality protein maize (QPM) varieties and hybrids, compared with normal maize hybrids. The genotypes were obtained from the Agricultural Research Council, Potchefstroom. Analysis of variance (ANOVA) on the data of the 15 maize genotypes showed significant differences among the QPM and normal maize genotypes for both amylose and starch content. The open-pollinated QPM variety SYNQWQPM had the highest amylose content and open-pollinated QPM variety SYN4QYQPM the lowest. The QPM genotype SYN2QWQPM had significantly higher starch content (66.20%) than the other genotypes. The genotype SYN4QYQPM had the lowest starch content (50.06%). This study indicated that the QPM genotypes did not yield starch and amylose content higher than the normal maize genotypes and that there was genetic variation for these characteristics in the tested material.

4.2 INTRODUCTION

Starch is composed primarily of branched glucans with an architecture that allows the formation of a semi-crystalline insoluble granule (Zeeman *et al*., 2007). Amylose is defined as a linear molecule of (1→4) linked a-D-glucopyranosyl units, but it is today well established that some molecules are slightly branched by (1→6)-a-linkages (Buléon *et al*., 1998). On the other hand, amylopectin has a branched structure with thousands of poly-glucose residues linked by α-D-(1→4) and α-D-(1→6) glycosidic linkages.

Maize serves as a very important source of starch producing approximately 60% of starch, which consists of approximately 25% amylose and 75% amylopectin (Anon, 2010). One of the newly emerging enhanced-value markets is for the production of high quality maize grain with high value of chemical compounds, including starch and amylose content (Paulsen *et al*., 2003). According to Cottrell *et al*. (1995) and Paulsen *et* *al*. (2003), the accumulation of chemical nutrients including starch and amylose content in the kernel is influenced by ecological growing conditions, and drying conditions. Wheat seeds exposed to water stress had low content of amylose and fat (Hurkman *et al*., 2003).

Maize genotypes vary widely in their starch and amylose contents, with both genetics and environment affecting the concentration of the compounds. Various starches including maize starches have been extensively studied and reported on (Chen *et al*., 2006). The majority of the starch within the seed is from sugars made by photosynthesis during the grain-filling period (Edwards, 2010). Van Twisk *et al*. (1976) reported that the starch yield of QPM does not vary greatly and can be compared favourably with the values obtained from normal maize. Gomez *et al*. (1990) indicated that QPM yielded starch and amylose amounts comparable to normal maize hybrids.

The production of maize cultivars with high amounts of starch and amylose or amylopectin (depending on the end use of the maize), as well as other desirable nutritional characteristics, is important. However, little is known about differences between normal maize and QPM with special reference to starch and amylose content. Maize starches have attracted particular scientific interests, because different amylose/amylopectin content can be used to manufacture renewable resources and various starch products (Chen *et al*., 2006; Liu *et al*., 2006; Chen *et al*., 2007; Corcuera, 2007). Measuring starch and amylose content is important to breeders focusing on high starch and amylose in their breeding programme (Polaske *et al*., 2005).

Maize breeders and agronomists have been striving to develop improved maize genotypes that are superior in various nutritional characteristics including starch and amylose content for a considerable time (Gajda *et al*., 2005). However, to date, there has been no study that compares normal maize and QPM in terms of starch and amylose content in South Africa. Thus, the aim of this study was to measure the starch and amylose content of South African open-pollinated and hybrid QPM varieties, and to compare them with some normal maize hybrids.

4.3 MATERIALS AND METHODS

4.3.1 Planting location

As described in Chapter 3 (section 3.3.1).

4.3.2 Plant material

As described in Chapter 3 (section 3.3.2).

4.3.3 Experimental design and procedures

As described in Chapter 3 (section 3.3.3).

4.3.4 Starch extraction and analyses

Starch content was determined according to the total starch assay procedure described by Megazyme International Ireland Limited (2009). The grain of freshly harvested materials was ground to a fine powder using a 1KA analysis grinder, A10 Yellowline (Merck Chemicals Pty Ltd) with a 1 mm sieve. A 100 mg sample of each entry was measured into a glass test tube (16x120 mm) and 0.2 ml of aqueous ethanol (80% v/v) was added to wet the samples to aid dispersion. The tube was stirred on a vortex mixer. Two ml of dimethyl sulphoxide (DMSO) was added immediately and the tube was stirred on a vortex mixer. After that, the tube was placed in a boiling water bath at 95^{o}C for 5 min. Three ml of thermostable α-amylase (300 U) was added in a 3-morpholinopropanesulfonic acid (MOPS) buffer (50 mM, pH 7.0) and the tubes were vigorously stirred on a vortex mixer. Sodium acetate buffer (4 ml, 200 mM, pH 4.5) was added to the tubes which were then incubated in a boiling water bath at 95^{o}C for 6 min. Amyloglucosidase (0.1 ml, 20 U) was added to the tubes before they were placed in a water bath at 50^{o}C for 30 min. The volume was adjusted from 7 ml to 10 ml with distilled water and then the tubes were centrifuged at 3000 rpm for 10 min. The duplicate aliquots (0.1 ml) of the diluted solution were transferred to glass test tubes (16 x 100 mm). Three ml of the glucose oxidase-peroxidase (GOPOD) reagent was added to each tube (including the glucose controls and reagent blanks), and the tubes were incubated at 50^{o}C for 20 min. A glucose control was prepared by mixing 0.1 m of glucose standard solution (1 mg/ml) and 3.0 ml of GOPOD reagent. A reagent blank solution was prepared by mixing 0.1 ml of water and 3.0 ml of GOPOD reagent. The absorbance was read in duplicate at 510 nm for each sample, and the glucose control was read against the reagent blank in duplicate. Total starch was measured as the glucose derived from hydrolysed starch and was expressed as a percentage of total sample weight on an “as is” basis:

Total starch = ΔA x F x FV/0.1 x 1/1000 x 100/W x 162/180 = ΔA x F/W x FV x 0.9

Where ΔA is the absorbance (reaction) read against the sample blank; F is a factor for the conversion from absorbance values to micrograms of glucose (100 µg of glucose/absorbance for 100 µg of glucose); FV = final volume; 0.1 = volume of sample analysed; 1/1000 is a conversion from micrograms to milligrams; 100/W = factor to express “starch” as a percentage of flour weight; W is the weight in milligrams (“as is” basis) of the flour analysed; and 162/180 is adjustment from free D-glucose to anhydro D-glucose (as occurs in starch).

4.3.5 Amylose determination

Amylose was extracted and estimated by the iodine binding method as described by Cruz and Khush (2000). One hundred milligram of maize flour sample was measured. The samples were wetted with 1 ml of 95% ethanol followed by 9 ml of 1 M Sodium hydroxide (NaOH) to aid dispersion and stirred using a vortex mixer. The samples were placed in a boiling water bath for 15 min and stirred using a vortex mixer every 5 min. The samples were cooled for 1 h at room temperature and centrifuged at 3000 rpm for 5 min. Duplicate 0.1 ml aliquots of the solution were transferred to clean test tubes and 0.1 ml of 1 M acetic acid was added to each test tube followed by the addition of 0.2 ml iodine solution and 9.6 ml distilled water. The contents were vortexed and left to stand for 20 min. The absorbance was read in duplicate against the reagent blank at 620 nm for each sample. The amylose percentage was calculated using the formula:

Amylose% = [Concentration (mg/ml) x 1000/mass of the sample (mg)] x 100

4.3.6 Data analysis

Analysis of variance was done using Agrobase (2005).

4.4 RESULTS AND DISCUSSION

The mean squares for genotype for both starch and amylose content were significant at P=0.05 (Table 4.1). The QPM genotypes contained slightly higher starch content than the normal maize whereas the normal maize contained a slightly higher amylose content than the QPM (neither difference was significant).

Table 4.1 Mean squares for measured characteristics of maize samples

Source	Amylose	Starch
Block	1.11	2.06
Entry	2.29*	2.23*

*P=0.05

The average amylose content of the tested maize genotypes ranged from 16.78% to 19.60%, but Batey and Curtin (1996) reported amylose content ranging from 25.3% to 30.2% in normal maize genotypes. This indicates that maize cultivars used in this study could contain low amylose content due to their type. In other maize nutritional value studies, the amylose content of maize ranged from 25% to 30% (Jane *et al*., 2010), a figure that is higher than that of the 15 maize genotypes used in this study, but lower than that of the 10 maize cultivars used in the study by Batey and Curtin (1996). The highest amylose content was 19.60% followed by 19.12% and were observed in the open-pollinated QPM genotypes SYN2QWQPM and SYN5QWQPM, respectively. The open-pollinated QPM genotype SYN4QYQPM yielded the lowest amylose content (16.78%) compared with both QPM and normal maize genotypes. The amylose content in both tested QPM and normal maize genotypes was different from genotype to genotype, proving that amylose content is not necessarily higher in QPM than in normal maize (Table 4.2).

The present values of amylose content in both QPM and normal maize genotypes with the highest being 19.60% were lower than those reported by Hasjim *et al*. (2009) in QPM genotypes which yielded 26% amylose. Similarly, the amylose content of normal maize genotypes obtained in this study were also not higher than those reported by Hernández-Uribe *et al*. (2004) and Chen *et al*. (2006) in normal maize genotypes (26.5% and 26.0%, respectively). However, the average amylase content for both QPM and normal maize genotypes nearly matched the amylose content of normal maize genotypes (21%) as reported by Wilson *et al*. (2004). In addition, the results obtained in this study, although little bit low, but they are in slight agreement with earlier reports and also in support of the findings of Sandhu and Singh (2007) in which amylose content in maize ranged from 16.9% to 21.3%.

Table 4.2 Means of amylose and starch content in maize genotypes (ranking in parenthesis)

No.	Genotype	Type	Amylose (%)	Starch (%)
1	CRW3505	Normal hybrid	17.62 (12)	50.06 (15)
2	QS7608	QPM hybrid	18.22 (7)	60.58 (8)
3	C3505	Normal hybrid	17.92 (9)	56.19 (13)
4	CB341xI37F2	Normal hybrid	18.73 (3)	62.64 (3)
5	CB346xI37F2	Normal hybrid	18.33 (6)	62.57 (4)
6	P6479F2	Normal hybrid	17.73 (10)	58.57 (9)
7	CB389xI37F2	Normal hybrid	18.18 (8)	62.56 (5)
8	SYN2QYQPM	Open-pollinated QPM	18.39 (5)	66.20 (1)
9	SYN4QYQPM	Open-pollinated QPM	16.78 (15)	63.21 (2)
10	SYN11QYQPM	Open-pollinated QPM	17.27 (13)	56.60 (12)
11	SYN13QYQPM	Open-pollinated QPM	17.72 (11)	58.56 (10)
12	SYN2QWQPM	Open-pollinated QPM	19.60 (1)	62.12 (6)
13	SYN5QWQPM	Open-pollinated QPM	19.12 (2)	60.80 (7)
14	SYN12QWQPM	Open-pollinated QPM	18.66 (4)	58.00 (11)
15	SYN15QWQPM	Open-pollinated QPM	16.97 (14)	53.15 (14)
	LSD (0.05)		1.47	8.19
	CV%		4.91	8.23
	Mean		18.10	59.91
	Mean for QPM		18.08	59.86
	Mean for normal hybrids		18.09	58.77

The maize amylose content is known to vary from cultivar to cultivar, but several studies have reported maize varieties with similar amylose content of about 26% which is higher than the results reported in this study. In fact, the majority of QPM genotypes were the ones with the amylose content of 26%, perhaps indicating that QPM could be having higher amylose content than normal maize hybrids as reported by Prasanna *et al*. (2001) and Hasjim *et al*. (2009). These observations could also mean that the insertion of opaque gene into maize landraces might have influenced the amylose content. However, the same amount of amylase content (26%) were also found in few normal maize genotypes as reported by Gerard *et al*. (2001) and Buckow *et al*. (2009) in a study of maize starches with different amylose contents.

The starch content ranged from 50.06% to 66.20%, a range that is below what most studies revealed in maize genotypes (Bewley and Black, 1978; Mellon *et al*., 2005). The open-pollinated QPM genotype SYN2QYQPM had significantly higher starch content (66.20%) than other 14 genotypes. However, it is not the highest compared with 72.4% starch found in QPM cultivar as reported by Hasjim *et al*. (2009). The normal maize genotype CRW3505 had the lowest starch content (50.06%), but the content is not comparable with 60%, the lowest starch content found in one the normal maize genotypes studied by Bewley and Black (1978). The starch content of above 66.20% in QPM has further been reported by Hasjim *et al*. (2009), and this is in agreement with the results obtained in this study. The average starch content obtained in this study slightly supports research evidence that maize comprises over 60% of starch (DiCostanzo, 2003). However, previous studies have showed that maize contains about 75% starch (Bae and Lim, 1998; Carriere, 1999; Mondragón *et al*., 2008).

There was no relationship between the starch and amylose content and the type of maize. This indicates that the endosperm characteristics of both types of maize were a function of the genotype rather than the type of maize. For example, one normal maize CB346xI37F2 ranked second and third, respectively, for starch and amylose contents, likewise QPM genotype SYN2QYQPM ranked first and fifth, respectively, for these two traits. This indicates that QPM breeding has managed to minimise differences between QPM and normal maize starch characteristics. The average amylose content for the QPM group was 18.18% compared with the 18.3% of the normal hybrids, and the starch was 58.76% for the normal hybrids and 59.86% for the QPM group. The low starch and amlylose contents under study could suggest that genotypes used just had lower amounts than amylose and starch contents obtained in previous studies of amylose and starch.

4.5 CONCLUSIONS

The results of this study showed that there were significant variations within normal maize and within QPM genotypes in terms of starch and amylose content. However, there were no significant differences between these two groups of material in terms of starch and amylose content. This indicates that the endosperm of QPM was not negatively influenced by the protein conversion to high lysine and tryptophan content. The variation expressed for both starch and amylose content offers broad opportunities for selecting genotypes with desired levels of starch and amylose in breeding programmes to develop varieties suitable for different purposes and for different markets such as in starch industry where starch is processed into bio-ethanol, for example.

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CHAPTER 5

THE INFLUENCE OF STORAGE CONDITIONS ON STARCH AND AMYLOSE CONTENT OF SOUTH AFRICAN QUALITY PROTEIN MAIZE AND NORMAL MAIZE HYBRIDS

5.1 ABSTRACT

The quality of maize grains during storage is affected by unfavourable storage conditions, resulting in physicochemical changes in specifically amylose and starch content that lead to significant product qualitative and quantitative losses. The objective of this study was to evaluate the starch and amylose content of normal maize and quality protein maize (QPM) seed samples at different temperature treatments: in cold storage at 3.6^{o}*C, at room temperature (18.5*^{o}*C) and at 30*^{o}*C for 0, 6 and 12 month storage periods. The genotypes were obtained from the Agricultural Research Council-Grain Crops Institute in Potchefstroom. The results of this study indicated that storage at 3.6*^{o}*C and 18.5*^{o}*C caused a reduction in amylose and starch content, although it was not significant. On the other hand, storage at 30*^{o}*C significantly reduced the starch and amylose content after both 6 and 12 month periods of storage.*

5.2 INTRODUCTION

Starch is the major reserve carbohydrate present in most seeds. Other major carbohydrates in seeds are cellulose, hemicelluloses, oligosaccharides and galactomannan (Desai, 2004). Starch, the chief source of carbohydrate in human diets, forms the major energy reserve in cereal grains. It is laid down in cereal endosperms in the form of discrete, insoluble particles called starch granules (MacGregor, 1983). Amylose is one of the two glucopolysaccharide components of starch (Corcuera, 2007). The amylose content in maize starch has an effect on many important physical, chemical and functional properties of starch, particularly during storage (Cheetham and Tao, 1998).

The starch and amylose contents of seed depend on the quality, physical structure and storage conditions (Buckow *et al*., 2009). From a seed quality preservation point of view, little is known about the response of starch and amylose of QPM when exposed to various storage conditions. Poor storage conditions often prevail and often lead to biochemical changes in chemical compounds of maize kernels including amylose and starch content during storage. The deterioration of maize kernels in terms of starch and amylose content during storage can be visually observed through the poor state of the kernels and through laboratory analysis (Kobayashi *et al*., 1986; Yuan *et al*., 1993; Hell *et al*., 2000).

The biochemical changes are not clear, but unfavourable storage conditions and the formation of free radicals during storage have been reported to play a role (McDonald, 1999; 2006). Free radical formation can be prevented by the presence of antioxidants, particularly during storage (McDonald, 1999; 2006). These biochemical changes may affect the starch and amylose content. During storage, some part of the starch and amylose has been reported to undergo aggregation process that leads to changes in their content (Liu *et al*., 2003; Pongsawatmamit *et al*., 2006). This change in content is the result of agglomeration of starch and amylose molecules (Daneke and Decker, 1988; Liu *et al*., 2003) which in turn causes problems for analytical methods with regards to the detection of exact amount of starch or amylose present in the seed during storage. The interaction of starch granules and high lipid content has also been reported to complex amylose content during storage (McCann *et al*., 2009).

There is a growing concern on how to best store seeds, including maize seeds, for a long time without biochemical changes, especially in developing countries worldwide (Thanapornpoonpong and Vearasilp, 1999; Doijode, 2001). Méndez-Albores *et al*. (2003) stated that in developing counties, efficient maintenance of grain quality is not possible due to the lack of adequate post-harvest technology, including suitable storage conditions. Despite ongoing research on maize quality improvement, little is known about how storage conditions affect both normal maize and QPM with special reference to starch and amylose content.

The biochemical changes in starch and amylose of maize seed during storage is a problem to small-scale farmers. Farmers lose large quantities of seeds due to their weakened value as a result of poor storage conditions. The consumers and the industrial companies, on the other hand, are indirectly affected. Nevertheless, decreasing storage losses is possible through the provision of suitable storage conditions. It is normal practice to implement preventive management, rather than to solve specific storage problems once they have occurred (Lopes *et al*., 2008). The measurement of starch and amylose content before and after storage should be able to provide the information that may be needed to help solve future storage problems. The aim of this study was to investigate the influence of different storage conditions on starch and amylose content extracted from South African open-pollinated QPM, a QPM hybrid and normal maize samples which were stored for 0, 6 and 12 months at 3.6°C, 18.5°C and 30°C.

5.3 MATERIALS AND METHODS

5.3.1 Planting location

As described in Chapter 3 (section 3.3.1).

5.3.2 Plant material

As described in Chapter 3 (section 3.3.2).

5.3.3 Experimental design and procedures

As described in Chapter 3 (section 3.3.3).

5.3.4 Storage conditions

The open-pollinated QPM, QPM hybrid and normal maize seed samples were sealed in brown paper bags and stored under the controlled conditions of a cold room (3.6°C), laboratory (18.5°C) and oven (30°C) for 0, 6, and 12 months. The relative humidity was 76.5% in the cold room, and 28% in the laboratory. In the oven the relative humidity was very low or close to zero due to the prolonged exposure to heat. Forty-five bags were placed in the cold room at 3.6°C for 6 and 12 months. Another 45 bags were placed in the laboratory at 18.5°C for 6 and 12 months. The last 45 bags were placed in an oven at 30°C for 6 and 12 months. Each bag consisted of 90 maize seeds. A randomized complete block design with three replicates was used. After 6 and 12 month storage periods, 45 seed samples per bag were taken and ground to a fine powder using a 1KA analysis grinder, A10 Yellowline (Merck Chemicals Pty Ltd) with a 1 mm sieve. The processed samples were directly transferred to containers to avoid moisture accumulation. The seeds were then evaluated for starch and amylose content.

5.3.5 Starch extraction and analysis

As described in Chapter 4 (section 4.3.4).

5.3.6 Amylose determination

As described in Chapter 4 (section 4.3.5).

5.3.7 Data analyses

The influence of storage conditions on starch and amylose content over time was analysed by analysis of variance (ANOVA) using Agrobase (2005).

5.4 RESULTS AND DISCUSSION

Amylose content of the maize genotypes at 3.6°C ranged between 16.66% and 19.49% after 6 months of storage and from 16.43% to 19.23% after 12 months of storage and varied significantly amongst tested maize genotypes (Table 5.1). Although the amylose content of both QPM and normal maize genotypes was negatively affected by storage, the open-pollinated QPM genotype SYN2QWQPM had the highest amylose content at 3.6°C, significantly higher than all QPM and normal maize genotypes after both 6 and 12 months of storage (Table 5.1). The second highest amylose content was observed in normal maize genotype CB341xI37F2 after 6 and 12 months of storage at 3.6°C, but it had the third highest amylose content before storage, which implies that during storage the genotype and relative humidity had an effect on the amylose content (Table 5.1). The lowest amylose content was observed in the open-pollinated QPM genotype SYN4QYQPM after both 6 and 12 months of storage at 3.6°C compared with normal maize and other QPM genotypes (Fig. 5.1).

The amylose content at 18.5°C ranged from 15.90% to 18.86% and from 15.35% to 17.81%, respectively, after 6 and 12 months of storage, a range that is lower than that of the control, indicating that the 18.5°C storage temperature significantly affected the amylose content of both QPM and normal maize genotypes. The highest amylose content at 18.5°C was observed in the open-pollinated QPM genotype SYN2QWQPM after both 6 and 12 months of storage (Fig. 5.2). The lowest amylose content at 18.5°C was observed in the open-pollinated QPM genotype SYN15QWQPM after 6 months and SYN4QYQPM after 12 months of storage. The 30°C temperature significantly reduced the amylose content of both QPM and normal maize genotypes after both 6 and 12 month of storage (Fig. 5.3). Highest amylose content at 30°C after both 6 and 12 months of storage was observed in the open-pollinated QPM genotype SYN2QWQPM.

Table 5.1 Effect of different storage conditions on amylose content over time of storage

		Amylose (%)						
Genotype	Type	Control	3.6°C		18.5°C		30°C	
		0 months	6 months	12 months	6 months	12 months	6 months	12 months
CRW3505	Normal hybrid	17.62	17.47	17.28	16.35	15.62	15.56	13.57
QS7608	QPM hybrid	18.22	18.87	18.65	17. 50	16.41	17.16	15.13
C3505	Normal hybrid	17.92	18.20	18.09	17.74	16.77	16.29	14.28
CB341xI37F2	Normal hybrid	18.73	19.35	19.09	18.72	17.03	17.43	15.41
CB346xI37F2	Normal hybrid	18.33	18.62	18.27	17.81	16.80	16.74	14.69
P6479F2	Normal hybrid	17.73	17.47	17.22	17.12	16.12	15.58	13.63
CB389xI37F2	Normal hybrid	18.18	17.97	17.78	17.50	16.53	16.09	14.12
SYN2QYQPM	Open-pollinated QPM	18.39	18.28	18.04	17.74	16.69	16.33	14.32
SYN4QYQPM	Open-pollinated QPM	16.78	16.66	16.43	16.24	15.22	14.72	14.14
SYN11QYQPM	Open-pollinated QPM	17.27	17.13	16.88	16.48	15.43	15.26	12.96
SYN13QYQPM	Open-pollinated QPM	17.72	17.51	17.15	16.81	15.80	15.69	13.75
SYN2QWQPM	Open-pollinated QPM	19.60	19.49	19.23	18.86	17.81	17.54	15.56
SYN5QWQPM	Open-pollinated QPM	19.12	19.04	18.96	18.68	16.37	17.18	15.15
SYN12QWQPM	Open-pollinated QPM	18.67	18.46	18.16	17.74	16.72	16.68	14.47
SYN15QWQPM	Open-pollinated QPM	16.97	16.83	16.62	15.90	15.35	14.93	12.64
LSD (0.05)		1.49	1.49	1.11	1.31	1.50	1.33	1.14
LSD (0.05) across treatments					0.42			
Mean		18.08	18.09	17.86	17.41	16.31	16.21	14.26
Mean for QPM		18.08	18.03	17.79	17.33	16.20	16.17	14.24
Mean for normal hybrids		18.09	18.18	17.96	17.54	16.48	16.28	14.28

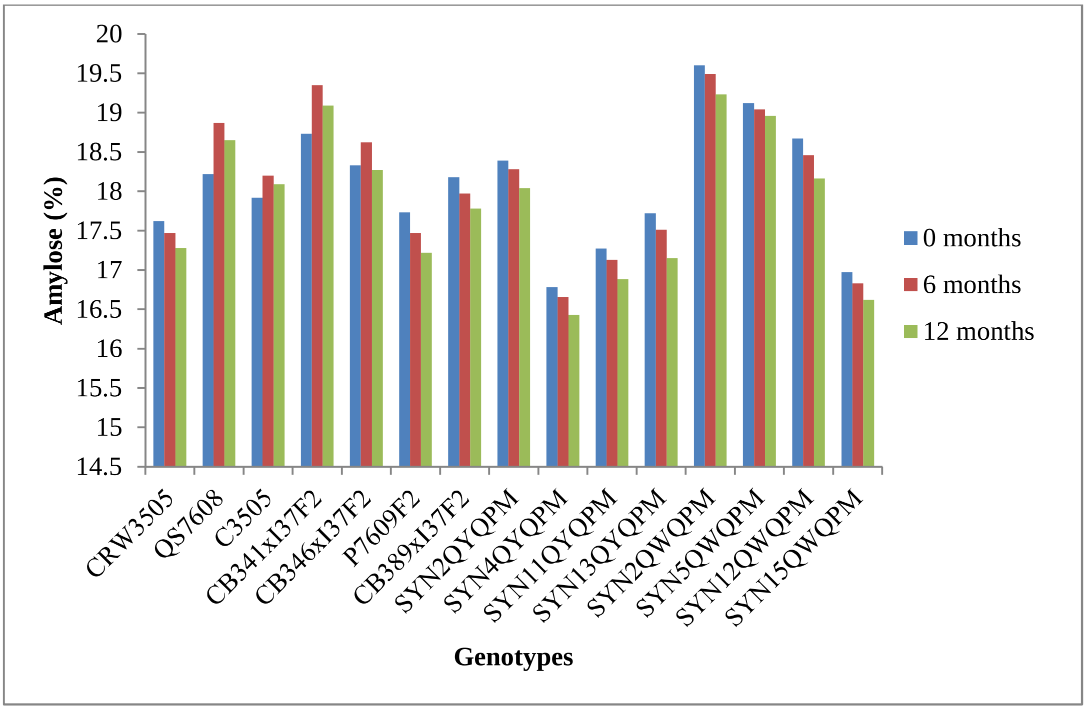

Figure 5.1 Amylose content of maize after storage at 3.6°C for 0, 6 and 12 months

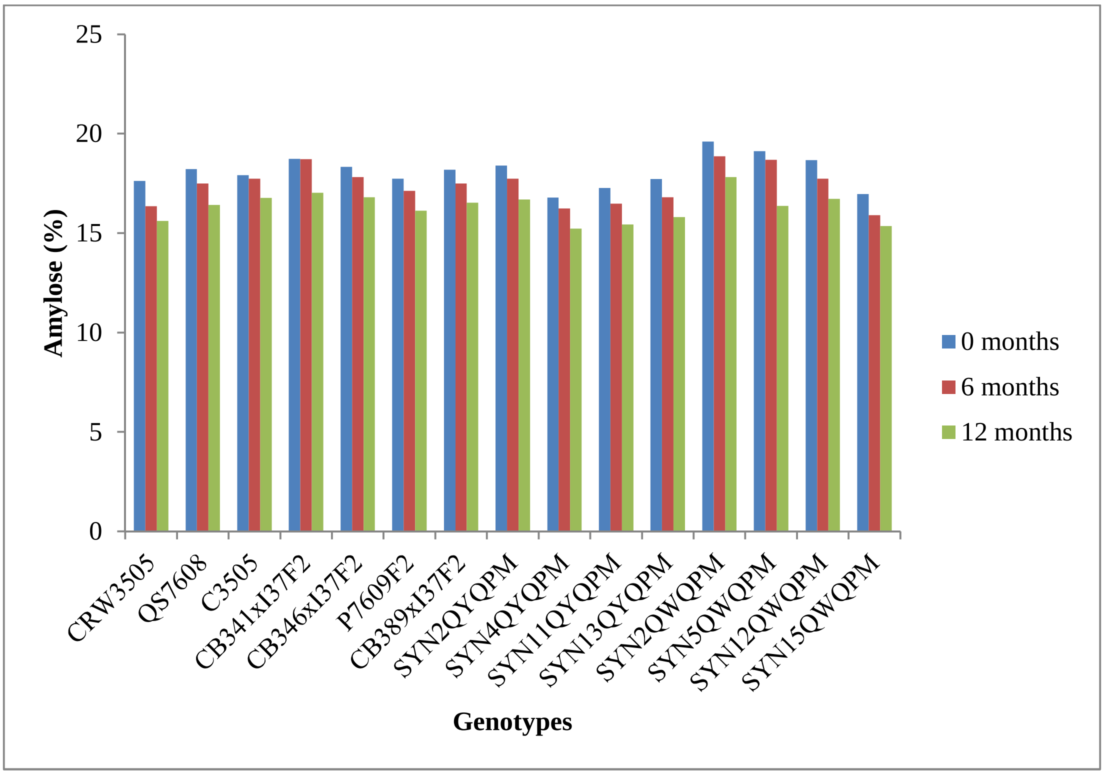

Figure 5.2 Amylose content of maize after storage at 18.5°C for 0, 6 and 12 months

The QPM genotypes seemed to have smaller reductions in amylose content than normal maize genotypes at 3.6°C, after both 6 and 12 months of storage (Table 5.1). At 18.5°C, the normal maize genotypes lost more amylose than the QPM genotypes after both 6 and 12 months of storage. The QPM genotypes lost higher amylose amounts at 30°C after 6 months of storage than normal maize genotypes, but after 12 months of storage more amylose was lost in normal maize hybrids than in QPM genotypes.

The storage time significantly influenced the amylose content (Table 5.1). The amylose content after 6 months of storage at 18.5°C was significantly different from the control. The amylose content after 6 months of storage at 3.6°C was similar to the control. High temperature storage for 6 and 12 months caused significant reduction in amylose content. Six months of storage at 30°C caused the amylose to decrease. A further decrease in amylose content was observed after 12 month storage at 30°C.

Starch content of the maize genotypes at 3.6°C ranged between 50.01% and 63.00% after 6 months of storage and from 49.88% to 65.64% after 12 months of storage and varied amongst tested maize genotypes, with statistically significant differences (Table 5.2). Starch content of both QPM and normal maize genotypes was negatively affected by storage at 3.6°C after both 6 and 12 months of storage (Fig. 5.4). The open-pollinated QPM genotype SYN2QYQPM had the highest starch content at 3.6°C, significantly higher than all QPM and normal maize hybrids after both 6 and 12 months of storage (Table 5.2). The third highest starch content was observed in both normal maize genotypes CB341xI37F2 and CB389xI37F2 after 6 months of storage, but after 12 months of storage CB341xI37F2 had higher starch content than CB389xI37F2, which implies that during storage the genotype and relative humidity had an effect on the starch content (Table 5.2). The drop in starch content could have been caused by the agglomeration of starch during storage under humid conditions as it was the case with the findings of Daneke and Decker (1988) where ambient humidity caused the seed starch to aggregate, preventing the actual content of starch present in the seed from being detected. The lowest starch content was observed in the open-pollinated QPM genotype SYN15QWQPM after 6 months of storage, but after 12 months of storage the genotype SYN15QWQPM had higher starch content than the normal maize genotype CRW3505.

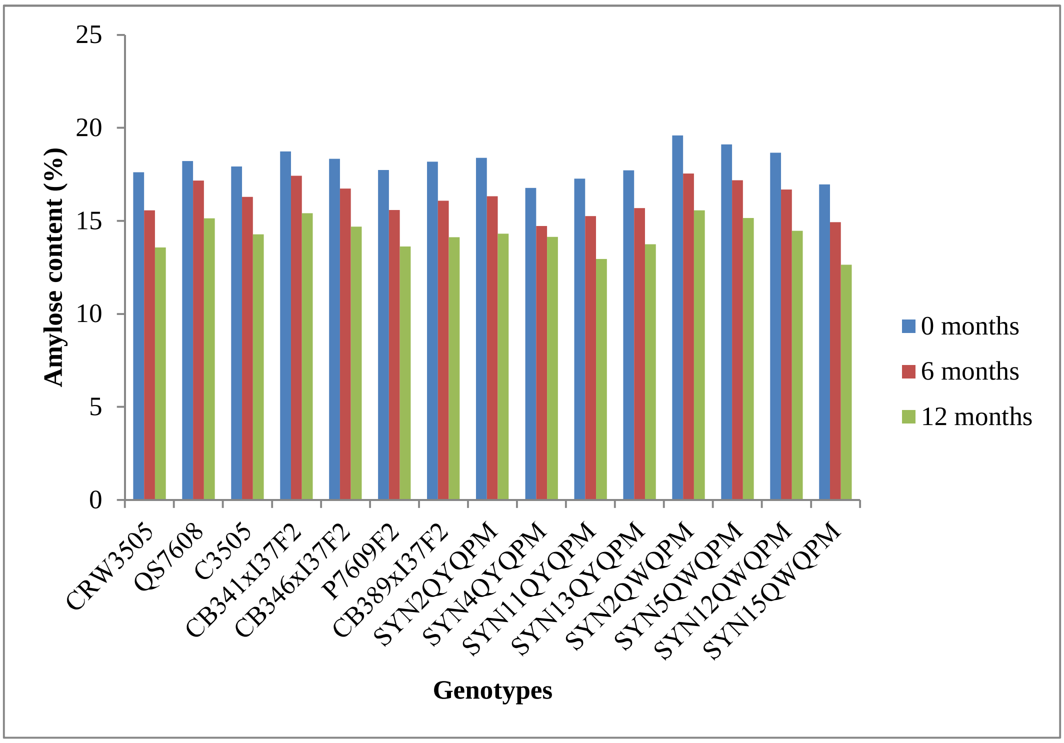

Figure 5.3 Amylose content of maize after storage at 30°C for 0, 6 and 12 months

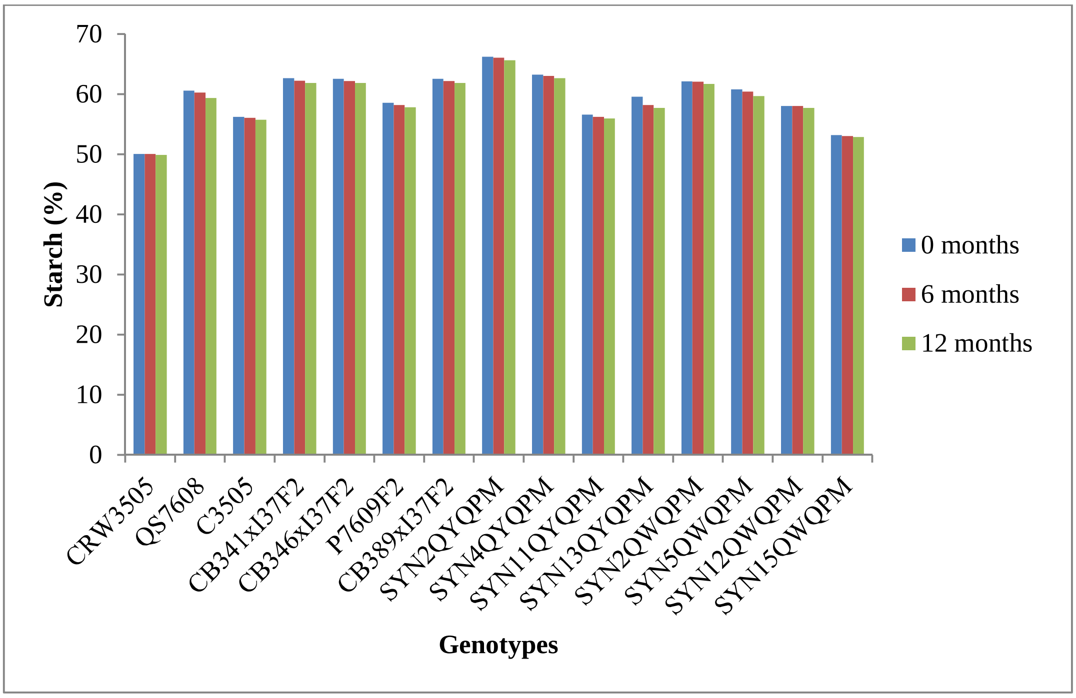

Figure 5.4 Starch content of maize after storage at 3.6°C for 0, 6 and 12 months

Table 5.2 Effect of different storage conditions on starch content over time of storage

		Starch (%)						
Genotype	Type	Control	3.6^{o}C		18.5^{o}C		30^{o}C	
		0 months	6 months	12 months	6 months	12 months	6 months	12 months
CRW3505	Normal hybrid	50.06	50.01	49.88	49.92	49.28	48.45	46.30
QS7608	QPM hybrid	60.58	60.25	59.37	59.49	58.95	57.28	55.64
C3505	Normal hybrid	56.19	56.06	55.75	55.84	55.45	53.91	51.16
CB341xI37F2	Normal hybrid	62.65	62.20	61.86	61.93	61.70	59.28	57.44
CB346xI37F2	Normal hybrid	62.56	62.17	61.82	61.89	61.48	59.02	57.07
P6479F2	Normal hybrid	58.57	58.18	57.78	57.85	57.32	55.87	53.74
CB389xI37F2	Normal hybrid	62.56	62.17	61.84	61.91	61.41	59.63	57.62
SYN2QYQPM	Open-pollinated QPM	66.20	66.03	65.64	65.73	65.24	63.50	61.27
SYN4QYQPM	Open-pollinated QPM	63.21	63.00	62.66	62.88	62.08	59.88	57.81
SYN11QYQPM	Open-pollinated QPM	56.60	56.20	55.93	56.07	55.95	53.71	51.39
SYN13QYQPM	Open-pollinated QPM	58.56	58.18	57.72	57.88	57.92	55.50	53.70
SYN2QWQPM	Open-pollinated QPM	62.12	62.04	61.69	61.85	61.46	59.05	57.98
SYN5QWQPM	Open-pollinated QPM	60.80	60.40	59.65	59.83	59.30	57.95	55.89
SYN12QWQPM	Open-pollinated QPM	58.00	58.00	57.70	57.77	57.32	55.66	53.34
SYN15QWQPM	Open-pollinated QPM	53.15	53.02	52.84	52.96	52.37	50.24	48.20
LSD (0.05)		8.19	1.77	2.11	2.03	1.93	1.48	1.92
LSD (0.05) across treatments					1.31			
Mean		59.45	59.19	58.81	58.92	58.48	56.60	54.57
Mean for QPM		59.91	59.68	59.24	59.38	58.95	56.97	55.02
Mean for normal hybrids		58.77	58.47	58.14	58.22	57.77	56.03	53.89

The starch content of the maize genotypes at 18.5°C ranged between 49.92% and 65.73% after 6 months of storage and from 49.28% to 65.24% after 12 months of storage, indicating that, compared to the control, the 18.5°C storage temperature significantly affected the starch content of both QPM and normal maize genotypes after both 6 and 12 months of storage (Fig. 5.5). The highest starch content at 18.5°C was observed in the open-pollinated QPM genotype SYN2QYQPM after both 6 and 12 months of storage. The lowest starch content was observed in normal maize genotype CRW3505 at 18.5°C after both 6 and 12 months of storage.

The starch content of the maize genotypes at 30°C ranged from 48.45% to 63.50% after 6 months of storage and from 46.30% to 61.27% after 12 months of storage, suggesting that the 30°C storage temperature significantly reduced the starch content of both QPM and normal maize genotypes after both 6 and 12 months of storage (Fig. 5.6). The highest starch content at 30°C after both 6 and 12 months of storage was observed in the open-pollinated QPM genotype SYN2QWQPM. The genotype SYN2QYQPM had constantly lost starch throughout the storage period.

There was no starch lost in both QPM and normal maize genotypes at 3.6°C, after both 6 and 12 months of storage (Table 5.2). At 18.5°C, the amount of starch lost in QPM genotypes was not significant, compared with normal maize genotypes after both 6 and 12 months of storage. Both normal maize and QPM genotypes had lost similar amounts of starch at 30°C after 6 months of storage compared with the control.

The storage time significantly influenced the starch content (Table 5.2). The effect of 6 month storage period on starch at 3.6°C was significantly different from the control. The effect of 6 months of storage on starch at 18.5°C was similar to 12 months of storage at 3.6°C which suggest that low temperature is better than high temperature during long term storage. Two periods of heat treatment caused significant reduction in starch content for all genotypes. Six months of storage at 30°C caused the starch to decrease, and a further decrease in starch content was observed after 12 months of storage. The findings are consistent with those reported by Rehman *et al.* (2002) where starch was reduced when exposed to high temperature (25°C) after 6 months of storage. This occurrence could be the effect of high temperature as the latter showed to have a negative effect on chemical compounds of various seeds including maize starch (Šimić *et al.*, 2007).

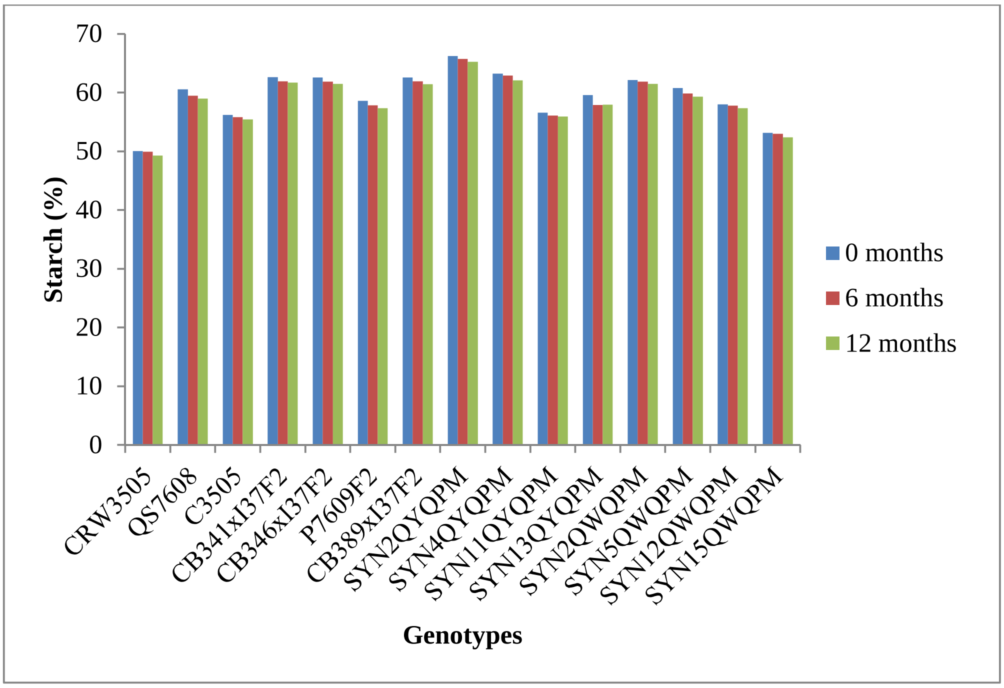

Figure 5.5 Starch content of maize after storage at 18.5°C for 0, 6 and 12 months

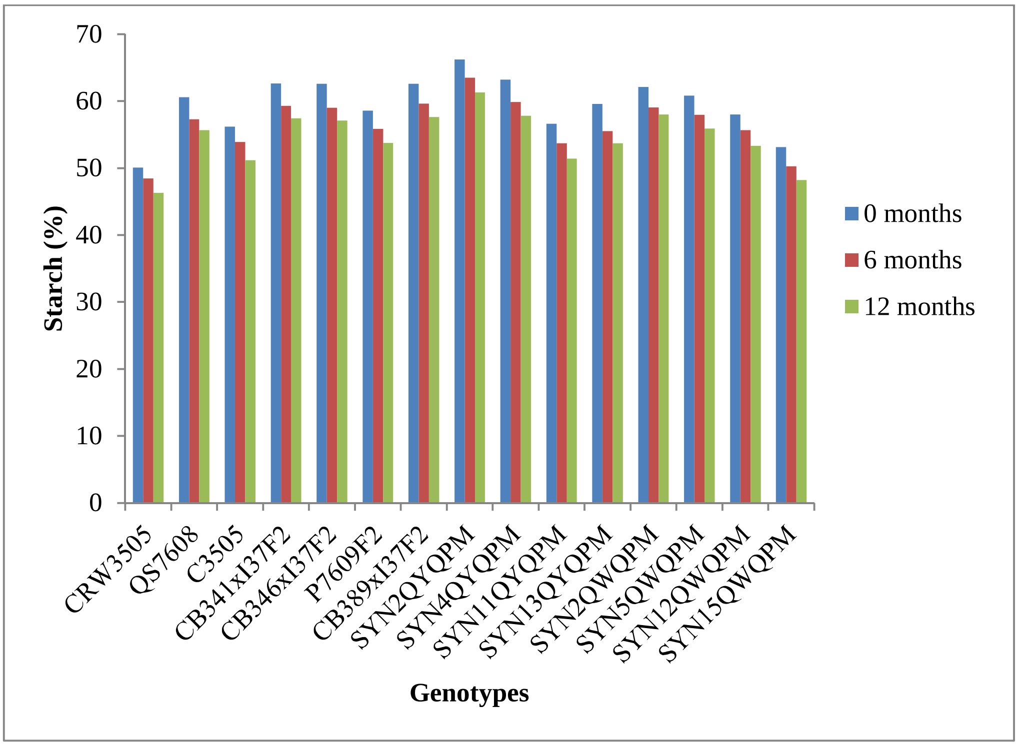

Figure 5.6 Starch content of maize after storage at 30°C for 0, 6 and 12 months

The QPM and normal maize genotypes showed a constant reduction in both amylose and starch content, producing rankings that were consistent over treatments. The amylose and starch content of open-pollinated QPM, QPM hybrid and normal maize genotypes were equally affected by the different storage conditions over time. In contrast, QPM genotypes were spread throughout the rankings and it is not clear if they contain higher amylose and starch content than normal maize genotypes. However, due to the considerable number of QPM genotypes showing higher amylose and starch content than normal maize under various storage conditions, this could indicate that QPM genotypes benefited from storage temperature.

The results of this study showed that storage conditions (temperature and humidity) and the time of storage were significant on the amounts of amylose and starch in maize, with significant differences between these genotypes in terms of the effect of the treatments. Thus, the amylose and starch content of both QPM and normal hybrids during storage were higher at 3.6^{o}C and 18.5^{o}C as opposed to 30^{o}C. High temperature of 30^{o}C had a negative effect on the stored QPM and normal maize genotypes in terms of starch and amylose content. These findings are in agreement with those reported elsewhere that unfavourable storage conditions (high temperature and relative humidity) cause seed quality losses (Daneke and Decker, 1988; Al-Yahya, 2001). In general, biochemical changes in amylose and starch content during the 360 days of storage indicated a gradual decline of amylose and starch content under all storage conditions. The observed reduced starch contents during storage at high temperature could be the possibility of occurrence of chemically cross-linking starch at relatively high temperature within the endosperm which can restrict access of amylase enzymes to starch and therefore less starch would be measured (Shukri, 2013).

Generally the results show that the amylose and starch content decrease over storage time. This is possible when one takes into consideration the analytical methods used to extract and analyse these nutritional characteristics, and the natural reaction of starch structure under certain circumstances. Many researchers have shown that chemical analytical methods have shortcomings when it comes to accuracy. Different amylose contents of cereals were observed using colorimetric iodine and high performance liquid chromatography (HPLC) methods (Batey and Curtin, 1996), which imply that the methods differ from one another when it comes to accuracy for amylose analysis. The average amylose contents obtained by Knutson and Grove (1994) and Mahmood *et al.* (2007) were different from the average amylose content obtained in this study when analysed by the same colorimetric method, revealing some inconvenience of using the analytical method for determining amylose in maize. That being said, storage conditions could also have influenced the different amounts of both starch and amylose contents observed in this study.

Under certain circumstances, particularly during storage, the semi-crystalline part of starch behaves in such a way that it is impossible to detect using the current analytical methods. The semi-crystalline regions may become more stable, such that the analytical method used, may provide “false” results (Rehman *et al*., 2002; Creek *et al*., 2007). This means that a part of the starch, although present, cannot be detected by the analytical method (Creek *et al*., 2007). This change in structure affects the digestibility of starch and has been, and still is, investigated by nutritionists (Rehman *et al*., 2002). In addition, changes in starch and amylose content could have been caused by other processes such as metabolism occurring during storage. During respiration, starch is converted into water, carbon dioxide and energy. Since a seed is a living organism which is able to respire, energy is used in this regard, which may be obtained by limited starch metabolism-a process accomplished by α-amylase enzyme which hydrolysis intact starch granules with the production of soluble starch fragments (MacGregor, 1983; Narayanasamy, 2006). With an increase in storage temperature, respiration rate is increased, resulting in loss of nutrients including starch present in the seed (Narayanasamy, 2006). The amount of starch lost through the metabolism process was reported to be the same as the amount of glucose required for respiration (Reed, 1992, Narayanasamy, 2006).

5.5 CONCLUSIONS

The results of this study indicated that storage at 3.6^{o}C had huge influence on starch and amylose content, which were stable for all storage periods. However, 18.5^{o}C storage temperature differently influenced both starch and amylose content. Amylose content was negatively influenced, compared with starch under the same storage conditions. On the other hand, storage at 30^{o}C for 6 and 12 months had a negative effect on starch and amylose content. The study indicated that storing maize seeds at low temperature is the best option if seeds are to be stored for a long period of time with minimal reduction in starch and amylose content. However, if low storage temperatures are not maintained, the chemical compounds of the seed including starch and amylose content will significantly be affected.

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CHAPTER 6

THE PROTEIN AND ZEIN CONTENTS OF SOUTH AFRICAN QUALITY PROTEIN MAIZE AND NORMAL MAIZE HYBRIDS

6.1 ABSTRACT

Quality protein maize (QPM) was developed for improved lysine and tryptophan content, but the extent of the differences in South African material between QPM and normal maize genotypes is not known. The objective of this study was to determine the protein and zein content of South African open-pollinated QPM varieties and a QPM hybrid, and to compare them to normal maize hybrids. The results showed that there were significant differences in protein and zein concentration of the QPM material compared with normal maize genotypes. Almost all QPM genotypes had higher protein content than normal maize genotypes. The zein peak that eluted at 7.9 min was much higher in QPM than other maize. The peak at 10.5 min was present almost exclusively in QPM. The peak at 11.8 min was present almost exclusively in normal maize. There were therefore clear differences between QPM and normal maize genotypes. The significance of each different peak is not known, but the differences in the peaks between QPM and non-QPM is probably related to the increased tryptophan and lysine in the QPM.

6.2 INTRODUCTION

Maize is one of the most important sources of protein in Africa. The provision of maize protein is important and its quality is as important as its quantity (Jugenheimer, 1961). The protein content is important for preventing diseases in humans and animals. The maize kernel consists of protein which is divided into four fractions. One of the protein fractions, found in the endosperm, is primarily zein. Zein is an alcohol-soluble protein that occurs principally in protein bodies of maize endosperm. Normal maize zein content has been reduced after the insertion of the opaque-2 gene which alters the protein profile. The introgression of the opaque-2 gene resulted in a new maize type called QPM which has a balanced zein content proportional to the other protein fractions.

There are considerable differences between maize cultivars for protein content (Arief, 2008), but the protein content is usually around 12%. QPM varieties, including opaque-2 and floury-2, have improved amino acid profiles but do not appear to yield higher protein content than conventional maize cultivars (Blair, 2007). The significant variation in protein content of different maize genotypes is brought about by many factors including the genotype itself, soil, nitrogen fertilizer and climatic conditions prevailing during grain development (Bushuk *et al*., 1969; Bajaj *et al*., 1994; Morris *et al*., 2003; Léder, 2004; Landry *et al*., 2005). The zein content of most maize genotypes ranges between 40% and 70% of the total protein found in maize endosperm (Wilson, 1992; Vivek *et al*., 2008).

The nutritional quality characteristics including protein content of maize genotypes have been investigated. Normal maize genotypes including yellow and white maize have been compared with respect to their protein content (Blair, 2007). However, to date, South African open-pollinated QPM varieties, QPM hybrid and normal maize genotypes have not been compared in terms of protein and zein contents. According to O'Kennedy (2011), protein and zein content are usually not considered when evaluating maize quality in South Africa. The aim of this study was to determine the protein and zein content of South African open-pollinated QPM varieties and a QPM hybrid, and to compare them with normal maize hybrids.

6.3 MATERIALS AND METHODS

6.3.1 Planting location

As described in Chapter 3 (section 3.3.1).

6.3.2 Plant material

As described in Chapter 3 (section 3.3.2).

6.3.3 Experimental design and procedures

As described in Chapter 3 (section 3.3.3).

6.3.4 Protein analysis

The total protein content was determined using the combustion method with a Leco FP-528 nitrogen analyser. Whole-grain samples were finely ground using a 1KA analysis grinder, A10 Yellowline (Merck Chemicals Pty Ltd) with a 1 mm sieve. Approximately 3 mg of maize flour was weighed into glass tubes and dried overnight at 95.5^{o}C. The samples were then placed in a desiccator (room temperature) to cool off. The dried

samples were individually removed from the desiccator and transferred to foils (which had their mass individually recorded) and immediately weighed in triplicate. Protein concentration was estimated from the nitrogen value as:
%protein= %nitrogen x 6.25 (conversion factor for maize).

6.3.5 Zein extraction and analysis

Zein was extracted using a 1 ml mixture of 60% (v/v) tert-butyl alcohol, 38% H_2O and 2% (v/v) beta-mercaptoethanol for protein reduction (Lee *et al.*, 2006). This was transferred to Eppendorf microfuge tubes containing 0.1 g of maize flour. The samples were then mixed on a vortex mixer for 1 h. The samples were centrifuged for 20 min at 12 000 rpm to separate supernatants from the pellets. The supernatants were then filtered through 45 µm filters into glass vials.

RP-HPLC

RP-HPLC was performed on a Shimadzu Prominence LC System using a Jupiter 300 C18 column (5 µm particle size, 300 Å pore size, 250 x 4.6 mm). Samples (100 µl) were injected and eluted with a solvent flow rate of 1 ml/min, using a column temperature of 55^{o}C. The two eluants used were: (A) 95% acetonitrile (ACN) containing 0.1% (v/v) trifluoacetic acid (TFA); (B) distilled water containing 5% (v/v) ACN and 0.11% (v/v) TFA.

A linear elution gradient was used for the zein analysis: 50-40% (B), 0-25 min; 20-25% (B), 25-40 min; 25-50% (A), 40-50 min. Protein was detected at 230 nm. Zein components were quantified by integrating the chromatogram areas using Shimadzu class-VP 6.14 SP1. Only the most distinct peaks were taken into account, and they were numbered according to their elution times.

6.3.6 Data analysis

Analysis of variance (ANOVA) was done for protein content and zein peak area (Agrobase, 2005).

6.4 RESULTS AND DISCUSSION

ANOVA on the data of the 15 tested genotypes indicated that there were significant differences (p=0.01) in protein content among the maize genotypes (Table 6.1). The protein content ranged from 5.41% to 13.40%, a protein range that nearly matched the 6-12% range which was reported by Watson (2003). The highest protein content was 13.40% followed by 11.97% and was recorded in open-pollinated QPM genotypes SYN4QYQPM and SYN15QWQPM, respectively. The highest protein content (13.40%) in this study is in agreement with the findings of Gibbon and Larkins (2005) that maize has an upper limit of about 13.5%. This value was higher than the highest values reported by Gupta (1994) and Prasanna *et al*. (2001) of 10.2% and 10.2%, respectively.

The lowest protein content was 5.41% and was observed in the normal maize genotype CB341xI37F2. This is in agreement with previous reports indicating that normal maize (9.22%) had lower protein content than QPM (10.01%) (Zhai and Zhang, 2007). The normal maize genotypes C3505 and CB389xI37F2 and open-pollinated QPM genotype SYN2QYQPM revealed intermediate protein content. The present results were lower than the earlier reports which recorded the lowest protein content of 8.1% in maize genotypes (Hernández-Uribe *et al*., 2004).

Table 6.1 Mean values of the protein content in maize genotypes (rank in parenthesis)

No.	Genotype	Type	Protein (%)
1	CRW3505	Normal hybrid	10.06 (8)
2	QS7608	QPM hybrid	7.10 (14)
3	C3505	Normal hybrid	7.86 (12)
4	CB341xI37F2	Normal hybrid	5.41 (15)
5	CB346xI37F2	Normal hybrid	7.78 (13)
6	P6479F2	Normal hybrid	9.87 (10)
7	CB389xI37F2	Normal hybrid	8.91 (11)
8	SYN2QYQPM	Open-pollinated QPM	9.95 (9)
9	SYN4QYQPM	Open-pollinated QPM	13.40 (1)
10	SYN11QYQPM	Open-pollinated QPM	10.91 (6)
11	SYN13QYQPM	Open-pollinated QPM	10.46 (7)
12	SYN2QWQPM	Open-pollinated QPM	11.97 (3)
13	SYN5QWQPM	Open-pollinated QPM	11.01 (5)
14	SYN12QWQPM	Open-pollinated QPM	11.70 (4)
15	SYN15QWQPM	Open-pollinated QPM	11.97 (2)
	LSD (0.05)		0.34
	CV (%)		2.07
	Mean		9.89
	QPM mean		8.32
	Normal maize mean		10.94

There was a significant difference between the normal maize and QPM genotypes in terms of protein content. This agrees with earlier reports where significant differences were observed between QPM and normal maize (Prasanna *et al*. 2001). The present results suggest that protein content was high in most QPM genotypes although relatively high protein content was observed in some normal maize types. This confirms findings by Sofi *et al*. (2009). There were significant differences within the QPM and the normal maize genotypes as well.

The zein peaks according to RP-HPLC-elution times (7.9, 9.4, 10.5, 11.8, 13.9, 15 and 18.4 minutes respectively) are given Table 6.2. Examples of zein profiles are given in Figs. 6.1-6.12. QPM genotypes had higher values than non-QPM genotypes for peak 7.9. Peak 9.4 was higher for all non-QPM genotypes with the exception of SYN4QY which had values similar to the non-QPM genotypes. The peak at 10.5 min was present in almost all QPM. Peak 11.8 was present in all the non-QPM but only in three of the QPM genotypes. Peak 15 was present and similar for both QPM and non-QPM genotypes with the exception of non-QPM genotype P6479 which had higher values than all QPM and non-QPM genotypes. Peaks 13.9 and 18.4 were similar for all QPM and non-QPM genotypes.

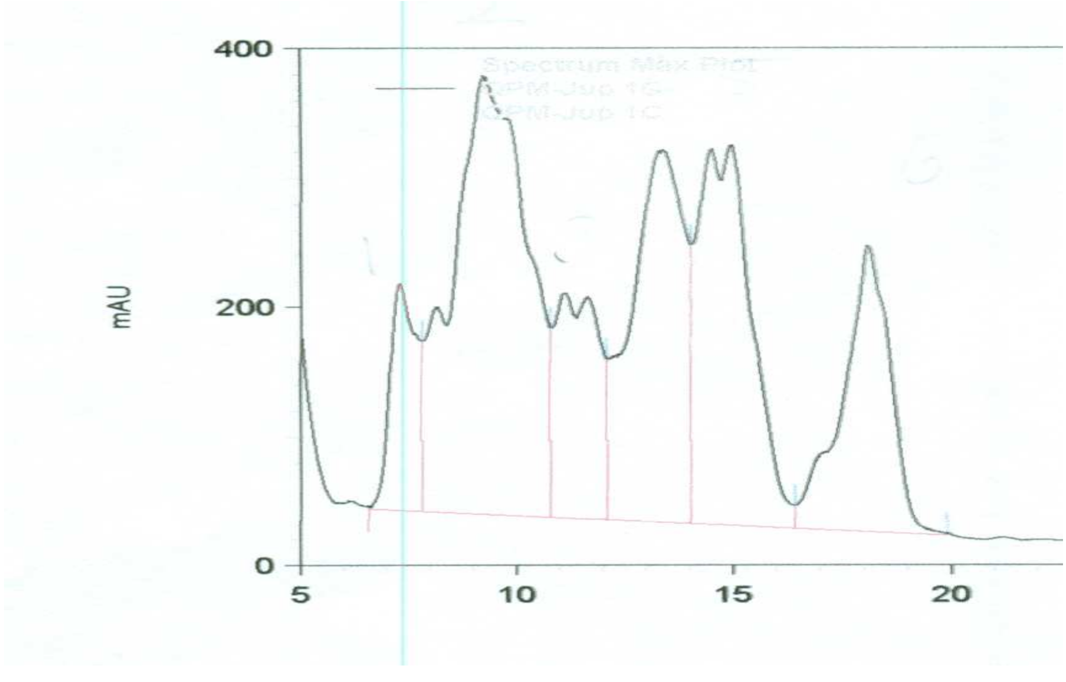

Figure 6.1 Zein profile of CRW3505 (normal hybrid)

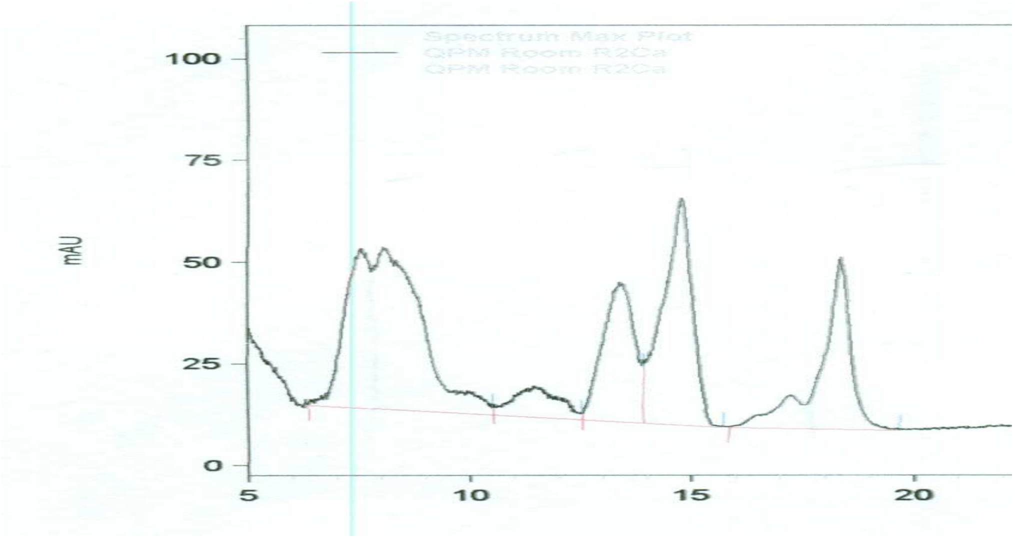

Figure 6.2 Zein profile of QPM hybrid QS7608

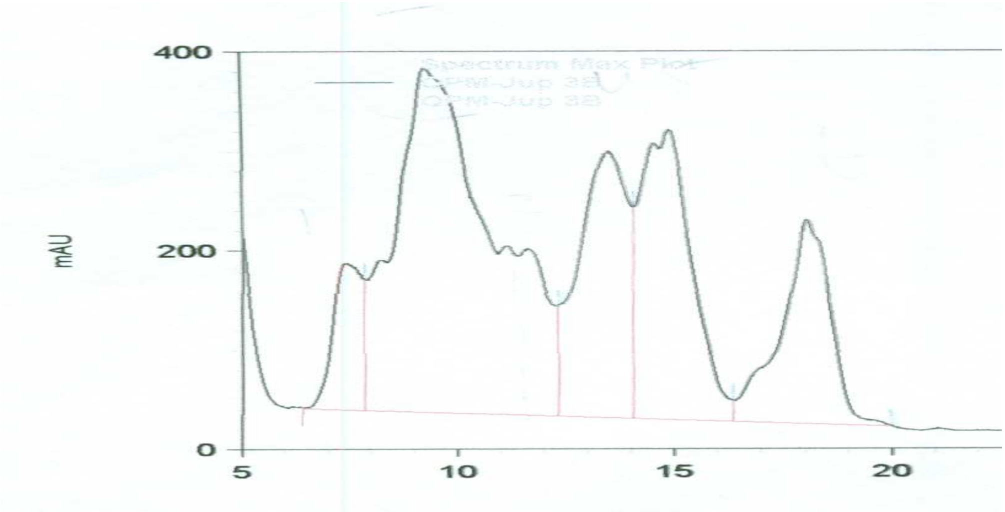

Figure 6.3 Zein profile of normal maize C3505

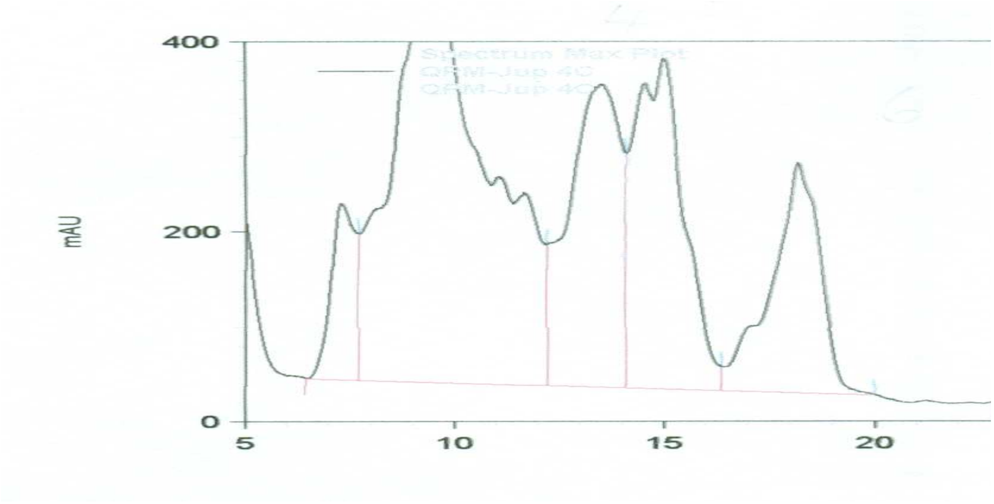

Figure 6.4 Zein profile of normal maize CB341xI37F2

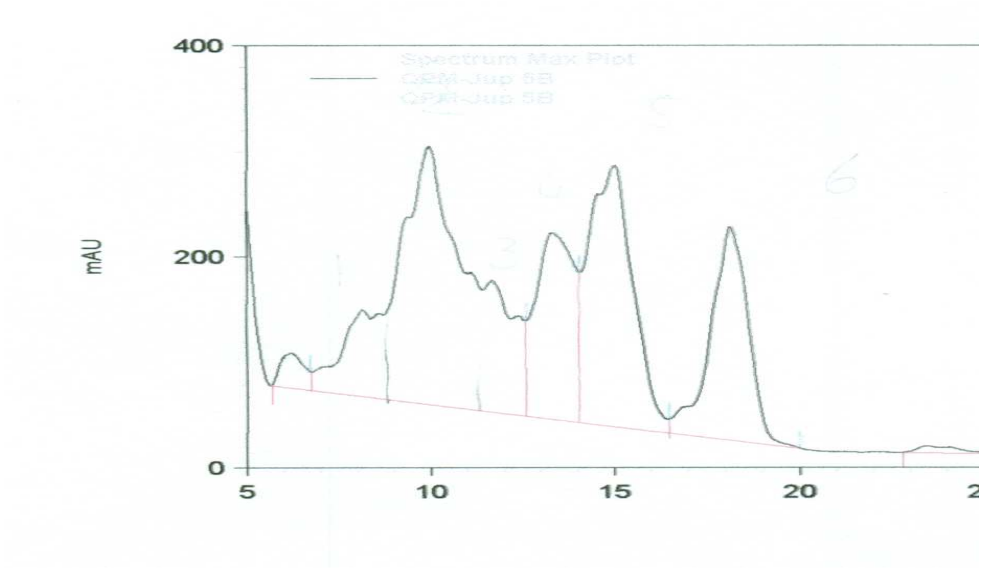

Figure 6.5 Zein profile of normal maize CB346xI37F2

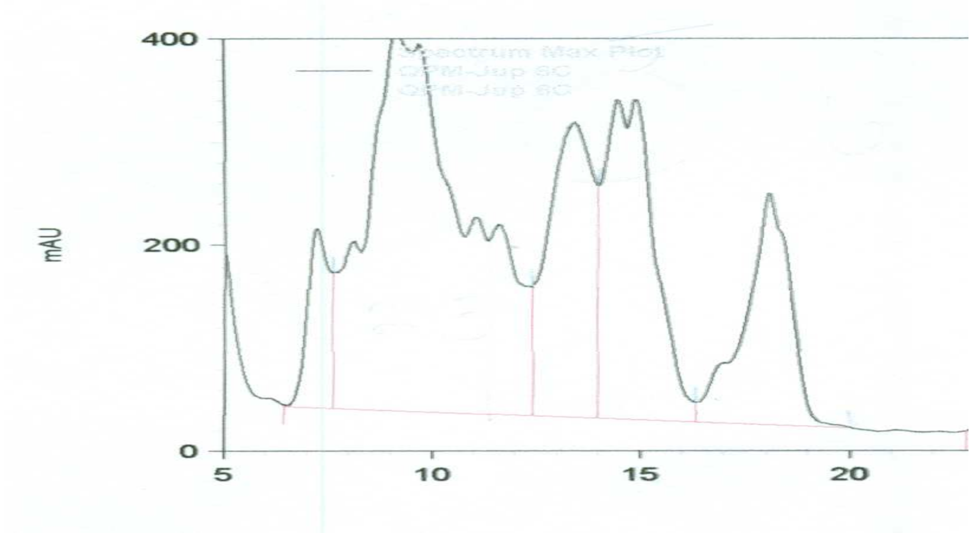

Figure 6.6 Zein profile of normal maize P6479F2

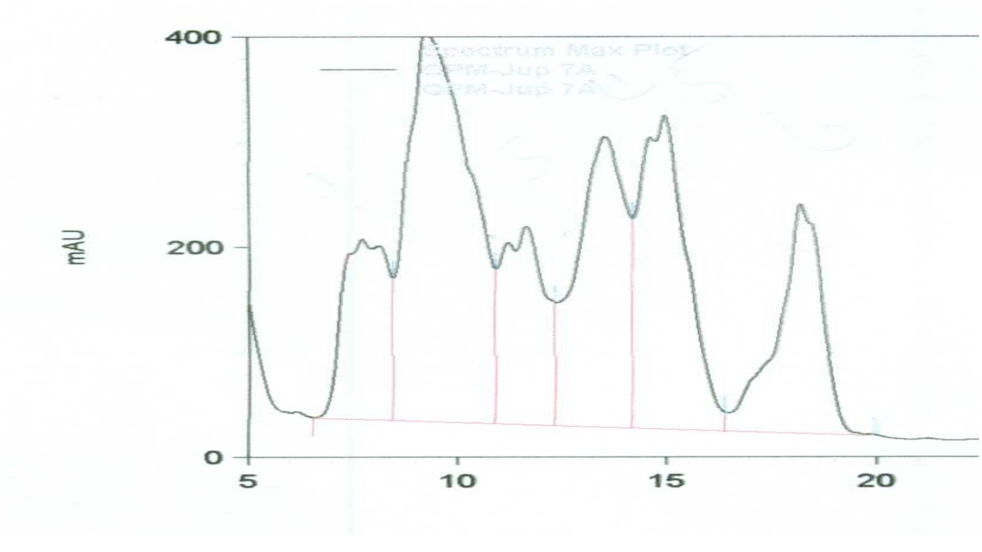

Figure 6.7 Zein profile of normal maize CB389xI37F2

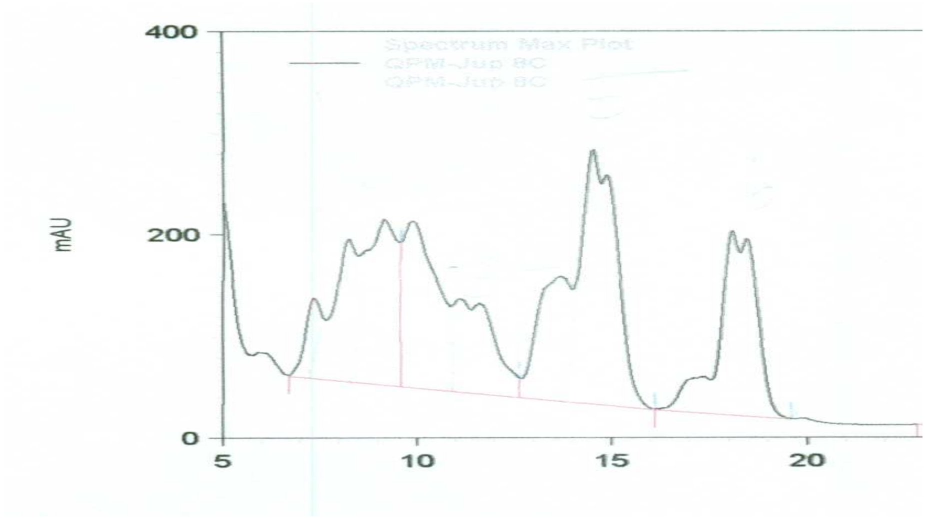

Figure 6.8 Zein profile of QPM open-pollinated variety SYN2QYQPM

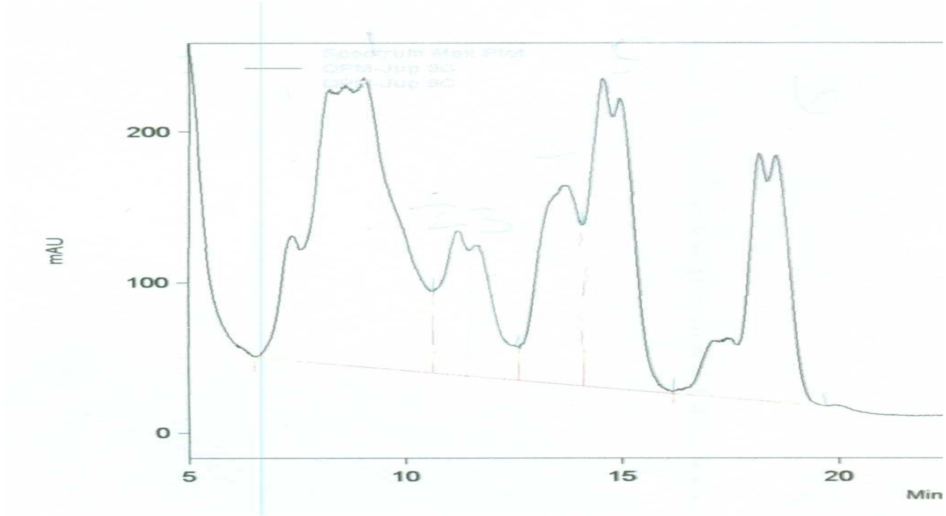

Figure 6.9 Zein profile of QPM open-pollinated variety SYN4QYQPM

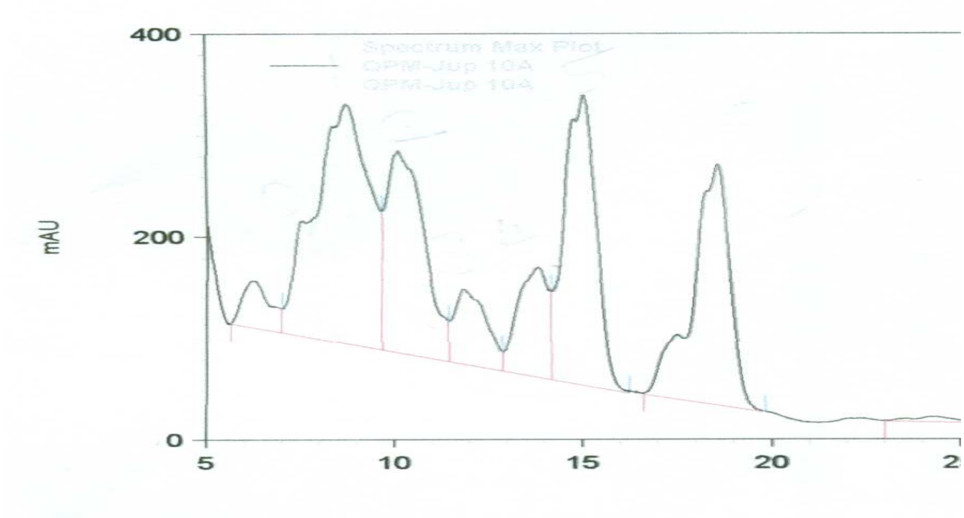

Figure 6.10 Zein profile of QPM open-pollinated variety SYN11QYQPM

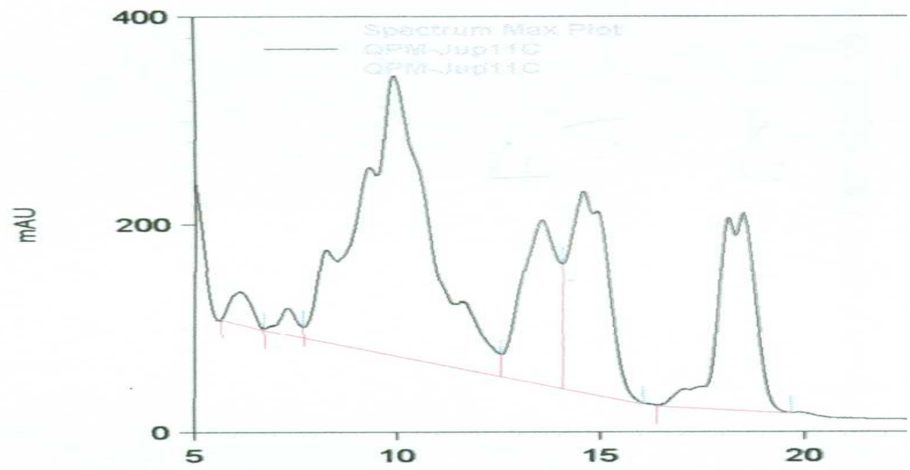

Figure 6.11 Zein profile of QPM open-pollinated variety SYN13QYQPM

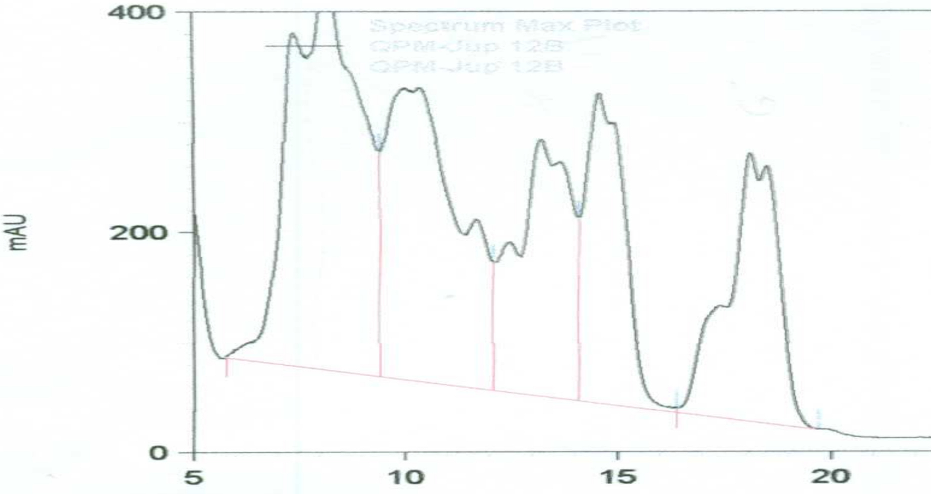

Figure 6.12 Zein profile of QPM open-pollinated variety SYN2QWQPM

Table 6.2 RP-HPLC zein peaks according to elution times in minutes (7.9, 9.4, 10.5, 11.8, 13.9, 15.0, 18.4)

	C3505 Normal	CB341 xI37F2 Normal	CB346 xI37F2 Normal	CB389 xI37F2 Normal	CRW 3505 Normal	P6479 F2 Normal	QS 7608 QPM	SYN 11QY QPM	SYN 12QW QPM	SYN 15QW QPM	SYN 13QY QPM	SYN 2QW QPM	SYN 2QY QPM	SYN 4QY QPM	SYN 5QW QPM
Peak 7.9	4.40 mi	3.57	5.75	6.93	7.10	3.86	3.82	6.45	12.18	12.33		12.67	10.33	7.33	15.67
Peak 9.4	29.06	31.99	24.67	30.63	29.44	28.19	3.70	18.37	20.39	15.80	21.44	16.96	11.88	27.63	18.63
Peak 10.5						7.67		14.52		19.84	20.00	23.86	13.45	3.79	
Peak 11.8	9.02	6.26	8.71	12.30	8.32	10.80		4.32					8.62	5.67	
Peak 13.9	18.24	15.91	12.74	17.03	18.30			6.21	14.55	13.24	11.09	13.64	9.76	11.19	16.04
Peak 15	18.56	18.48	21.05	18.12	18.29	40.16	11.28	17.51	10.37	15.19	16.37	12.57	21.11	17.99	17.18
Peak 18.4	13.07	14.18	14.44	12.37	12.22	9.94	10.41	17.00	14.66	15.25	15.39	14.71	16.92	16.24	18.19

6.5 CONCLUSIONS

The results showed that the protein content of QPM is significantly higher than that of normal maize. There was considerable genetic variation among maize genotypes for protein and zein contents. This variation offers opportunities for using the genotypes with desired levels of protein and zein in a maize breeding programme to develop improved protein content and manipulate zein fractions. Specific peaks could not be identified as specific proteins, but there were clear differences reflected in the presence or absence of some peaks between QPM and normal maize, and also in the amount of protein expressed as an area of some peaks. In addition, the different peaks could also mean that QPM and normal maize genotypes used in this study separate in terms of size of the peaks.

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CHAPTER 7

THE INFLUENCE OF STORAGE CONDITIONS ON PROTEIN AND ZEIN CONTENT OF SOUTH AFRICAN QUALITY PROTEIN MAIZE AND NORMAL MAIZE HYBRIDS

7.1 ABSTRACT

The grain composition of normal maize and quality protein maize (QPM) genotypes may be affected by storage conditions, leading to changes in protein and zein content. The objective of this study was to examine the protein and zein content of South African open-pollinated QPM varieties and one QPM hybrid compared with normal maize hybrids at 3.6^o*C, 18.5*^{o}*C and 30*^{o}*C after 0, 6 and 12 months of storage. The results of this study indicated that there were significant differences in protein and zein of South African open-pollinated QPM varieties and one QPM hybrid compared with normal maize hybrids. Storage at 3.6*^{o}*C and 18.5*^{o}*C kept the protein content stable during the storage period. Storage at 30*^{o}*C reduced the protein content after both 6 and 12 months of storage. Zein peak 9.4 was very sensitive to most storage conditions, leading to a reduction in this peak. Peak 13.9 in QPM entries was very sensitive to heat treatment and long term storage under room temperature conditions, which caused an increase in this peak. Peak 15 was consistently very sensitive to storage at 30*^{o}*C for 6 and 12 months, which caused an increase in the peak.*

7.2 INTRODUCTION

Maize kernels contain differing amounts of protein and zein. QPM is nutritionally enhanced maize that has a better protein quality in that it possesses double the quantity of lysine and tryptophan found in non-QPM. Maize researchers at the International Maize and Wheat Improvement Center (CIMMYT) developed hard endosperm QPM with improved lysine and tryptophan content. Cereals, including maize, are the main source of protein consumed by humans and thus the need for better nutritionally balanced cereal proteins is important (Ingversen, 1983). Zein, the major protein fraction of maize endosperm tissue, is soluble in aqueous alcohol solutions and contains almost no lysine or tryptophan (Paulis and Bietz, 1986).

From a nutritional quality preservation point of view, little is known about the reaction of protein and zein of QPM when exposed to different storage conditions. During storage, nutritional aspects of the grain including protein and zein content may be changed as a result of exposure to unfavourable storage conditions (Reed, 1992). The most important external factors influencing protein and zein content in maize kernels during storage are storage temperature, time of storage, seed moisture content and relative humidity (McDonald, 1999). Therefore, protein and zein content of QPM and normal maize hybrids are likely to vary and to be influenced by different storage conditions.

During storage, protein and zein content can remain at the initial amount or decline to a point that may make the kernel undesirable for human consumption. The production of essential amino acids plays a role in protein degradation (Bagley *et al*., 1963). The free amino acid groups of protein spontaneously form complex intermediate compounds resulting in the activity, leading to reduction in protein and zein content during storage (Marshall and Chrastil, 1992; Rehman *et al*., 2002). According to Kalpana and Rao (1997), a decrease in protein content is associated with a rise in the level of amino acids during the ageing process.

González-Pérez and Arellano (2009) reported that protein fractions undergo reversible and irreversible association-dissociation phenomena, which may complicate their isolation and characterization. For instance, the majority of 7S globulins from monocotyledonous seeds (e.g. maize) associate to larger molecular weight species (González-Pérez and Arellano, 2009). Little biochemical changes in protein occur in dry seeds during storage than in seeds with high moisture content (British Nutrition Foundation, 2004). According to Serna-Saldivar (2010), protein content does not change when determined with the Kjeldahl method even when exposed to extreme storage conditions or normal conditions, but if it does change, then the changes are due to the carbohydrate losses from respiration. During storage, the agglomeration of starch and protein within the endosperm may affect the final protein content (Pomeranz and Williams, 1990).

During extended storage, changes in the chemical composition and nutritive value of grain may occur (Mitchell and Breadles, 1949). Although the processes involved in the biochemical changes are unclear, available experimental evidence indicates that storage conditions may contribute to the reduction in nutritional composition, including protein content (Prestley, 1986; McDonald, 1999). Successful storage of seeds, therefore, requires certain conditions to be met and thus, the aim of this study was to investigate the influence of different storage conditions on protein and zein content extracted from QPM and normal maize seed samples stored for 0, 6 and 12 months at 3.6°C, 18.5°C and 30°C.

7.3 MATERIALS AND METHODS

7.3.1 Planting location

As described in Chapter 3 (section 3.3.1).

7.3.2 Plant material

As described in Chapter 3 (section 3.3.2).

7.3.3 Experimental design and procedures

As described in Chapter 3 (section 3.3.3).

7.3.4 Storage conditions

The storage conditions of the South African open-pollinated QPM, QPM hybrid and normal maize seed samples were the same as that described in Chapter 5 (section 5.3.4).

7.3.5 Protein analysis

As described in Chapter 6 (section 6.3.4).

7.3.6 Zein extraction and analysis

As described in Chapter 6 (section 6.3.5).

RP-HPLC

As described in Chapter 6 (section 6.3.5.1).

7.3.7 Data analyses

The effect of storage conditions on protein content and zein peak area over time was analysed by analysis of variance (ANOVA) using Agrobase (2005). For statistical comparisons over storage time and storage temperature, the average peak sizes were determined from three profiles.

7.4 RESULTS AND DISCUSSION

The means of protein content of tested maize genotypes under different storage conditions over time are presented in Table 7.1. Protein content varied amongst tested maize genotypes, with statistically significant differences. Almost all QPM genotypes had higher protein content than normal maize genotypes at 3.6^{o}C and 18.5^{o}C after 0, 6 and 12 months of storage (Figs. 7.1 and 7.2). At 30^{o}C, QPM genotypes still had higher protein content than normal maize although the 30^{o}C environment significantly reduced the protein content after 6 and 12 months of storage (Fig. 7.3). A higher amount of total protein was obtained in the QPM genotypes (SYN4QYQPM, SYN15QWQPM, SYN2QWQPM, SYN12QWQPM, SYN5QWQPM and SYN11QYQPM) than in the normal maize across all treatments; these results are in agreement with previous reports indicating that QPM had higher protein content than normal maize (Prasanna *et al.*, 2001). Although QPM showed to have higher protein content than normal maize genotypes under different conditions, the research aimed at looking at the effect of storage conditions on nutritional content showed that protein content of seeds does not change under any storage conditions (Serna-Saldivar, 2010).

The storage time significantly influenced the protein content (Table 7.1). Maize samples stored for six months at had lower protein content than the control, suggesting that 3.6^{o}C temperature and length of storage contributed to the decrease of protein content. However, protein content after 12 months of storage at 3.6^{o}C was similar to 6 months of storage at 18.5^{o}C, which imply that low storage temperature of 3.6^{o}C played an important role in keeping the protein content intact. During the 12 month storage period at 30^{o}C, protein content was significantly different from the control, implying that storage period under the storage conditions of high temperature had a severe impact on the protein content of both QPM and normal maize genotypes.

Table 7.1 Effect of different storage conditions on protein content over time

		Protein (%)						
Genotype	Type	Control	3.6°C		18.5°C		30°C	
		0 months	6 months	12 months	6 months	12 months	6 months	12 months
CRW3505	Normal hybrid	10.06	9.91	9.76	9.75	9.44	9.42	8.79
QS7608	QPM hybrid	7.10	6.95	6.80	6.79	6.48	6.46	5.83
C3505	Normal hybrid	7.86	7.71	7.56	7.55	7.24	7.22	6.59
CB341xI37F2	Normal hybrid	5.41	5.26	5.11	5.10	4.79	4.77	4.16
CB346xI37F2	Normal hybrid	7.78	7.63	7.48	7.47	7.16	7.14	6.51
P6479F2	Normal hybrid	9.87	9.72	9.57	9.56	9.25	9.23	8.60
CB389xI37F2	Normal hybrid	8.91	8.76	8.61	8.60	8.29	8.27	7.64
SYN2QYQPM	Open-pollinated QPM	9.95	9.80	9.65	9.64	9.33	9.31	8.67
SYN4QYQPM	Open-pollinated QPM	13.40	13.25	13.10	13.09	12.78	12.76	12.13
SYN11QYQPM	Open-pollinated QPM	10.91	10.76	10.61	10.60	10.29	10.27	9.63
SYN13QYQPM	Open-pollinated QPM	10.46	10.31	10.16	10.15	9.84	9.82	9.18
SYN2QWQPM	Open-pollinated QPM	11.97	11.69	11.54	11.59	11.21	11.25	10.55
SYN5QWQPM	Open-pollinated QPM	11.01	10.86	10.71	10.70	10.39	10.37	9.74
SYN12QWQPM	Open-pollinated QPM	11.70	11.55	11.40	11.39	11.08	11.06	10.42
SYN15QWQPM	Open-pollinated QPM	11.97	11.82	11.67	11.66	11.35	11.33	10.69
LSD (0.05)		0.34	1.09	0.52	1.07	0.92	0.77	0.70
LSD (0.05) across treatments					0.40			
Mean		9.89	9.73	9.58	9.58	9.26	9.25	8.61
Mean for QPM		10.94	10.78	10.63	10.62	10.31	10.29	9.65
Mean for normal hybrids		8.32	8.17	8.02	8.01	7.70	7.68	7.05

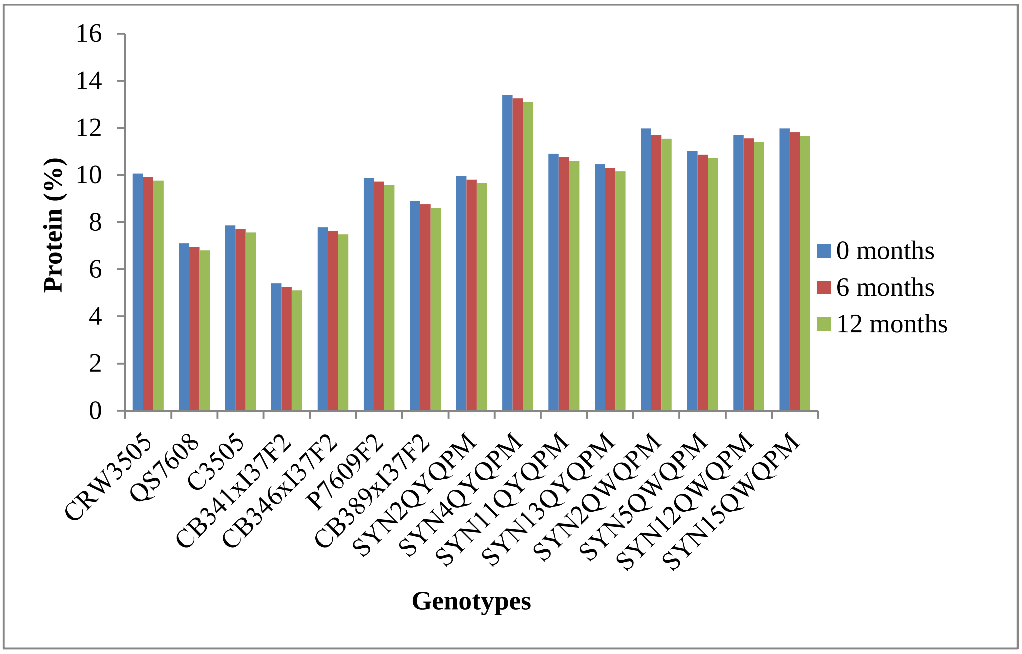

Figure 7.1 Protein content of maize after storage at 3.6°C for 0, 6 and 12 months

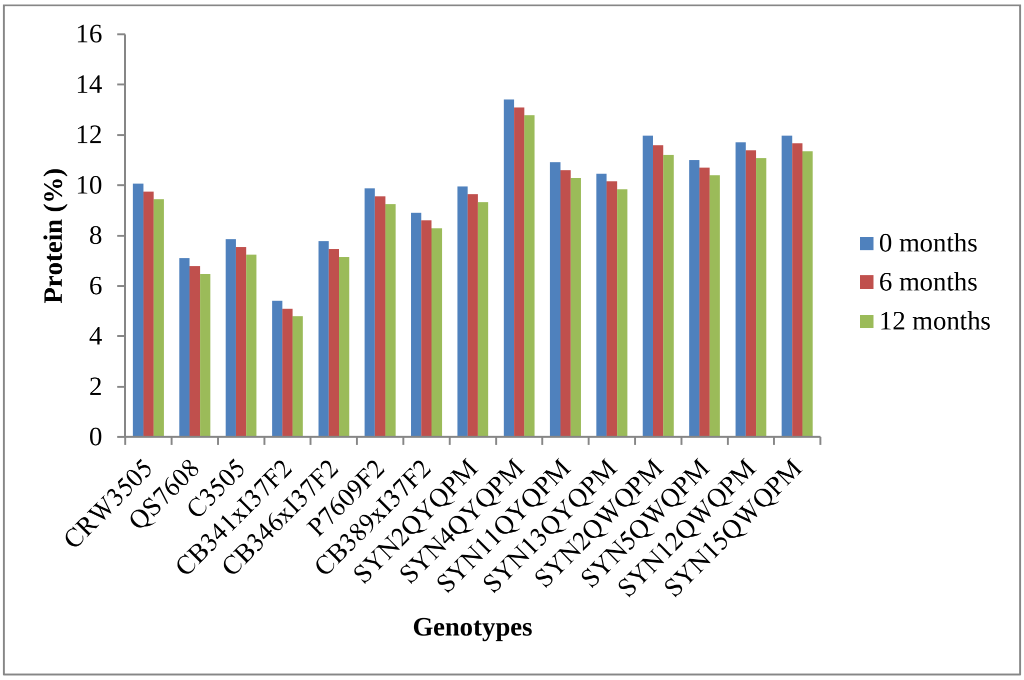

Figure 7.2 Protein content of maize after storage at 18.5°C for 0, 6 and 12 months

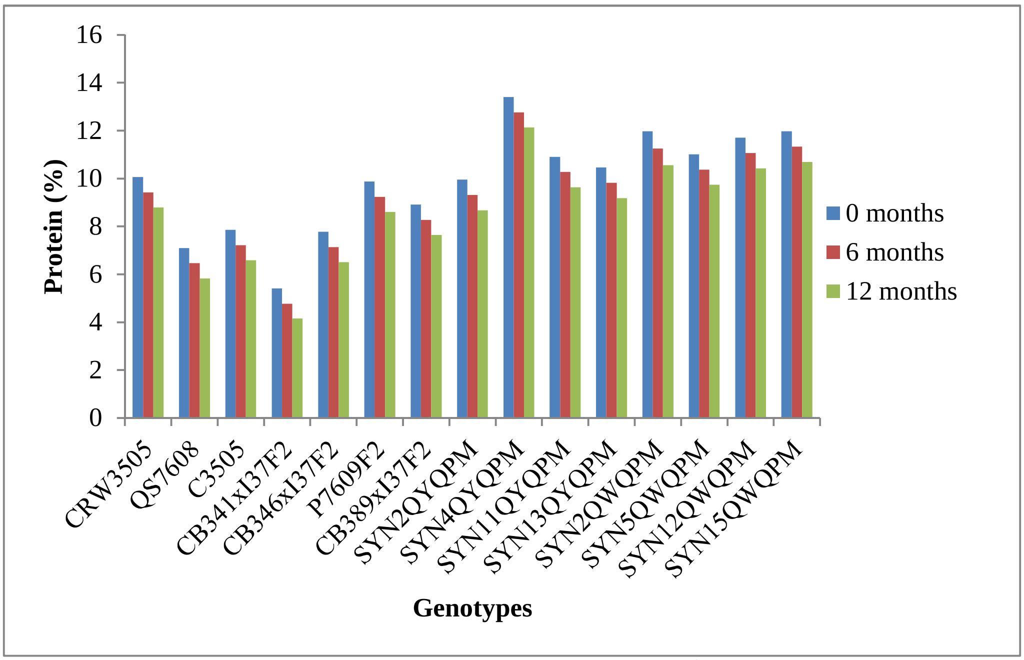

Figure 7.3 Protein content of maize after storage at 30°C for 0, 6 and 12 months

Two heat treatment periods caused significant reduction in protein content for all genotypes. Six months of storage at 30°C on protein caused the protein content to decrease, while a further decrease in protein content was observed after 12 months of storage at 30°C. The results of protein content observed in this study are not necessarily consistent with the findings reported in other studies which demonstrated that protein content does not change under storage conditions, unless as a result of respiration which causes carbohydrate loss which in turn affects the overall protein content (Mezei *et al*., 2007; Strelec *et al*., 2010; Serna-Saldivar, 2010). In contrast, Bhullar and Jenner (1985) observed a significant increase in protein content of seeds exposed to high temperatures.

Although the protein content has shown to change when exposed to storage conditions over time, the possible reasons for this change are complex and range from the chemical methods used to determine the content to the behaviour of some of the protein fractions during storage (Serna-Saldivar, 2010). The high lipid content often agglomerates with the protein fractions during milling, and therefore making the protein content appear to have dropped (González-Pérez and Arellano, 2009). In contrast, the protein, under some circumstances of ageing, bio-degrades, leading to a slight drop in its content (British Nutrition Foundation, 2004). This is in agreement with the findings of Mezei *et al*. (2007) which showed that protein content of soybean cultivars was lost due to degradation. Another possible cause of protein deterioration in field crops, especially in soybeans during storage is the occurrence of reactions between the proteins or the proteinogenous amino acids and the sugars, mainly pentoses, which occur to the extent of about 10% (MacMasters *et al.*, 1941). In addition, the observed reduced protein content during storage could be the possibility of occurrence of protein cross-linking within the endosperm matrix proteins which can restrict access of protease enzymes to protein and therefore less protein would be measured (Ioerger *et al.*, 2007).

Zein

The mean squares for HPLC zein peaks common to all entries are presented in Table 7.2. The mean squares for entry were highly significant for peaks 7.9, 9.4, 13.9, 15 and 18.4. The treatment mean squares were also highly significant for peaks 7.9, 9.4, 13.9, 15 and 18.4. This indicates that different storage conditions significantly influenced the peaks. The entry x treatment mean squares were highly significant for peaks 7.9, 9.4, 13.9, 15 and 18.4, indicating that the genotypes reacted differently to different storage conditions.

Table 7.2 Mean squares for HPLC zein peaks (in minutes elution time) common to all entries

	7.9	9.4	13.9	15.0	18.4
Entry	282.87**	1350.45**	612.34**	1150.91**	194.93**
Treatment	92.56**	525.96**	110.70**	257.80**	227.65**
Entry x Treatment	689.23**	27.51**	20.77**	16.86**	22.92**

** $P \leq 0.01$

The HPLC zein peaks according to elution times in minutes after 0, 6 and 12 month storage periods at 3.6^{o}C, 18.5^{o}C and 30^{o}C are presented in Table 7.3. Examples of extractable zein profiles are given in Fig. 7.4a-7.5g. Peak 7.9 at 3.6^{o}C was present in both non-QPM and QPM genotypes after 6 and 12 months of storage, but only absent in one QPM genotype SYN13QY. At 18.5^{o}C, peak 7.9 as at 3.6^{o}C, was absent in QPM genotype SYN13QY, but present in other QPM and normal maize genotypes. At 30^{o}C, peak 7.9 was also missing in QPM genotype SYN13QY, but appeared in other QPM and normal maize genotypes after both 6 and 12 months of storage.

Table 7.3 HPLC zein peaks according to elution times in minutes after 0, 6 and 12 month storage periods

	C3505	CB341 xI37F2	CB346 xI37F2	CB389 xI37F2	CRW 3505	P6479 F2	QS 7608	SYN 11QY	SYN 12QW	SYN 15QW	SYN 13QY	SYN 2QW	SYN 2QY	SYN 4QY	SYN 5QW
	Normal	Normal	Normal	Normal	Normal	Normal	QPM	QPM	QPM	QPM	QPM	QPM	QPM	QPM	QPM
Peak 7.9															
Control	4.40	3.57	5.75	6.93	7.10	3.86	3.82	6.45	12.18	12.33		12.67	10.33	7.33	15.67
3.6^{o}C 6M	2.73	6.08	3.72	9.27	3.88	2.67	6.37	3.30	12.14	11.49		9.87	10.75	7.99	9.33
3.6^{o}C 12M	6.09	2.33	2.73	6.45	4.47	2.67	3.09	5.71	12.36	9.22		12.85	5.08	4.72	7.82
18.5^{o}C 6M	6.48	9.08	7.98	12.79	8.42	5.40	11.99	6.67	16.82	13.62		16.54	5.74	8.85	16.96
18.5^{o}C 12M	3.54	3.82	4.05	8.67	4.57	3.33	5.14	4.52	13.77	9.76		10.83	7.84	6.00	12.50
30^{o}C 6M	2.86	3.76	3.33	9.35	4.29	2.00	2.79	2.78	11.70	10.30		11.82	7.07	4.00	7.15
30^{o}C 12M	4.36	5.57	2.95	11.62	6.79	3.63	7.34	8.72	12.09	8.25		9.71	9.67	5.58	10.98
LSD	2.45	2.78	2.42	4.18	2.66	1.32	6.35	4.85	5.50	3.26		3.3	4.17	4.10	3.12
Peak 9.4															
Control	29.06	31.99	24.67	30.63	29.44	28.19	3.70	18.37	20.39	15.80	21.44	16.96	11.88	27.63	18.63
3.6^{o}C 6M	22.95	21.36	23.34	18.68	23.87	15.29	6.17	8.66	14.46	9.85	23.81	9.48	8.51	9.28	5.86
3.6^{o}C 12M	24.39	21.86	18.87	14.14	22.32	14.68	3.10	12.97	9.49	7.19	24.10	9.93	5.93	9.99	5.44
18.5^{o}C 6M	29.73	26.82	21.17	19.40	25.83	16.58	12.01	12.65	18.01	12.33	41.16	12.75	11.18	21.55	14.39
18.5^{o}C 12M	25.43	31.88	21.71	18.90	25.18	15.76	2.51	14.29	10.45	8.76	35.73	7.38	8.96	10.00	10.17
30^{o}C 6M	21.28	23.87	21.59	13.79	22.14	13.94	3.16	9.88	9.88	12.07	34.58	11.70	8.08	9.03	8.50
30^{o}C 12M	23.36	26.89	26.23	17.12	26.99	18.01	7.93	15.43	13.91	11.42	43.99	12.87	10.33	17.16	12.27
LSD	9.36	8.90	6.27	3.83	5.45	4.59	6.40	3.00	4.76	4.21	4.29	2.86	1.98	2.80	3.12
Peak 10.5															
Control						7.67		14.52		19.84	20.00	23.86	13.45	3.79	
3.6^{o}C 6M						15.29		8.66		9.85		19.17	7.51	6.55	
3.6^{o}C 12M						14.68		12.97		7.19		23.25	7.25	5.96	
18.5^{o}C 6M						16.58		12.65		12.33		23.57	9.92	5.76	
18.5^{o}C 12M						15.76		14.29		8.76		21.52	13.37	8.35	
30^{o}C 6M						13.94		9.88		12.07		17.29	7.45	11.19	
30^{o}C 12M						18.01		15.43		11.42		24.15	11.23	7.75	
LSD						4.59		3.00		4.21		4.90	3.71	3.54	

Table 7.3 Continued

Peak 11.8															
Control	9.02	6.26	8.71	12.30	8.32	10.80		4.32					8.62	5.67	
3.6^{o}C 6M	8.59	8.66	12.72	9.51	8.18	6.9		2.33					4.70	6.46	
3.6^{o}C 12M	6.71	3.29	5.11	7.15	5.35	6.92		5.50					3.20	5.52	
18.5^{o}C 6M	7.69	7.09	9.25	10.10	7.19	6.67		5.89					9.87	9.08	
18.5^{o}C 12M	5.97	5.40	8.27	8.28	5.83	6.22		4.98					7.18	7.56	
30^{o}C 6M	9.06	6.67	11.42	12.99	10.12	9.35		2.78					7.12	9.58	
30^{o}C 12M	8.38	6.58	12.47	12.11	8.94	8.84		7.95					10.06	11.72	
LSD	2.5	4.34	2.18	2.70	3.33	4.72		1.52					1.29	1.58	
Peak 13.9															
Control	18.24	15.91	12.74	17.03	18.30			6.21	14.55	13.24	11.09	13.64	9.76	11.19	16.04
3.6^{o}C 6M	22.63	11.74	10.97	17.14	20.37			7.54	14.17	16.20	5.53	11.40	12.01	9.16	20.43
3.6^{o}C 12M	19.69	15.00	22.69	18.27	21.30			11.72	16.97	14.31	7.50	12.80	9.69	10.17	15.65
18.5^{o}C 6M	20.21	10.47	13.99	16.06	20.10			7.65	20.14	13.69	13.21	12.97	10.13	14.95	19.23
18.5^{o}C 12M	21.94	13.43	16.45	16.50	21.54			10.08	19.48	17.67	10.13	15.05	14.58	14.36	21.96
30^{o}C 6M	25.79	12.63	15.55	17.25	22.77			13.73	20.02	13.73	11.03	15.38	14.51	16.43	24.71
30^{o}C 12M	23.42	10.81	9.7	13.64	21.94			17.93	25.58	16.81	16.82	16.19	14.75	16.27	25.00
LSD	2.88	3.72	6.20	3.01	2.67			2.49	4.96	3.78	3.03	2.90	3.15	1.77	6.48
Peak 15															
Control	18.56	18.48	21.05	18.12	18.29	40.16	11.28	17.51	10.37	15.19	16.37	12.57	21.11	17.99	17.18
3.6^{o}C 6M	22.95	20.94	21.71	21.17	17.63	44.84	14.28	19.67	11.14	15.04	12.14	15.59	21.00	18.37	18.74
3.6^{o}C 12M	20.57	20.54	22.31	26.86	17.74	42.24	11.12	18.25	15.69	15.34	13.25	15.73	22.00	18.33	14.67
18.5^{o}C 6M	18.55	20.12	20.64	25.68	16.41	45.63	14.88	23.96	13.46	16.98	16.95	14.32	15.40	17.24	17.34
18.5^{o}C 12M	23.17	21.75	24.44	27.90	21.08	52.01	15.55	18.85	17.70	19.31	19.79	18.77	22.30	20.80	20.36
30^{o}C 6M	19.14	24.79	21.82	20.81	24.83	43.46	20.25	24.59	12.91	19.44	25.86	15.69	23.66	23.09	20.20
30^{o}C 12M	23.39	28.55	25.05	24.48	21.18	53.44	19.12	25.35	15.37	22.08	20.46	21.82	22.21	24.96	26.65
LSD	5.39	5.62	2.19	4.98	4.20	7.81	5.50	5.98	2.36	3.13	3.68	2.80	4.47	3.23	6.02
Peak 18.4															
Control	13.07	14.18	14.44	12.37	12.22	9.94	10.41	17.00	14.66	15.25	15.39	14.71	16.92	16.24	18.19
3.6^{o}C 6M	13.44	15.44	14.60	14.54	11.83	7.74	15.83	25.93	14.15	12.66	17.10	19.52	21.29	17.18	26.91
3.6^{o}C 12M	12.41	16.59	17.59	15.43	12.64	11.73	7.88	14.99	24.79	18.39	12.84	17.74	19.66	16.99	17.88
18.5^{o}C 6M	10.77	13.10	14.44	12.08	13.38	10.80	10.03	15.21	17.68	14.22	15.56	16.33	17.72	14.53	18.49
18.5^{o}C 12M	14.71	15.57	16.75	15.53	15.13	8.28	14.64	17.00	24.68	23.40	18.62	21.09	20.41	21.00	25.35
30^{o}C 6M	19.63	20.14	14.52	12.81	12.38	6.69	15.29	23.95	17.35	17.85	23.08	16.67	21.99	16.69	24.70
30^{o}C 12M	13.41	12.33	14.02	12.69	10.04	5.13	10.58	7.40	19.01	13.91	9.34	9.54	12.61	10.02	14.38
LSD	3.47	4.47	3.50	2.76	4.20	3.62	4.09	4.89	5.25	3.62	4.63	3.10	5.57	2.91	8.35

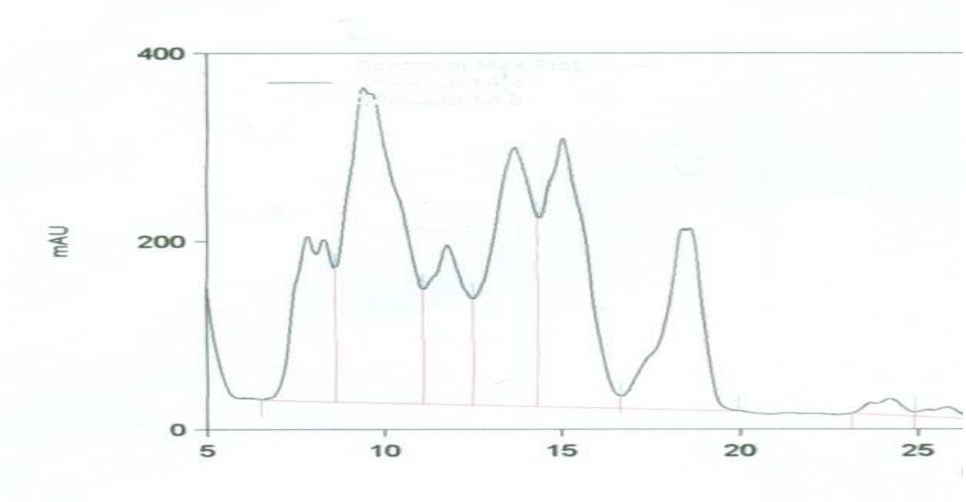

Figure 7.4a Zein profile of CRW3505, control

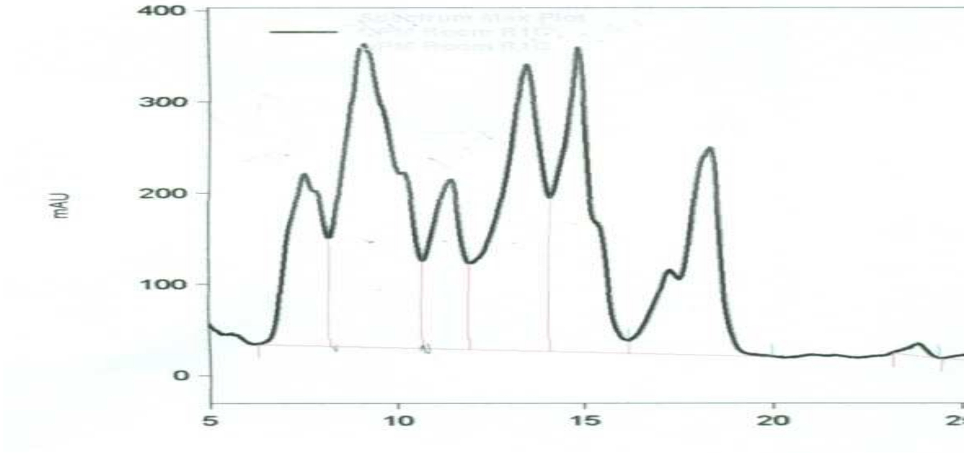

Figure 7.4b Zein profile of CRW3505 after storage at 18.5°C for 6 months

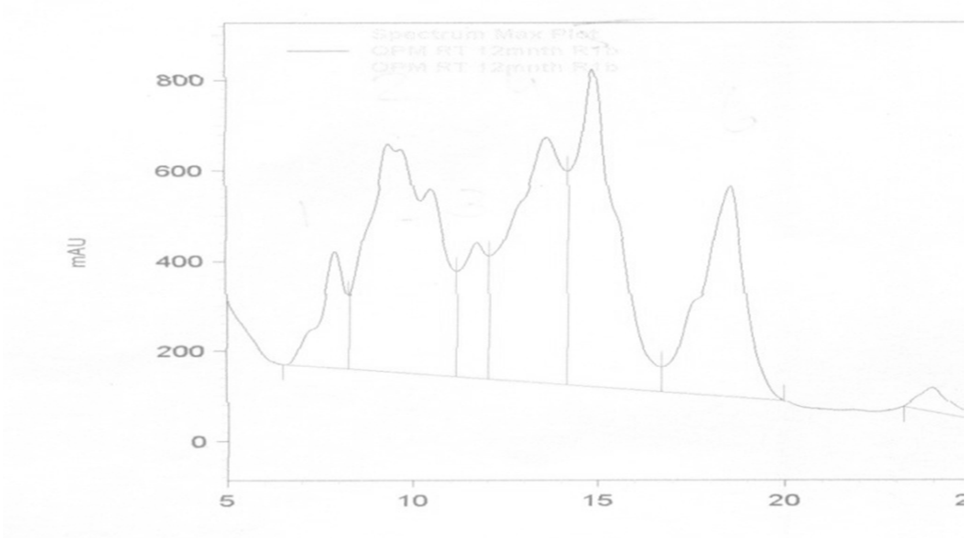

Figure 7.4c Zein profile of CRW3505 after storage at 18.5°C for 12 months

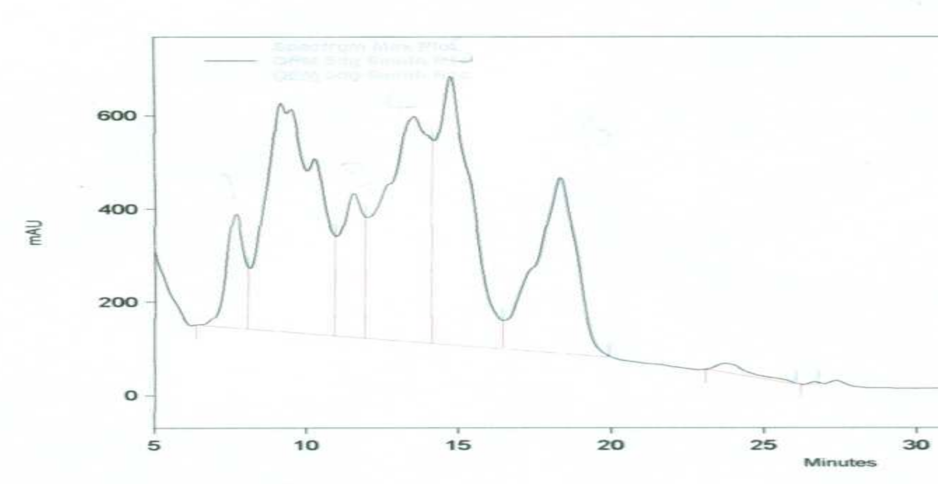

Figure 7.4d Zein profile of CRW3505 after storage at 3.6^{o}C for 6 months

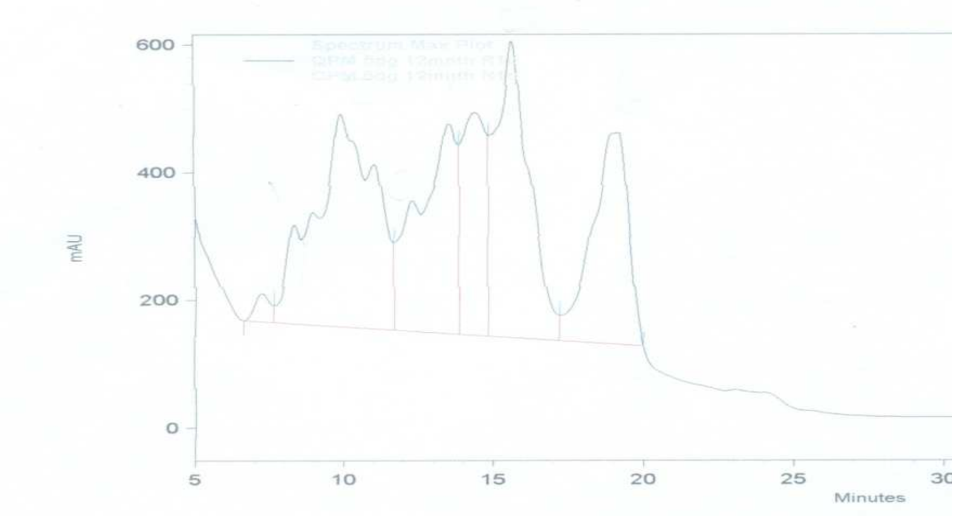

Figure 7.4e Zein profile of CRW3505 after storage at 3.6^{o}C for 12 months

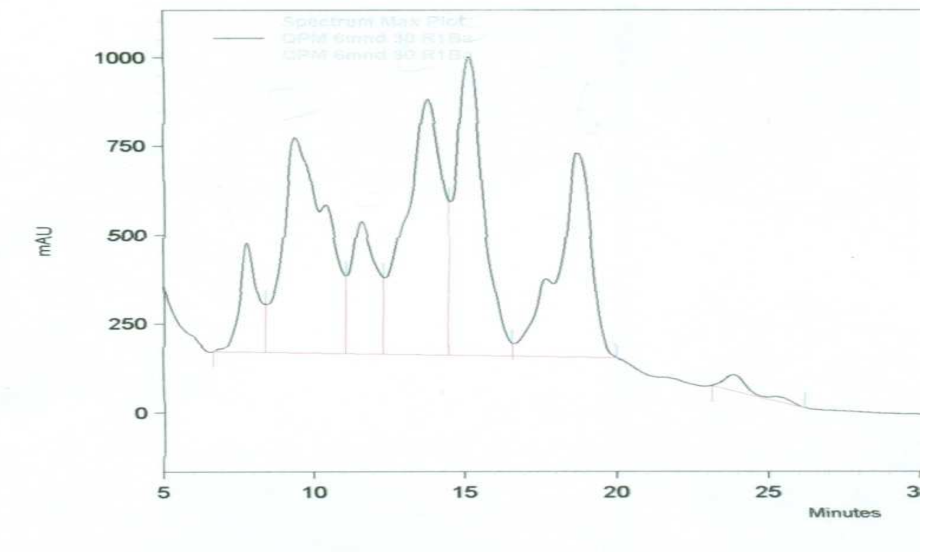

Figure 7.4f Zein profile of CRW3505 after storage at 30^{o}C for 6 months

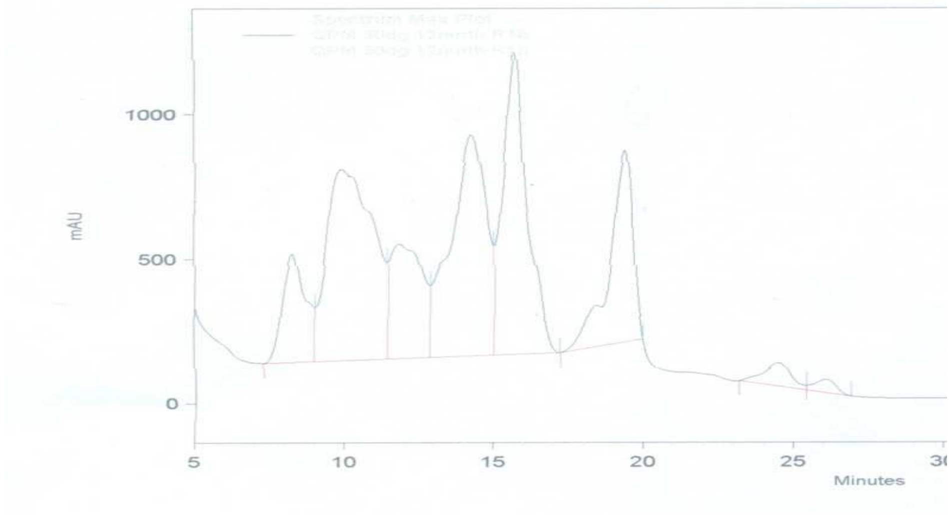

Figure 7.4g Zein profile of CRW3505 after storage at 30^{o}C for 12 months

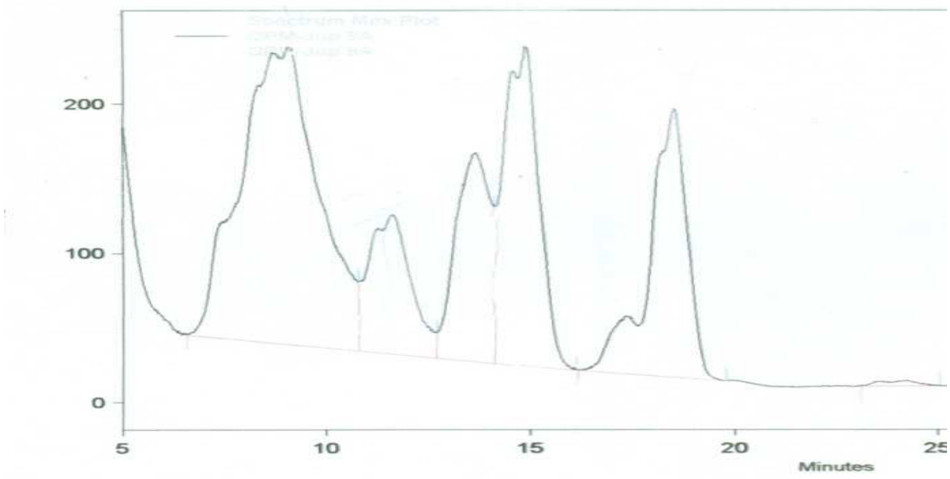

Figure 7.5a Zein profile of SYN4QYQPM, control

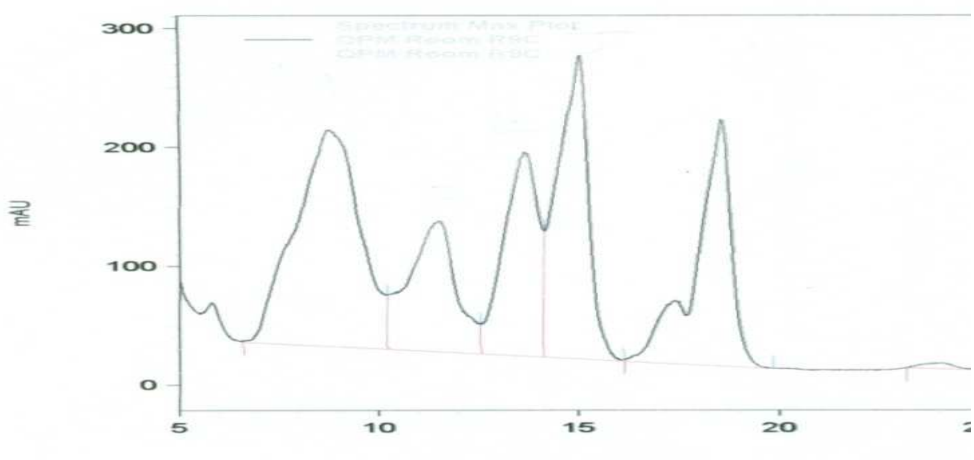

Figure 7.5b Zein profile of SYN4QYQPM after storage at 18.5^{o}C for 6 months

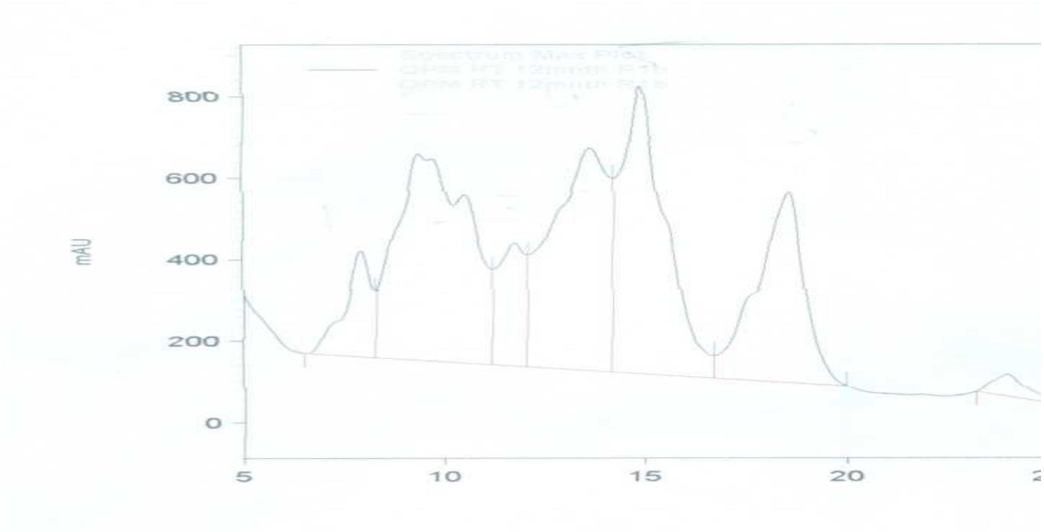

Figure 7.5c Zein profile of SYN4QYQPM after storage at 18.5°C for 12 months

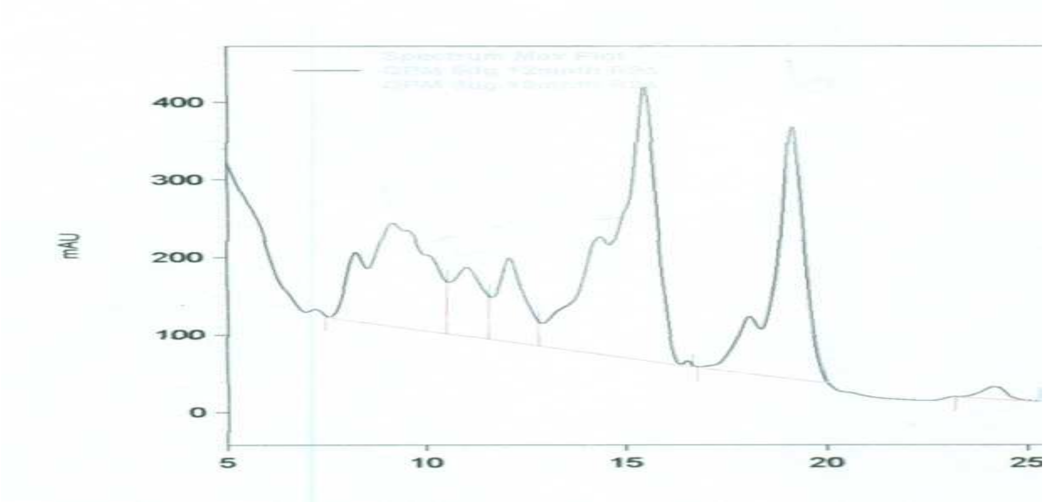

Figure 7.5d Zein profile of SYN4QYQPM after storage at 3.6°C for 6 months

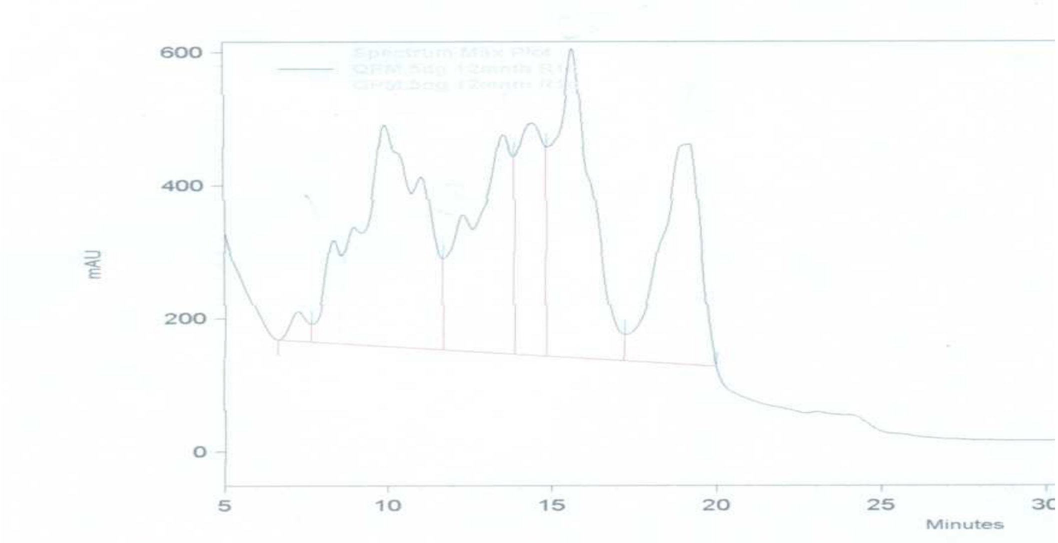

Figure 7.5e Zein profile of SYN4QYQPM after storage at 3.6°C for 12 months

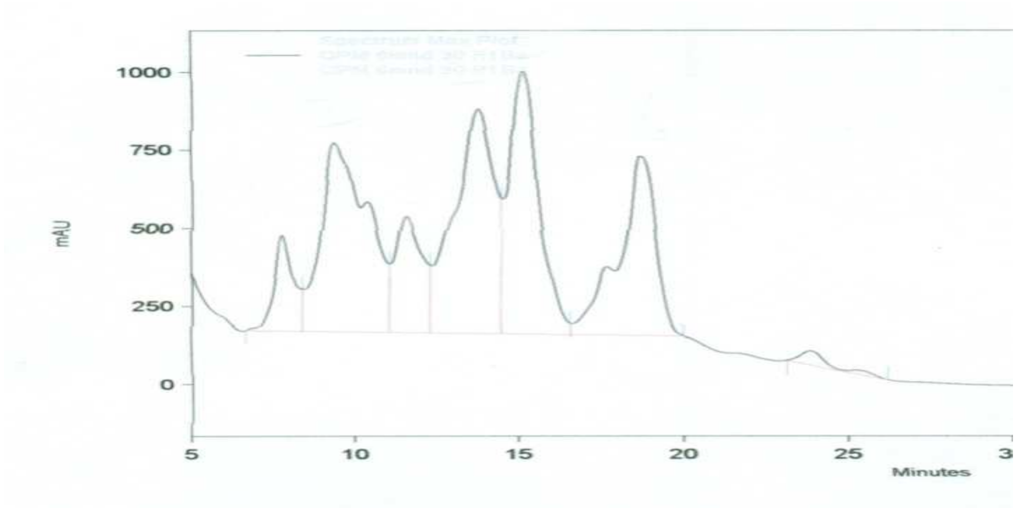

Figure 7.5f Zein profile of SYN4QYQPM after storage at 30°C for 6 months

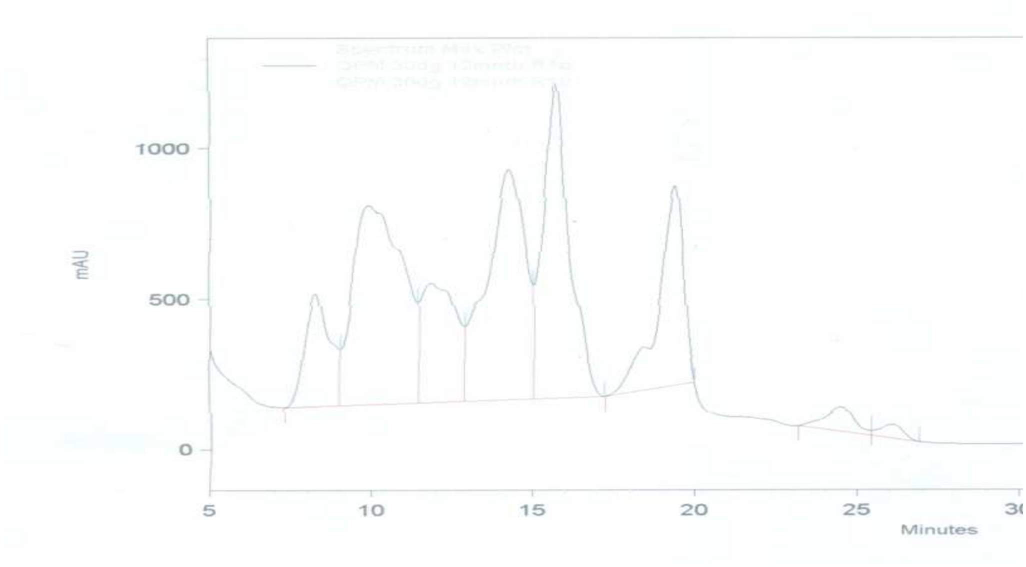

Figure 7.5g Zein profile of SYN4QYQPM after storage at 30°C for 12 months

Peak 9.4 was similar at 3.6°C for both QPM and normal maize genotypes after 6 and months of storage, but higher for QPM and normal maize genotypes at 18.5°C and 30°C after 6 and 12 months of storage. The observed high peak under higher temperatures of 18°C and 30°C could suggest that high temperatures during storage had a positive influence on the extractable zein content of both QPM and normal maize genotypes (Bhullar and Jenner, 1985; Wilhelm *et al.*, 1999). Peak 10.5 was absent in almost all normal maize at 3.6°C, 18.5°C and 30°C after 6 and 12 months of storage with an exception of P6479F2, but present in some QPM genotypes with an exception of four QPM genotypes. Peak 11.8 was present in all non-QPM genotypes at 3.6°C, 18.5°C and 30°C after 6 and 12 months of storage, but absent in QPM genotypes with an exception of three QPM genotypes. The absence of proteins in the HPLC profile may not necessarily mean that the protein has been degraded. Proteins including extractable maize zein proteins may also agglomerate during storage which makes them unextractable by the applied method (Palzer, 2005; Kim, 2008; Dhanalakshmi *et al*., 2011).

Peak 13.9 was present in almost all normal maize and QPM genotypes at 3.6^{o}C, 18.5^{o}C and 30^{o}C after 6 and 12 months of storage with an exception of one normal maize genotype and one QPM genotype. Peak 15 was higher in normal maize genotypes than in QPM genotypes at 3.6^{o}C after 6 and 12 months of storage, but lower in normal maize genotypes at 18.5^{o}C after 6 and 12 months of storage. At 30^{o}C, peak 15 was high in both QPM and normal genotypes after 6 and 12 months of storage. Peak 18.4 was, at 3.6^{o}C, stable for all normal maize and QPM genotypes after 6 and 12 months of storage compared to the control. Peak 18.4 at 18.5^{o}C had higher values after 6 months of storage, but after 12 months the values increased even further. At 30^{o}C, peak 18.4 was high in both QPM and normal maize genotypes after 6 months of storage but low after 12 months of storage in both QPM and normal maize genotypes compared to the control, and this is in agreement with earlier reports that persistent heat stress negatively affects proteins including extractable zein (Rehman *et al*., 2002).

Peak 7.9 was not largely influenced by storage conditions and mostly at 18.5^{o}C after 6 months of storage there was a significant increase in this peak against the control for six of the tested genotypes. SYN5QW was an exception where all but two treatments caused a significant reduction in this peak. Peak 9.4 was very sensitive to storage in most of the entries. In CB389xI37F2, P6479F2, SYN12QW, SYN2QW, SYN4QY and SYN5QW all storage treatments caused a significant reduction in this peak. C3505 and CB346xI37F2 were not significantly influenced by storage.

Very few entries had peak 10.5 but in the one normal maize variety all the treatments caused a significant increase in this peak. In SYN15QW all treatments caused a significant reduction in this peak and in SYN2QY three of the treatments caused a significant reduction in this peak. For peak 11.8 there were variable reactions to storage, there were some significant differences but there was no consistent pattern.

For peak 13.9 especially the heat treatment (30°C) for both 6 and 12 months and the 18.5°C treatment for 12 months caused a significant increase in this peak for most of the QPM entries. For peak 15 there was a clear pattern with heat treatment for 12 months causing a significant increase in this peak for all but three entries. Heat treatment for 6 months also caused a significant increase in this peak for eight of the entries. This peak was also significantly increased in six of the entries after 12 months of storage at 18.5°C. For peak 18.4 during 12 month storage at 18.5°C a significant increase was seen for six of the nine QPM entries.

The results of this study indicated that seed storage at consistently high temperature and the duration of storage lead to a significant reduction in protein content and significantly influenced some of the zein peaks. The data indicated that maize seeds stored at 3.6°C and 18.5°C for 6 months had more protein and zein content as opposed to the maize seeds stored at 18.5°C for 12 months and 30°C for 6 and 12 months. This is not necessarily in agreement with the previous reports that protein content was increased due to the effect of persistent heat (Monjardino *et al.*, 2005) and that storage at low temperature had a negative effect on chemical compounds including protein and zein content (Rehman *et al.*, 2002). Smith and Smith (1986) also confirmed that environmental effects can markedly affect both the quantitative and qualitative constitution of proteins that are formed in the seed. Nevertheless, losses in these chemical compounds occurred to a lesser extent during storage at 18.5°C after 6 months of storage. In contrast, no significant changes in protein content were observed during storage of maize seeds at 3.6°C after both 6 and 12 months of storage.

7.5 CONCLUSIONS

The results from this study showed that high temperature during storage has a negative impact on the protein content. Therefore, it is suggested that maize grains should not be stored at high temperatures above 16-20°C in order to minimize protein losses during storage. In addition, heat treatment showed to have negatively affected the zein peaks, suggesting that low temperature is ideal for zein storage. The results of this study also highlights the need for further investigations on the changes in protein content and zein peaks of various maize genotypes during storage so that informed decisions about changing chemical compounds can be made available and shared between those who make recommendations on storing grain with minimal quantity losses.

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CHAPTER 8

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Maize is a versatile crop and many people, particularly those in rural communities, depend largely on it as their main food source for carbohydrates and proteins. It is used to manufacture a wide variety of products. As a result, end-use quality traits are very important in the food industry. Despite the importance of these traits in the livelihoods of people, small-scale maize farmers face challenges related to storage. Maize deteriorates to such an extent that it does not meet the acceptable levels of nutrition. The problem of deterioration of the grain during storage is perpetuated by unfavourable storage conditions and the length of storage period. The demand for maize varieties with high nutritional quality, particularly in rural communities, pushes the small-scale farmers to find ways of storing maize with less storage losses.

In this study, open-pollinated QPM genotypes, one QPM hybrid and non-QPM hybrids were compared for various endosperm and nutritional characteristics. The Ca content was highest in the open-pollinated QPM genotype SYN2QYQPM and lowest in SYN5QWQPM. The Fe content was highest in normal maize genotype CRW3505, but lowest in the open-pollinated QPM genotype SYN13QYQPM. The K content was highest in QPM genotype QS7608, while the lowest K content was observed in normal maize genotype P6479F2. QS7608 had the highest Mg content while normal maize genotype had the lowest Mg content. The highest Mn content was observed in the open-pollinated QPM genotype SYN15QWQPM, while open-pollinated QPM genotype SYN11QYQPM had the lowest Mn content. The open-pollinated QPM genotype SYN4QYQPM had the highest Na content, while the lowest Na content was observed in the open-pollinated SYN13QYQPM. The highest P content was observed in the open-pollinated SYN15QWQPM, while the lowest P content was observed in normal maize genotype P6479F2. The principal component analysis revealed that a relationship exists between some mineral elements, particularly between K and Zn, P and Mn, Zn and P, and K and P, and these mineral elements could be concurrently improved without lowering the concentrations of Na, Ca and Mg. In this study, the overall content of the minerals was lower in normal maize than in QPM, but the content of Ca, Na, Mg and Fe was higher in normal maize than in QPM. Although the QPM in this case has shown to outperform normal maize, these findings do not necessarily suggest that QPM has higher mineral content than normal maize as it has been reported in other studies that the overall content of the minerals in the seed depends largely on the amount of existing minerals present in the soil, the amount of fertilizers applied and other environmental factors. In other studies, some normal maize genotypes showed to have higher mineral content than some of the maize genotypes under study.

In this study, the overall content of the starch and amylose was lower in normal maize genotypes compared with QPM genotypes after storage. The content of starch and amylose reported in other studies was higher than the content of starch and amylose observed in this study, suggesting that starch and amylose content could have been influenced by the environmental factors. The open-pollinated QPM genotypes SYN2QWQPM and SYN4QYQPM had the highest and lowest amylose content, respectively. The average amylose content of normal maize dropped from 18.18% to 17.96% at 3.6°C during 6 and 12 months of storage. In QPM, it dropped from 18.87% to 18.65% at 3.6°C during 6 and 12 months of storage at 3.6°C. In other studies, the amylose content did not change significantly in QPM, open-pollinated QPM and normal maize genotypes. The amylose content of normal maize dropped from 17.54% to 16.48% at 18.5°C during 6 and 12 months of storage. In QPM, the average content dropped from 17.33% to 16.20% at 18.5°C during 6 and 12 months of storage at 18.5°C. In other studies, the amylose content did not change significantly in QPM, open-pollinated QPM and normal maize genotypes. The amylose content of normal maize dropped from 16.28% to 14.28% at 30°C during 6 and 12 months of storage. In QPM, it dropped from 16.17% to 14.24% at 30°C during 6 and 12 months of storage. In other studies, the amylose content did not change significantly in QPM, open-pollinated QPM and normal maize genotypes. The open-pollinated QPM genotype SYN2QWQPM had the highest amylose content during 6 and 12 months of storage at 3.6°C, 18.5°C and 30°C. The open-pollinated QPM genotype SYN4QYQPM had the lowest amylose content at 3.6°C, 18.5°C and 30°C after 6 and 12 months of storage. High temperature of 30°C negatively affected the amylose content whereas 3.6°C and 18.5°C were ideal for stabilising the amylose content.

The starch content of normal maize dropped from 58.47% to 58.14% at 3.6°C during 6 and 12 months of storage. In QPM, it dropped from 60.25% to 59.24% at 3.6°C during 6 and 12 months of storage. The starch content did not change significantly in QPM, open-pollinated QPM and normal maize genotypes. The starch content of normal maize dropped from 58.22% to 57.77% at 18.5^{o}C during 6 and 12 months of storage. In QPM, it dropped from 59.49% to 58.95% at 18.5^{o}C during 6 and 12 months of storage at 18.5^{o}C. In other studies, starch content did not change significantly in both QPM, open-pollinated QPM and normal maize genotypes. The starch content of normal maize dropped from 56.03% to 53.89% at 30^{o}C during 6 and 12 months of storage. In QPM, it dropped from 57.28% to 55.64% at 30^{o}C during 6 and 12 months of storage at 30^{o}C. In other studies, starch content did not change significantly in QPM, open-pollinated QPM and normal maize genotypes.

The open-pollinated QPM genotype SYN2QYQPM also had the highest starch content at 3.6^{o}C, 18.5^{o}C and 30^{o}C after 6 and 12 months of storage. The normal maize genotype CRW3505 had the lowest starch content at 3.6^{o}C, 18.5^{o}C and 30^{o}C after 6 and 12 months of storage. The low temperatures of 3.6^{o}C and 18.5^{o}C had a minimal impact on the starch content as opposed to 30^{o}C which significantly reduced starch content. Although starch and amylose dropped significantly in non-QPM compared with QPM genotypes during storage, the research evidence in other studies suggest that content of starch and amylose does not change significantly during storage. In addition, storage does not seem to induce nutritional changes in maize seed during storage, however, a decrease in starch and amylose content has been observed in soybean seeds though the changes could have been influenced by mycoflora inoculation. However, the observed changes in starch, amylase, protein and zein, could be due to the extreme storage conditions (e.g. relative humidity) that the seeds were exposed to. That is, relative humidity could indeed be a reason for these observations, since in other studies it was controlled.

The protein content of normal maize dropped from 8.17% to 8.02% at 3.6^{o}C during 6 and 12 months of storage. In QPM, it dropped from 6.95% to 6.80% at 3.6^{o}C during 6 and 12 months of storage at 3.6^{o}C. During 6 and 12 months of storage at 3.6^{o}C, the protein content of open-pollinated QPM dropped from 11.26% to 11.11%. In other studies the protein content did not change significantly in both QPM, open-pollinated QPM and normal maize genotypes, with the exception of where the mycoflora were used. The protein content of normal maize dropped from 8.01% to 7.70% at 18.5^{o}C during 6 and 12 months of storage. During 6 and 12 months of storage at 18.5^{o}C, the average protein content of open-pollinated QPM dropped from 10.62% to 10.31%. In other studies, protein content did not change significantly in both QPM, open-pollinated QPM and normal maize genotypes, unless the mycoflora and other destructive insects were present. The protein content of normal maize dropped from 7.68% to 7.05% at 30°C during 6 and 12 months of storage. During 6 and 12 months of storage at 30°C, the protein content of open-pollinated QPM dropped from 10.29% to 9.65%. In other studies, protein content did not change significantly in both QPM, open-pollinated QPM and normal maize genotypes, with the exception of where the mycoflora were used.

South African open-pollinated QPM and a QPM hybrid had significantly higher protein content than the normal maize. The open-pollinated QPM genotype had the highest protein content whereas the normal maize genotype CB341xI37F2 had the lowest protein content. The overall content of the protein was low in non-QPM compared with QPM before storage, but normal maize genotype CRW3505, had higher protein content than only two QPM genotypes (QS7608 and SYN2QYQPM). In previous studies, the content of other chemical compounds including that of protein, has shown to be higher than the protein content observed in this study. At different storage conditions of 3.6°C, 18.5°C and 30°C, the protein content and composition differed. Some general guidelines can be formulated to optimise protein content and composition during storage, especially in QPM. Although storage does not seem to induce nutritional changes in maize seed during storage, biochemical changes have been observed in soybean seeds though the changes have been influenced by mycoflora inoculation. In this study, the content of the protein has also been significantly reduced over storage time under different storage conditions, and this tendency has not been observed in other studies. The storage conditions do not in all cases lead to the reduction of protein content. Protein content has shown to be positively influenced by high temperature storage conditions in other research studies on seed storage.

QPM genotypes had higher values than the non-QPM genotypes for the zein peak eluting at 7.9 minutes. The peak eluting at 9.4 minutes was higher for all non-QPM genotypes than QPM genotypes with the exception of SYN4QYQPM which had values similar to the non-QPM genotypes. The peak at 10.5 minutes was present in almost all QPM. The peak eluting at 11.8 minutes was present in all non-QPM but only in three of the QPM genotypes. The peak eluting at 15 minutes was present and similar for both QPM and non-QPM genotypes with the exception of non-QPM genotype P6479F2 which had higher values than all QPM and non-QPM genotypes. Peaks eluting at 13.9 and 18.4 minutes were similar for all QPM and non-QPM genotypes.

Peak 7.9 was not largely influenced by storage conditions and mostly at 18.5°C during 6 months of storage there was a significant increase in this peak against the control for six of the tested genotypes. SYN5QW was an exception where all but two treatments caused a significant reduction in this peak. Peak 9.4 was very sensitive to storage in most of the entries. In CB389xI37F2, P6479F2, SYN12QW, SYN2QW, SYN4QY and SYN5QW all storage treatments caused a significant reduction in this peak. C3505 and CB346xI37F2 were not significantly influenced by storage. Very few entries had peak 10.5 but in the one normal maize variety all the treatments caused a significant increase in this peak. In SYN15QW all treatments caused a significant reduction in this peak and in SYN2QY three of the treatments caused a significant reduction in this peak. For peak 11.8 there were variable reactions to storage, there were some significant differences but there was no consistent pattern. For peak 13.9 especially the heat treatment (30°C) for both 6 and 12 months and the 18.5°C treatment for 12 months caused a significant increase in this peak for most of the QPM entries. For peak 15 there was a clear pattern with heat treatment for 12 months causing a significant increase in this peak for all but three entries. Heat treatment for 6 months also caused a significant increase in this peak for eight of the entries. This peak was also significantly increased in six of the entries after 12 months of storage at 18.5°C. For peak 18.4 during 12 month storage at 18.5°C a significant increase was seen for six of the nine QPM entries.

The results showed that the starch, amylose, protein and zein content were negatively influenced with increase in storage time at 30°C during 6 and 12 months of storage and at 18.5°C during the 12 month storage period, and this tendency has been confirmed in other studies. A reduction of these negative effects is possible through the provision of suitable storage conditions although it was shown that natural deterioration occurs and varies according to the storage period. Therefore, even with the best storage facilities, seed deterioration is inevitable as it was shown to influence grain composition in both types of maize. The 6 and 12 months of storage at 3.6°C had a little impact on the grain composition, suggesting that there is a need for adoption of cold storage for both normal maize and QPM genotypes. However, this is usually expensive for long-term storage as the operational costs are too high. This means that a cheap and efficient cold storage method must be initiated. The study has revealed the importance of assessing the nutritional quality characteristic of the seeds obtained from and stored at different environments. Based on this study, it is advisable that the detailed records on the condition of the grain before and after storage be kept to help in identifying potential storage problems and in planning preventative action.

In conclusion, there were differences between QPM and normal maize genotypes in terms of nutritional characteristics. QPM genotypes had higher protein content than other tested normal maize genotypes. During 6 and 12 months of storage at 3.6°C, 18.5°C and 30°C, the same QPM genotypes retained their high protein content in terms of the rankings and the normal maize genotypes retained their low protein content. Some zein peaks were shown to be specific for QPM or non-QPM. QPM genotypes were not necessarily higher in amylose and starch contents than non-QPM genotypes before and after storage at 3.6°C, 18.5°C and 30°C.

The present investigations show that during storage, seeds were affected by storage conditions such as high temperature (30°C) and storage time (12 months), resulting in change of nutritional composition. All the chemical compounds including starch, amylose and protein determined in this study under different storage conditions and before storage showed to have been consistently reduced compared with other chemical compounds determined under more or less the same conditions, and the reason behind this change was unclear. However, the conditions that all these seeds have been exposed to, particularly during storage, were different. In addition, this study and other studies mainly differ from one another by the use of mycoflora during storage. That is, where these compounds have been reduced, especially during storage, mycoflora were a number one suspect for those observations. In addition, these compounds did not seem to change under storage conditions where mycoflora were not used in many other studies. However, only few studies led to the discovery of little biochemical changes during seed storage.

SUMMARY

Maize is the main source of proteins, minerals, carbohydrates and lipids for many people, and the world-wide demand for these primary nutrients is growing continuously. The physical and compositional characteristics of maize are of utmost importance. Quality protein maize (QPM) has a high nutritional value, but its nutritional content may be affected by storage conditions. The objectives of this study were to determine the nutritional composition of South African open-pollinated QPM varieties and a QPM hybrid, compared with normal maize hybrids and finally to evaluate the influence of different storage conditions on these measured nutritional characteristics. This was realised by exposing the seed of these genotypes to 3.6^{o}C, 18.5^{o}C and 30^{o}C for 0, 6 and 12 months of storage. The nutritional quality and endosperm characteristics of these genotypes were measured first before storage and then after 6 and 12 months of storage, respectively. Before storage, open-pollinated QPM, a QPM hybrid and maize genotypes had comparable amounts of minerals, amylose and starch, revealing that the two types of tested maize genotypes were not necessarily different from each other. However, protein content in QPM was significantly higher than in normal maize genotypes. Some zein peaks were also different in QPM than in normal maize genotypes. Zein peaks of both QPM and normal maize were significantly increased by warm storage conditions (18.5^{o}C and 30^{o}C) for 6 and 12 months of storage. A 5^{o}C temperature did not significantly influence the zein peaks of both QPM and normal maize genotypes for 6 and 12 months of storage. During storage, one open-pollinated QPM genotype SYN2QWQPM retained higher amylose, starch and protein content than non-QPM and other QPM genotypes. This suggests that optimal conditions for storage are distinctive for different genotypes, and it is possible to suggest some general guidelines for storage to maintain endosperm and nutritional characteristics. The protein and endosperm characteristics were negatively influenced by storage at 3.6^{o}C, 18.5^{o}C and 30^{o}C, but SYN15QWQPM was least affected. The 3.6^{o}C for 12 months and 18.5^{o}C for 6 months seemed to be the most favourable conditions to retain kernel stability in terms of the measured characteristics. The results suggest that there is a need for adoption of QPM as it had better protein quantity and quality than the normal maize hybrids.

Keywords: QPM, maize, nutritional composition, storage, minerals, starch, amylose, protein, zein.

OPSOMMING

Mielies is die hoof bron van proteïene, minerale, koolhidrate en vette vit baie mense, en die wêreldwye aanvraag na hierdie primêre voedingstowwe groei voortdurend. Die fisiese eienskappe en smaestelling van mielies is baie belangrik. Kwaliteit proteïen mielies (QPM) het ‘n verbeterde voedingswaarde, maar die voedingswaarde kan deur storingstoestande beïnvloed word. Die doel van hierdie studie was om die voedingswaarde en samestelling van Suid Afrikaanse oopbestuifde QPM genotipes en ‘n QPM baster, in vergelyking met normale mielie basters te bepaal en om dan die invloed van verskillende storingstoestande op sekere voedingswaardes te bepaal. Dit is gedoen deur die saad bloot te stel aan 3.6^{o}C, 18.5^{o}C en 30^{o}C vir 0, 6 en 12 maande van storing. Die voedings en endosperm eienskappe van hierdie genotipes is gemeet voor storing en daarna by 6 en 12 maande van storing onderskeidelik. Voor storing het die oopbestuifde QPM, die QPM baster en die gewone mielie genotipes vergelykbare hoeveelhede minerale, amilose en stysel gehad, wat getoon het dat die twee tipes mielies nie noodwendig van mekaar verskil het nie. Die proteïeninhoud van die QPM genotipes was egter betekenisvol hoër as die van die normale mielie genotipes. Seker zeïen pieke was verskillend tussen QPM en gewone mielie genotipes. Zeïen pieke van beide QPM en gewone mielies is betekenisvol verhoog by warm storingstoestande (18.5^{o}C en 30^{o}C) vir 6 en 12 maande van storing. Die 5^{o}C temperatuur het geen betekenisvolle invloed op die zeïen pieke van beide gewone en QPM mielies gehad na 6 en 12 maande van storing gehad nie. Tydens storing het een oopbestuifde QPM genotipe, SYN2QWQPM, hoër amilose, stysel en proteïeninhoud as gewone mielies en ander QPM genotipes behou. Dit is ‘n aanduiding dat optimale storingstoestande verskil van een na die ander genotipe, maar dit is moontlik om algemene riglyne voor te stel vir storing om endosperm en voedingseienskappe te behou. Die proteïen en endosperm eienskappe is negatief beïnvloed deur storing by 3.6^{o}C, 18.5^{o}C en 30^{o}C, maar SYN15QWQPM is die minste beïnvloed. Die 3.6^{o}C temperatuur vir 12 maande en 18.5^{o}C vir 6 maande was die beste toestande om saad stabiliteit vir die gemeette eienskappe te behou. Die resultate het aangedui dat QPM gebruik moet word, omdat dit beter proteïen kwantiteit en kwaliteit het as gewone mielie basters.

Sleutelwoordes: QPM, mielies, voedingssamestelling, storing, minerale, stysel, amilose, proteïen, zeïen.