Journal of Stored Products Research 56 (2014) 16–20

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

The influence of storage conditions on starch and amylose content of South African quality protein maize and normal maize hybrids

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ARTICLE INFO

Article history:
Accepted 19 November 2013

Keywords:
Quality
Protein
Maize
Storage
Starch
Amylose

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 °C, at room temperature (18.5 °C) and at 30 °C for 0, 6 and 12 month storage periods, respectively. Sixteen genotypes were tested in a single trial at Potchefstroom in South Africa. Due to optimal growing conditions the seed was of excellent quality. Storage at 3.6 °C and 18.5 °C caused some reduction in amylose and starch content, although for starch it was not significant. On the other hand, storage at 30 °C significantly ($P \leq 0.05$) reduced the starch and amylose content after both 6 and 12 month periods of storage. QPM and non-QPM seed reacted similarly to storage conditions, and there were larger differences between cultivars than between QPM and non-QPM material. Even at relatively high relative humidity, low temperature storage maintained seed quality the best. Therefore high temperature storage is detrimental to starch and amylose content of both normal maize and QPM, especially after 6 months or more of storage. Maize should therefore be stored under low temperatures (around 4 °C) or if not possible, at least under 19 °C.

1. Introduction

Starch, the chief source of carbohydrate in human diets, forms the major energy reserve in cereal grains. Amylose and amylopectin are the two glucopolysaccharide components of starch. Starch granule physical properties are influenced by the distribution of amylose and amylopectin in starch (Corcuera et al., 2007).

The starch and amylose contents of seed depend on the quality, physical structure and storage conditions of the seed after harvest (Buckow et al., 2009). Poor storage conditions often prevail and can lead to biochemical changes in chemical compounds of maize kernels including amylose and starch content during storage. The possible causes of these changes are not clear, but high relative humidity (r.h.) and the formation of free radicals during storage have been reported to play a role (MacDonald, 1999, 2006). These changes may affect the starch and amylose content. During storage, part of the starch and amylose has been reported to undergo an aggregation process that leads to changes in their content (Liu et al., 2003; Pongsawatmamit et al., 2006).

There is a concern in terms of food security, on how to best store seeds, including maize seeds, for a long time without compositional changes, especially in developing countries worldwide. In these countries, efficient maintenance of grain quality is often not possible due to the lack of adequate post-harvest technology, including suitable storage conditions (Doijode, 2001).

Quality protein maize (QPM) is nutritionally enhanced and has 70–100% more lysine and tryptophan than normal maize. QPM cultivars are being introduced in a number of African countries to reduce protein deficiencies in areas where maize is the staple food (Sofi et al., 2009). Little is known about how storage conditions affect both normal maize and QPM with special reference to starch and amylose content.

It is better to implement preventive management, rather than to solve specific storage problems once they have occurred (Lopes et al., 2008). The aim of this study was to investigate the influence of different storage conditions on starch and amylose content extracted from the South African open pollinated QPM, a QPM hybrid and normal maize samples which were stored for 6 and 12

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0022-474X/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.jspr.2013.11.004

months at 3.6 °C, 18.5 °C and 30 °C compared to an untreated control.

2. Materials and methods

2.1. 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 and works on a broad diversity of cereal crops grown in South Africa. Potchefstroom is located at an altitude of 1344 m above sea level with an average minimum and maximum temperature of 9.6 °C and 25.5 °C respectively with an average annual total rainfall of 619 mm (Weather South Africa, 2013). The rainfall during the growing season (November to April) was 409 mm which was well distributed over the season. The temperatures for the last two months of the growing season when grain filling took place ranged between a minimum of 12.6 °C and a maximum of 27.9 °C. Grain quality and yield was very good for this season.

2.2. Plant material

The genotypes used in this study were eight South African open pollinated QPM varieties (SYN2QYQPM, SYN4QYQPM, SYN11-QYQPM, SYN13QYQPM, SYN2QWQPM, SYN5QWQPM, SYN12Q WQPM 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.

2.3. Experimental design and procedures

The trial was planted for one season at a density of 50,000 plants per hectare on sandy clay loam. The experimental design was a randomised complete block design with three replications. The genotypes were grown in two-row plots. The rows were 5 m long with 0.3 m spacing apart, and row width was 90 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). 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.

2.4. Storage conditions

Seed sampling was done randomly from each of the three replications after checking for QPM status. All samples were sealed in brown paper bags and stored under controlled conditions in a cold room (3.6 °C), laboratory (18.5 °C) and oven (30 °C) for 6 and 12 months and were compared with an untreated control. The humidity was 76.5% r.h. in the cold room (3.6 °C), and 28% r.h. in the laboratory (18.5 °C). The laboratory was air conditioned which maintained a constant temperature. In the oven (30 °C) moisture was very low or close to zero due to the prolonged exposure to heat. Forty-five bags (15 entries with three replications) 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 contained 90 maize seeds (45 seeds for the 6 months sampling, and 45 for the 12 month sampling). A randomized complete block design was used for the layout with three replications where each replication consisted of 45 seeds. After 6 and 12 month storage periods, 45 seed samples per bag were sampled and bulked and ground to a fine powder using a 1 kA 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.

2.5. Starch extraction and analysis

Starch content was determined according to the Megazyme total starch assay procedure (Megazyme, 2009). A homogenized milled sample (100 mg) of three replications of each genotype was measured into glass test tubes 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 °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. The tubes were incubated in a boiling water bath at 95 °C for 6 min and stirred vigorously after 2, 4 and 6 min. The tubes were then placed in a water bath at 50 °C after which sodium acetate buffer (4 ml, 200 mM, pH 4.5) was added, followed by amyloglucosidase (0.1 ml, 20 U). 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. 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 °C for 20 min. A glucose control was prepared by mixing 0.1 ml of glucose standard solution (1 mg ml^{-1}) 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 hydrolyzed starch and was expressed as a percentage of total sample weight on an "as is" basis:

$$\text{Total starch} = \Delta A \times F \times \text{FV}/0.1 \times 1/1000 \times 100/W \times 162/180$$
$$= \Delta A \times F/W \times \text{FV} \times 0.9$$

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).

2.6. Amylose determination

Amylose was extracted and estimated by the iodine binding method (Cruz and Khush, 2000). One hundred milligram of homogenized maize flour sample was measured for three replications of each genotype. The samples were wetted with 1 ml of 95%