Variations in fatty acid proportions during desiccation of *Telfairia occidentalis* seeds harvested at physiological and agronomic maturity

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The effect of desiccation on lipid content, fatty acid composition and the antioxidative enzymic capacity was investigated in seeds of *Telfairia occidentalis*, harvested at physiological and agronomic maturity. Seeds were dried at 5 and 28 °C, environments that induced different drying and metabolic rates. Desiccation of seeds was associated with decreased antioxidative enzymic capacity (of peroxidase and polyphenoloxidase), and thus increased likelihood of free radical attack and decreased viability (germinability). Agronomically mature seeds contained predominantly saturated fatty acids (tridecanoic), with very low levels of the major fatty acids of edible oilseeds (palmitic, stearic or the unsaturated C18 fatty acids). There was increased accumulation of the mono-unsaturated (oleic) and polyunsaturated (linoleic) fatty acids when seeds were dried at 28 °C and moisture contents have reduced to about 42 % or lower. In contrast, seeds dried at 5 °C maintained high levels of saturated fatty acids and lower levels of monounsaturated or polyunsaturated fatty acids. Results suggest the need to develop different post-harvest protocols for seed storage, and for processing *T. occidentalis* to 'improve' the seed fatty acid profile as an oilseed for human and animal food.

Key words: Agronomic maturity, desiccation, fatty acid, lipid peroxidation, oilseed.

INTRODUCTION

Telfairia occidentalis Hoof (Family Cucurbitaceae), commonly called fluted pumpkin, is a crop of commercial importance grown across the lowland humid tropics in West Africa (Nigeria, Ghana and Sierra Leone being the major producers). However, there are no identifiable information on the crop in terms of varieties, harvesting methods, preservation, storage methods, oil composition and processing methods (FAO, 1992). The crop is grown mainly for the leaves, which constitute an important component of the diet in many West African countries (Gill, 1988). Seeds of *T. occidentalis* are recalcitrant or intolerant of desiccation. Consequently, nuclear stock for the next planting season is maintained locally by storing fully mature fruits (Nkang et al., 2000). Due to their high moisture content, they can only be maintained in short-term storage (about three months) as seeds held in pods usually rot or progress directly to germination, without developmental arrest. Although pumpkin seeds are recalcitrant, limited desiccation enhances their germination (Nkang et al., 2000). Plant tissues experience oxidative stresses under moisture stress, with

accumulation of free radicals and build up of hydroperoxides (Hendry et al., 1992). These peroxidative reactions may be influenced by the rate of drying (Li and Sun, 1999; Pammenter et al., 2000). Increased activities of free radical scavenging systems (Hendry et al., 1992) and/or the accumulation of substances (such as sugars) that improve structural stability (Buitink et al., 2000) will reduce damage by peroxidative reactions.

Although pumpkin seeds are rich in oil storage reserves, it presently has very low commercial value as an oilseed but is potentially valuable as a high protein oilseed for human and animal food (Giami et al., 1999). In local practice, seeds of melon (*Colocynthis vulgaris*, Family Cucurbitaceae) are used in cooking only after being sun-dried and/or fried. Perhaps the potential utilitarian benefits of pumpkin seeds could be improved similarly. To the best of our knowledge, there have been no studies on the effects of drying on nutritional quality and antioxidant systems of pumpkin seeds but there are a few reports on *in vitro* protein digestibility and anti-nutritional factors in extracts from raw, fermented and germinated seeds (e.g. Giami et al., 1999). As part of studies aimed at improving processing of pumpkin seeds for storage and consumption, this paper reports on the changes in the antioxidative enzymic capacity, storage

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reserves and fatty acid composition during desiccation, under environments that induced different drying rates, of *T. occidentalis* seeds harvested at physiological and agronomic maturity.

MATERIALS AND METHODS

Plant material and desiccation treatments

Ten plants of *T. occidentalis* were raised from seeds obtained from a single provenance and grown to maturity in a completely randomized design in two experimental plots located within the University of Calabar, Calabar (8° 20$^/$ E, 4° 57$^/$ N), Nigeria. Each experimental plot was considered a replicate. Following pod set, which occurred approximately 17 weeks after planting, fruits were harvested at 2-3 weekly intervals for observation of fruit/seed characteristics and determination of seed moisture contents. Harvesting of fruits for assessment of seed germinability, storage reserves and assays of enzymic activity was started at 9 weeks after pod set. This interval was considered as corresponding, approximately, to the period when seeds attained physiological maturity (attainment of seed maximum dry weight). Agronomic maturity occurred later and is defined as the point of 'natural' dispersal. At harvest, 4-5 fruit pods of each experimental plot were cut open and the seeds extracted, pooled together and cleaned free of fruit pulp. Batches of 250 seeds (from each experimental plot) were treated with captan (2g/kg seed) and dried over $CaCl_2$ in desiccators maintained at 5°C (± 1°C; effective relative humidity 55%) and 28°C (± 2°C; effective relative humidity 47%). The desiccation treatments were carried out over a nine-day period. Samples of 50 seeds per replicate were analyzed at harvest and following desiccation.

Germination tests were carried out on 20 seeds per replicate under diurnal illumination at 25°C (± 2°C). Germination percentages were calculated only on seeds whose radicles had emerged through the seed coat by more than 2 mm. Moisture contents of intact seeds were determined on a fresh weight basis, after oven-drying at 103°C for 17 hours (ISTA, 1985).

Extraction of enzymes

Testae and small embryonic axes were routinely removed from 10 seeds before any extractions were effected. Four grams fresh weight of cotyledonary tissue was homogenized in 20 ml of 20 mM mixed potassium phosphate buffer (pH 7.5) containing 2% polyvinyl polypyrrolidone. The homogenate was centrifuged at 2000 rpm and the supernatant passed through two layers of cheesecloth. The filtrate was further centrifuged at 12000 rpm for 10 min and the supernatant used as crude enzyme source. Enzyme fractions were partially purified as follows: Five millilitres of the crude enzyme was brought to 80% saturation with $(NH_4)_2SO_4$. The precipitate was collected by centrifugation, dissolved in 0.5 ml of 20 mM mixed potassium phosphate buffer (pH 7.5) and desalted by passage through a Sepharose 4B column (10 cm x 1.8 cm) previously equilibrated with the same buffer. All operations were carried out at 5°C.

Enzyme assays

Polyphenol oxidase: To 3 ml of assay buffer (20 mM mixed potassium phosphate buffer, pH 7.0 at 30 oC) was added 100 µl 20mM DOPA (dihydroxy phenylalanine), followed by 300 µl of partially purified enzyme preparation. The mixture was incubated for 1 min at 30°C and the reaction started with the addition of 7 µmol

H_2O_2 (30% v/v). Activity was measured colorimetrically at 475 nm (Kahn, 1983) and calculated using an extinction coefficient of 1,433 mM/cm for the quinone product (Jimenez and Garcia-Carmona, 1995).

Peroxidase: To 3 ml of assay buffer (20 mM mixed phosphate buffer, pH 7.0 at 30°C) was added 100 µl 100 mM guaiacol and 300 µl of partially purified enzyme preparation. The mixture was incubated for 1 min at 30 ^{o}C and the reaction initiated with the addition of 1 µmol H_2O_2 (30% v/v). Activity was measured colorimetrically at 436 nm and calculated using an extinction coefficient of 6.39 mM/cm for the guaiacol dehydrogenation product (Putter, 1974).

Extraction of sugars and starch

Soluble sugars were extracted from 1 g of fresh cotyledonary tissues in 20 ml boiling 80% ethanol. Extraction of the sample was repeated twice and the supernatants pooled and concentrated to 10 ml by evaporation. Starch in the ethanol insoluble residue was solubilized in 1M NaOH and neutralized with 1M acetic acid. Soluble sugars and starch content (after acid hydrolysis at 100°C for 15 min in 0.1 M H_2SO_4) (Adams et al., 1980) were estimated colorimetrically as the reducing sugars at 540 nm using the dintrosalicylic acid reagent. Glucose solution (1%) was used as standard.

Data for moisture content, germination, starch/sugar contents and enzyme activities are presented as means (± SE) of duplicate determinations on two replicates.

Extraction and analyses of lipids

Total lipids were extracted from fresh tissues using the solvent system of Khor and Chan (1985). Two grams fresh weight of cotyledonary tissue (obtained from the same samples used for enzymic analysis) was extracted for 30 min with 20 ml of methylene chloride/methanol (2/1, v/v) containing 0.006% of the anti-oxidant butylated hydroxytoluene. Following centrifugation at 1500 rpm, the solvent was washed with a quarter volume of 1% NaCl, recentrifuged, the lower phase aspirated and dried down at 60°C. Fatty acid methyl esters were obtained by dissolving an aliquot of lipid in 1 ml of diethyl ether and adding 200 µl Tri-deuter 8 (Pierce Chemical Company). The mixture was shaken for 1 minute, warmed in boiling water and an aliquot (5 µl) analyzed using a Carlo Erba Fractovap 4200 gas chromatograph equipped with a flame ionization detector. Separation was achieved on a stainless steel column (1.5 m x 4 mm) packed with 10% Silar 5CP on Supelcoport 100 G, and operated isothermally at 240°C with nitrogen (3.2 Kg/cm^2) as carrier gas. Total lipids and area percentages of fatty acids are given as the mean of two determinations on two replicates.

RESULTS

Fruits lost the light to pale green colouration of immature fruits, becoming creamy yellow (at physiological maturity) or deep green (at agronomic maturity) (Table 1). Dry matter accumulation was rapid during seed filling. The cotyledons expanded from about 11 to 47% of the seed's final dry weight within 2 weeks (between weeks 5 and 7 following pod set). Immature seeds (at weeks 5 and 7) exhibited the highest relative moisture contents (more than 70%) and this decreased with development. At about 9 weeks after pod set, seeds were considered

Table 1. Characteristics of *T. occidentalis* fruits/seeds harvested at different developmental stages.

Time after pod Set (weeks)	Fruit colour/seed characteristics	Seed dry weight (g/seed)	Relative moisture content (%)
5	Fruits ashy green, seeds of milky Consistency	0.42 ± 0.05	82.0 ± 6.0
7	Fruits greenish yellow, seeds semi-solid in consistency	1.83 ± 0.17	70.4 ± 2.8
9	Fruits pale green, seeds fully developed, with soft seed coats	3.72 ± 0.27	65.1 ± 3.5
13	Fruits deep green, seeds fully developed, with fibrous seed coats	3.89 ± 0.35	58.3 ± 3.0

Physiological and agronomic maturity are identified as the developmental stages with seed moisture contents of about 65 % and 58 %, respectively. Values shown are means (± SE) of duplicate determinations from two independent determinations.
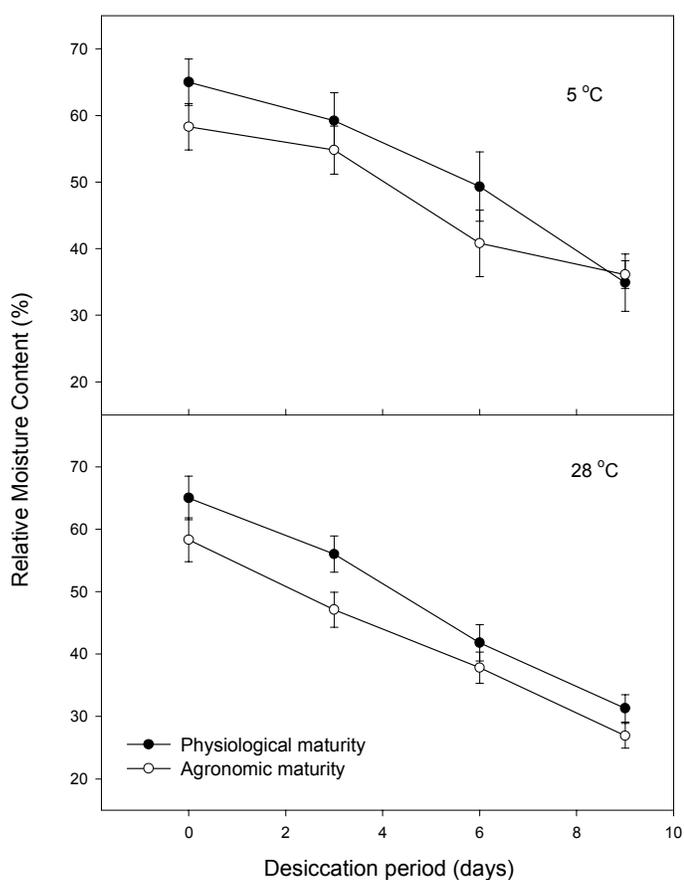

Figure 1. Changes in relative moisture content during desiccation, at 5 or 28°C, of *T. occidentalis* seeds harvested at physiological and agronomic maturity.
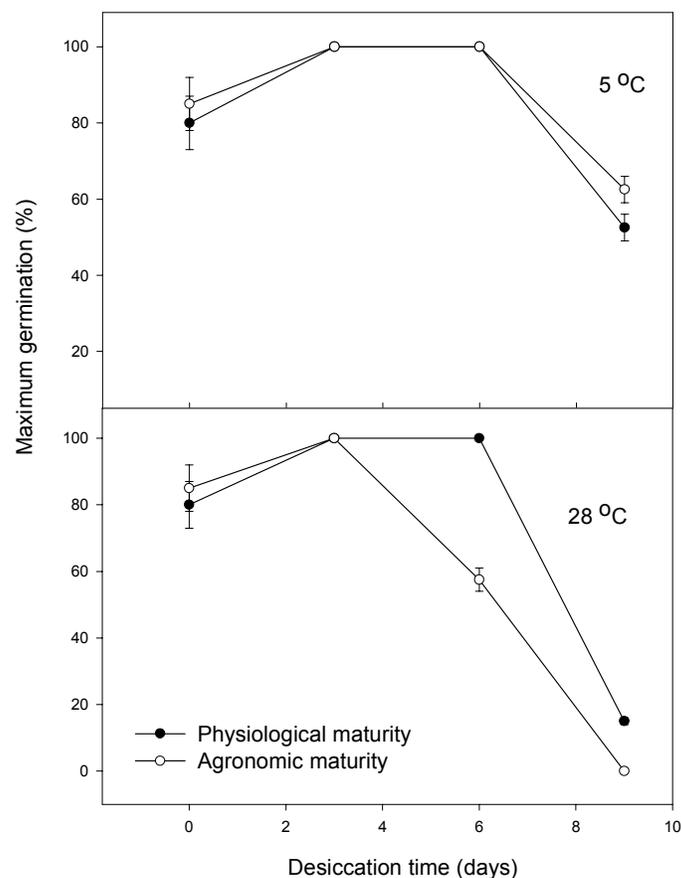

Figure 2. Influence of desiccation time, at 5 or 28°C, on the maximum germination potential of *T. occidentalis* seeds harvested at physiological and agronomic maturity.

physiologically mature having achieved relatively steady dry weights and moisture content had declined to about 65% (wet mass basis, Table 1). Agronomic maturity or point of 'natural dispersal' occurred about 3-4 weeks later, with further reduction in moisture content to about 58%. During the desiccation treatment at 5°C, moisture loss was lower in seeds that attained agronomic maturity (about 38%) relative to the physiologically mature seeds (about 46%) (Figure 1). Moisture loss was relatively greater at 28°C. Seeds harvested at, or after physiological maturity demonstrated high germination values (80-85%) (Figure 2). Limited desiccation (for 3 days) maximised germination, but prolonged desiccation especially at 28 °C led to rapid loss of germinability.
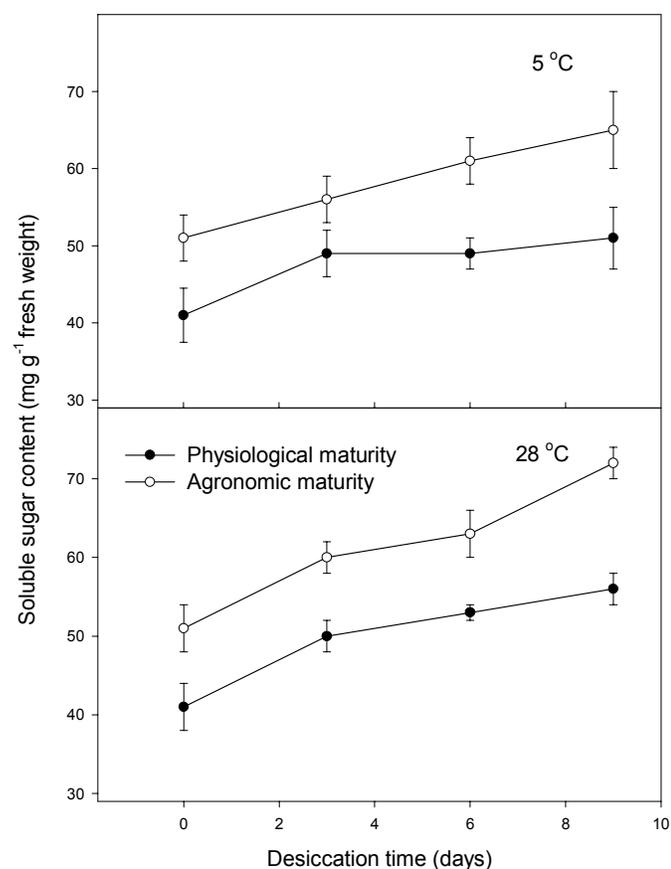

Figure 3. Changes in soluble sugar content during desiccation, at 5 or 28°C, of *T. occidentalis* seeds harvested at physiological and agronomic maturity.

Seed soluble sugar and lipid content increased with developmental maturity and during desiccation (Figures 3 and 4, respectively). Total lipid content was up to 30% (wet mass basis) and soluble sugars up to 7% (wet mass basis) in seeds that attained agronomic maturity (Figures 3 and 4). Seeds, particularly those harvested at agronomic maturity, exhibited higher soluble sugar
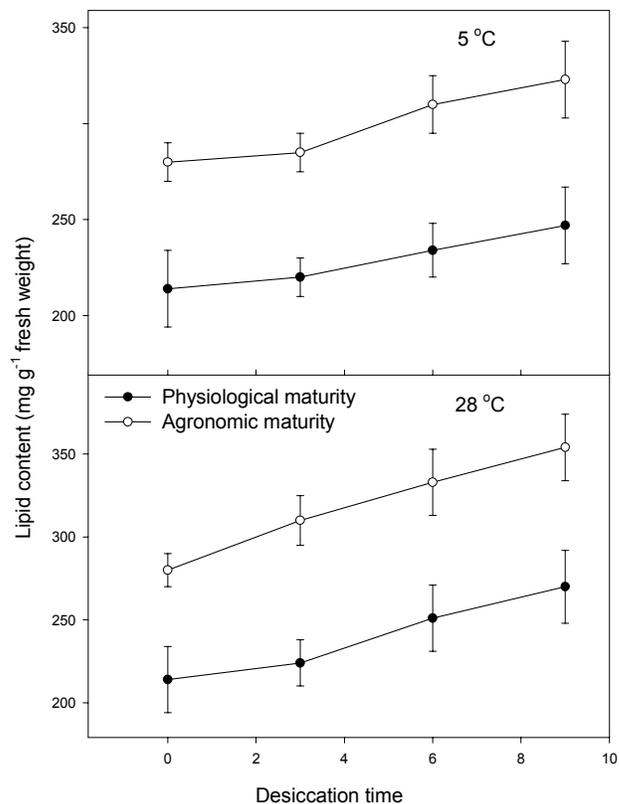

Figure 4. Changes in lipid content during desiccation, at 5 or 28°C, of *T. occidentalis* seeds harvested at physiological and agronomic maturity.

contents when dried at 28°C than at 5°C. Starch content increased with developmental maturity reaching up to 3%, wet mass basis, at agronomic maturity. Starch levels decreased, especially at 28°C, during desiccation (Figure 5).

The fatty acid profile of total lipids extracted from agronomically mature seeds showed a predominance of the saturated fatty acids tridecanoic (13:0) and palmitic, with low levels of stearic (18:0) and the unsaturated fatty acids oleic (18:1) and linoleic (18:2) (Table 2). There were variations in fatty acid composition of seeds during desiccation at different temperatures, with increased accumulation of oleic and linoleic in seeds dried at the higher temperature (28°C). In contrast, seeds dried at the lower temperature (5°C) maintained high levels of saturated fatty acids [stearic and heptadecanoic (17:0)], with low levels of the monounsaturated fatty acids (oleic) (Table 2).

The initial desiccation period was associated with slightly increased levels of peroxidase (15-30%) and PPO activities (20-40%) (Figures 6 and 7, respectively). Activities of both enzymes decreased in the later desiccation period. Developmental maturity in seeds was associated with increased peroxidase activity (about 60%) and slightly decreased (-29.5%) PPO activity.

Table 2. Percentage fatty acid content of lipid fractions of *T. occidentalis* harvested at agronomic maturity and following desiccation at 5°C and 28°C.

Treatment	Moisture content (%)	Tridecanoic	Palmitic	Stearic	Oleic	Linoleic	Heptadecanoic	Hydroperoxide content (absorbance units)
Fresh, mature seeds	64	90.9	4.68	1.06	0.35	3.02	0	0.02
Dried 9 days @ 5°C	49	97.8	2.15	0	0.05	0	0	0.24
Dried 9 days @ 28°C	42	0	0	0	97.2	2.78	0	0.15
Dried 42 days @ 5°C	19	0	0	60.97	15.7	0	23.37	1.20
Dried 42 days @28°C	11	0	0.25	6.15	52.9	40.65	0	0.10

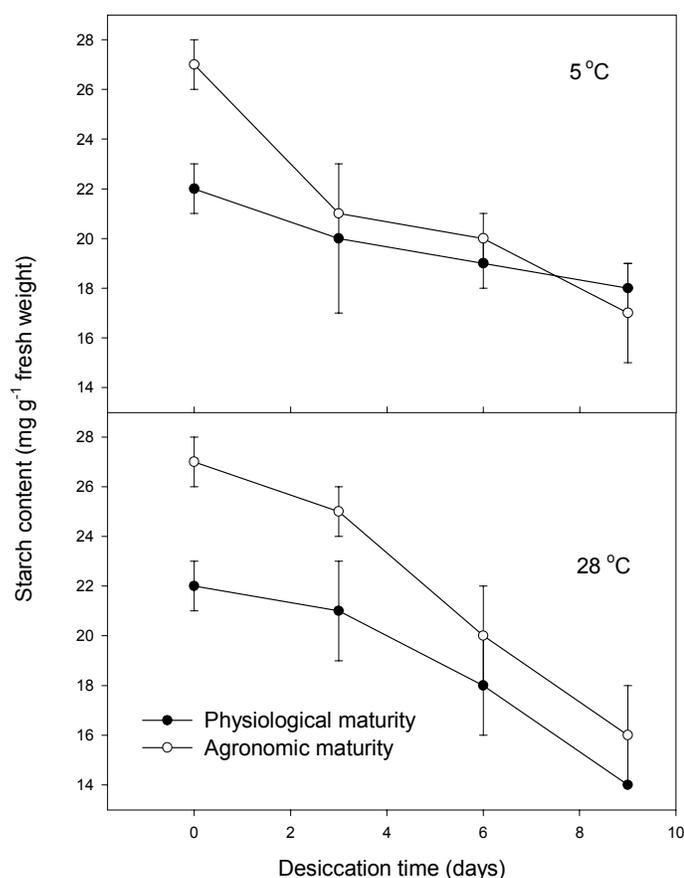**Values shown are means of two independent determinations.**

Figure 5. Changes in starch content during desiccation, at 5 or 28°C, of *T. occidentalis* seeds harvested at physiological and agronomic maturity.

DISCUSSION

Fruit development of *T. occidentalis* followed the more general pattern found in tropical fruits (Janzen, 1983), with seed filling occurring rapidly in a few weeks following pod set. Seed maturation and optimal seed quality has been reported to occur at physiological maturity, approximately 9 weeks after pod setting, and thereafter declined (Adetunji, 1997). The present results agree with the earlier report, having identified physiological maturity on the basis of moisture content, visual characteristics and seed dry weight as occurring at about 9 weeks after pod setting (Table 1).

Seeds at physiological or agronomic maturity have high moisture contents, enmeshed in fruit pulp and thus with the potential to germinate immediately. Limited desiccation enhanced germination of *T. occidentalis* as has been reported in other recalcitrant seeds (Finch-Savage and Blake, 1994; Tompsett and Pritchard, 1998). Our results, therefore, support the local practice of sun-drying seeds for some hours before sowing. Seeds dried at the lower temperature maintained viability (as assessed by % germination) for longer periods during the desiccation treatments, probably due to a slower rate of moisture loss. Moisture loss was relatively lower in seeds that attained agronomic maturity. Drying seeds, which have attained agronomic maturity at low temperatures could be exploited in efforts to develop storage protocols for *T. occidentalis* seeds. However, the low temperature limit to germination (base temperature, T_b) needs to be established as storage of recalcitrant seeds at
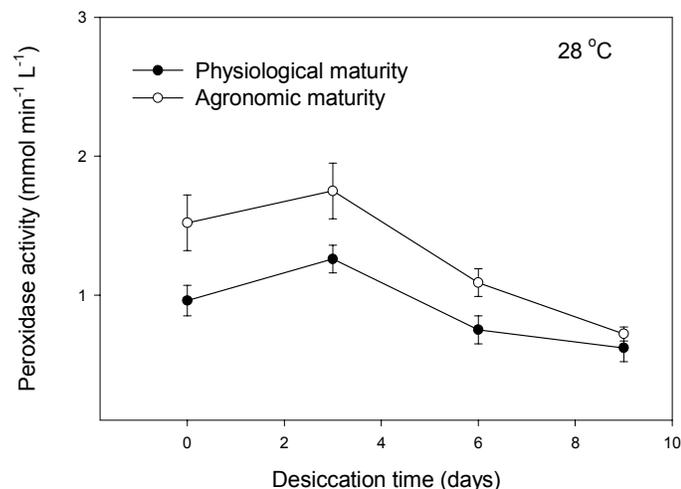
Figure 6. Activities of peroxidases during desiccation, at 5 or 28°C, of *T. occidentalis* seeds harvested at physiological and agronomic maturity.
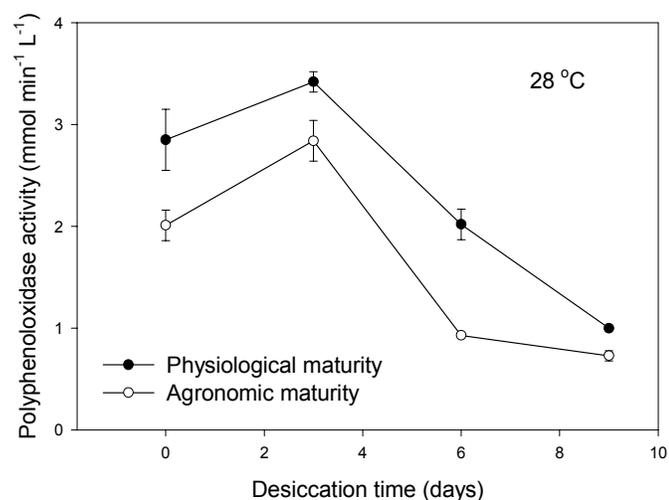

Figure 7. Activities of polyphenoloxidases during desiccation, at 5 or 28°C, of *T. occidentalis* seeds harvested at physiological and agronomic maturity.

temperatures that retard germination generally enhances longevity (Tompsett and Pritchard, 1998).

Seeds of *T. occidentalis* might be expected to maintain high metabolic activities at maturity due to their high level of hydration. Increased carbohydrate and lipid contents with developmental age indicated a continuous flow of nutrients from the fruit to the developing seeds (sinks). Sugar and lipid levels increased during desiccation, as starch levels decreased, and suggested that starch might be utilized in the biosynthesis of lipids, the major storage reserves. Lipids could also be functioning as "cotyledonary sinks",

thereby avoiding the build up of starch hydrolysis products.

The major enzymic protective processes against damage by products of peroxidation include the main enzymes of hydroperoxide metabolism (superoxide dismutase, catalase and guaiacol-type peroxidases) (Bernal-Lugo et al., 2000). Peroxidase, which has been reported in many biological systems as possessing in parallel polyphenoloxidase activity (Okpuzor and Omidiji, 1998; Nkang, 2001), may be involved in reducing high contents of auxin and phenolics (Nikolaeva et al., 1978; Nkang, 1996). The partial adaptive advantage of high initial peroxidase levels in seeds of *T. occidentalis* is having a high capacity to eliminate harmful metabolic products and progress towards germination. Increased activities of peroxidase and polyphenoloxidase and decreased germination inhibition have been reported as parallel phenomena in a number of species (Kudret et al., 1997; Andarwulan et al., 1999), especially during slow drying. The conditions employed in this study would more closely approximate to slow drying (drying at temperatures at or below 30 °C and relative humidities of less than 60 % over several days) (Hailstones and Smith, 1988; Tommasi et al., 1999). Our observations also agree with the argument that decreased peroxidase and polyphenoloxidase activity levels during desiccation (Figures 6 and 7) increased the likelihood of free-radical attack, and thus decreased germination responses (Li and Sun, 1999; Song and Fu, 1999; Tommasi et al., 1999). Decreased polyphenoloxidase activity of seeds might also minimize the development of off-flavour and decrease browning reactions associated with post harvest storage or processing.

Membrane as well as storage lipids are important targets of peroxidative attack. Other than direct detection of free radicals, lipid peroxidation in seeds can also be detected through the relative changes in fatty acids of differing levels of saturation (Wilson and McDonald, 1986). Different fatty acids possess different susceptibilities to peroxidation, with polyunsaturated fatty acids being more prone to auto-oxidation. The fatty acid profile of *T. occidentalis* seeds during desiccation showed shifts towards increased accumulation of mono-unsaturated (oleic) and polyunsaturated (linoleic) fatty acids. Similar variations in fatty acid profiles have previously been reported in plant tissues, the level of unsaturated fatty acids changing with growth temperature (Nishida and Murata, 1996). These changes might be due to the sustained activity of fatty acid desaturases, especially at the higher temperature. Fatty acid desaturase enzymes capable of converting saturated to unsaturated fatty acids occur in soluble form in plant tissues (Shanklin and Cahoon, 1998). During slow drying, seeds would be exposed to relatively high moisture contents, and consequently high metabolic rates, with the possibility of continued

synthesis of triacylglycerols and thus the increased lipid contents. It is possible that the fatty acid content of *T. occidentalis* seeds during desiccation, within certain moisture content limits, reflects a balance between synthetic events (and modifications) and lipid peroxidation.

At harvest, mature seeds of *T. occidentalis* contained predominantly saturated fatty acids (tridecanoic). This is in contrast with the report (Martin, 1984) that most cucurbit oil is made up of non-saturated fatty acids and thus of high nutritional value. In the present study, the predominance of unsaturated fatty acids during slow drying at 28 ^{o}C and at moisture contents below 40 % (the critical moisture content limit to maximum germination) suggest the possibility of developing post-harvest treatments to 'improve' *T. occidentalis* seed fatty acid profile and its potential value as an edible oilseed of commerce.

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