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Effects of accelerated aging upon the lipid composition of seeds from two soft wheat varieties from Morocco

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Abbreviations: DAG: diacylglycerol, MAG: monacylglycerol DGDG: digalactosyldiacylglycerol, FFA: free fatty acids, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine, MGDG: monogalactosyldiacylglycerol, NL: neutral lipids, PC: phosphadidylcholine, PL: polar lipids, SFA: saturated fatty acids, TAG: triacylglycerol, TFA: total fatty acids, UFA: unsaturated fatty acids. MDA: Malondialdehyde, DM: dry matter.

RESUMEN

Efecto del envejecimiento acelerado sobre la composición lipídica de semillas de dos variedades de trigo blando de Marruecos.

La composición lipídica de semillas de dos variedades de trigo blando (*Triticum aestivum*, cv. Marchouche and Mahdia) fueron analizadas antes y después del envejecimiento acelerado. Ocho días de envejecimiento acelerado provoco una inhibición total de la geminabilidad, así como un descenso en el contenido total de ácidos grasos, en especial de los ácidos grasos insaturados. Los contenidos del ácido oleico y linoleico disminuyeron particularmente, en la fosfatidilcolina de las semillas de ambas variedades. La proporción de lípidos polares también decreció después del envejecimiento en comparación con los lípidos neutros: un descenso del 5.8% y 7.2% de los lípidos polares fueron observados en los cultivos de Mahdia y Marchouche, respectivamente. En los lípidos de las semillas de la variedad Marchouche, el porcentaje de ácidos grasos libres aumento, mientras que los triglicéridos decrecieron. Después del envejecimiento, la composición de los ácidos grasos de todas las clases de lípidos fue modificada de la misma manera que la composición de los ácidos grasos totales. Entre los lípidos polares, la proporción de fosfolípidos fue la que disminuyo principalmente, en especial el porcentaje de fosfatidilcolina: un 18.1% y 19.1% para las variedades Mahdia y Marchouche, respectivamente. Por otra parte, los porcentajes de MGDG aumentaron, en especial en las semillas de la variedad Marchouche: un 15.5% en comparación con las semillas no envejecidas. Al mismo tiempo, los porcentajes de DGDG mostraron un descenso del 16.6% después del envejecimiento acelerado de semillas de la variedad Marchouche. De todos estos resultados, se concluye que el descenso del contenido lipídico observado en semillas después del envejecimiento acelerado podría estar relacionado con una pérdida de germinación y de vigor de semillas de trigo.

PALABRAS CLAVE: Ácido graso – Deterioro – Envejecimiento acelerado – Trigo.

SUMMARY

Effects of accelerated aging upon the lipid composition of seeds from two soft wheat varieties from Morocco.

The lipid composition of the seeds from two soft wheat varieties (*Triticum aestivum*, cv. Marchouche and Mahdia) were analyzed before and after accelerated aging. Eight days of accelerated aging resulted in a total inhibition of seed germinability as well as a decrease in their total and especially unsaturated fatty acid contents. Oleic and linoleic acid contents decreased particularly in the phosphatidylcholine of the seeds from both varieties. The proportion of polar lipids also decreased after aging as compared to neutral lipids: a 5.8% and 7.2% decrease in polar lipids was e observed in Mahdia and Marchouche cultivars, respectively. In the neutral lipids of the seeds from the Marchouche variety, the percentage of free fatty acids increased whereas the triacylglycerols decreased. After aging, the fatty acid compositions of all lipid classes were modified in the same manner as total fatty acid compositions. Among polar lipids, phospholipid proportions were particularly small, especially the phosphatidylcholine percentages with an 18.1% and 19.1% decrease in Mahdia and Marchouche varieties, respectively. In contrast, MGDG percentages increased, especially in the seeds from the Marchouche variety. A 15.5% increase was noticed when compared with seeds which were not aged. At the same time, the DGDG percentage showed a 16.6% decrease after accelerated aging of the seeds from the Marchouche variety. From these results we concluded that the lipid content decrease observed in seeds after accelerated aging could be linked to a loss in the germination and vigor of wheat seeds.

KEY-WORDS: Accelerated aging – Deterioration – Fatty acids – Wheat.

1. INTRODUCTION

Seed storage in conditions which are able to entirely preserve the germination capacity of the seeds is a constant problem in agriculture. Wheat lipids, although minor constituents, contribute to the technological, rheological and nutritional qualities of flour (Ruibal-Mendieta *et al.*, 2002) as well as to the quality of the primary or secondary transformation

products of soft or durum wheat. Bread volume, during baking, would be strongly linked to the non polar / polar lipid ratio and the galactolipid content of the flour (Goesaert *et al*., 2005). Lipids in wheat embryos are mainly neutral lipids whereas in endosperm, lipids are associated with membranes and are glyco- and phospho-lipids (Konopka *et al*., 2005).

Several theories have been proposed to explain the loss in seed viability during storage. Lipid deterioration and especially lipid peroxidation, is the most frequently suggested cause of loss in viability (Mazliak, 1983, Bailly *et al*., 1996 and Corbineau *et al*., 2002). McDonald (1999) suggested that the secondary of lipid peroxidation, such as malondialdehyde, was likely to be involved in the breakdown of proteins and nucleic acids through the Amadori and Maillard reactions. However, the involvement of lipid peroxidation in seed degradation during storage is still subject to debate (Narayana Murthy and Sun, 2000).

During seed aging, the oxidation of polyunsaturated fatty acids would alter membrane permeability and fluidity and reduce cellular compartimentation, thus leading to changes in enzymatic activities (Mazliak, 1983). Such changes depend on the level of aging and on the storability of the seed species. The most common way to determine seed storability is to use accelerated aging techniques, which consist of subjecting the seeds to high temperatures and relative humidity (Delouche and Baskin, 1973). Such methods provide information on both putative seed storability and on seed vigor; seeds able to tolerate this treatment are generally of good germinative quality.

Damage at the level of cellular membranes can be appreciated by determining the changes in the membrane lipid composition or indirectly by measuring electrolyte leakage from the seed tissues (Palta *et al*., 1977; McKerzie *et al*., 1990). The quantification of seed conductivity is generally accepted as a good indicator of seed vigor and deterioration during storage (Gaudillat, 1985).

The aim of the present work was to determine whether the deterioration of the seeds of two soft wheat varieties from Morocco during aging was related to changes in their lipid content and composition and in membrane properties.

2. MATERIAL AND METHODS

The seeds from two soft wheat varieties from Morocco: *Triticum aestivum*, var. Marchouche and Mahdia were furnished by DPA (Direction provinciale d'agriculture; Oujda, Morocco). The two varieties of wheat were cultivated in 2004 in the same agricultural station, near Oujda (eastern Morocco). After harvest, the seeds were stored in the laboratory at 6 °C.

2.1. Accelerated aging

Accelerated aging (AA) was carried out following the method of Delouche and Baskin (1973). Batches of seeds were placed in an oven heated to 40 °C and under a fully saturated humid atmosphere (100% relative humidity) for 2, 4, 6 or 8 days. Aged seed batches were designated AA2d, AA4d, AA6d and AA8d, respectively.

2.2. Germination test

Seeds were disinfected with sodium hypochloride (80 %) and rinsed twice with sterile distilled water. Germination was performed in Petri dishes on a filter paper moistened with distilled water, at 25 °C in the dark. A grain was regarded as germinated when the radicle had pierced the seed coat. The results presented here are the means of the germination percentages obtained from 3 replicates. They are expressed as the germination percentages obtained after 2, 4 and 7 days.

2.3. Solute leachate test

Twenty seeds from each lot were soaked in 50 mL deionized water and kept in the incubator at 30 $\pm$ 1 °C for 24 h and the electrical conductivity of the seed leachates was recorded using a direct reading conductivity meter (Thermo Orion). The conductivity was expressed as µs/g DM (Halder and Gupta, 1980).

2.4. Lipid extraction and separation

After different aging periods, seeds were rinsed with distilled water and lipids were extracted following the Bligh and Dyer method (1959). Seeds (2 g) were fixed in 15 mL of boiling water for 3 min and then ground with a Turax homogenizer, first in methanol, then in chloroform and finally in water (containing 1% dissolved NaCl) (1/1/1,v/v/v). After a 1000 g centrifugation for 15 min, the chloroform phase was recuperated and dried under nitrogen.

The different lipid classes were separated by thin-layer chromatography on a silicagel G60 (Merck). The separation of neutral lipids was carried out using the solvent (Petroleum ether/ ethylic ether/ acetic acid: 70/30/0.4; V/V/V) recommended by Mangold (1961) whereas polar lipids were separated using the solvent mixture (chloroform/ acetone/ methanol/ acetic acid/ water: 50/ 20/ 10/ 10/ 5; V/V/V/V/V) according to Lepage (1967). Lipid spots were revealed by spraying a Primuline solution (0.1% in 80% acetone-water) and observed under UV light. Lipid classes were identified by comparison with standards and by means of specific colorings

2.5. Fatty acid analysis

After the addition of heptadecanoic acid as an internal standard, fatty acids were methylated and separated by gas-liquid chromatography (Varian 3300 gas-liquid chromatograph, 30 m x 0.32 mm

column filled with CP-wax 52 CB).) according to Demandre *et al* 1994.

2.6. Statistical test

All data were subjected to an analysis of variance, and LSD (least significant difference) values were calculated at $P \leq 0.05$.

3. RESULTS

As far as germination capacities are concerned, the seeds from the soft wheat varieties Mahdia or Marchouche were affected differently by accelerated aging (Figure 1). Maximal germination rate (observed without any aging treatment) was 66% for Mahdia seeds and 56% for Marchouche seeds. After two days of accelerated aging, it was reduced to 25% for Marchouche seeds which represented a 52.95% decrease in germination capacity; in contrast, Mahdia seeds displayed only a 12.5% decrease in germination capacity after the same treatment. After four days of accelerated aging, germination rates were 15% and 9% for Mahdia and Marchouche seeds, respectively. After six days of accelerated aging, followed by seven days at 25 °C in Petri dishes, no germination was obtained at all, with any seed from both varieties.

The total germination inhibition observed after 8 days of accelerated aging was accompanied by a 30.43% increase in solute leakage for Marchouche seeds and a 50% increase for Mahdia seeds (Table 1). At the same time, the total fatty acids contents decreased in seeds after 8 days of accelerated aging: the reductions were 16.92% (as compared to standards without any aging) for seeds from the Mahdia variety and 31.05% for seeds from the Marchouche variety (Table 1). These decreases in total fatty acid contents were accompanied by a significant ($P < 0.05$) lowering of the ratio of unsaturated to saturated fatty acids: the reductions in this ratio were 23.2% and 27.7% respectively for the seeds from Mahdia and Marchouche varieties. The total fatty acid content decreased after the second day of accelerated aging in Marchouche seeds whereas this decrease was only observed after the fourth day of treatment in Mahdia seeds (results not shown).

In Figure 2, the quantitative changes in the main fatty acid contents of the seeds during accelerated aging are shown and it can be clearly observed that the decrease in linoleic acid content was the greatest. The percentage of decrease was 26% for

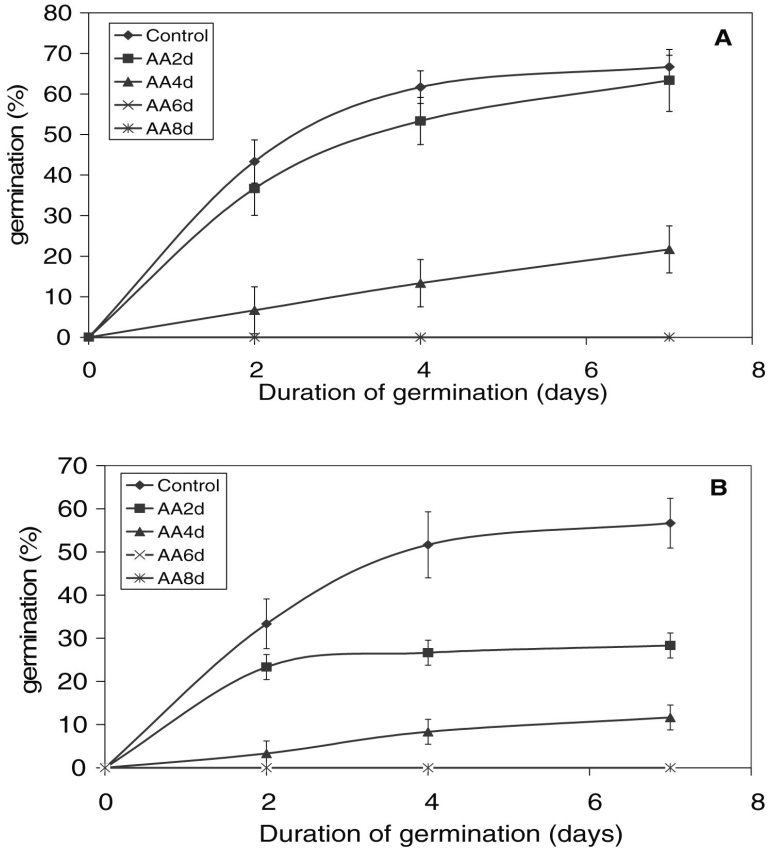

Figure 1
Changes with accelerated aging time (control, AA2d, AA4d, AA6d and AA8d) in the germination rates of the seeds from two soft wheat varieties (Mahdia, A and Marchouche, B). Results are the means of three independents assays; bars correspond to ± standard error. AA2d, AA4d, AA6d and AA8d refer to Batches of seeds placed in an oven heated to 40 °C and under a fully saturated humid atmosphere (100% relative humidity) for 2, 4, 6 or 8 days.

Table 1
Effects of eight days of accelerated aging on the solute leaking (SL) and the total fatty acid (TFA) contents of the seeds from two soft wheat varieties (Mahdia ant Marchouche)*

	Mahdia		Marchouche	
	Control	**AA 8d**	**Control**	**AA 8d**
SL (µS/DM)	4.9 ± 0,76	7.35 ± 1.05	8.05 ± 1.74	10.5 ± 0.14
TFA (mg · g^{-1})	4.55 ± 0.12	3.78 ± 0.09	5.54 ± 0.02	3.82 ± 0,05
UFA/SFA	2.64 ± 0.03	2.03 ± 0.02	3.82 ± 0.01	2.76 ± 0.01

*Results are the means of three independent assays ± standard errors. (AA8d: accelerated aging for 8 days).

Mahdia seeds and 28% for Marchouche seeds and this decrease in linoleic acid content was observed since the second day of aging for Marchouche seeds and only after four days of aging for Mahdia seeds.

Accelerated aging only slightly modified the relative proportions of neutral and polar lipids in the seeds (Table 2), but within these two lipid classes, different changes occurred in the seeds of both varieties (Figures 3 and 4). In the seeds from the Marchouche variety, accelerated aging induced a 9.8% decrease in the proportion of TAG accompanied by a 6.5% increase in the proportions of FFA (Figure 3). No significant variation was observed at the level of DAG. In the seeds from the Mahdia variety, accelerated aging induced no change in neutral lipid proportions.

Polar lipid analysis (Figure 4) showed that in the seeds from both varieties, phospholipids and especially PC were mostly affected by the decrease in lipid content induced by accelerated aging. PC percentages were 18.1% and 19.1% lower in the seeds from Mahdia and Marchouche varieties respectively. However PE decreases of 21% were detected only in Mahdia seeds. In contrast, MGDG percentages increased, above all in the seeds from the Marchouche variety: a 15.5% increase was noticed when compared with not aged seeds. At the

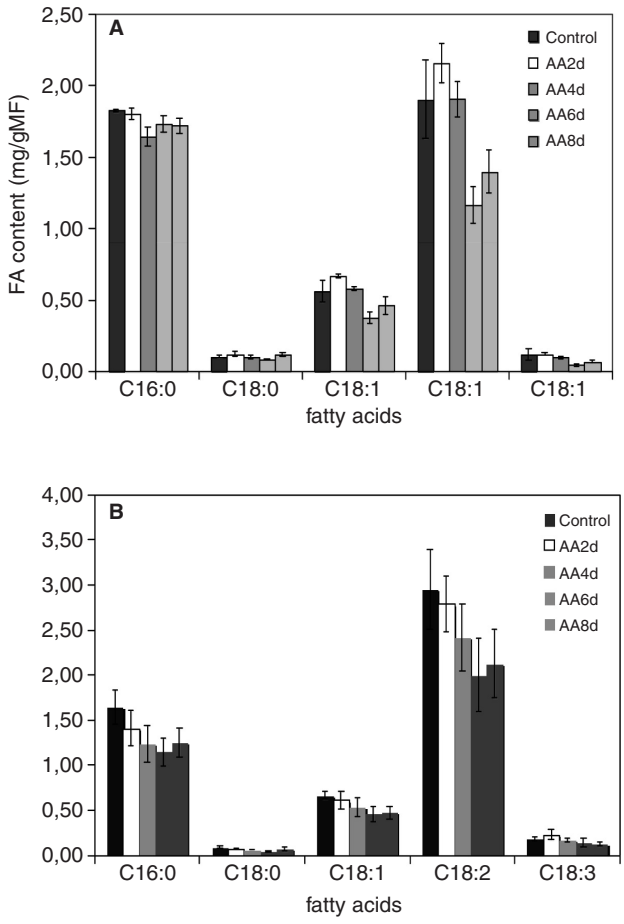

Figure 2
Changes with accelerated aging time in the fatty acid contents of the seeds of two soft wheat varieties (Mahdia, A and Marchouche, B). Results are the means of three independents assays; bars correspond to ± standard error.

Table 2
Changes in PL (polar lipid) and NL (neutral lipids) proportions in the seeds of two soft varieties (Mahdia and Marchouche) after 8 days of accelerated aging (AA 8d)

	Madhia		Marchouche	
%	control	AA 8d	control	AA8j
PL	21.28 ± 0.04	20.04 ± 1.94	18.24 ± 1.52	17.53 ± 1.52
NL	78.72 ± 0.04	79.96 ± 1.94	81.76 ± 0.25	82.46 ± 0.25

*Results are the means of three independent assays ± standard errors.

same time, DGDG percentage showed a 16.6% decrease ($P < 0.05$) after accelerated aging of the seeds from the Marchouche variety.

4. DISCUSSION

In the seeds from two soft wheat varieties from Morocco (Mahdia and Marchouche), eight days of accelerated aging induced a total inhibition of germination capacity, an increase in solute leaking and a decrease in total fatty acid content. These results are agreed with those of Gidrol *et al* (1989) and Corbineau *et al* (2002) obtained with sunflower seeds. Unsaturated fatty acid and phosphatidylcholine contents were particularly low. The proportions of all polar lipids decreased in the seeds but the percentage of MGDG increased at the expense of DGDG. These changes were mainly observed in the seeds from the Marchouche variety.

Loss in seed germinability has already been shown to be associated with electrolyte leakage in the seeds of various species (Bailly *et al*., 1996,

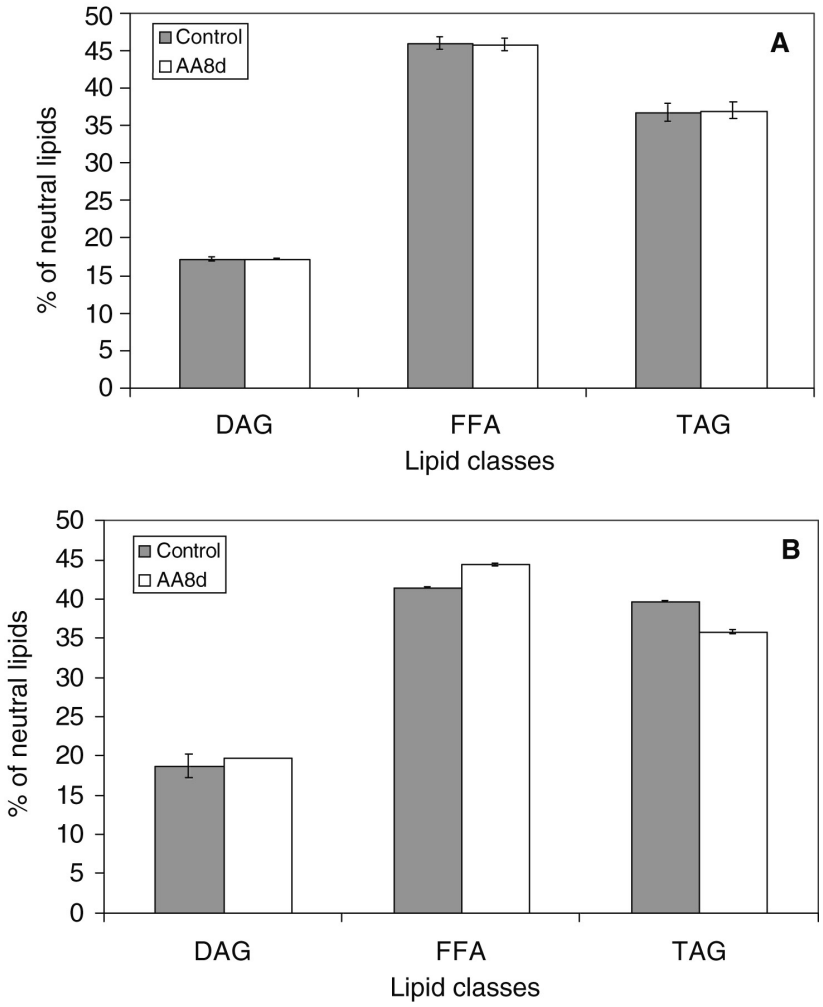

Figure 3
Changes in neutral lipid composition of seeds from two soft wheat varieties (Mahdia, A and Marchouche, B) after 8 days of accelerated aging: DAG, diacylglycerol, FFA, free fatty acid and TAG, triacylglycerol. Results are the means of three independents assays; bars correspond to ± standard error.

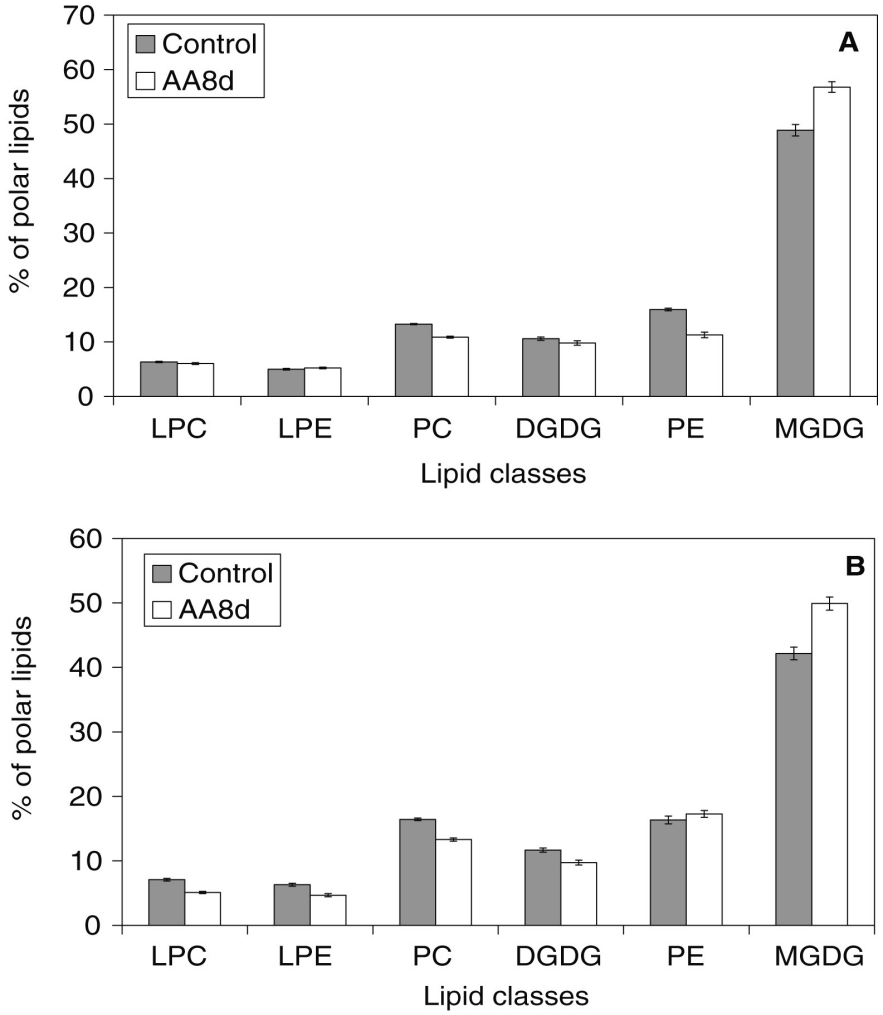

Figure 4
Changes induced by 8 days of accelerated aging in polar lipid proportions in the seeds of two soft wheat varieties (Mahdia, A and Marchouche,B). Results are the means of three independant assays; bars correspond to ± standard error. DGDG: digalactosyldiacylglycerol, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine, MGDG: monogalactosyldiacylglycerol, PC: phosphadidylcholine.

Corbineau *et al.,* 2002 and Goel *et al.,* 2003). However, the use of conductivity measurements as a marker of seed deterioration has mainly been associated with seeds with lipid reserves (Harman and Mattick, 1976; Stewart and Bewley, 1980, Gidrol *et al*., 1989, Tammela *et al.,* 2000 and Freitas *et al*., 2006) and few studies of electrolyte leakage have been carried out during the aging of wheat seeds.

The decrease in fatty acid content, and particularly in linoleic acid, might result from lipid oxidation which is known to be directed against the double bonds of polyunsaturated fatty acids (Mazliak, 1983 and McDonald, 1999). Such a decrease has already been observed during the natural or artificial aging of almond seeds (Zacheo *et al*., 1998 and Zacheo *et al*., 2000) but is not yet documented in wheat seeds. A measurement of malondialdehyde has often been taken in order to reveal lipid peroxidation in seeds, but results appear contradictory. Indeed, depending on the species MDA accumulate or not during aging. For example, Gidrol *et al.* (1989) and Lehner *et al.* (2007) did not find any increase in this compound during the accelerated aging of sunflower or wheat seeds. On the contrary, Goel *et al.* (2003) or Kibinza *et al*. (2006), for example, demonstrated that MDA accumulated during seed aging. The use of MDA as a marker of lipid oxidation has often been often subject to debate because this assay may be non specific and it does not measure hydroperoxides but the product of their degradation (Zacheo *et al*., 2000). Our results suggest that the measurement of lipid composition is probably a better way of estimating the oxidative alteration of the lipid fraction in seeds.

In pine seeds stored for 29 years, Tammela *et al.* (2000) had also observed a general lipid degradation mainly marked by decreases in PL and TAG contents and simultaneous increases in FFA, MAG and DAG contents. Similar results have been obtained with other plant species; for instance, a decrease in polar lipid content, after accelerated aging, has been observed in seeds from pea (Powell and Matthews,

1981), soybean (Stewart and Bewley, 1980), peanut (Pearce and Abdel Samad, 1980), tomato (Francis and Coolbear, 1984), sunflower (Gidrol *et al*., 1989) and cotton (Freitas *et al*., 2006). These decreases could result either from metabolic degradations affecting seeds during storage (Parrish and Leopold, 1978) or simply from the increase in temperature applied during accelerated aging. As a matter of fact, several studies have shown that the lipid composition of plants was altered by an increase in temperature (Harwood *et al*., 1994 and Falcone *et al*., 2004). Somerville and Browse (1991) have shown, with *Arabidopsis thaliana*, that the total lipid content of the plant is 50% less and the ratio of unsaturated/saturated fatty acid is decreased by 30% after an exposure of plants to high temperatures. Together with our data, this again underlines the fact that the conditions used for the accelerated aging treatment are critical and that different combinations of temperature and relative humidity during the treatment may trigger different kinds of cellular injuries.

In our study, the decrease in TAG content simultaneous to FFA and DAG increases in the seeds from the Marchouche variety, as well as the relative changes in MGDG and DGDG proportions, could indicate an enhancement of lipase and galactolipase activities during accelerated aging. Similarly, several researchers have noticed an increase in lipase activity resulting in an FFA content increase during accelerated aging (Leshem, 1987). These FFA, especially linoleic and linolenic acids, may be subsequently oxidized by lipoxygenases, producing hydroperoxides and active oxygen forms. Devaiah *et al*. (2007) have noticed that Arabidopsis thaliana seeds, deprived of phospholipase D gene (PLPα 1), do present, as compared to the wild type, a better germination capacity associated with a great stability and, in particular, a lower decrease in unsaturated fatty acid content during storage. However, other results have shown, on potato tubers, a decrease in phospholipase activity after 20 months of natural storage (Kumar and Knowles (1993). Freitas *et al*. (2006) demonstrated that the accelerated aging of cotton seeds was associated with a simultaneous decrease in lipid content and in lipoxygenase and phosphatase activities.

5. CONCLUSION

Accelerated aging is a good test to predict the longevity of the seeds, as suggested by Delouche and Baskin (1973). Our results suggest that, at least within the first 8 days of treatment, lipid degradation, particularly unsaturated fatty acid degradation, might act as probable cause of the loss in vigour and germination of wheat seeds. These degradations, if occurring during natural aging, might be responsible for the decrease in technological and rheological qualities of stored flour (Barnes, 1983).

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