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Storage stability of three genotypes of sunflower seeds

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ABSTRACT

Sunflower seeds (*Helianthus annuus L.*), due to their high oil content, are among the raw materials cited by the National Program of Biodiesel Production and Use (NPBP) with potential for the production of biodiesel. On the other side, seeds with high lipid content could be more sensitive to degradation that non-oil seeds. The objective of this project was to evaluate the stability of three genotypes of sunflower seeds, kept in two different raffia packages under two storage conditions: a constant temperature and humidity reference condition set at 25 °C/75% RH (Ref) and a cyclic, accelerated aging test (CW), built to reproduce the variation of temperature and humidity of a Center West production region, which was in average of 24 °C/71% RH, but with extreme temperatures of 17–32 °C and humidity of 24–96% RH. As all samples evaluated had almost the same fatty acid composition up to the end of ten monthsístorage, it was concluded that seed can be kept without significant loss of quality in the packaging materials and storage conditions evaluated. Cyclic accelerated aging generated different evolution profiles of the peroxide values and moisture levels when compared to the reference condition, with constant temperature and humidity. All seeds showed dehydration when undergoing the (CW) condition during the winter simulation period, the driest season among the evaluated. During fall, winter and spring simulation, the (Ref) condition generated higher peroxide values than the accelerated aging test. However, in the summer period simulation of the cyclic aging, the peroxide values of seeds packed in uncoated raffia increased by 32% (OL5), 9% (He250) and 90% (He 253). Laminated raffia showed a slightly higher performance than uncoated for seed preservation. The results found in this study show that storage at constant temperature and humidity conditions does not reproduce the sequence of degradation reactions which occurs due to the daily cyclic variation in temperature and humidity. This highlights, the importance of carrying out studies in laboratory conditions closer to reality for studying seed aging.

1. Introduction

1.1. Background

In 2000–2010 decade oil crop production has been among the most vibrant activities in world agriculture. The sector grew by almost 5 percent per annum; the major part as a vegetable oil. This expansion is of oils with high protein content which also produces oilmeals employed as feed. Sunflower seed is among the four most important oil crops: oil-palm, soybean and rapeseed that account for approximately 75 percent of world production (FAO, 2013).

Sunflower planting has grown in various parts of the world that have warm weather to semi arid as it has good drought resistance and a relatively short growing season, ranging from 90 to 160 days. It also adapts to a wide variety of soils (FAO, 2010).

Brazilian production of sunflower seed (*Helianthus annuus L.*) has grown significantly in recent years. Between 2005 and 2013, there was an increase of about 80% when 109,000 tons were produced. This growth is due mostly to planting in the Center-West region of Brazil, currently responsible for 83% of the country's production (IBGE, 2015).

The National Program of Biodiesel Production and Use—NPBP which has been in operation in Brazil since 2005, is based on the possibility of producing biodiesel from different oleaginous sources (ANP, 2014). Sunflower seeds, due to their high oil content, are

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among the raw materials cited by NPBP with potential use for the production of biodiesel. Most biodiesel is produced using soybean through a complex solvent extraction process. Sunflower oil production is much simpler with just pressing required. As sunflower can be grown in varied soil and climatic conditions, small farmers have the advantage of a cheap biodiesel production source for their machinery and sell off the surplus for extra income (Porte et al., 2010).

1.2. Sunflower seed storage studies

Due to the high content of lipids, these seeds are more sensitive to deterioration than other non-oil seeds (Balešević-Tubić et al., 2010). Considering also the seasonality of any biomass, it is extremely important to know the stability of this raw material during storage. The deterioration of seeds with loss of their germination over time is a well-known fact. Understanding the mechanisms that lead to this deterioration has been studied by many researchers, since the efficiency of germination is of great economic importance. Walters (1998) states that the factors that determine the rate of this "aging" are temperature, moisture content and the intrinsic quality of seeds.

High temperature (45 °C) and high humidity (100% RH) inhibit seed germination and seedling sunflower (Corbineau et al., 1988). Bailly et al. (1996) suggest that sunflower seed deterioration during accelerated aging (45 °C and humidity between 76 and 100% RH) is closely related to a decrease in activities of detoxifying enzymes and to lipid peroxidation.

Aging experiments with maize and sunflower seed (15, 20 and 25% RH, and afterwards maintenance at 40 °C for periods from 0 to 96 h) showed a greater loss of germination the higher the humidity (Stan, 1997).

Few changes in the content of lipids, in the proportion of saturated and unsaturated fatty acids, and in the percentage of free fatty acids were observed in sunflower seeds undergoing priming (7 days, 15 °C, solution of polyethylene glycol) or accelerated aging (5 days, 45 °C, water, 100% RH). Electron microscopy of these seeds, however, revealed that lipid bodies became smaller and more dispersed throughout the cytoplasm during priming and aging (Walters et al., 2005).

A study on the effect of storage conditions on the quality of sunflower seeds for the purpose of preservation of germplasm was carried out by José et al. (2010), a group of researchers at Embrapa. Seeds dried by various drying processes (chamber at 22 °C and 18% RH) and silica gel up to final moisture values ranging between 2.1 and 10.2% were stored in liquid nitrogen (−196 °C) and a freezer (−20 °C). All seeds showed high levels of germination after three months of storage. It was found that seeds stored in normal atmosphere (peroxide value between 14.16 and 24.06 mEq kg^{-1}) had a higher degree of oxidation than those stored in liquid nitrogen (peroxide value between 2.6 and 12.7 mEq kg^{-1}) probably due to the larger concentration of oxygen in the chamber atmosphere. In this work, although large variations in peroxide value were observed it was not possible to correlate them with high or low moisture content of the seeds.

Sunflower seed (cv. BRS 122) kept in paper and vacuum-sealed plastic bags at three different temperatures (10, 25 and 30 °C), and up to four months of storage, maintained its physiological quality. After this period seed quality decreased. Oil content, enzyme reduction and disintegration of cellular components were observed. The best preservation condition was Kraft paper sacks kept at 10 °C (Lins et al., 2014).

Aging of sunflower seed at 35 °C and moisture contents (MC) ranging from 0.04 to 0.48 g H_2O g^{-1} dry matter showed that seed viability is affected by MC and is related to accumulation of hydrogen peroxide and changes in energy metabolism (El-Maarouf-Bouteau et al., 2011). These authors proposed the following mechanism for seed death: when the moisture content of seed increases, lypoxygenase, an enzyme that produces free radicals is activated and respiration resumes at 0.08 g H_2O. These events produce ROS (radical oxygen species) which leads to lipid peroxidation by-products, inducing factors for PCD (controlled programmed cell death) through DNA alteration and mitochondrial dysfunctioning.

These studies reveal that there is still no complete understanding of the mechanisms that lead to the deterioration of stored seeds, but there is a general consensus that the moisture content and temperature conditions of storage play key issues in these processes.

Another aspect that is important to consider is the availability of water in the seeds to promote any subsequent reaction. Labuza (1971) demonstrates that the degradation of various foods is closely linked to water activity and their moisture content. The oxidation of lipids is faster in both low ($0 \sim a_w$) and high water activity ($a_w > 5$) than in intermediate situations ($0.2 < a_w < 0.5$).

The influence of humidity on the properties of cellulose fibers is well known and has been studied for many purposes. The moisture content of cellulose increases with relative humidity which affects many fiber properties such as dimension, stiffness, strength, stretch, water dependent reactions, exudation and shelf life, among others. When water penetrates into the fibers, it breaks the secondary interactions between cellulose macromolecules and is absorbed into the cell walls by hydrogen bonds, causing swelling of the fibers and loss of mechanical resistance (Rostic et al., 2008), and consequently, changing the permeation and solubility properties of its walls. The kinetic of absorption depends on the water content and the temperature. Irreversible mechanisms occur when the fibers are dried and re-humidified for long periods, such as in the processes that occur in natural environments (Baley et al., 2005). In the cellulose packaging area, the phenomenon of creep deformation is well known. Creep is affected by environmental conditions and only laboratory cyclic variations in humidity and temperature (accelerated creep test), within natural limits, can reproduce the types of failures found for packaging in real conditions. The deformations obtained after cycles of humidity and temperature exceed those in any condition of constant humidity (Alfthan et al., 2002).

Considering the results of these previous studies and with the aim of evaluating the storage stability of three genotypes in two different ambient conditions, the following experimental design was selected. The seeds were kept in two different packaging materials under two storage conditions that could be used by the industrial biodiesel sector. Low cost storage conditions and packaging materials were selected in order to avoid significant impact on the costs of biodiesel production.

2. Materials and methods

2.1. Seeds evaluated

The three seeds with genotypes identified as OL5, He 250 and He 253, shown in Fig. 1, were selected from a previous study (Regitano Neto et al., 2013), based on their profiles of fatty acid composition. These seeds were produced during the 2011/2012 crop season (sowed in December 2011, harvested in April 2012). Sunflower OL5 is a triple way cross hybrid—high oleic acid content from Atlantica Seeds and He250 and He 253 are single cross hybrids, standard fatty acid profile, from Heliagro Seeds.

2.2. Storage conditions

Two conditions were selected to study the behavior of seeds in this study. The first was a cyclic accelerated aging designed to

Fig. 1. Genotypes of sunflower seeds used in this shelf life evaluation.

reproduce the sequence of chemical reactions that follow the typical oscillations of temperature and humidity of the main seed production region of Brazil. The second was a constant reference, as explained in subsequent sections.

Chemical properties were monitored after the following periods of storage:

CW: 0, 36, 74, 110, 135 and 181 days.

Ref: 0, 62, 121, 181, 244 and 301 days.

2.2.1. Cyclic accelerated aging—Center West region (CW)

Due to the representativeness of the Center West Region for sunflower production, typical cyclic variation of CW temperature and humidity were used to build the profile for accelerated aging storage. In this area, the main sunflower production hub is the city of Campo Novo de Parecis, located in the state of Mato Grosso (MT).

Crude climate data from the Campo Novo de Parecis Automatic Weather Station were provided by the National Institute of Meteorology (INMET) of the Ministry of Agriculture.

The maximum and minimum temperatures and relative humidity of the air between June 2010 and December 2011, collected hourly, were provided for this study. The raw data were processed and separated for all seasons.

The average values of maximum and minimum temperature and humidity were used to build the simulation curve of each season, set for hourly variations. As the storage can be maintained for long periods, an accelerated storage was performed, reducing the actual intervals of 60 to 30 min. Thus, one year of 360 days was simulated in 180 days. The three months (90 days) of each season were simulated in 45 days of accelerated storage in a climatic chamber Vötsch Model VC-0060.

Fig. 2 shows the values of temperature and humidity in the climatic chamber obtained for each simulated weather season, measured by a thermohygrometer. The following sequence of simulation was performed: fall, winter, spring and summer, simulating the periods of normal harvesting. The average condition of cyclic accelerated aging considering the four-season simulation was 24 °C/71% RH with extreme temperatures of 17 and 32 °C and

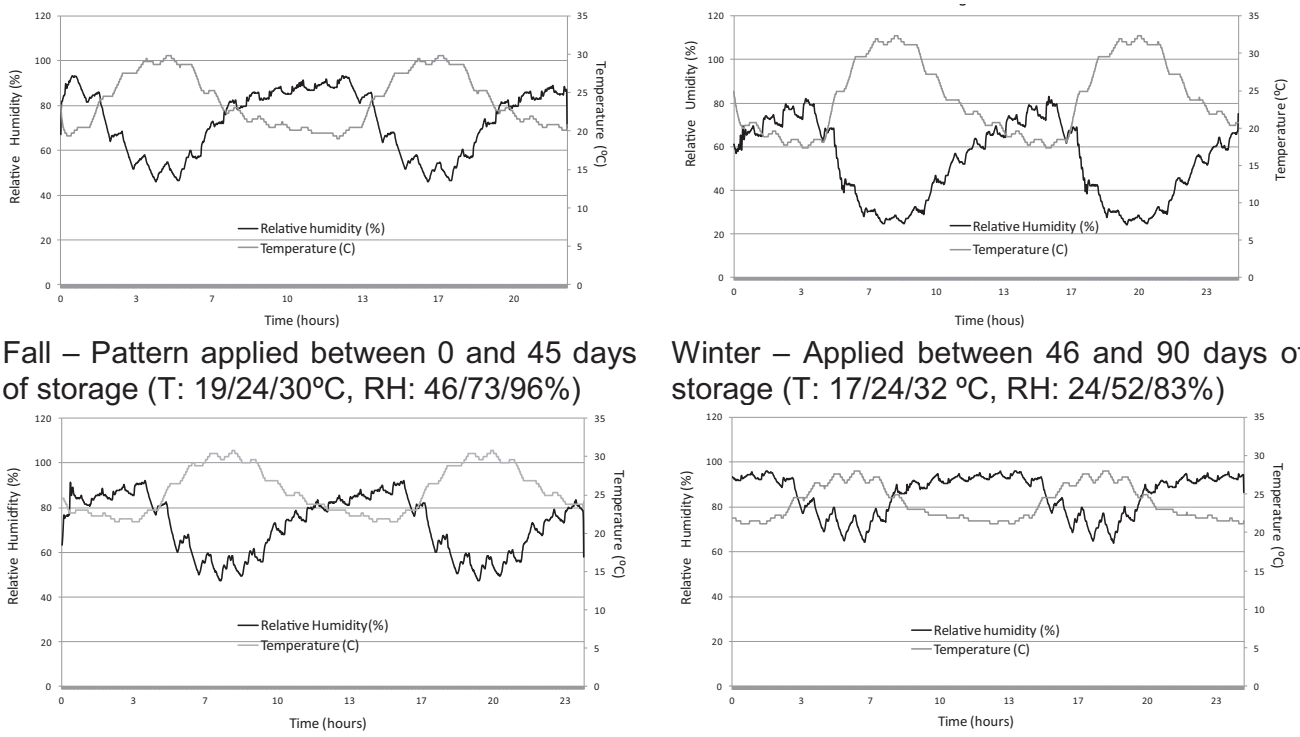

Fig. 2. Pattern of temperature and humidity variation in the Vötsch chamber. Records refer to measurements performed during approximately 24 h which represents simulation of 2 days of real variations, in each season indicated. The numbers after T and RH represent the minimum, the average and the maximum values of each season.

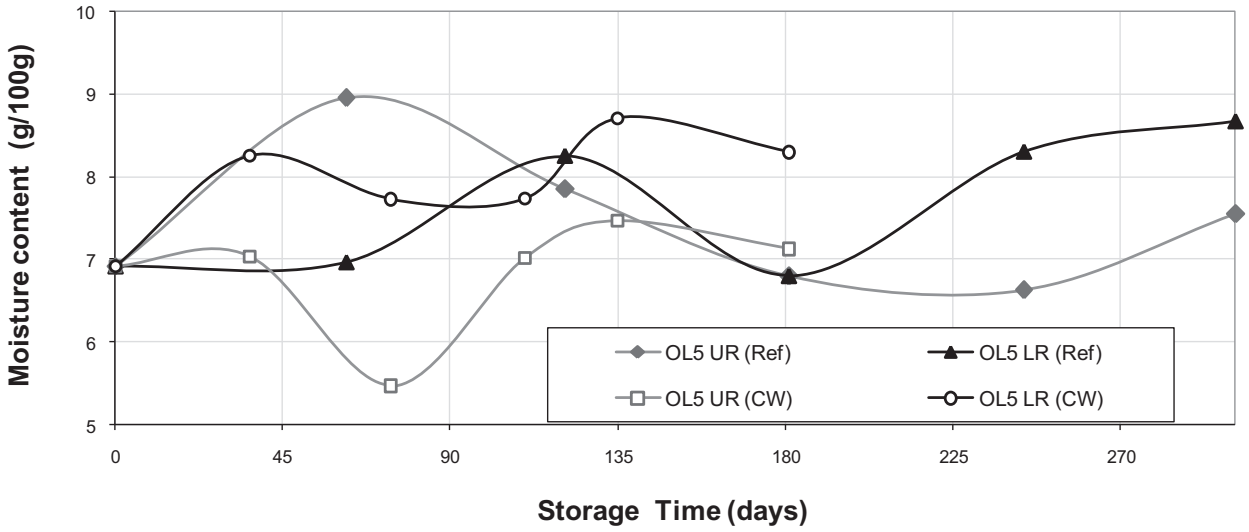

Fig. 3. Variation of moisture content of OL5 seed packaged in UR and LR stored in Center west (CW) and reference (Ref) conditions. CW simulates each season in different periods: fall (0–45) days, winter (46–90) days, spring (91–135) days and summer (136–181) days.

humidity of 24 and 96%RH. The specific ranges of each season are shown in Fig. 2.

2.2.2. Constant reference condition (Ref)

The second storage condition was performed in a storage chamber maintained at the constant reference condition of (25 ± 4 °C) and (75 ± 7%) RH for 10 months.

2.3. Seed characterization

The seeds were initially characterized by the following parameters:

2.3.1. Rancimat oxidative stability

The resistance of oils and fats to oxidation was carried out using the equipment Methrom Rancimat, model 679, with 5 g of sample to 110 °C and 10 L/h air flow. The result is expressed in hours of the induction period (Horwitz and Latimer, 2010).

2.3.2. Moisture and volatile content

Samples of 10 ± 0.5 g were dried at 130 °C for 3 h (Firestone, 2012). The final moisture content (%) was calculated relative to the initial mass of wet sample.

Ash content was measured in an oven previously set at 550 ± 10 °C and was expressed relative to initial mass of seeds (Zenebon and Pascuet, 2005).

2.3.3. Total lipid content

Samples of 5 ± 0.5 g were subjected to extraction with petroleum ether for 8 h. The extracted lipids were dried in an oven maintained at 100 ± 5 °C for 1 h, cooled and weighed. Total lipid content was calculated as the mass of oil extracted per 100 g of initial sample (Firestone, 2012; ANVISA, 2005; Mapa, 2006; Horwitz and Latimer, 2010).

2.3.4. Protein content

The determination was made according to the Kjeldahl titration method that determines the amount of total nitrogen to calculate the protein content of the sample (Zenebon and Pascuet, 2005). The protein content of the samples was calculated by multiplying the total nitrogen content by a specific product factor, which in the case of this project was 5.75. This factor is a reference for vegetal products according to Zenebon and Pascuet (2005) and also follows the legislation for nutritional labeling (ANVISA, 2003).

2.4. Packaging characterization

After characterizing, seeds were dried in an oven maintained at 45 °C up to approximately 7% of moisture. Then, about 300 g of each type of seed were placed in plastic bags manually made in the laboratory with two different materials: uncoated raffia (UR) and laminated raffia (LR) with low density polyethylene. Raffia is an intertwined structure made of polypropylene (PP) threads, and therefore a hollow structure which allows gas exchange between the interior and the exterior of the package, widely used for containing agricultural products. The lamination of raffia with polyethylene provides a moisture barrier to packaging. The films have the following thicknesses: 90 μm for UR (only PP) and 90 μm (PP) + 20 μm (LDPE) for LR.

The materials used to prepare the packages of the study were characterized according to the following test:

Water vapor transmission rate (WVTR) of raffia films were determined at 38 °C/90% RH condition through the gravimetric method, according to ASTM E96/E96 M-12 ASTM (2012).

2.5. Monitoring of seed quality during storage

Changes in seed quality during storage were monitored by determining the moisture and volatile content and peroxide value. Crude oils were obtained by pressing the clean seeds in a mini press expeller type, brand Ecirtec MPE 40. The fatty acid composition of their lipid fraction was assessed initially and after the last collection in each storage condition.

2.5.1. Peroxide value

This method determines all substances present in the oil which oxidize potassium iodide under the conditions of the test (Firestone, 2012). These substances are generally considered as peroxides or other similar products of fat oxidation. The peroxide value is calculated as the number of milliequivalents of sodium thiosulfate per kilogram of sample.

Table 1
Water vapor transmission rate (WVTR) of the packaging materials used for seed storage.

Packaging	WVTR at 38 °C/90% RH (g water × m^{-2} × day^{-1})		
	Average	Minimum–maximum	CV (%)
Uncoated raffia	1473 (*)	1381–1579	4.6
Laminated raffia	4.44 (**)	3.22–5.78	27.2

(*, **) = Average for 6 and 5 measurements; CV = coefficient of variation; CV(%) = standard deviation/average × 100.

2.5.2. Fatty acid composition

Original oils were saponified, esterified and the methyl esters of the fatty acids were quantified by gas chromatography (Hartman and Lago, 1973). A Varian model 3900 was used with a capillary column Chrompack CP-Sil 88, 100 meters long, 0.25 mm internal diameter, 0.20 μm film thickness. The centesimal composition of each acid was calculated considering the area of each peak in relation to the area of all peaks, considered representative of 100% of the sample.

2.6. Moisture sorption isotherm

The seeds were placed on small glass weighing containers inside the climatic chamber Vötsch Model VC-0060 at 25 °C under the following conditions: 25, 50, 75 and 95% relative humidity until reaching equilibrium. Their weight was determined on an analytical balance and the moisture content calculated on a dry mass basis, measured at the same temperature of moisture content.

2.7. Statistical analysis

The nonparametric Kruskal–Wallis test (Hollander and Wolfe, 1973) was used rather than that corresponding to ANOVA because only two replicates of data did not follow a normal probability distribution, even when performing Box-Cox transformations (Ryan, 2009). Furthermore, the data obtained fulfill the statistical prerequisites of the Kruskal–Wallis test, since the same types of analyses were performed for various periods of storage. The test was used to check for significant differences in moisture absorption and peroxide values due to the following factors:

- three types of seeds: OL5, He 250 and He 253
- two types of storage: CW and Ref
- two types of packaging: UR and LR

3. Results and discussion

The results are presented and discussed using the results of statistical analysis performed.

3.1. Characterization of packaging materials

Table 1 shows the experimental results of the characterization of the materials used in this study. Due to voids in the entwined structure of uncoated raffia, the exchange of gases with the atmosphere is high. The laminated raffia has on its inner side a layer of polyethylene which seals the voids and, for this reason, it constitutes a barrier to moisture penetration compared to the uncoated. Table 1 shows that the WVTR of uncoated raffia is about 332 times larger than the laminated raffia.

3.2. Characterization of seeds

Table 2 shows the results of the initial centesimal composition and also a Rancimat oil characterization of seeds studied. Small variations, characteristic and normal, between different genotypes were observed, particularly with respect to the total lipid content and protein content. The Rancimat results obtained, however, are quite pronounced between the seeds evaluated, with OL5 oil (63.6 h) having five times higher stability than that of He 250 (11.3 h) and He 253(12.3 h). Oxidation stability is a very important parameter for fuels in engines working at high temperatures and depends on the composition of fatty acids present. The values of stability Rancimat for oils of three genotypes are well above the minimum amounts required for the European biodiesel standard EN 14214 that limits to a minimum of 6 h (EU, 2003).

Table 2
Characterization of genotypes OL 5, He 250 and He 253 of sunflower seeds.

Parameter (*)	OL 5	He 250	He 253
Ash (g kg^{-1})	2.55 (0.01)	3.01 (0.01)	2.58 (0.01)
Total lipid (g kg^{-1})	44.35 (0.01)	43.07 (0.60)	42.28 (0.04)
Protein (N x 5.75) (g kg^{-1})	14.43 (0.19)	15.57 (0.39)	13.31 (0.28)
Rancimat oxidative stability (h)	63.6 (2.1)	11.3 (0.14)	12.3 (0.14)

* = Average of two determinations and respective standard deviation.

3.3. Moisture measurement during storage

Figs. 3–5 show the variation of moisture and volatile content of seeds in the study on the Center West and Reference conditions.

The statistical analysis shows that no significant differences in moisture content were found due to the genotype origin. This analysis showed that the storage conditions of Center West and Reference influence differently the moisture content and that the packaging materials generated significant differences in moisture content. All seeds showed periods of dehydration when undergoing the CW condition during the winter simulation period, the driest season among the evaluated.

Seeds on uncoated raffia reached the lowest levels of moisture because these packages allow vapor escaping. It is observed in Figs. 3–6 that the seed moisture kept in laminated raffia (8.3–8.7) g kg^{-1}, at the end of storage, is slightly above the values obtained for the common raffia (7.6–8.1) g kg^{-1}. This fact can be explained by its higher moisture barrier, which hinders the speed of the exchanges of moisture between the inside and outside of the packaging.

In common raffia, variations in humidity are basically due to physico-chemical interactions that usually succeed during seed storage without packaging. In the Center West condition, periods of dehydration followed by hydration were noted which are not observed in the Reference condition.

A study on the power of germination of sunflower seeds (Balešević-Tubić et al., 2010) also shows rise and fall curves during storage, which are dependent on the genotype. Three hybrids exhibit rise with a peak between 4 and 8 months, and one of them first shows a decline in germination at 3 months. Decreases in the power of germinating sunflower seeds were higher in uncontrolled than in controlled conditions (4 °C, RH 80–85%). While the former lost 4.2% and 11.8% after 6 and 12 months of storage, in controlled conditions the losses were 3% and 8.4% for the same periods of storage.

3.4. Monitoring of the peroxide value during storage

The peroxide during storage was monitored as an indicator of oxidative integrity of the seeds.

Figs. 6–8 show the evolution of the peroxide value during storage in the packages studied in Center West and Reference conditions.

The statistical analysis shows that peroxide values of the OL5 seed differ significantly from the He 250 and 253 seeds, which may

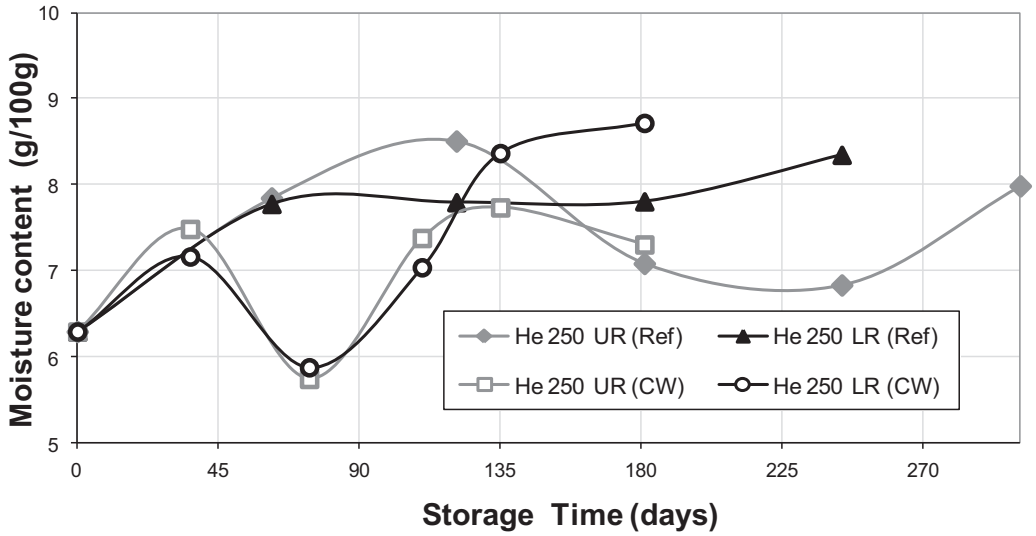

Fig. 4. Variation of moisture content of He 250 seed packaged in UR and LR stored in Center west (CW) and reference (Ref) conditions. CW simulates each season in different periods: fall (0–45) days, winter (46–90) days, spring (91–135) days and summer (136–181) days.

be considered statistically similar. The OL5 seed packages showed different behavior with maximum values of peroxide value around 1 meq/kg while the remaining were between 2.6 and 3.1 mEq/kg. The fatty acid composition of the lipid fraction of the sample OL5, rich in monounsaturated oleic acid (C 18:1), confirms the consistency of these results.

During fall, winter and spring simulation, the constant reference condition generated higher peroxide values than the accelerated aging test. A summer deterioration effect was observed with significant increase of peroxide values for seeds evaluated in the cyclic accelerated aging condition. This fact shows that storage at constant temperature and humidity induces different mechanisms of degradation of seeds compared to cyclic accelerated storage as confirmed by statistical analysis summarized in Table 6.

Considering the degradation mechanism proposed by El-Maarouf-Bouteau et al. (2011), with enzyme activation by moisture increase, it can be hypothesized than constant reference condition is more favorable for enzyme activation than continuous cyclic variation of temperature and humidity.

An interesting point is the behavior of seeds packed in uncoated raffia in the period of summer simulation in the Center West region (Figs. 7 and 8, in the period between 135 and 180 days). The peroxide values increased by 32% (OL5), 9% (He250) and 90% (He 253), a fact that shows the sensitivity of the seeds with hot and humid external climatic conditions.

A study carried out by Telles (2006), found values between 0.2 and 7.2 mEq/kg during 6 months of oil storage in ambient temperature. All the peroxide values measured in this work are lower than the limit of 15 mEq/kg established by Brazilian legislation (MAPA, 2006) for crude oils.

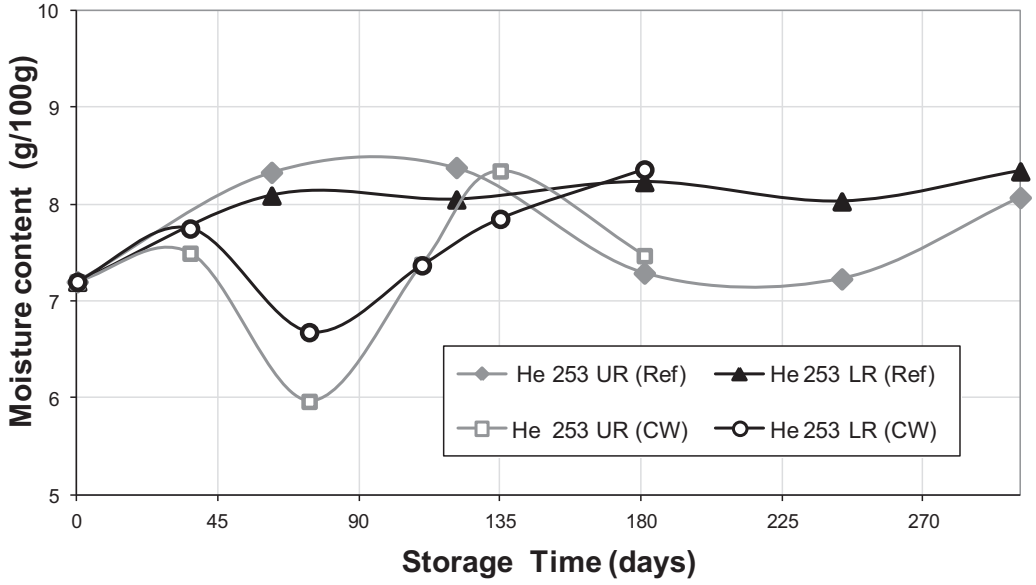

Fig. 5. Variation of moisture content of He 253 seed packaged in UR and LR stored in Center west (CW) and reference (Ref) conditions. CW simulates each season in different periods: fall (0–45) days, winter (46–90) days, spring (91–135) days and summer (136–181) days.

Table 3
Composition, total saturated, monounsaturated and polyunsaturated (g kg^{-1}) of the lipid fraction of sunflower genotype OL 5, at the beginning and end of storage time in each packaging.

Fatty acid (*)	Storage condition				
	Initial	CW/UR final	CW/LR final	Ref/UR final	Ref/LR final
C 14:0 Myristic	0.00	0.00	0.00	0.00	0.00
C 16:0 Palmitic	1.28	1.35	1.30	1.31	1.31
C 16:1 Palmitoleic w7	0.03	0.04	0.03	0.03	0.04
C 18:0 Stearic	1.06	1.06	1.06	1.08	1.08
C 18:1 Oleic w9	38.15	38.11	38.18	38.00	37.89
C 18:2 Linoleic w6	1.16	1.21	1.15	1.25	1.30
C 20:0 Araquidic	0.11	0.10	0.11	0.12	0.12
C 20:1 w11 *cis*-11-eicosenoic	0.11	0.10	0.10	0.03	0.05
C 18:3 Alpha linolenic w3	0.03	0.00	0.00	0.11	0.12
C 22:0 Behenic	0.33	0.31	0.33	0.33	0.34
C 24:0 Lignoceric	0.14	0.12	0.14	0.13	0.15
Total saturated	2.91	2.94	2.93	2.98	3.00
Total monounsaturated	38.30	38.25	38.31	38.06	37.98
Total polyunsaturated	1.19	1.21	1.15	1.36	1.42
Total w6	1.16	1.21	1.15	1.25	1.30
NI	0.00	0.00	0.00	0.00	0.00

CW = Center west; Ref = reference constant; UR = uncoated raffia; LR = laminated raffia; NI = not identified (*) = according to Codex Alimentarius (2005).

Table 4
Composition, total saturated, monounsaturated and polyunsaturated (g kg^{-1}) of the lipid fraction of sunflower genotype He 250, at the beginning and end of storage time in each packaging.

Fatty acid	Storage condition				
	Initial	CW/UR final	CW/LR final	Ref/UR final	Ref/LR final
C 14:0 Myristic	0.02	0.00	0.02	0.00	0.03
C 16:0 Palmitic	2.10	2.03	2.08	2.05	2.14
C 16:1 Palmitoleic w7	0.04	0.04	0.04	0.05	0.04
C 18:0 Stearic	1.35	1.32	1.32	1.33	1.29
C 18:1 Oleic w9	14.84	14.88	14.77	15.16	14.73
C 18:2 Linoleic w6	22.18	22.28	22.33	21.76	22.37
C 20:0 Araquidic	0.11	0.11	0.11	0.12	0.10
C 20:1 w11 *cis*-11-eicosenoic	0.06	0.06	0.06	0.04	0.05
C 18:3 Alpha linolenic w3	0.02	0.00	0.02	0.07	0.00
C 22:0 Behenic	0.32	0.33	0.32	0.35	0.31
C 24:0 Lignoceric	0.12	0.12	0.12	0.12	0.11
Total saturated	4.02	3.91	3.96	3.97	3.99
Total monounsaturated	14.94	14.98	14.97	15.25	14.82
Total polyunsaturated	22.21	22.28	22.35	21.83	22.37
Total w3	0.02	0.00	0.02	0.07	0.00
Total w6	22.19	22.28	22.33	21.76	22.37
NI	0.00	0.00	0.00	0.12	0.00

CW = Center west; Ref = reference constant; UR = uncoated raffia; LR = laminated raffia; NI = not identified (*) = according to Codex Alimentarius (2005).

Table 5
Composition, total saturated, monounsaturated and polyunsaturated (g kg^{-1}) of the lipid fraction of sunflower genotype He 253, at the beginning and end of storage time in each packaging.

Fatty acid	Storage condition				
	Initial	CW/UR final	CW/LR final	Ref/UR final	Ref/LR final
C 14:0 Myristic	0.03	0.03	0.03	0.03	0.03
C 16:0 Palmitic	2.10	2.09	2.02	2.04	2.04
C 16:1 Palmitoleic w7	0.03	0.03	0.03	0.04	0.04
C 18:0 Stearic	1.11	1.01	0.95	1.10	1.09
C 18:1 Oleic w9	18.12	17.68	18.39	18.24	18.44
C 18:2 Linoleic w6	18.46	19.01	18.54	18.14	17.98
C 20:0 Araquidic	0.09	0.09	0.07	0.11	0.10
C 20:1 w11 *cis*-11-eicosenoic	0.07	0.07	0.06	0.04	0.04
C 18:3 Alpha linolenic w3	0.00	0.00	0.00	0.08	0.08
C 22:0 Behenic	0.28	0.28	0.23	0.31	0.32
C 24:0 Lignoceric	0.11	0.12	0.10	0.14	0.16
Total saturated	3.72	3.62	3.40	3.73	3.73
Total monounsaturated	18.23	17.78	18.48	18.31	18.51
Total polyunsaturated	18.46	19.01	18.54	18.22	18.06
Total w3	0.00	0.00	0.00	0.08	0.08
Total w6	18.46	19.01	18.54	18.14	17.98
NI	0.00	0.00	0.00	0.16	0.11

CW = Center west; Ref = reference constant; UR = uncoated raffia; LR = laminated raffia; NI = not identified (*) = according to Codex Alimentarius (2005).

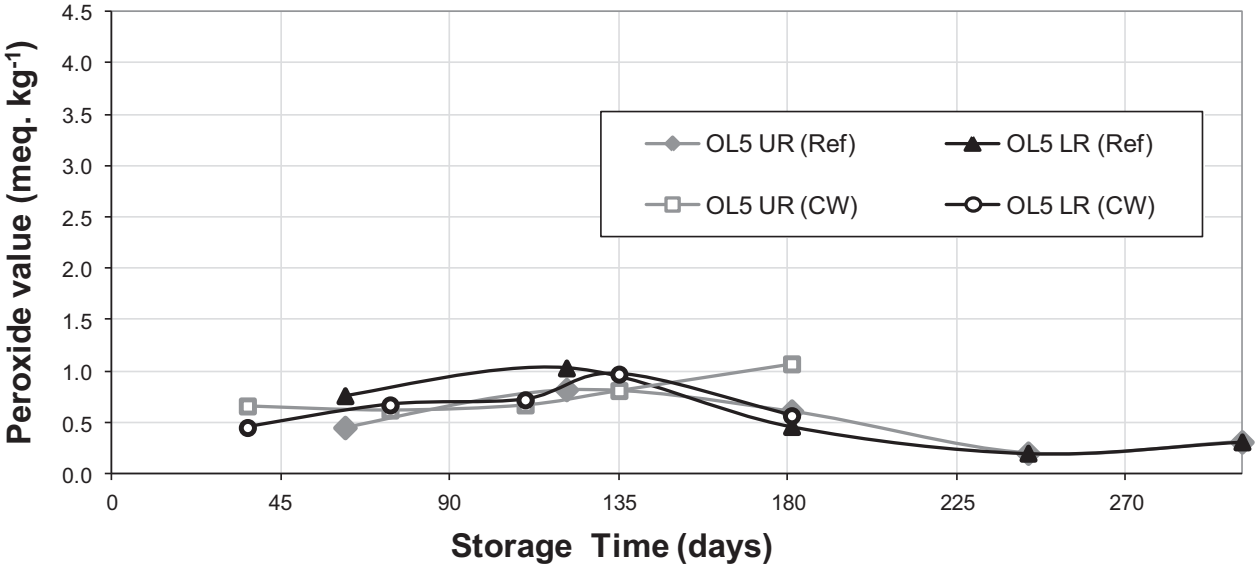

Fig. 6. Peroxide value evolution of OL5 seeds packed on uncoated (UR) and laminated (LR) raffia during storage in Center west and reference conditions. CW simulates each season in different periods: fall (0–45) days, winter (46–90) days, spring (91–135) days and summer (136–181) days.

3.5. Fatty acid composition variation

Tables 3–5 show the initial and final fatty acid composition of the lipid fraction of the sunflower genotypes studied, in each storage condition and packaging evaluated.

It is observed that the genotype OL 5 has a high (~38 g kg^{-1}) content of oleic acid (C 18:1) when compared to He 250 (~15 g kg^{-1}) and He 253 (~18 g kg^{-1}). The He 250 and He 253 genotypes show higher percentages of linoleic acid (C 18:2) than OL 5. This characteristic of higher content of monounsaturated fatty of OL 5 may confer greater oxidative stability to the oil extracted from these seeds, which was actually observed through tests to monitor the shelf life of products.

This fact can be explained by the relative oxidation rate between oleic acid and linoleic acid which is 1/10, or by the presence of more than one double bond in the hydrocarbon chain which increases by 10 times the rate of occurrence of the oxidation product. Furthermore, the position of monounsaturated acid in the triglyceride chain, can positively affect the process, providing greater protection against oxidation (Allen and Hamilton, 1989). Comparing all results, we can conclude that there was no change in the fatty acid composition of the lipid fraction during storage.

The numerical results of statistical treatment are presented in Table 6.

3.6. Moisture sorption isotherms

Fig. 9 shows the isotherms of sorption of the 3 types of seeds, measured at 25 °C. It is observed that He250 and OL5 seeds exhibit a very similar profile, different to the He253 genotype, which for the same moisture content, has lower water activities than OL5 and He250 seeds. According to Fig. 9, water activities ranged from

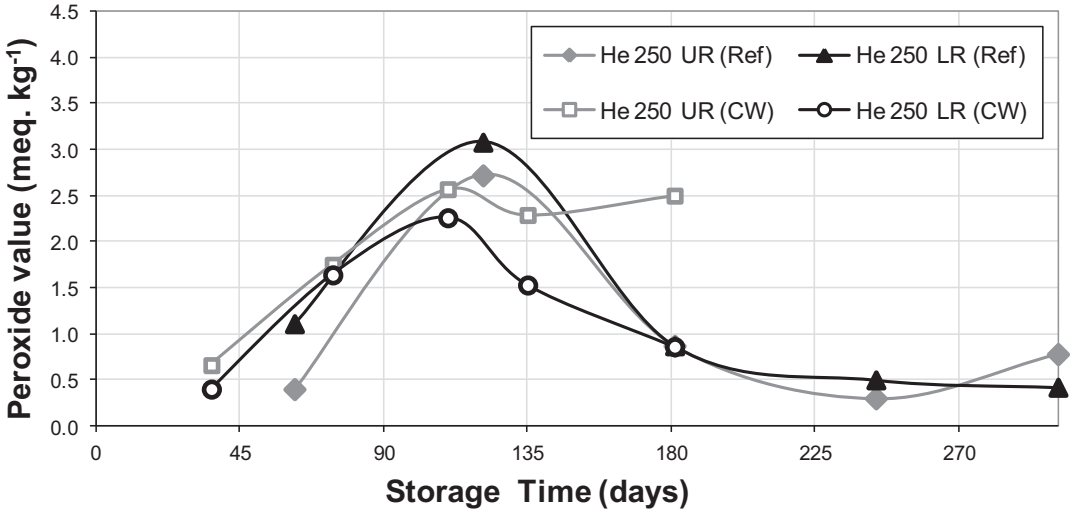

Fig. 7. Peroxide value evolution of He 250 seeds packed on uncoated (UR) and laminated (LR) raffia during storage in Center west and reference conditions. CW simulates each season in different periods: fall (0–45) days, winter (46–90) days, spring (91–135) days and summer (136–181) days.

Table 6
Results of nonparametric Kruskal-Wallis test statistical analysis applied to all data collected during storage.

Question 1: There are differences between peroxide values among the three seeds?

Test information	KW_{cs}	DF	*P*-value
	20.66	2	3.26E-05
Compared factors	Obs	Crit	Difference
He 250 peroxide–He 253 peroxide	3.52	18.55	No
He 250 peroxide–OL5 peroxide	31.88	18.55	Yes
He 253 peroxide–OL5 peroxide	28.35	18.31	Yes

Question 2: There are differences between humidity content among the three seeds?

Test information	KW_{cs}	DF	*P*-value
	2.57	2	0.28
Compared factors	Obs	Crit	Difference
He 250 moisture–He 253 moisture	8.07	19.01	No
He 250 moisture–OL5 moisture	4.31	19.01	No
He 253 moisture–OL5 moisture	12.38	18.78	No

Question 3: There are differences between CW and Ref conditions in the peroxide value?

Test information	KW_{cs}	DF	*P*-value
	12.64	1	0.00038
Compared factors	Obs	Crit	Difference
CW peroxide–Ref peroxide	22.38	12.34	Yes

Question 4: There are differences between CW and Ref conditions in the moisture content?

Test information	KW_{cs}	DF	*P*-value
	5.18	1	0.02
Compared factors	Obs	Crit	Difference
CW moisture–Ref moisture	14.72	12.68	Yes

Question 5: There are differences between LR and UR packaging in the peroxide value?

Test information	KW_{cs}	DF	*P*-value
	0.11	1	0.74
Compared factors	Obs	Crit	Difference
LR peroxide–UR peroxide	2.10	12.34	No

Question 6: There are differences between LR and UR packaging in the moisture content?

Test information	KW_{cs}	DF	*P*-value
	8.16	1	0.0043
Compared factors	Obs	Crit	Difference
LR moisture–UR moisture	18.48	12.68	Yes

KW_{cs} = Kruskal–Wallis chi-square; DF = degrees of freedom; Obs = observed difference; Crit = critical difference.

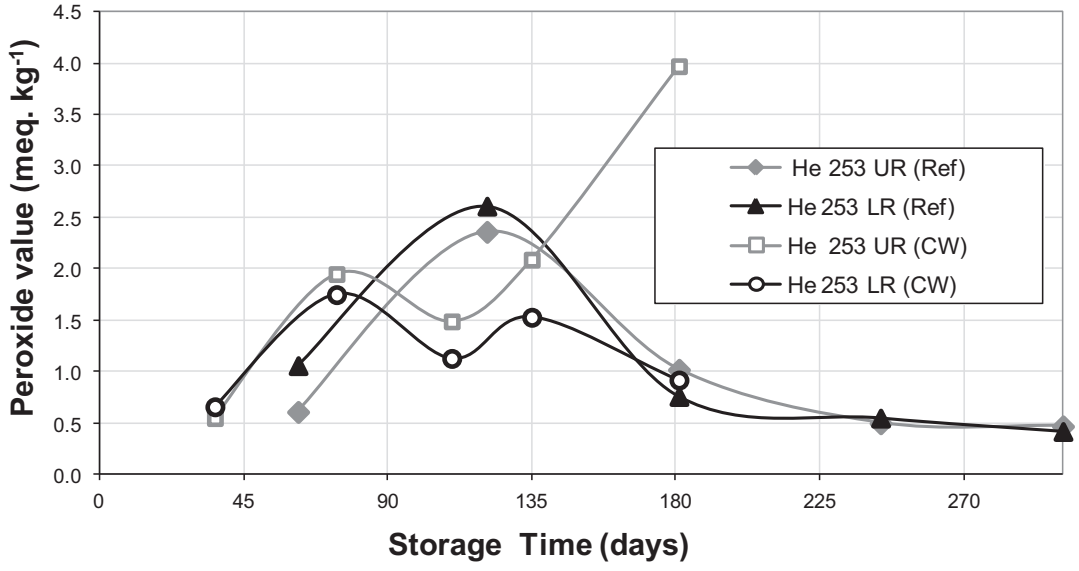

Fig. 8. Peroxide value evolution of He 253 seeds packed on uncoated (UR) and laminated (LR) raffia during storage in Center west and reference conditions. CW simulates each season in different periods: fall (0–45) days, winter (46–90) days, spring (91–135) days and summer (136–181) days.

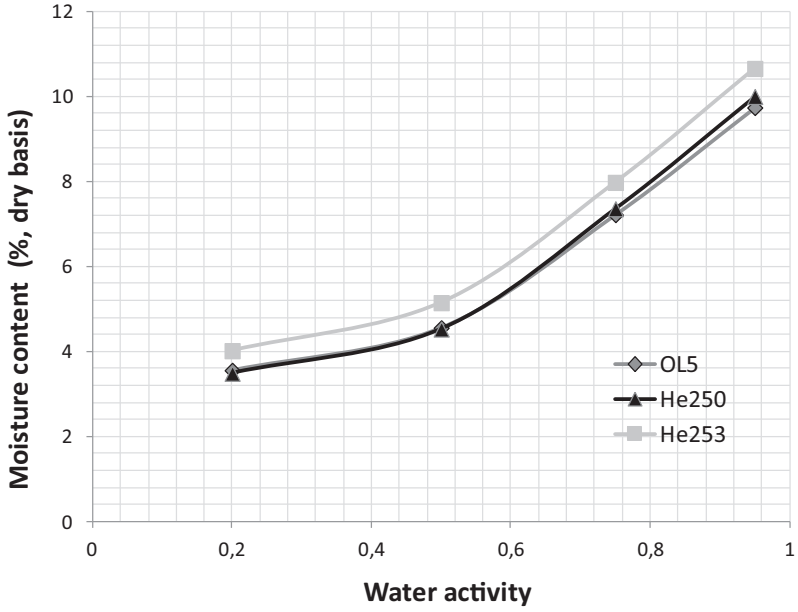

Fig. 9. Moisture sorption isotherm of seeds measured at 25 °C.

0.58 and 0.82 during storage of the sunflower seeds, probably an intensive range for lipid oxidation.

According to Labuza and Altunakar (2007) lipid oxidation shows a minimum in the 0.2 to 0.35 a_w range and increases in rate on both sides, i.e., an increase or a decrease in a_w. Similar behavior was found by Baker et al. (2002): High oleic content peanuts developed lower levels of peroxide after 14 weeks of storage when stored in environments with $0.33 < a_w < 0.44$ than outside of this range.

The results found in this study show that the differences in fatty acid composition were probably more important influent factors in the degradation rate observed, than the slight differences in water activities of the genototypes evaluated.

3.7. *Viability of genotypes for biodiesel production*

As previously discussed the higher content of the monounsaturated oleic acid (C 18:1) of OL5 genotype probably generates biodiesel with higher oxidation stability than the other two seeds analyzed. Currently, some researchers are doing a partial hydrogenation in order to convert the polyunsaturated acids to monounsaturated ones (Nikolaou et al., 2009; Papadopoulos et al., 2010). A high degree of unsaturation affects negatively the performance of fuel in diesel engines. Iodine value is the property defined in the European biodiesel standard EN 14214 that establishes a maximum limit for fatty acids methyl esters (FAME) fuels unsaturation (IV), of a maximum limit of 120gI_2/100 g FAME. From the seeds evaluated, only two present an estimate of iodine value lower than this limit during the storage of 181 days(Center West) and 301 days (Reference Constant): 82.2–82.9 gI_2/100 g (OL5), 124.0–124.9 gI_2/100 g (He 250) and 117.1–119.1 gI_2/100 g (He 253). These numbers were estimated using one of the most widely accepted methods to determine the iodine value adapted from the AOCS recommended practice Cd 1c-85 AOCS (1998) for the determination of the iodine value of edible oil from its fatty acid composition.

The cetane number measures the ignition quality of a fuel and has influence on the ignition delay. The lower the cetane number the greater will be the delay in ignition. The minimum cetane number is 51 for European biodiesel standard UNE-EN 14214. The cetane number of biodiesel also depends on the fatty acid composition of the original oil or fat. The cetane number increases with the length of the carbon chain and with the saturation of molecules. Among the seeds examined only the OL5 had an estimated cetane number (58.5–58.7) higher than the European minimum. The other genotypes, He 250 (43.3–43. 4) and He 253 (45.4–46.0) did not meet this value. In Brazil, however, as climatic conditions favor the ignition due to higher temperatures and commercialized diesel only contains 5% biodiesel, 3 genotypes could probably be used. The cetane number was estimated using the same methodology used by researchers in Spain (Ramos et al., 2009).

4. Conclusions

Based on the all results obtained, it can be stated that: OL5 genotype has the highest oxidative stability, followed by He 253 which is similar to He 250. These results are in agreement with the fatty acid composition of total lipids of each genotype, since the OL 5 sample has a higher content of unsaturates. The results are also in agreement with the reached peroxide value of the samples during storage, since OL5 seeds differed from the others, with maximum values of around 1 meq/kg while the others were between 2.6 and 3.1 meq/kg.

The study concluded that as all samples evaluated practically showed the same fatty acid composition up to the end storage, seed can be stored without loss of quality in the packaging materials and storage conditions evaluated, for the biodiesel or food industry, in the two ambient storage conditions studied.

All seeds showed dehydration when undergoing the CW condition during the winter simulation period, the driest season among the evaluated. During fall, winter and spring simulation, the constant reference condition generated higher peroxide values than the accelerated aging test. A summer deterioration effect was observed with significant increase of peroxide values for seeds evaluated in the cyclic accelerated aging condition.

The major difference between the uncoated and laminated raffia was their characteristics of moisture permeation. As the kinetics of degradation was different in each aging condition, the lowest peroxide values were found for laminated raffia in the Center West region and in uncoated raffia in the Reference condition. In general, laminated raffia showed a slightly higher performance than uncoated for seed preservation.

The three genotype seeds with 42.3–44.4 g kg^{-1} of oil content and Rancimat oxidative stability (11.3–66.6 h) have fatty acid composition adequate to fulfill the estimate requirements for Brazilian B5 (5% biodiesel) diesel, but only genotype OL5 could comply with B100 biodiesel European standard.

The storage in the chamber under constant reference condition generated different results from those obtained in the cyclic accelerated aging, designed for reproduction of the variations of temperature and humidity of the Center West region. This fact confirms that storage in constant temperature and humidity conditions does not reproduce the same sequence of degradation reactions. This highlights the importance of carrying out studies in conditions closer to reality for the estimation of the shelf life of products.

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