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Comparative assessment of bioactive components, antioxidant effects from 15 cultivars of sesame (*Sesamum indicum* .L) for different crop years

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Abstract— Sesame has been considered as one of the most important health crops in the world with greet potential nutraceutical compounds, the compositional components like lignans with HPLC technique, phenolic, flavonoids contents as well as antioxidant properties with the DPPH and ABTS methods of 15 cultivars of sesame seeds from different years were evaluated with the aim of the characterization of cultivars and their nutritional quality with the change of time, weather. The distribution of sesamin and sesamolin contents in the two years was different for the same sample, the values were ranging between 3,97-5,45mg/g.3,64-5,07mg/g in the crop years for sesamin, and the same results were shown in sesamol. As for antioxidant activity, in all extracts; the activity against ABTS radical was higher when compared to DPPH radical. But, the scavenging capacities still has been considered high which make sesame seeds a natural potent antioxidant. The phenolic contents and flavonoids were ranging between 3,58-3,98mg/g and 0, 14-0,148mg/g respectively. Thus, antioxidant effects of sesame are correlated very well with all the bioactive compounds.

Index Terms— Sesame, Lignans, Cultivars,Sscavenging capacities

1 INTRODUCTION

Sesame seeds (Sesamum indicum .L) from Pedaliaceae family have been considered as one of the oldest cultivated plants in the world, it is renowned as an important oil seeds from tropics and temperate zones, and as a nutritious food for human health. Sesame is also known as sim-sim, gingelly, beniseed, he has multifarious purposes containing about 50-56,2 % of edible oil in seed with high quality nearly matching olive oil and also stable against prolonged storage and heating which make foods fried in sesame oil have a long shelf life which could be attributed to an endogenous antioxidants components called lignins and tocopherols [1], [2].The sesame lignans appear to have an important functional components, the main ones are sesamin and sesamolin, they both can be converted to other lignans more powerful antioxidative constituent like sesamol during manufacturing or sesamol and sesaminol during chemical refining and beaching, as well they have an important role in plant defense so they have been commercially employed as potent antioxidant.

It was also shown that sesame cakes, seeds, spots and oil exhibited high activities in reducing power and radical scavenging methods as well as protection against oxidative deterioration [3], [4], [5].

Sesame seeds due to their high antioxidants activity have a pharmaceutical properties and a health promoting effects. They are valuable in treating respiratory disorders like preventing airway spasm in asthma, pneumonia, and also because of their good source of minerals compounds like magnesium which have a great support in respiratory health and calcium which helps prevent bone less and menopause [6].

The tocopherols in sesame are believed to promote the integrity of body tissues in the presence of oxidizing compounds specially gamma tocopherols which influence vitamin E activity that is believed to prevent cancer and heart disease [7].

Until now, numerous studies have established that the great oxidative stability and antioxidant activity of sesame are due to the contents of phenolic compounds, Sesame has attracted the attention of public due to its beneficial constituents to the health. But except in a few countries of Asia, the production of sesame seeds and oil are still suffering the constraints on its valuation and profitability. In addition, little information is available on the determination and comparison of antioxidants effects and other components of sesame seeds. Therefore, this study was designed to analyze 15 cultivars grown in different regions from Tadla- Azilal location and compare the compositional compounds in various crop years and their antioxidant activities through DPPH and ABTS radical scavenging methods.

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2 Material and methods

2.1 Plant Material

Fifteen sesame cultivars have been used in this study collected from locations where sesame grows in the area Tadla-Azilal (Fig 1), Morocco. These cultivars were grown during 2013 and 2014 in the experimental fields of Regional Agricultural Research Centre of Afourer, Morocco (RARC). After harvesting, sesame seeds were immediately dried and stored until analysis.

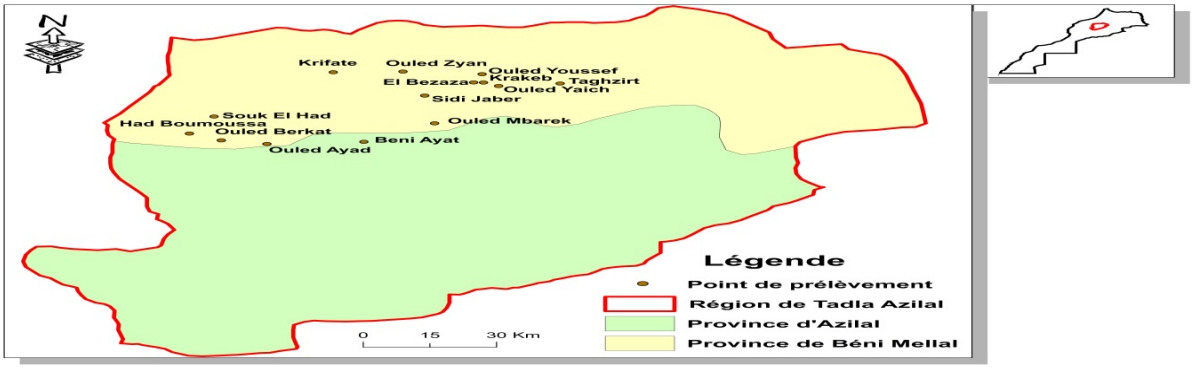

Fig. 1. Map shows the areas of sampling in the region Tadla-Azilal.

2.2 Chemicals and reagents

The solvents and the chemicals used were of analytical grade, ethanol and distilled water were used as solvent for extraction of antioxidants compounds. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS),sodium bicarbonate, Folin-Ciocalteu, gallic acid, aluminum trichlorid, quercetin, persulphate de sodium and ABTS were of analytical grade and stored at prescribed conditions in the laboratory.

2.3 Assessment of Bioactive Activity

2.3.1. Preparation of Seed Extracts

The seeds of each cultivar were ground in the mixer separately. 10g of the powder was weighed and suspended in 100ml of 90% ethanol and kept for shaking for2 hours.

After filtration, the samples were subjected for vacuum evaporation [13]. The extract was dissolved in 2 ml of 90% ethanol and assayed for its antioxidant activities, phenolic content and flavonoids.

2.3.2. DPPH Radical Scavenging Activity

For determination of the antioxidant activity of sesame extracts, the stable, 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical was used. An aliquot 0.5ml of DPPH solution was diluted in 4.5 ml of methanol, and 30 µl of ethanolic solution sesame extract of various concentrations (100,90,70,50,40,20,10,5µg/ml) was added. A control without extract was also maintained. The mixture was shaken vigorously and allowed to stand for 45 minutes in the dark and the absorbance was measured at 515nm [8]. The antioxidant activity of the extract was calculated using the formula,

% scavenging activity= ((Absorbance sample – Absorbance control)/ Absorbance control) X 100.

2.3.3 ABTS radical scavenging activity

ABTS radical scavenging activity of sesame extracts was measured by the ABTS cation decolorization assay as described by Re and al., [9] with some modifications. The ABTS radical cation (ABTS•+) was produced by reaction of 7 mM stock solution of ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in dark at room temperature for 12 h before use. The ABTS•+ solution was diluted with methanol to give an absorbance of 0.7 ± 0.01 at 734 nm. The extracts fractions (1 ml) of various concentrations (100, 90, 70, 50, 40, 20, 10, 5µg/ml) were allowed to react with 2 ml of the ABTS•+ solution and the absorbance was measured at 734 nm after 6 minutes. The results were expressed as Trolox equivalent antioxidant capacity (TEAC) values and calculated as mean value ± standard deviation (SD) (n = 3).

2.3.4 Total Phenolic Content

The amount of total phenolic compounds was measured [10]. 15mg of extract was dissolved in 1ml of 90% ethanol. A 10µl aliquot of the resulting solution was added to 2ml of 2% Na2CO3 and after 2 minutes 100µl of Folin-ciocalteu reagent (diluted with water 1:1) was added. After a further 30 minutes, the absorbance was measured at 750nm. The concentration was calculated using gallic acid as standard, and the results were expressed as mg gallic acid equivalents per mg extract.

2.3.5 Total Flavonoid Content

The flavonoid content was determined [11]; 1ml of the extract was added to 1ml of aluminum trichlorid ALCL3 (2%).After 15 min of incubation. The absorbance was measured at 430 nm and the results were expressed an mg quercetin equivalents per mg extract.

2.4 Lignans analysis:

For the extraction of sesamin and sesamolin, 0,4 of air-dried sesame seeds were ground into powder and added into 15 ml plastic tubes. 5 ml of 80% methanol was added and the whole extracted for
30 min. The samples were centrifuged at 2000g, for 3 min at 25°C. The supernatant was transferred into a 10 ml volumetric flask. The residues were then re-extracted with 4.0 ml of 80% methanol. All extracts were combined, volume adjusted with 80%
methanol, and filtered through a 0.45µm PVDF membrane prior to HPLC analysis.
The HPLC analysis of sesamin and sesamolin was performed using the external standard method by Agilent 1100 high-performance liquid chromatography. A reversed-phase column, Hypersil BDS C18 5ml, 150*4 mm i.d. The mobile phase consisted of water (solvent A) and methanol
(solvent B) with a gradient system: 0 min, 5%B; 0–5 min, 5–18%B; 5–10 min, 18–35%B; 10–15 min, 35–62%B; 15–18 min, 62–80%B, 18–22 min, 80%B; 22–23 min, 80–5%B, and equilibrated at this condition (5%B) for 3 min. The flow rate was 1.0 ml/min (injection volume 20µl) with detection at 280 nm. The total running time was 25 min and the column temperature were maintained at 25°C.

2.5 Statistical Analysis

Statistical analyses were conducted using SPSS (Statistical Program for Social sciences) version 20.0 (Version 20, SPSS Inc., Chicago, IL, USA). All analyses were performed in triplicate and data reported as means ± standard deviation (SD).

3 Results and Discussion

Comparison of antioxidant activities against DPPH and ABTS radicals from the seeds of sesame cultivars of different crop years
In many screening methods of antioxidant properties, the DPPH and ABTS radical scavenging activities are used to evaluate the antioxidant effects of different plants. The free radical scavenging ability of sesame seeds (Sesamum indicum .L) extracts were analyzed against DPPH and ABTS, the DPPH assay is rapid, sensitive method to survey the antioxidant activity of specific compounds; he is a very stable organic free radical with violet color which gives absorption maxima within 515-530nm range. When he receive a proton from hydrogen donors like phenolic compounds, the color became yellow indicating the reaction of antioxidants with DPPH, resulting a decrease of number of DPPH molecules.
The ABTS decolorization method is based on the ability of different substances to scavenge the ABTS.+, it is a protonated radical with the absorption maxima 734 nm which decrease with the scavenging of the proton radical. Many studies have identified phenolic compounds in sesame having high antioxidant activity and even responsible of the stability of sesame seeds and oil in thermal process and a large storage condition [12]. In this study, we investigated antioxidant activities by comparing percentage inhibition in the two methods and also comparing the change of scavenging capacity of the same cultivars in 2 successive years.
For the ABTS assay, the yield of ethanolic extracts of sesame with ABTS radical of all samples increased with increasing concentrations ranging between 64,69%-78,69% at 70µg/ml for the first year and 62,17%-74,64 at 70µg/ml for the second year (Fig 2). The IC50 were as follows: 49 µg/ml for sample 2, 66 µg/ml for sample 5 in the first year and 50µg/ml, 66µg/ml for samples 7 and 9 respectively in the second year (Fig 3).
According to our experiments, the antioxidant activity with ABTS shows a large difference in the same sample for the 2 years, as for sample 1; the antioxidant activity was 67,6 % and 63,61% for the first and second year respectively. The same observation for the rest of samples, which can be due to the temperature changes or mineral loss in soil, the test of ANOVA shows that the difference between the 2 years was significantly high ($p<0,05$).

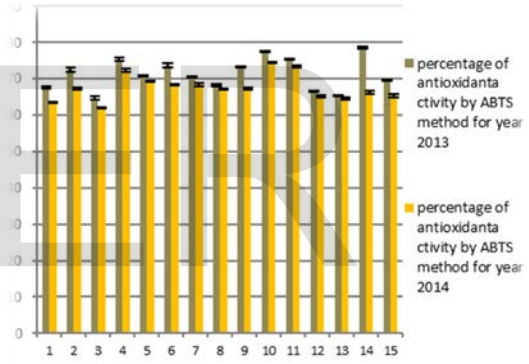

Fig. 2. Percentage of antioxidant activity of sesame by ABTS radical scavenging method in the years 2013 &2014.

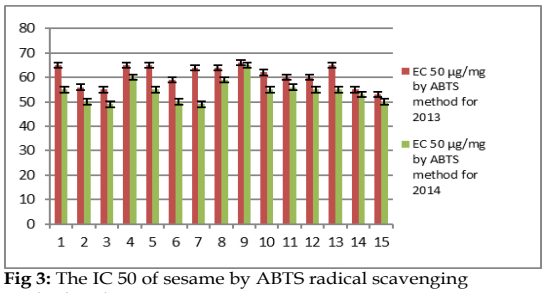

Fig 3: The IC 50 of sesame by ABTS radical scavenging method in the years 2013 &2014.

The samples from the first year exhibited higher ABTS radical scavenging properties than those of the second year. However, the activity against this radical differed with seed crop years, our results exhibited similar antioxidant activity for some samples, and also higher

activity for other samples by comparing the data of earlier research.

This difference may be also due to the extraction method which could affect the phytochemical and physicochemical contents [13].

The scavenging effects against DPPH radical of all extracts in the two years with the different concentrations (100, 90, 70, 50, 40, 20, 10, 5µg/ml) were observed, lower radical scavenging activities with DPPH when compared to the ABTS radical, it was reported that DPPH radical is used to investigate the scavenging effects of hydrogen donating abilities of phytochemicals in sesame.

The scavenging effects against DPPH radical shows also considerable differences in cultivars of the crop years as those of ABTS radical with the test ANOVA, the effects of antioxidant activity of DPPH radical increased with increasing concentrations, which confirm the results with ABTS radical method. The highest and lowest activities were observed in the cultivars 5 and 4 with the values 61,45%-69,52% respectively in the first year and 61,02%-69,46% in the cultivars 5 and 15 in the second year at the concentrations of 70µg/ml (Fig 4). The IC50 values were ranging between 49-65 µg/ml and 52-65µg/ml for the two years respectively (Fig 5). According to Vishwanath and al., [13]and Rizki and al., [14], the scavenging activities of sesame extracts were higher than BHT and BHA, so this plant could be considered as excellent natural antioxidant and may be a natural source as therapeutic and nutraceutical agents.

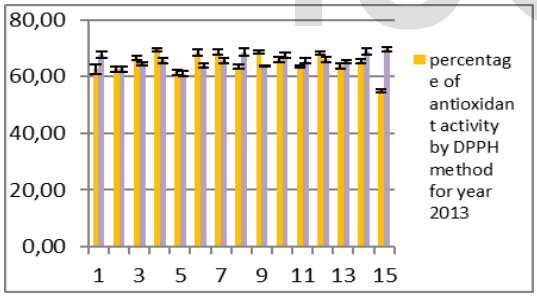

Fig 4 : Percentage of antioxidant activity of sesame by DPPH radical scavenging method in the years 2013 &2014.

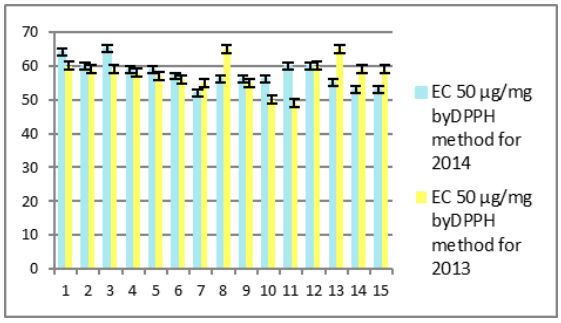

Fig 5: The IC 50 of sesame by DPPH radical scavenging method in the years 2013 &2014.

Total phenol, flavonoids contents:

The phenolic compounds are distributed in the plant, it was reported that they are responsible for the high antioxidant activity of sesame seeds due to their capacity to donate a hydrogen atom or electron in order to form stable radical. The presence of those bioactive components is attributed to this high activity of seeds and oils, which help reducing the danger of several diseases and increase the probability of using the phenolic in sesame products as natural antioxidant.

The total phenolic contents of sesame seeds extracts was ranging between 3,58-3,98 mg GAE/g for the samples 2-3 in the first year. As for the second, the phenolic compounds were ranging between 3,53-3,93 mg GAE/g for the samples 2 and 13 (Fig 6).

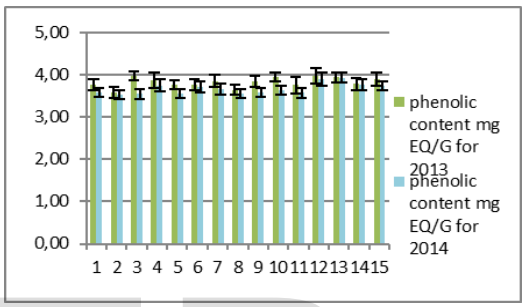

Fig. 6. Phenolic compounds (mg gallic acid/g dry matter) in the years 2013&2014.

The flavonoids contents are expressed in the same way as in the Fig 7; the samples of the first year shows higher flavonoids contents than the second year (0,145 mgQE/g against 0,13 mgQE/g for the sample 1). This difference is observed in the all bioactive compounds which also could be due to the temperature changes, soil, color of seeds …ect.

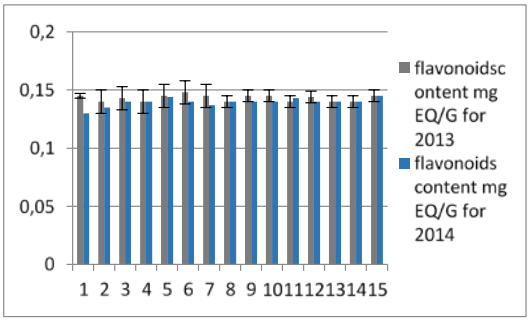

Fig. 7. Flavonoids content (mg quercetin/g dry matter) in the years 2013&2014.

Even with this difference in the two years, phenolic compounds in sesame seeds was found to be higher than banana (2,32mg/g) and carrot (1,52mg/g) [15].

The values obtained corresponded with antioxidant activity and correlated well in all the extracts studied ($p<0,01$). The R2 (coefficient of correlation) of relations between total phenolic content, flavonoids and DPPH-scavenging activity was 0.8899 and 0.8678 for the year 2013. As for 2014, the same results was found with R2 was 0.7599 and 0.679 for phenolic and flavonoids respectively.

Those results suggests that phenolic are important components of sesame and could be responsible for the scavenging activity possessing potential antioxidant power, compared to synthetics ones BHT and BHA. In fact, many oilseeds have been investigated for the use of their phenolic compounds like safe sources of natural antioxidant preventing oxidation of biological molecules.

Lignans contents:

The lignans of sesame are a group of natural compounds contains sesamin and sesamolin, which have many pharmacological activities considered as potent phenolic antioxidants.

In this study,the Hplc analysis indicated that the lignans in sesame extracts in seeds are sesamin and sesamolin. The other lignin's like sesaminol are produced from sesamolin during the bleaching process, and sesamil which is formed from sesamolin during the roasting process.

The sesamin and sesamolin were determined in 15 various cultivars at 290nm, the retention time of sesamolin was 18,1 min and sesamin 17,5 min (Fig 8)

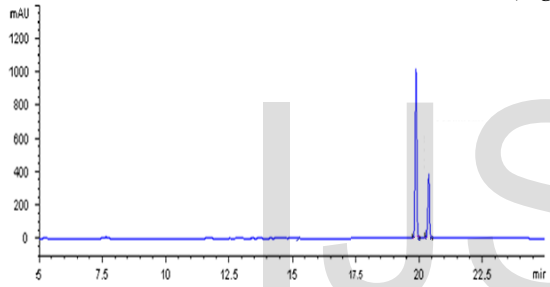

Fig.8. HPLC fingerprints of lignans of sesame seed; sesamin and sesamolin.

The values of lignans shows significant differences between cultivars in the crop years with the sesamin having the highest values years found in large amounts followed by sesamolin found in small amount in sesame.

The difference between the compounds for the same samples in the 2 years could be attributed to the growing conditions, weather which could influence the irrigation and also seed, capsules size and position or environmental stresses.

The range of sesamin content was between 3,97-5, 45mg against 3,64-5,07mg/g for in the two years, and the sesamolin content was ranging between 2,95-4,10mg/g against 2,3-3,72mg/g, these results shows that the lignans contents like the other components decreased from the year 2013 to 2014 (Fig9&10) .

The highest content in sesamin were 5,45 mg/g (2013), 5,07mg/g (2014) for sample 14 and the lowest were 3,93 (2013), 3,64 (2014) for sample 13. The same observations were for the sesamolin contents.

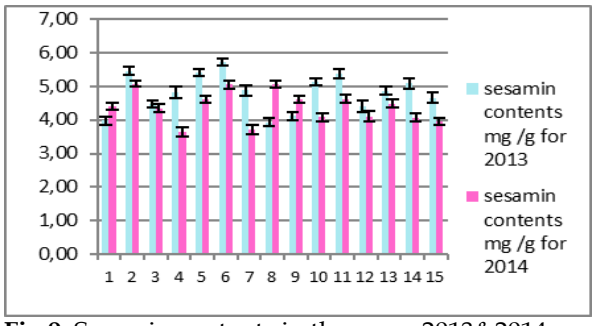

Fig 9: Sesamin contents in the years 2013&2014.

The sesamin and sesamolin values were correlated with R=0,876 and the values of lignans were significantly correlated with antioxidants activity p<0,05 (R=0,821) and (R=0,933) for the two years respectively. The results also show that the total lignans contents are mainly influenced by sesamin and sesamolin compounds.

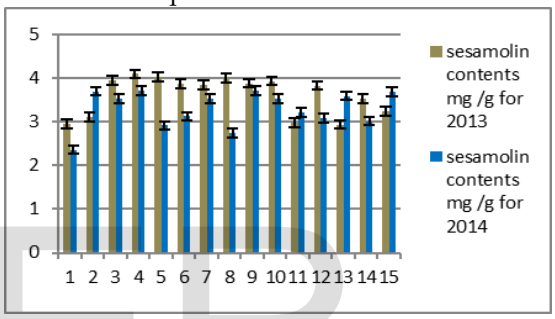

Fig.10. Sesamolin contents in the years 2013&2014.

The high amount of lignans assure to sesame a great scavenging activity, they have unique bioactive, physiological and nutritional properties and could be used as natural antioxidant. When added, they increase greatly the oxidative stability.

4 Conclusion:

In conclusion, the evaluation of the antioxidant activity with DPPH and ABTS methods, sesamin, sesamolin and phenolic compounds present in 15 sesame seeds cultivars in the 2 years 2013, 2014 may be beneficial for the selection of the best sesame line to develop an excellent sesame cultivar with high levels of lignans and phenolic, those compounds exhibited remarkable differences along the crop years which could be due to environmental and weather changes. Even with the differences between the cultivars, the sesame products could be used as functional foods considered as a natural source for nutraceutical foods owing to their strong radical scavenging capacities and high lignan and phenolic contents.

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