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Assessment of Antioxidant Capacity of 16 Cultivars of Sesame (Sesamum indicum.L) from Different Areas

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ABSTRACT: Sesame (Sesamum indicum.L) is one of the most important oilseed crops, having seeds and oil that are highly valued as a traditional health food and recently, natural antioxidants have gained increased interest because natural food ingredients are safer than synthetic ones. Antioxidant activity and bioactive molecular tests were carried out on 16cultivars of sesame seeds from different areas in the world. This activity was found to be high ranging between 59 and 64% for the samples; this result can be related to the large amount of phenolic and flavonoid contents with the values 3.79-3.97 mg/g and 0.127-0.147mg/g respectively. The flavonols content was ranging between 0.400 and 0.421mg/g EQ per mg extract. those results strongly suggest that phenolics compounds are a good natural antioxidant and also put the American cultivar in the first choice for the customer with the Indian and Morocco ones. Due to its all favorable properties; sesame seeds could be used in either food or cosmetic and pharmaceutical products.

KEYWORDS: Sesame (sesamum indicum), Antioxidant activity, Phenolic, Flavonoid, Flavonols.

1 INTRODUCTION

Sesame (Sesamum indicum L.) is one of the most important oilseed crops in the world which is due to its high content of lipid [1], it is not only a source of edible oil, but also widely used in baked goods and confectionery products [2].It is also consumed as a nutritious food, beneficial to health in oriental counties. Many studies have been conducted to investigate the health-promoting effects of sesame [1], It has been cultivated for centuries, especially in Asia and fibrous husk. As such the meal is only useful as cattle Africa. In 2009, the world production of sesame seed was feed. 3,976,968 tons and the major production areas were Asia (2,489,518 tons) and Africa (1,316,690 tons), constituting about 62.6 and 33.1% of the total world production [3]. Sesame had also been used throughout East Africa where it is mainly grown for grain and oil [4]. So, many countries produce and export this product, mainly China, Japan, India, Cameroon, Egypt, Senegal, Brasilia and Iran[5][6][7][8]. As for Tunisia, 80% of the needed sesame seed is imported from Sudan and 20% from Egypt) [9]. Sesame meal has a composition of 7.92% moisture, 27.83% about 62.6 and 33.1% fat, 30.56% protein, 6.22% fiber, 5.27% ash and 28.14% carbohydrate. Extraction of oil has led to increased protein content of defatted sesame meal (41.15-49.58%). This meal can be used as a protein source ingredient in the food industry, the residue sesame oil cake contains on an average 32% crude protein, 8-10% oil, total oil and albuminoids of 40-42% and rich in essential amino acids namely methionine and cystine [10]. And the sesame shows great antioxidant stability, this could be attributed to endogenous antioxidants namely lignins and tocopherols [11] [12] .It showed that the addition of unsaponifiable matter extracted from sesame seed increases the stability of sunflower oil. This stability is more pronounced in the case of unsaponifiable matter extracted from roasted sesame seeds due to a synergistic role which was related to sesamol formed by the decomposition of sesamolin during roasting [13].

Sesame seeds, for the major part, essentially transformed to halaweh, obtained after mixing the white tehineh (white sesame seed dehulled, roasted and grinded), saponin and nougat (hete-treated sucrose).the sesame seeds also present an essential material to manufacture “Halwa Chamia” which was prepared from “Tahina” and from Nougat; with an addition of some such flavoring ingredients as vanilla and walnut. “Tahina” is a paste exclusively elaborated from milled and decorticated sesame seed and for the preservation and the conservation of the by-products for a long time, great antioxidant stability is needed, to minimize any sort of deterioration or alteration. It has been proven also that sesame seed and oil may have potential as a cancer fighter. It contains large amounts of linoleate in triglyceride form which selectively inhibited malignant melanoma growth [14], Sesame seeds are valuable in treating respiratory disorders like preventing airway spasm in asthma, pneumonia, acute and chronic bronchitis. Sesame seeds are a good source of magnesium which supports respiratory health. Black sesame seeds, as rich source of iron, are valuable in treating anemia [15], Sesame seeds are a good source of calcium which helps prevent bone loss that can occur as a result of menopause. Another reason for older men to make zinc rich foods such as sesame seeds as a regular part of their healthy way of eating will increase bone mineral density although osteoporosis is often found in postmenopausal women, it is also a potential problem for older men. The beneficial properties of sesame seeds are due to their oxidative stability which is attributed to endogenous antioxidants (lignans) together with tocopherols. The stability is more pronounced in case of unsaponifiable matter extracted from roasted sesame seeds due to synergestic role. Lignans and lignan glycosides present in sesame appear to be the important functional components. The main sesame lignans are sesamin and sesamolin which are found in sesame oil, those compounds are believed to play an important role in the oxidative stability of sesame, this stability is different between the black sesame seeds and white, yellow sesame seed, and also the difference can be due to the origin of the seeds. The aim of this study was to evaluate the antioxidant stability and phenolic compounds of different cultivars from different area in the world.

2 Materials and Methods

2.1 Plant Material

The cultivars studied represented material collected from locations where sesame grows in the world, with 16 different cultivars which are from India (I), Turkey (T), Arabi Saudi (A S), Egypt (E), Morocco (M), and 11 accessions from United states (USA), The accessions of the sesame (sesamum indicum.L) of USA were imported and grown in Morocco in the same cultural practices during the rainy season in the year 2013-2014 at the INRA, and phytochemical properties were evaluated.

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’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) 1,1-diphenyl-2-picrylhydrazyl (DPPH),Trolox Na_2CO_3, phenol reagent, gallic acid, aluminium trichlorid, quercetin, were 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 for 2 hours. After filtration with whatman paper, the samples were subjected for vacuum evaporation. The extract was redissolved in a 2 ml of 90% ethanol and assayed for its antioxidant activity, phenolic, flavonoids and flavonols contents [16].

2.3.2 DPPH Radical Scavenging Activity

For determination of the antioxidant activity of sesame extracts, the stable, 1 diphenyl-2-picryl hydrazyl (DPPH) radical was used [17]. An aliquot 0.5ml of DPPH solution was diluted in 4.5 ml of methanol, and 30µl of ethanolic solution sesame extract 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. 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 [18] 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) were allowed to react with 2 ml of the ABTS•+ solution and the absorbance was measured at 734 nm after 30 minute. Trolox was used as a reference compound. 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 using the method [19]. 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 [20]; 1ml of the extract was added to 1ml of aluminium 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.

2.3.6 TOTAL FLAVONOLS

Total flavonol content was determined [21]. To 2.0ml of extract solution, 2.0ml of 2% AlCl3 ethanol and 3.0 ml (50g/l) sodium acetate solutions were added. The absorption at 440 nm was recorded after 2h30 min at 20°C. Extract samples were evaluated at a final concentration of 0.1mg/ml. total flavonols content expressed as quercetin equivalent (QE).

2.4 STATISTICAL ANALYSIS

Statistical analyses were conducted using SPSS (Statistical Program for Social sciences) version 17.0 for window. All analyses were performed in triplicate and data reported as means ± standard deviation (SD).

3 RESULTS AND DISCUSSION

3.1 DPPH RADICAL SCAVENGING ACTIVITY

The DPPH radical is considered to be a model of a stable lipophilic radical. A chain reaction in lipophilic radicals was initiated by the lipid autoxidation. Antioxidants react with DPPH, reducing number of DPPH molecules equal to the number of their available hydroxyl groups.

The free radical scavenging ability of sesame seeds (sesamum indicum) extracts were analyzed by DPPH method. The DPPH radical is commonly used for the assessment of antioxidant activity in vitro and is foreign to biological systems; he is a very stable organic free radical with deep violet color which gives absorption maxima within 515-528 nm range.

Upon receiving proton from any hydrogen donors, mainly from phenolic, it loses it chromophore and became yellow, which mean that the antioxidants react with DPPH, reducing a number of DPPH molecules equal to the number of their available hydroxyl groups. The DPPH radical is considered to be a model of a stable lipophilic radical [22].

Total antioxidant (TAS) of different sesame cultivars, expressed as Trolox equivalents, is shown in Figure 1. The total antioxidant activity of the samples tested was in the decreasing order of American 47 with the 64.17%> American 45 and 50 with 63.17% > Morocco, India, American 43 with 62.83% and 46, 48 with the value 62.33%> American 44 with 61.83% and 49 with 61.50%, American 42 with 61.17% > Egypt, American 40 and 41 with the value 60.33% and Turkey with 60.17%> Saudi 59.67%. DPPH radical scavenging activity of sesame extracts may primarily be related to their hydrogen donation ability. The values obtained for DPPH radical scavenging capacity corresponded well with those of the total antioxidant activity and total phenolics. Thus, sesame extracts may also possess a strong antimutagenic activity which is attributed to the ability of the molecules involved to scavenge free radicals [23].

The results of the DPPH free radical scavenging suggest that the different extracts from the different cultivars and area are capable of scavenging free radicals via electron- or hydrogen-donating mechanisms, the higher antioxidant activity was found in the American cultivars , and low activity was found in Saudi cultivar but the sesame seeds still have a great antioxidant activity compared to other seeds and thus should be able to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices. This result showed the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage.

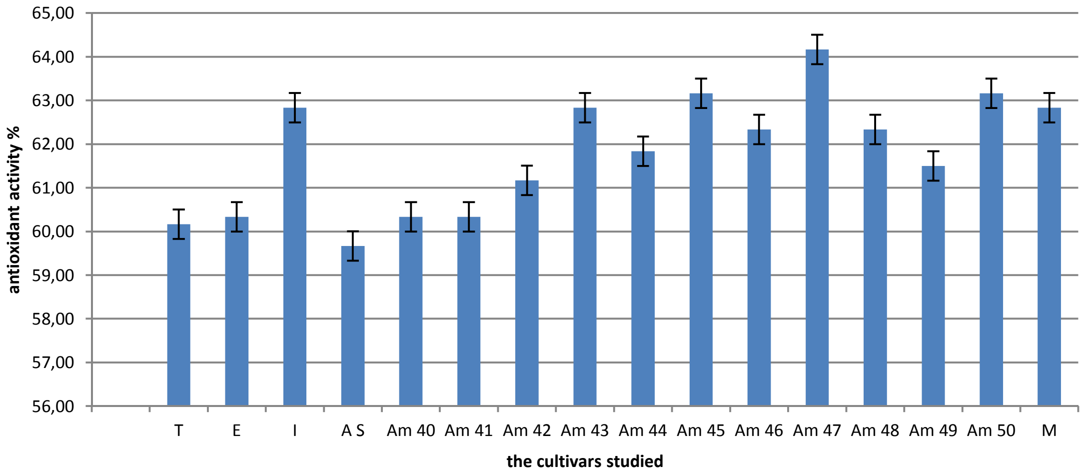

Fig. 1. Antioxidant activity by DPPH method of the differents cultivars

3.2 ABTS RADICAL SCAVENGING ACTIVITY

Although the DPPH free radical is ubiquitously used to estimate the potential free radical scavenging activity of natural products, the ABTS free radical is commonly used when issues of solubility or interference arise and the use of DPPH based assays becomes inappropriate. Having considered the solubility of the test sample and the advantages and disadvantages of the use of the DPPH free radical, it was considered necessary to further assess the extracts against the ABTS free radical. Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima 734nm which decrease with the scavenging of the proton radicals.

In our study, most of the tested cultivars of sesame extracts exhibited antioxidant activity and showed comparable or higher activity to the synthetic antioxidants BHA, BHT [22].

With the values 57 and 48% respectively, the scavenging of the ABTS radical by the extract was found to be higher than the DPPH radical. The total antioxidant activity of the samples tested was in the decreasing order of American 47 and 45 with the value 66.7%> American 50,48, India,46and 43 with 65.9%, 65.7%, 65.72%, 65.2%, 65.1% > Morocco,44 and 42 with the values 64.9%, 64.5%, 64%> American 40,Saudi, Egypt, American 41 and Turkey with the value 63.9%, 63.8%, 63.6%, 63.5%, 63.2% and finally American 49 with the value 62.4% (Fig2). Factors like stereo selectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react different radicals. With those results, sesame seeds of different cultivars can be considered as a potent radical scavenger and a good natural source for functional food and nutraceuticals.

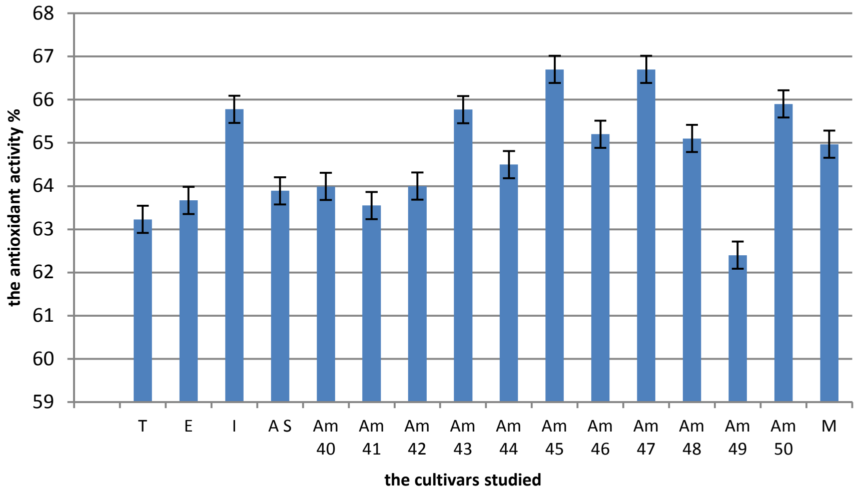

Fig. 2 . Antioxidant activity by ABTS method of the differents cultivars

3.3 TOTAL PHENOL, FLAVONOIDS AND FLAVONOLS CONTENTS

TPC were determined by using Folin-Ciocalteu reagent Folin-Ciocalteu reagent react nonspecifically with phenolic compounds as it can be reduced by a number of nonphenolic compounds e.g., vitamin C, Cu (II), etc. Although exact reaction of the reagent with reducing species is not known, but it is considered that a complex is formed between phospho-molybdic tungstate and reducing species, phenolate ion, changing color from yellow to blue where absorbance is measured [24].

The phenolic compounds are considered a natural antioxidant; they have potential sources of natural antioxidant have been searched in many plant sources such as vegetables, fruits, leaves, oilseeds, cereal crops, barks, and roots, spices and herbs, and crude plant drugs [25]. Besides role of natural antioxidants as preventative components towards diseases, they also prevent oxidative deterioration of vegetable oils and fats during processing, distribution, and storage [26].

The total phenolic compound was determined by the Folin-Ciocalteu phenol method, this method measures the reduction of the reagent by phenolic compounds with the formation of a blue complex that can be measured at 760 nm. Also the flavonoids possess a broad spectrum of chemical and biological activities including radical scavenging properties. Such properties are especially distinct for flavonols which present the most ubiquitous flavonoids in foods, and the main representatives are quercetin and kaempferol. They are generally present at relatively low concentrations. These compounds are present in glycosylated forms, often associated with glucose and rhamnose sugar, but other sugar type may also be involved, the flavonols contents biosynthesis is stimulated by light. The values of phenolics content and flavonoids were ranging between 3.75mg/g± 0. 05, 0.127± 0.08 mg/g for the saudi cultivar and 3.97mg/g ±0. 03, 0.147 ±0.05 mg/g respectively for the American 47 cultivar (Fig 2, Fig 3), still all the cultivars have higher concentrations on phenolic compound, which could have a role in the prevention of degenerative diseases such as cancer and cardiovascular diseases. The results in this study proved that the difference of the nature and contents of the various polyphenols, flavonoids may be due to the influence of agricultural practices and industrial processes. The results suggest also that phenolics compounds are important for this plant and others, the values obtained corresponded well with the antioxidant activity.

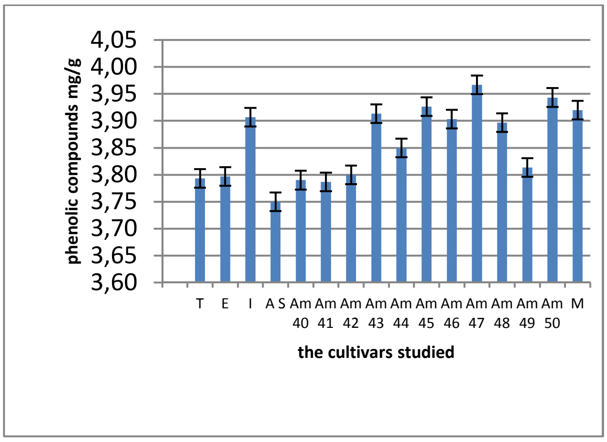

Fig.3. phenolic compounds (mg gallic acid/g dry matter) of the different cultivars.

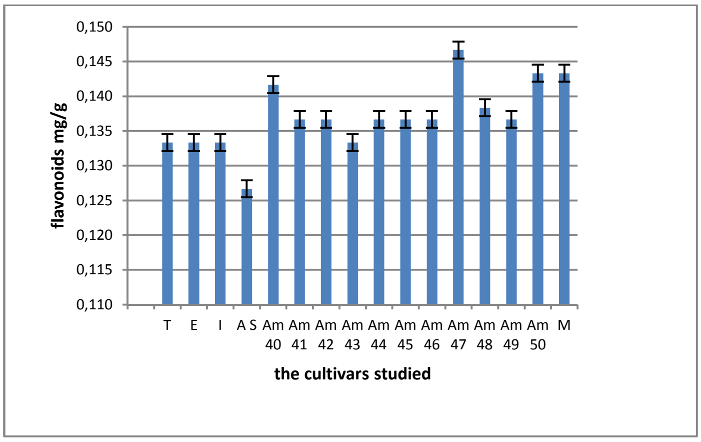

Fig.4. Flavonoids compounds (mg quercetin/g dry matter) of the different cultivars

As for the flavonols, the values of the compound were ranging between 0.412 mg quercetin acid/g dry matter for the American 50, Morocco cultivars and 0.4 mg/g for the egyptian cultivar (Fig 4). Growing and processing may influence the concentrations of flavononols in foods, which are produced in direct response to environmental conditions such as carbon dioxide levels and ultraviolet light. This means there are differences in flavonoid concentrations in similar foods depending on the region and time of year in which they are grown. In this study, great correlations existed between total phenolic contents and antioxidant activity for each cultivars in all the extracts studied with $p<0.05$, the coefficient of correlation was 0.8983, which is may be related to the chemistry behind these methods based on the same redox properties, even with the difference of environment and agricultural techniques in the differents areas.

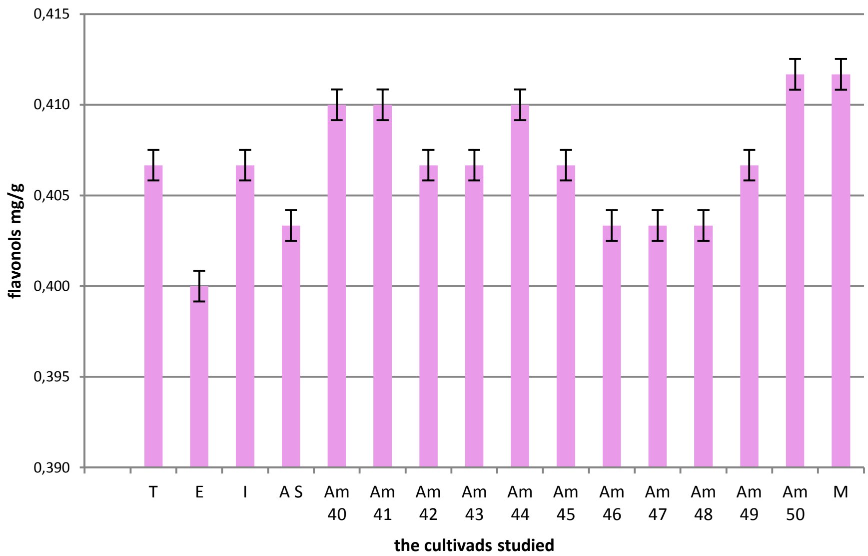

Fig .5. flavonols compounds (mg quercetin per / g dry matter) of the different cultivars

4 CONCLUSION

This study researched the contents of bioactive compounds and the antioxidants activities of 16 cultivars from different areas in the world. A large variability in these contents was observed among the cultivars which allow us to create a differentiation between the species, still, in all the extracts, we could say that sesame extracts has stabilization efficiency comparable to other seeds and also the synthetic antioxidant, the phenolic compounds appear to be responsible for the antioxidant activity of sesame seeds, those seeds could be part of the nutritional for human. The seeds of sesame could be considered as an excellent source of natural bioactive and may be used as a functional ingredient.

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