الجمـــهوريـــة الجـــزائريـــة الديـمقراطيـــــــة الشـــعبيـــــة

THE PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

وزارة التعـلـيـم الـعـالـــي والـبـحث الـعـلـمي

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

جـامـعـة عمّـــار ثـلـيـــجـــي بالأغـــواط

AMAR TELIDJI UNIVERSITY OF LAGHOUAT

كـلـيـــــة الـعـلـــــوم

FACULTY OF SCIENCES

قسم علوم المادة

DEPARTMENT OF MATERIAL SCIENCES

Master's memory

Field: Material Sciences

Major: Chemistry

Option: Organic Chemistry

By: Ms. ZIDANE Khadidja

THEME

The variability of morphological characters, chemical composition of oil and the antioxidant activity of the lipid from the seeds of sorghum (*Sorghum bicolor* L. Moench) from the south of Algeria.

Defended publicly before a jury composed of:

Mr. Benalia Mohamed	**MCA**	**President**
Mr. Belhadi Badreddine	**MCA**	**Examiner**
Mrs. Hadbaoui Zineb	**MCB**	**Rapporteur**

2024/2025

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Dedication

Praise be to God, first and last, outwardly and inwardly.

Praise be to God, who taught with the pen, taught man what he did not know.

Praise be to God for guidance and success, and for blessings too numerous to count.

To my dear mother, who has always been and continues to be our protective shield, especially after the death of my father, may God have mercy on him. This accomplishment is a reflection of her sacrifices, her prayers, and her belief in me.

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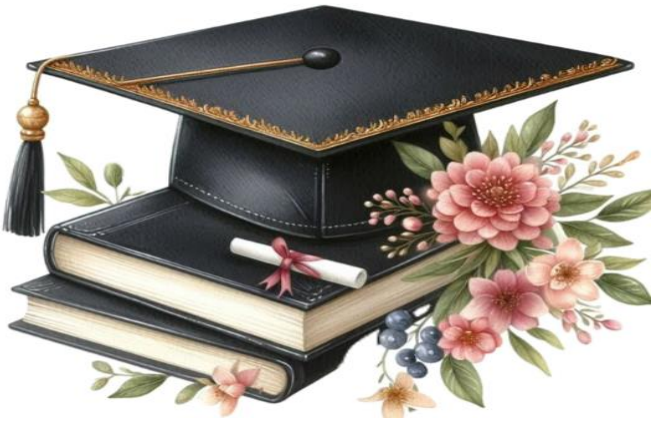

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Abbreviations	The name
SB	White sorghum
SR	Red sorghum
FRAP	Ferric Reducing Antioxidant Power
DPPH	1,1-diphenyl-2-picrylhydrazyl
EMAG	Fatty Acid Methyl Esters
TPTZ	2,4,6-tripyridyls-Triazine
PH	Potential of Hydrogen
AAEC	Ascorbic Acid Equivalents Concentration
PI	Pourcentage d'Inhibition
SD	Standard Deviations
TE	Tocopherol
R^2	Coefficient correlation
PUFA	The polyunsaturated fatty acids

Introduction

I. Introduction

Sorghum (*Sorghum bicolor* L. Moench) is the fifth most important cereal crop globally, following maize, rice, wheat, and barley. (Hayden et al, 2021) Originally domesticated in northeastern Africa, it is now extensively cultivated in semi-arid and arid tropical regions across Africa, Asia, and the Americas, due to its remarkable tolerance to drought, high temperatures, and low-fertility soils. These adaptive traits make sorghum a critical crop for ensuring food and nutritional security in areas increasingly affected by climate change and water scarcity. **(Anna Przybylska et al 2024; Taylor et al., 2006)**

In Algeria, sorghum is grown primarily in the steppe and southern regions, such as Biskra, El Oued, Ghardaïa, Tamanrasset, Adrar, and In Saleh, where harsh climatic conditions limit the cultivation of other cereals. Research has highlighted sorghum's agronomic potential under local environmental constraints, supporting its inclusion in sustainable cropping systems. Yet beyond its role in animal feed and traditional food products like couscous and porridge, the grain remains underutilized regarding its broader nutritional and industrial potential. **(Hadbaoui et al., 2010; Zine El Abidine Fellahin et al., 2016)**

Sorghum is a cane-like grass with stout and erect stems (culms), 0.5 - 6 m tall. Most types used in grain production have a terminal compact or semi-compact head (Figure 01). Cultivated sorghum is generally treated as an annual crop, but may be maintained over several seasons under suitable conditions and has been described as annual or weakly perennial. **(Raj Bhula, 2018)**

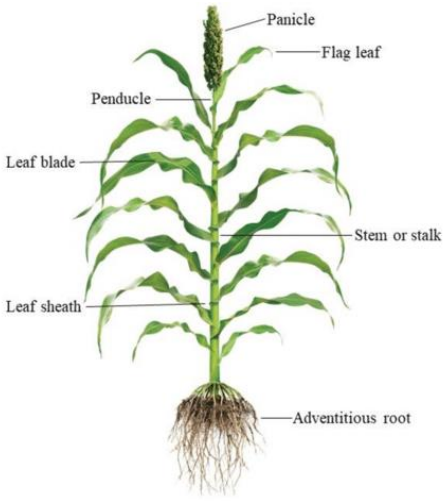


Figure 01: Diagram of a sorghum plant with a single main stem.

Sorghum grains can be variable in shape, size, and color. The seed is generally spherical, but may be flattened on one side. Sorghum grain contains the embryo, the endosperm (starch-rich core), and the testa and is surrounded by the pericarp (Figure 02) **(Dayanandan, 2021)**. The

testa and pericarp form the seed coat. Seed colors range from white and cream to brown, red, purple, and black, depending on the color of the pericarp and testa. Seed size varies from 1-6 g per 100 seeds **(Spenceley et al. 2005; Pacific Seeds 2008; Raj Bhula, 2018)**

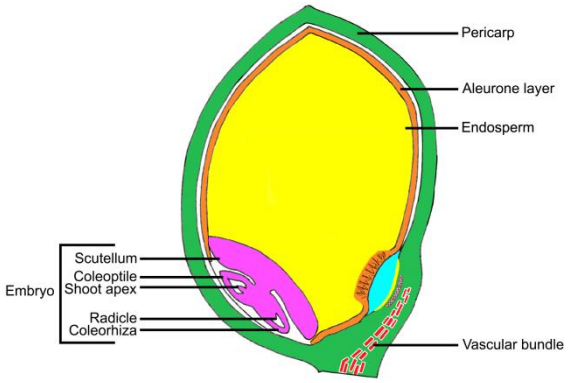


Figure 02: Sorghum Grain Structure.

Sorghum (Sorghum bicolor L. Moench) is classified into several types based on grain color, primarily white, red, brown, yellow, and black, each with distinct chemical and nutritional characteristics. (Figure 03) **(Sureshkumar et al., 2024).**

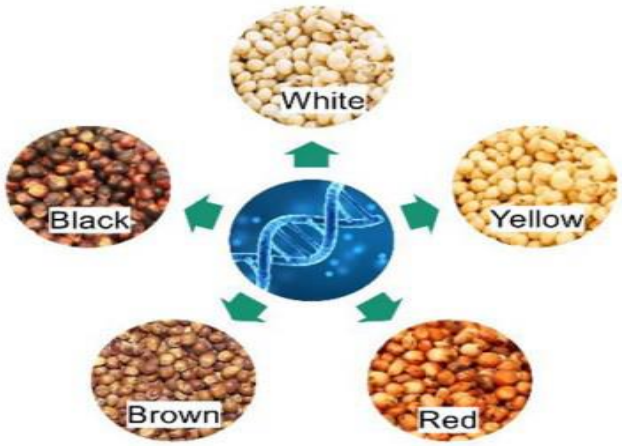


Figure 03: Sorghum types based on grain color.

White sorghum, which is low in tannins and has a lipid content of ~2.8–3.4%, is widely used in human food due to its mild flavor and light color. Red and brown sorghums contain higher levels of condensed tannins and polyphenols, making them more suitable for animal feed and brewing, though they have lower lipid contents (~2.0–3.2%). Yellow sorghum is notable for its carotenoid content, offering provitamin A activity, while black sorghum is rich in anthocyanins, giving it strong antioxidant potential. These differences in pigment and

phytochemical profiles affect the grains' applications in food, feed, and functional products. **(Espitia-Hernández et al..,2022)** (Table 01).

Main (Most Common) Color of Sorghum: Globally, the most commonly cultivated and used sorghum is white sorghum, especially for human consumption because of its neutral flavor, light color, and low tannin content. Red sorghum is also widely grown, especially in Africa and Asia, for feed, brewing, and industrial uses**. (Mohammad Zarei et al.,2022)**

Table 01: Comparison of Sorghum Grain Types by Color

Colors	Tannin Content	Lipid Content (% dry weight)	Common Uses	Notes
White	Low	~2.8–3.4%	Food (flour, porridge, bakery)	Preferred for human food; low polyphenols
Red	Medium–High	~2.6–3.2%	Feed, brewing, some food	Rich in polyphenols and antioxidants
Brown	Very High	~2.0–2.8%	Brewing, feed	High in condensed tannins; bitter taste
Yellow	Low	~3.0–3.6%	Food, feed	Contains carotenoids (provitamin A activity)
Black	Variable	~3.0–3.5%	Functional/health foods	Rich in anthocyanins (strong antioxidants)

Starch is the main component of sorghum grain (60–75 g/100 g), followed by proteins (7–15 g/100 g). Sorghum contains various phytochemicals, including phenolic compounds, fatty acids, sterols, and policosanols (fatty alcohols). Phenols help in the natural defense of plants against pests and diseases, while the plant sterols and fatty alcohols are mostly components of wax and plant oil. The fatty acids contribute to human physiology in different ways. **(Jingwen, 2020; Fred Kwame, 2020; Awika & Rooney, 2004).** (Table 02)

Table 02: Nutrient composition of Sorghum

Component	Content (Range)	Remarks	Reference
Carbohydrates	70–80%	Mainly starch; the amylose and amylopectin ratio influences gelatinization and digestibility	Taylor et al., 2006
Proteins	8–18%	Dominated by kafirins (prolamins); low digestibility due to disulfide cross-linking	Awika& Rooney, 2004
Lipids	2–4%	Triacylglycerols, phospholipids, and free fatty acids, mainly in the germ and pericarp	Rooney &Waniska, 2000
DietaryFiber	6–19%	High insoluble fiber (cellulose, hemicellulose, lignin); promotes gastrointestinal health	Dykes & Rooney, 2006
Ash (Total Minerals)	1–2%	Represents total inorganic residue; depends on genotype and soil	Călinoiu et al., 2021
Moisture	8–13%	Influences storage stability	Codex Alimentarius, 1995

Although lipids constitute only 2–4% of the grain's weight, they are mainly concentrated in the germ and outer layers parts typically removed during processing and discarded as by-products **(Liu et al., 2011).** These lipids are composed predominantly of unsaturated fatty acids (Table 02) and bioactive molecules such as tocopherols (chromanol-based vitamin E compounds) and phytosterols, including β-sitosterol and campesterol, plant-derived sterols structurally similar to cholesterol. These compounds are of particular interest in organic

chemistry due to their diverse structural features and well-documented antioxidant, cholesterol-lowering, and anti-inflammatory properties **(Ingh et al.2024; Moreau et al., 2002; Shahidi et al, 2015)**.

The oxidative stability of sorghum oil is further enhanced by its natural antioxidants, making it a promising candidate for functional food, cosmetic, and pharmaceutical applications.

Despite this potential, limited research has been conducted on the lipid composition of Algerian sorghum varieties. Few studies have investigated the extraction and functional characterization of these lipid fractions, leaving a significant gap in understanding their chemical and biological properties.

In light of Algeria's growing emphasis on sustainable agriculture and bioresource valorization, studying the bioactive lipid content of local sorghum cultivars presents a timely opportunity for innovation and value-added development.

Table 03: Fatty acid compositions of the lipid fractions obtained from the seeds of sorghum.

Fatty Acid	Chemical Formula	Content (% of total fatty acids)	Type	Function/Remarks	Reference
Linoleic acid (C18:2)	$C_{18}H_{32}O_2$	42–52%	Polyunsaturated (PUFA)	Essential fatty acid; contributes to membrane fluidity; precursor to eicosanoids	Taylor et al., 2006
Oleic acid (C18:1)	$C_{18}H_{34}O_2$	30–45%	Monounsaturated (MUFA)	Enhances the oxidative stability of oil; cardio-protective properties	Dykes & Rooney, 2006
Palmitic acid (C16:0)	$C_{16}H_{32}O_2$	10–20%	Saturated (SFA)	Common in plant oils; contributes to energy storage	Rooney &Waniska, 2000
Stearic acid (C18:0)	$C_{18}H_{36}O_2$	1–3%	Saturated (SFA)	Less atherogenic than other saturated fats	Codex Alimentarius
Linolenic acid (C18:3)	$C_{18}H_{30}O_2$	<1%	Polyunsaturated (PUFA)	Omega-3 fatty acid; sensitive to oxidation	Taylor et al., 2006
Myristic acid (C14:0)	$C_{14}H_{28}O_2$	Trace amounts (<0.5%)	Saturated (SFA)	Minor component	Awika& Rooney, 2004

The principal aim of this research is to characterize morphological variability among sorghum landraces and to examine the lipid fraction, with a focus on evaluating their biological functionality. Specifically, the study seeks to extract total lipids from selected Algerian sorghum cultivars; quantify tocopherols and sterols, and assess the antioxidant capacity of the extracted lipids using two widely recognized assays: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the ferric reducing antioxidant power (FRAP) assay. These evaluations aim to determine the nutraceutical potential of sorghum lipids and contribute to the broader effort of valorizing underutilized crop components within sustainable, health-oriented agro-industrial systems in Algeria.

Materials and Methods

II. Materials and methods

1. Materials :

Plant material:

Nine landraces of sorghum have been collected from seed farmers in In Saleh, south of Algeria. The study was conducted on white and red seeds of sorghum varieties collected during the 2020,2021, 2022, and 2023 seasons.

The names and abbreviations of sorghum landraces used in this experiment are summarized in the table 04 below:

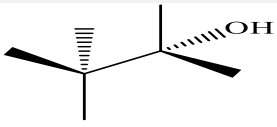
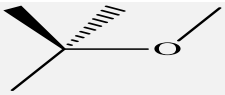
Table 04: The names and abbreviations of sorghum landraces used.

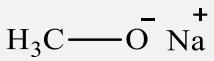
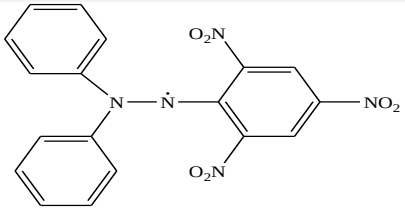
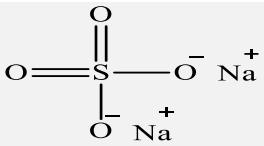
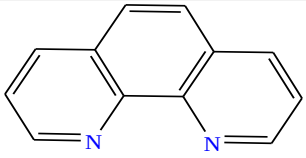
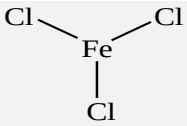
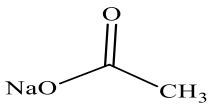
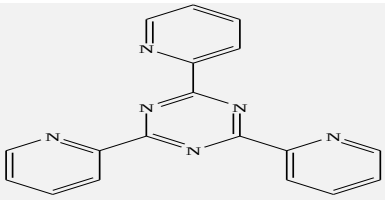
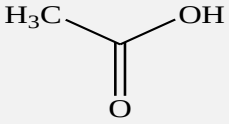
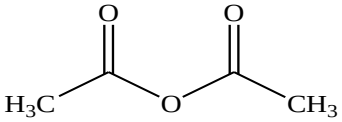
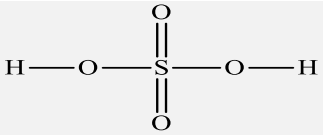
Abbreviations	The name
SB 01	White sorghum -Nadjmi Saleh-(2022)
SB 02	White sorghum -Salamat Abdullah (2020)
SB 03	White sorghum -Barkat-(2023)
SB 04	White sorghum -Babi-(2020)
SR 01	Red sorghum -Nadjmi Saleh-(2022)
SR 02	Red sorghum -Salamat Abdullah (2020)
SR 03	Red sorghum -Salamat Abdullah (2020)
SR 04	Red sorghum -Barkat-(2023)
SR 05	Red sorghum -Babi- (2020)

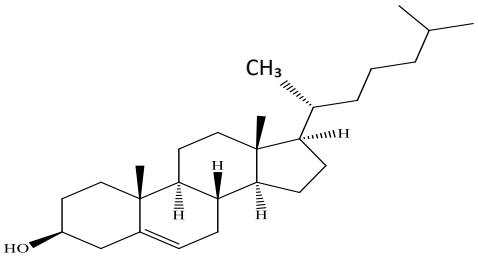
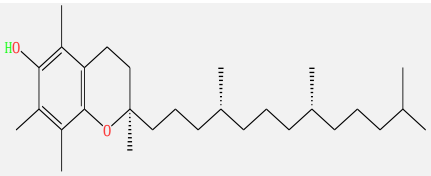
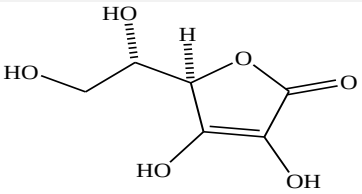
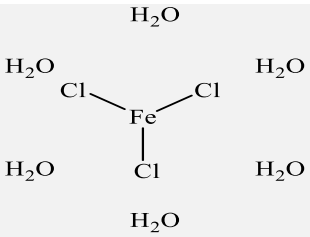
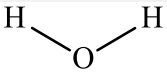
Chemicals and reagents:

Materials, chemicals, and reagents used in this work are summarized in Table 05 **(structures given by the Material Studio app).**

Table 05: The materials, chemicals, and reagents

Materials	Chemicals	Strectures	Product source
Glassware Rotavaporator Balance (Pioneer (DHAUS)), UV-visible spectrophotometry	Hexane (C_6H_{14})		Sigma Aldrich
	Ethanol (C_2H_5OH)		Sigma Aldrich
	Methanol (CH_3OH)		Sigma Aldrich

(SP-3000nano (OPTIMA)), Soxhlet Vernier caliper	Butanol ($C_4H_{10}O$)		Sigma Aldrich
	Sodium Methylate (CH_3ONa)		
	1,1-diphenyl-2-picrylhydrazyl (DPPH)		Sigma Aldrich
	Anhydrous Sodium Sulphate (Na_2SO_4)		AnalaR Norma purprolab
	1,10-phenanthroline ($C_{12}H_8N_2$)		Sigma Aldrich
	Iron III Chloride ($FeCl_3$)		Sigma Aldrich
	Sodium Acetate (CH_3COONa)		Sigma Aldrich
	, 2,4,6-tripyridyls-Triazine (Tptz)		Sigma Aldrich
	Hydrochloric Acid - HCl- (37%)		Sigma Aldrich
	Acetic Acid (CH_3COOH)		Sigma Aldrich
	Acetic Anhydride ($(CH_3CO)_2O$)		Sigma Aldrich
	Sulfuric Acid (H_2SO_4)		Sigma Aldrich

Cholesterol		Sigma Aldrich
Commercial Vitamin E		AnalaR Norma purprolab
Vitamin C		AnalaR Norma purprolab
Iron III Chloride Hexahydrate ($FeCl_3.6H_2O$)		Sigma Aldrich
distilled water		

All chemicals and reagents used were of analytical grade.

2. Methods:

a) Morphological characterization:

Morphological characterization of 09 landraces of sorghum (Figure 04) was undertaken for quantitative characters like panicle length, panicle width, length and diameter of penducle , and the weight of the panicle was measured and recorded.

Grain Isolation and Physical Characterization. After harvesting, the panicles were manually threshed to obtain clean grains. A sample of 100 grains was weighed, and the length, width, and height of individual grains were measured using a vernier caliper. Additionally, the weight of a 10 mL volume of grains was measured to assess density.

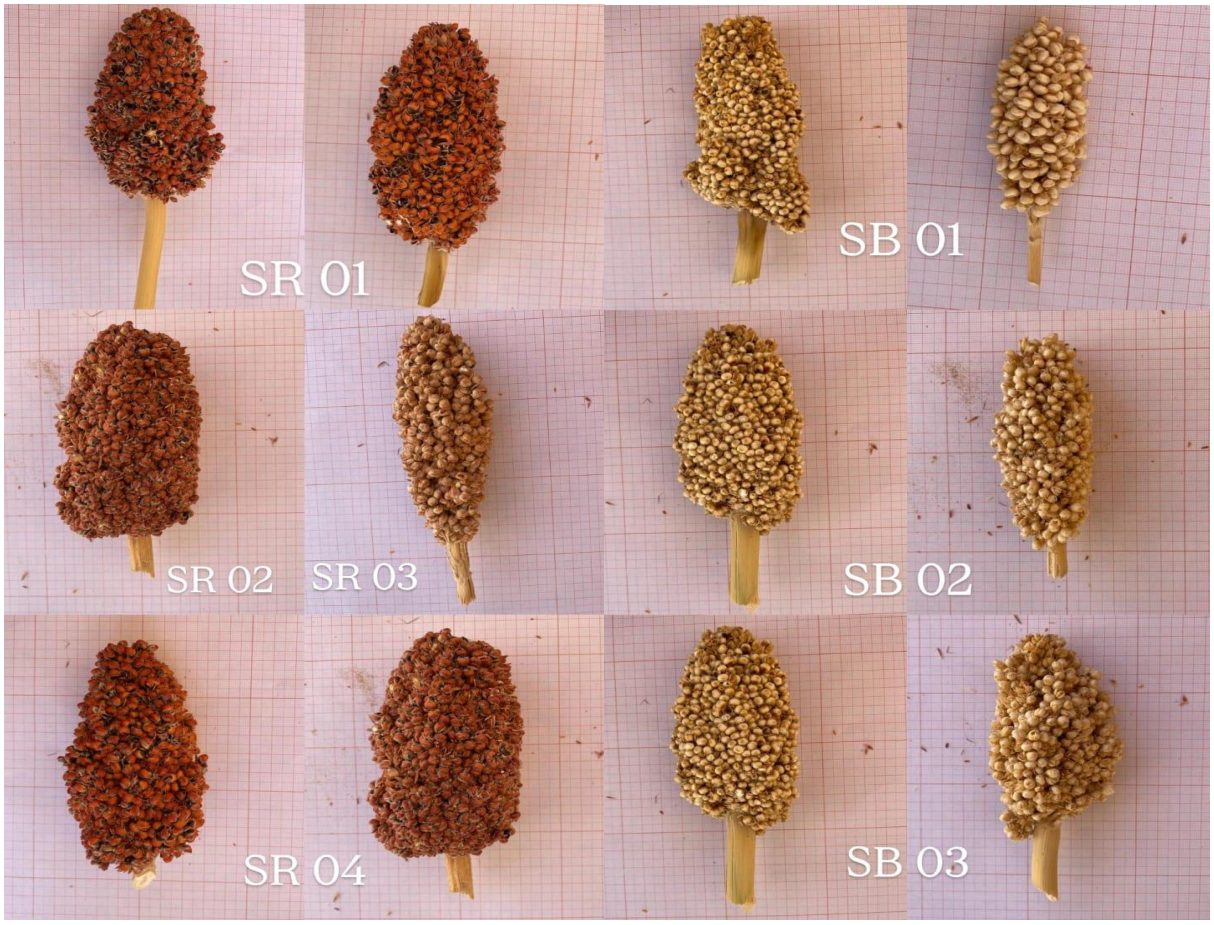


Figure 04: Samples of sorghum.

b) Lipid compounds extraction:

1. Grinding and Sieving:

The seed samples of Sorghum varieties were analyzed for their total oil contents, which were further fractionated into saturated and unsaturated fatty acids, α-tocopherol, and sterol content. We also evaluated its possible antioxidant activity.

The collected grains were ground using a specialized mill at the Génie Civil Laboratory. The ground material was then sieved to obtain a uniform particle size suitable for solvent extraction. We grind the sorghum mixture for five minutes three times, and then let it rest to prevent overheating the grinder and damaging the sample. Once we get a fine powder, we sift it to obtain samples of consistent sizes. (Figure 05)

Figure 05: The grinding and Sieving steps

2. Oil Extraction:

The fine powder seeds of sorghum (100 g) were extracted with 300 mL n-hexane using a Soxhlet extraction apparatus for 8 hours. Samples were then dehydrated with anhydrous sodium sulfate and filtered. The solvent was evaporated under reduced pressure using a rotavaporator at 60°C. The dried crude oils were stored until use.

Yield in lipid extraction refers to the percentage of oil (lipids) recovered from a raw material (sorghum) relative to the initial mass of that material. It provides comparative insight into which samples are richer in oil.

Mathematical Definition

:

$$Yield\ (\%) = (\frac{Mass\ of\ \ oil\ extracted(g)}{Mass\ of\ sample\ used\ (g)}) \times 100$$

b.3. Fatty acid composition:

The fatty acid composition of the oils was determined after converting the fatty acids into fatty acid methyl esters (FAMEs), which were then analyzed by gas chromatography (GC). The methyl esters were prepared by the following procedure: Lipids (0.4 g) were refluxed for 20 min in 10 mL of 0.5% sodium methylate (NaOMe), and then 20 mL of water was added. The

fatty acid methyl esters (FAMEs) were extracted with n-hexane and washed with distilled water. The combined extracts were dried over anhydrous sodium sulfate (Na_2SO_4) and then evaporated under vacuum before being analyzed by capillary gas chromatography.

b.4. Dosage of α-tocopherol (vit E):

The total tocopherol content of the lipids was determined by the spectrometric method described in reference **(Harrath et al, 2018)** with a few modifications. This method is based on the redox reaction between tocopherols and ferric iron (Fe^{3+}), which is reduced to ferrous iron (Fe^{2+}). The latter, in the presence of specific reagents such as ortho-phenanthroline (1,10-phenanthroline), forms a stable red-orange complex with a very high molar extinction coefficient at 510 nm. From the standard solution of α-tocopherol in hexane, we prepared aliquot solutions with different concentrations. To 1 mL of each solution was added 1 mL of 1,10-phenanthroline reagent (0.4% in ethanol), then 0.5 mL of ferric chloride reagent (0.12% in ethanol) was added, and the mixture was shaken for 5 min. The absorbance of the mixture was read at 510 nm. A blank was run, using 1 mL of hexane, 1 mL of 1,10-phenantroline reagent, and 0.5 mL of ferric chloride reagent. The above-described procedure was followed by using sample solutions of lipids. The total tocopherols in the lipids were calculated from the regression equation of the standard curve. The results were expressed as mg α-tocopherol equivalent in g of oil.

Processes that Occur During the Dosage of α-Tocopherol (Colorimetric Method):

The colorimetric dosage of α-tocopherol (Vitamin E) using $FeCl_3$ (ferric chloride) and 1,10-phenanthroline is based on the reduction of Fe^{3+} to Fe^{2+} by α-tocopherol, followed by complexation of Fe^{2+} with 1,10-phenanthroline to form a red-orange colored complex that can be measured spectrophotometrically. (Figure 06)**(Panikumar Du 2020)**

✓ Oxidation of α-Tocopherol:

α-Tocopherol acts as a reducing agent. It reduces Fe^{3+} (from $FeCl_3$) to Fe^{2+}:

$$\mathbf{Fe^{3+} + (\alpha\text{-tocopherol})1e^- \rightarrow Fe^{2+}}$$

✓ Formation of Fe^{2+}–Phenanthroline Complex:

The Fe^{2+} ions formed react with 1,10-phenanthroline, a chelating agent, forming a colored complex:

$$\mathbf{Fe^{2+} + 3\ (1,10\text{-phenanthroline}) \rightarrow [Fe(phen)_3]^{2+}}$$

This complex has an intense red-orange color and shows a maximum absorbance at 510 nm

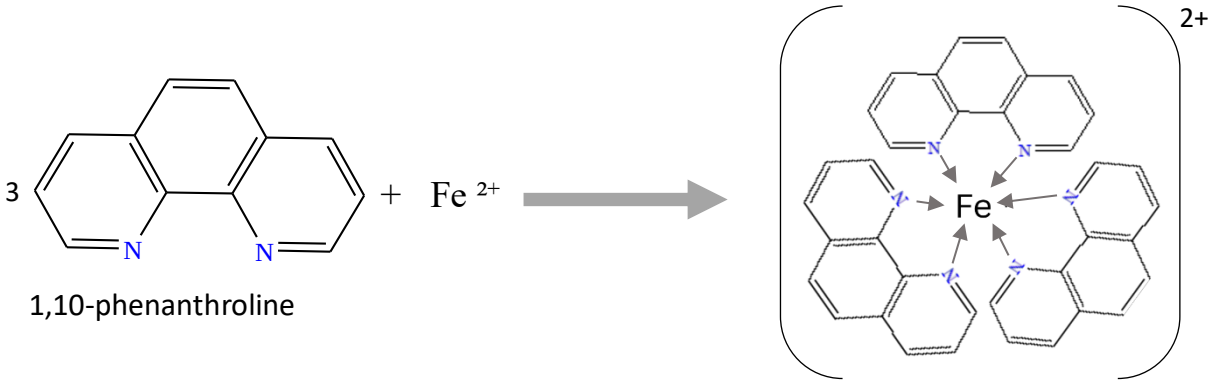


Figure 06: The processes that occur during a colorimetric determination of α-tocopherol

5. Dosage of sterol:

This method is based on spectrophotometric absorption according to the Liebermann-Burchard test **(Harrath et al, (2018))**, based on a specific-colored reaction of the 3-hydroxysteroids having a double bond in position 5-6. The sterols form a stable complex with the acetic anhydride in an acid medium, which absorbs in the visible at a wavelength of 550 nm (the Liebermann spectral reagent consists of 60 mL of acetic anhydride and 10 mL of concentrated sulfuric acid, and 30 mL of acetic acid). From a hexane solution of cholesterol with a concentration of 1 g/L, we have prepared a series of solution dilutions in order to plot a calibration curve linking the optical density to the concentration. 1 mL of each diluted solution was mixed with 2 mL of the Liebermann reagent, then kept for 25 min in order to allow the reagent color to fully develop and stabilize. The above-described procedure was followed by using sample solutions of lipids. The total sterols in the sorghum oil were calculated from the regression equation of the standard curve. The results were expressed as mg cholesterol equivalent in g of oil.

Processes That Occur During the Dosage of Cholesterol (Liebermann–Burchard Method)

The principle of the method is based on the formation of a colored complex through a series of chemical reactions(Figure 07) **(Uvindu Thilanka, 2020).**

Step 01: Cholesterol, when dissolved in acetic anhydride and treated with concentrated sulfuric acid, undergoes oxidation and rearrangement to generate highly conjugated structures.

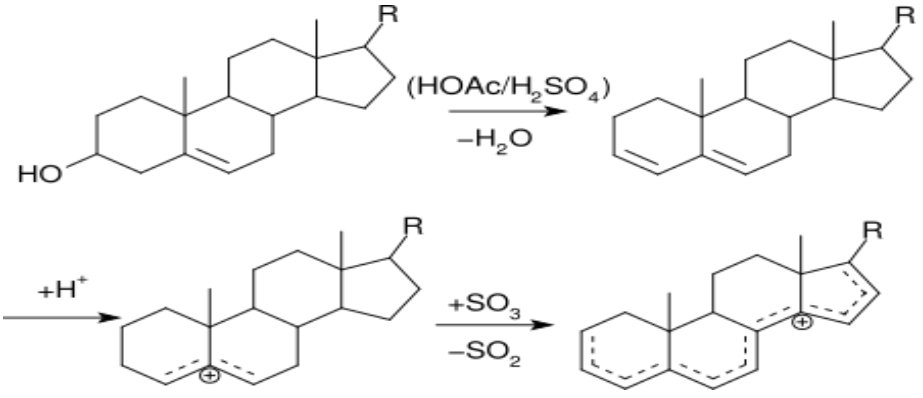


Figure 07: Processes That Occur During the Dosage of Cholesterol (Liebermann–Burchard Method)

Step 02: These newly formed chromophores interact with the reaction medium, resulting in the development of a blue-green complex whose intensity is directly proportional to the concentration of cholesterol present in the sample. The absorbance of this complex is typically measured at a wavelength of 550 nm using a spectrophotometer

Equation Representation (Simplified Form):

Cholesterol + Acetic Anhydride + H_2SO_4 → Green Chromophore (absorbs at 550 nm)

This method, while simple and widely used, must be performed under controlled conditions (temperature, light, and reaction time) to ensure reproducibility and accuracy. Despite its limited specificity, since other sterols may also react, it remains a valuable tool in lipid analysis due to its sensitivity and ease of implementation.

c) Antioxidant activity:

1) Test DPPH:

The antioxidant activity of crude oil extracts was evaluated using DPPH free radical scavenging modified method described in reference (**Chalghoum et al., 2021**). 1 mL of DPPH solution prepared in methanol (0.0039 g of DPPH in 100 mL of methanol) was mixed with 500 µL of sample solutions diluted in butanol at different concentrations, then incubated for 30 min in obscurity and at room temperature, absorbances were measured at 517 nm against a blank, a control containing butanol and DPPH solution was also realized. The lipid extracts were expressed as mg equivant Vitamin C per g oil (mg Vit C / g oil). The radical scavenging capacity using the free DPPH radical was calculated from the following equation:

$$AAEC/weight.oil = \frac{\frac{A_\circ - A}{A_\circ} \times 100 \times \frac{\mathrm{nd}}{Slope} \times \frac{V}{1000}}{m}$$

With:

AAEC/weight.oil: ascorbic acid equivalents concentration per weight of oil.

A: absorbance value of the tested sample

$A_{\circ}$: absorbance value of control (DPPH)

n_d: dilution factor

Slope: slope of the ascorbic acid curve

V: solvent volume

m: weight of oil

✓ The more antioxidants present, → the more DPPH˙ is reduced, → the greater the decrease in absorbance.

This data is used to build a calibration curve using ascorbic acid as a standard

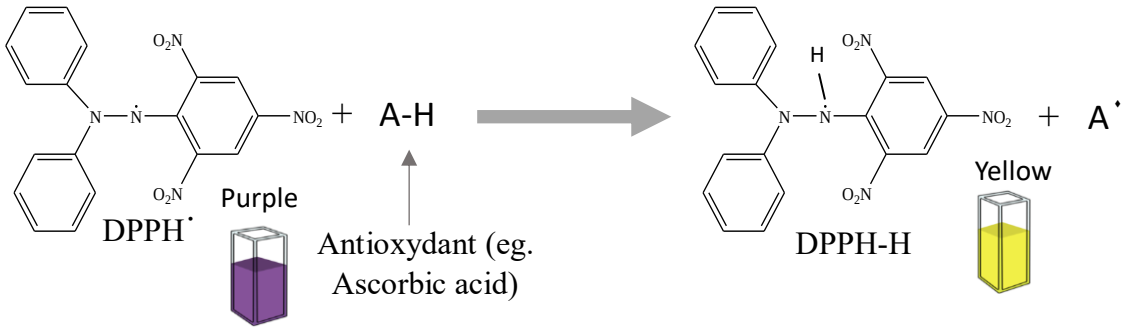
Mechanism of DPPH test with Ascorbic Acid (Vitamin C):

Ascorbic acid ($C_6H_8O_6$) is a water-soluble antioxidant that easily donates hydrogen atoms due to its enediol structure on the lactone ring. To explain this, we suggest this Step-by-step Mechanism(Figure08)

1. **Hydrogen Atom Transfer (HAT)**:

Ascorbic acid donates a hydrogen atom (H˙) from its enediol group to the DPPH radical.

DPPH˙ + Ascorbic Acid ($C_6H_8O_6$) → DPPH-H + Semidehydroascorbate ($C_6H_7O_6$˙)



2. **Further oxidation**: The semi dehydroascorbate radical is relatively stable and can disproportionate (two radicals reacting together) to form dehydroascorbic acid and ascorbic acid:

$$2C_6H_7O_6^{\cdot} \rightarrow C_6H_8O_6 + C_6H_6O_6$$

Figure 08: Processes that Occur During the Dosage of DPPH

2) Test FRAP:

The antioxidant capacity was also measured with the colorimetric method of FRAP assay, which was based on the reduction of tripyridyltriazine (Fe^{+3}-TPTZ) complex to the ferrous form (Fe^{+2}), at low pH. The procedures of the FRAP assay were done according to Fanali et al. (**I.F.F. Benzie, 1996**) with the following modifications. The fresh working solution was prepared by mixing 25 mL of acetate buffer 300 mM (pH 3.6), 2.5 mL of 2,4,6-tripyridyl-s-triazine TPTZ solution (10 mM), and 2.5 mL of iron III chloride hexahydrate ($FeCl_3.6H_2O$) solution (20 mM). Fifty microliters of the sample diluted in ethanol were allowed to react with 1 mL of the FRAP solution for 10 min in the dark at room temperature. The ferrous tripyridyltriazine complex developed an intense blue color measured at 593 nm against a blank. The reducing antioxidant activity was expressed relative to the value obtained with vitamin C from the calibration curve and then expressed as mg equivalent vitamin C per g oil (AAEC).

Mechanism of FRAP assay:

The main reaction with antioxidants in the first step was the formation of the Fe^{3+}-TPTZ complex (Figure 09) **(Benzie & Strain, 2020)**. In the reactive solution, Fe^{3+} forms a complex with TPTZ, which is colorless or faintly colored:

$$\mathbf{Fe^{3+} + TPTZ \rightarrow [Fe(TPTZ)]^{3+}}$$

Secondly, Reduction by an antioxidant (e.g., vitamin C). It reduces the ferric complex to the intensely blue ferrous complex.

The product $[Fe^{2+}(TPTZ)]$ is responsible for the maximum absorbance at 593 nm.

$$\mathbf{[Fe(TPTZ)]^{3+} + (C_6H_8O_6)\ 1e^- \rightarrow [Fe(TPTZ)]^{2+}}$$

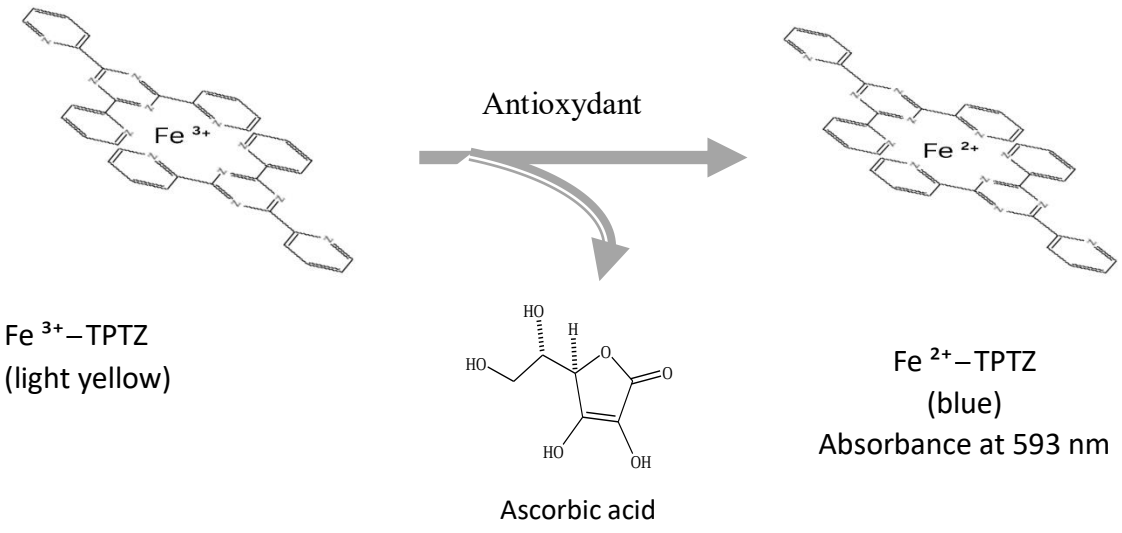


Figure 09: Processes That Occur During the Dosage of FRAP

AAEC of FRAP was calculated according to the following equation:

$$\text{AAEC/weight.oil} = \frac{\frac{A}{A_{\circ}} \times 100 \times \frac{n_d}{Slope} \times \frac{V}{1000}}{m}$$

With:

AAEC/weight.oil: ascorbic acid equivalents concentration per weight of oil.

A: absorbance value of the tested sample

$A_{\circ}$: absorbance value of control (FRAP)

n_d: dilution factor

Slope: slope of the ascorbic acid curve

V: solvent volume

m: weight of oil

3) Statistical analysis

All analyses of phytochemical and antioxidant activity were performed in triplicate, and the data were reported as means ± standard deviations (SD).

Results and discussion

III. Results and discussion:

1. Morphological Traits:

Quantitative traits: The mean values of quantitative characters of sorghum landraces are given in Table 6. The results of the present study showed that panicle weight was the most varied trait, giving a very less degree of uniformity (51.75%), followed by panicle branch length (23.18%) and panicle branch width (22.46%). Among the studied traits, seed density showed the lowest variation (3.98 %). The yield contributing characters like panicle length, panicle branch length, and 100 grain weight were evaluated. An average 100 grain weight was 3.48 g and ranged between 2.94 g (SR02) to 3.86 g (SB01). 12.79 cm and 10.06 cm were the average panicle length and panicle branch length, respectively.

Table 06: Mean values of quantitative characters of sorghum landraces observed during 2020,2021,2022, and 2023.

Verities	Panicle		Panicle	Penducle	Panicle	Weight	Test	Grain measurements		
	Length (cm)	Width (cm)	Length (cm)	diameter (mm)	Weight (g)	100 grains (g)	weight (g/L)	Length (mm)	Width (mm)	Height (mm)
SB 01	7.25	3.51	9.79	0.39	12.45	3.863	684,80	0.51	0.438	0.259
SB 02	9.55	5.31	11.88	0.76	28.03	3.023	639,03	0.46	0.360	0.282
SB 03	9.83	5.88	14.27	1.21	60.20	3.718	667,60	0.48	0.421	0.275
SB04	ND	ND	ND	ND	ND	4.140	ND	ND	ND	ND
SR 01	8.62	3.84	12.29	0.55	27.71	3.779	714,17	0.54	0.454	0.293
SR 02	10.32	6.88	12.88	1.06	86.87	2.944	704,29	0.46	0.429	0.249
SR 03	14.78	5.52	16.1	0.79	42.15	3.440	687,99	0.43	0.480	0.281
SR 04	10.07	5.55	12.36	0.87	56.37	3.580	726,90	0.57	0.439	0.217
SR05	ND	ND	ND	ND	ND	**3.850**	ND	ND	ND	ND
means	**10,06**	**5,21**	**12.79**	**0.80**	**44,83**	**3,480**	**689,00**	**0,49**	**0,430**	**0,260**
standard deviation	**2,33**	**1,17**	**1.98**	**0.26**	**23,2**	**0,350**	**27,00**	**0,04**	**0,034**	**0,024**
degree of uniformity%	**23,18**	**22,46**	**15.45**	**32.5**	**51,75**	**10,075**	**3,98**	**9,56**	**7,92**	**9,04**

The panicle length was found highest in SR03 (16.1 cm) and lowest in SB 01(9.79 cm) while panicle branch length was found highest in SR03 (14.78 cm) and lowest in SB01 (7.25 cm).

Observations also showed the variation in panicle weight (12.45 g to 86.87 g) and panicle branch width (3.51 cm – 6.88cm).

The characteristics of sorghum grain are also given in Table 6. The degrees of uniformity of grain measurements were 7.929.%, 9.04%, and 9.56% width, height, and length, respectively. An average grain length was 0.49cm and ranged between 0.43 cm (SR03) to 0.57 cm (SR04). 0.43 cm and 0.26 cm were the average grain width and grain height, respectively.

Characterization of sorghum landraces during the present evaluation based on 09 agro-morphological traits in sorghum for testing indicated that evaluated genotypes showed variation in the studied traits, particularly for panicle weight, panicle branch length, and panicle branch width. Some landraces of sorghum exhibited desirable characteristics such a greater panicle length (SR03 and SB03), panicle branch length (SR03), and higher 100 grain weight (SR05). These traits are useful in developing trait-specific varieties in Sorghum. Such an unexploited genepool existing found in remote areas like Ain Saleh may be utilized by plant breeders when planning a targeted sorghum breeding program.

2. Extraction yield:

The oils (total lipids) content is summarized in Table 07. The results showed that the yields of extraction obtained from white sorghum were higher than those obtained from red sorghum. The yield values were in the range of 3.77-6.014% w/w for the white sorghum extraction, and were in the range of 1.73-3.77% w/w in red sorghum.

We can attribute the variation in oil yield in sorghum samples to the grain measurements, as the average oil yield in white sorghum (4.64%) is greater than that in red sorghum (2.97%), which is matched by average weight of 100 grains 3.6864g and 3.5217g of white and red sorghum respectively.

The oil's content decreases in the following order: SB04› SB03› SB02 ›SB01= SR05 ›SR02› SR01› SR03 › SR04. (Figure 10)

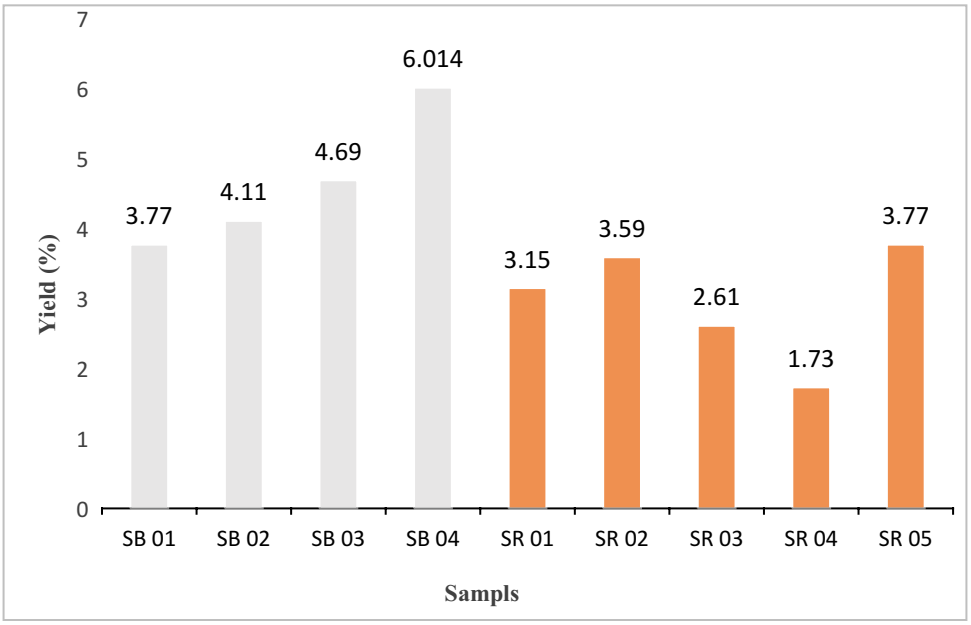


Figure 10: yield of sorghum oil.

The yields of oils extracted from sorghum seeds found here in this investigation are in good agreement with those of Vijay Singh (3.22%), but when we compare our work with that of Hadbaoui (6.3%),2010 and Sajid (6.3%), 2009 oil contents of the sorghum seeds, our results are slightly inferior.

3. Total tocopherol and total sterol contents:

The stability of vegetable oils under the conditions of oxidation is due to the presence of high levels of natural antioxidants, the most important are the tocopherols and sterols. The total tocopherols and sterols contents identified in the studied total lipids are listed in the table 7, which clearly shows that the seed oils are rich in tocopherols. The total tocopherols contents varied significantly from 3.09 ± 0.0015 mg α-tocopherol equivalent per g of lipids (for SB01) to 4.99 ± 0.0012 mg α-tocopherol equivalent per g of lipids (for SB02) with an average of 3.91 ± 0.0008 mg α- tocopherol equivalent per g of lipids While, in red sorghum, the values ranged from 1.175 ± 0.00 mg α--tocopherol equivalent per g of lipids (for SR04) to 4.65 ± 0.00 mg α-tocopherol equivalent per g of lipids (for SR03) with an average of 2.52 ± 0.0002 mg α-tocopherol equivalent per g of g lipids. Among all extracts, the highest amount of tocopherols was recorded for white sorghum, whose an average is 1.5 that of the red sorghum.

Table 07: The total tocopherols and sterols contents in the studied total lipids

Extract	Yield (%)	Tocopherols (mg eq α-Tocopherol. g^{-1} Oil)	sterol (mg eq cholesterol. g−1 Oil)
SB 01	3.77	3.09 ± 1.54×10^{-3}	63.10 ± 3.86×10^{-4}
SB 02	4.11	4.99 ± 1.23×10^{-3}	37.55 ± 1.23×10^{-3}
SB 03	4.69	3.69 ± 0	40.21 ± 1.35×10^{-4}
SB 04	6.01	3.86 ± 4.36×10^{-4}	23.10 ± 4.36×10^{-4}
SR 01	3.15	1.74 ± 3.68×10^{-4}	27.08 ± 0
SR 02	3.59	2.60 ± 4.15×10^{-4}	36.44 ± 0
SR 03	2.61	4.65 ± 0	54.10 ± 0
SR 04	1.73	1.17 ± 0	74.72 ± 3.33×10^{-4}
SR 05	3.77	2.43 ± 4.34×10^{-4}	24.07 ± 0
Average	3.5442	3.14 ± 4.92×10^{-4}	42.26 ± 1.52×10^{-4}

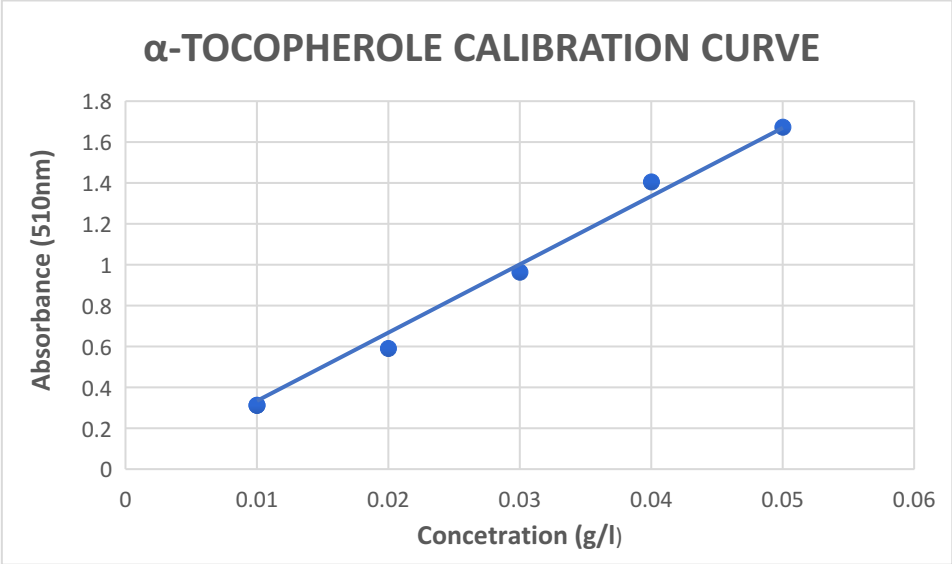


Figure 11: α-Tocopherol calibration curve.

The total contents of sterols were much higher than the content of total tocopherols. The quantification of sterols (cholesterol) in sorghum lipid extracts revealed significant variability across different samples, indicating the influence of varietal and possibly environmental factors on sterol content. The contents of sterols per gram of oil ranged from 23.1 ±0.0004 mg equivalent cholesterol per g of oil in SB 04 to a maximum of 74.72 ±0.0003 mg equivalent cholesterol per g of oil in SR 04, which exhibited the highest cholesterol content among all samples. In contrast, samples such as SB 04 and SR 05 exhibited relatively low sterol

concentrations, suggesting a lower nutritional or industrial value in terms of sterol content. The SR group (especially SR 03 and SR 04) generally demonstrated higher sterol concentrations compared to the SB group, implying that these varieties may be more suitable for functional food or nutraceutical applications targeting sterol enrichment. The measurement uncertainty remained low across the dataset, confirming the reliability and precision of the spectrophotometric method used, despite some extreme values. Overall, this analysis underscores the importance of sorghum genotype selection in optimizing lipid extract composition for targeted health-related applications.

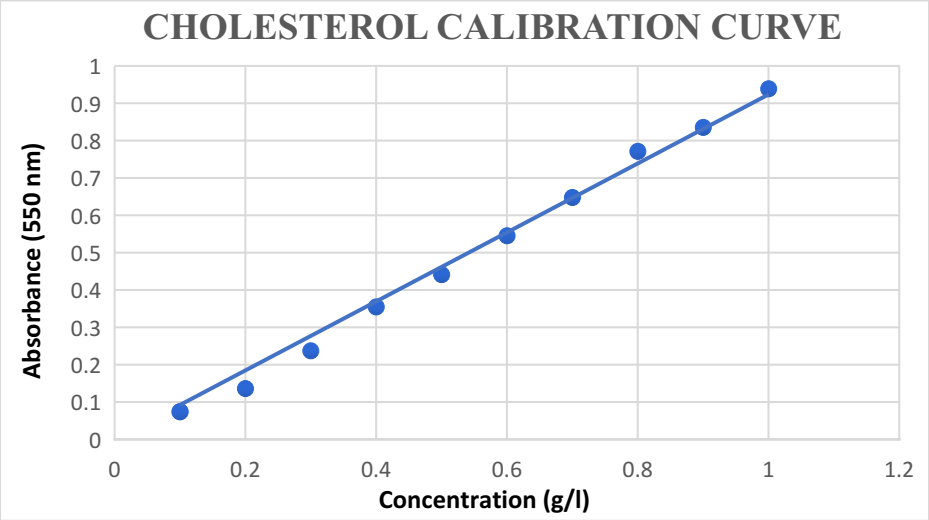


Figure 12: Cholesterol calibration curve

4. Antioxidant Activities:

The antioxidant properties, particularly the neutralization of free radicals, are crucial due to the harmful effects of these radicals in biological systems. The antioxidant activity of sorghum oil was estimated by two antioxidant tests, DPPH assay (free radical scavenging activity) and FRAP (Ferric Reducing Antioxidant Power), which are commonly used in biological samples. The antioxidant activities are shown in Table 9.

The DPPH and FRAP test results for oil lipid extracts from sorghum reveal important insights into the antioxidant behavior of different samples. Both assays measure antioxidant capacity, but via different mechanisms: DPPH evaluates free radical scavenging, while FRAP assesses the reducing power of the samples.

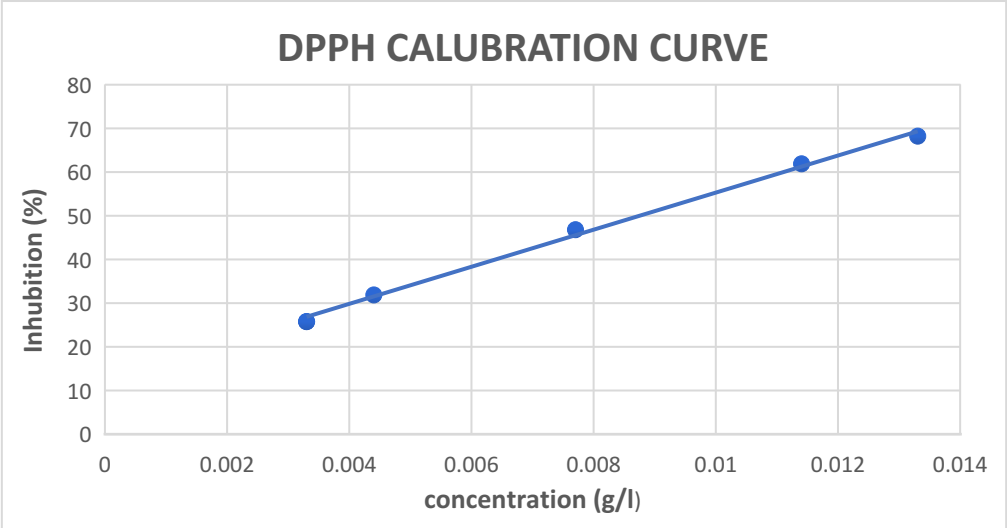


Figure 13: The DPPH calibration curve.

The DPPH˙ radical scavenging is a widely used method to evaluate the capacity of plant extracts to scavenge free radicals generated from DPPH reagent **(Hefeid et al., 2020**). The white sorghum had an average of 0.438 ± 0.033 mg AAEC per g of oil (values between 0.0935 ± 0.058 mg AAEC per g of oil (for SB02) and 1.0007 ± 0.074 mg AAEC per g of oil (for SB01)). On the other hand, the red sorghums had an average of 0.1915 ± 0.0804 mg AAEC per g of oil (values between 0.0425 ± 0.0001 mg AAEC per g of oil (for SR02) and 0.4021 ± 0.040 mg AAEC per g of oil (for SR05)). According to the results, the amount of antioxidants in white sorghum was twice as rich as that of red sorghum.

The different sorghum lipid extracts possess varying degrees of antioxidant activity, with some (like SB01) being more promising candidates for functional food or nutraceutical applications due to their higher DPPH radical scavenging capacity. In the literature, the inhibition of DPPH radical scavenging by plant oils can be attributed to the polyunsaturated fatty acids (PUFA), phospholipids, total phytosterols, total tocopherols, carotenoids, and phenolics.

All the samples gave good values of antioxidant activities compared to **(Hadbaoui et al 2010)** The difference in values of antioxidant activity may be due to several factors that affect the production and stability of antioxidants, such as location, harvest period (although in our case these two factors are not significant), the meteorological conditions and the molecular geometry of the antioxidant compounds. Indeed, some small molecules have a better ability to access the radical site.

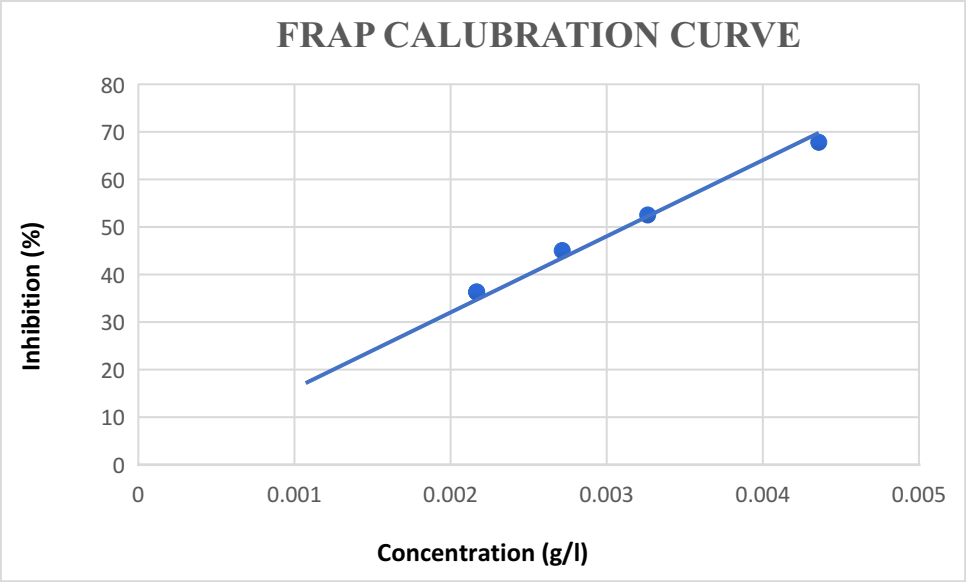


Figure 14: The FRAP calibration curve.

For FRAP, reducing power is generally associated with the presence of reductants in the antioxidant samples. Reductants cause the reduction of Fe^{3+}/Fe^{2+} **(Hefied et al.2020)**. The reducing power in white sorghum varied from 0.6937 ± 0.0091 mg AAEC per g of oil (for SB03) to 0.466 ± 0.012 mg AAEC per g of oil (for SB02), with an average of 0.391 ± 0.033 mg AAEC per g of oil. While in red sorghum, the values ranged from 0.1285 ± 0.0072 mg AAEC per g of oil (for SR04) to 0.7237 ± 0.0040 mg AAEC per g of oil (for SR02), with an average of 0.3082 ± 0.041 mg AAEC per g of oil.
All extracts of sorghum oil have shown a positive effect on scavenging free radicals, reducing power, and can be used as a source of natural antioxidants.

The divergence between DPPH and FRAP results in some samples (e.g., SB 02 vs. SR 05) highlights the complexity of antioxidant mechanisms. Some compounds act more effectively through electron transfer (detected by FRAP), while others are better at radical scavenging (detected by DPPH) (Figure 15). This difference might be attributed to the nature and polarity of phenolic compounds, the presence of lipophilic antioxidants, or extract-specific constituents.

No single antioxidant assay provides a complete picture. The combined DPPH and FRAP results suggest that SR samples, especially SR 04 and SR 05, are more potent in free radical neutralization (Figure 15). These complementary assays underline the importance of using multiple methods to evaluate antioxidant potential in complex lipid matrices like sorghum extracts.mg AAEC per g of oil.

Table 08: The antioxidant activity, DPPH, and FRAP contents in the studied total lipids

Excerpts	DPPH(mg eq Vit C.g^{-1} Oil)	FRAP(mg eq Vit C.g^{-1} Oil)	FRAP(g/l. eq Vit C.g^{-1} Oil)	DPPH(g/ml eq Vit C.g^{-1} Oil)
SB 01	1.0007 ± 7.42×10^{-2}	0.1244 ± 0	0.01073	0.0863
SB 02	0.0935 ± 5.84×10^{-2}	0.4666 ± 1.23×10^{-3}	0.02509	0.0050
SB 03	0.5551 ± 0	0.6937 ± 9.10×10^{-4}	0.05076	0.0406
SB 04	0.1045 ± 3.05×10^{-4}	0.2831 ± 0	0.02161	0.0079
SR 01	0.1849 ± 0	0.2399 ± 2.02×10^{-3}	0.02170	0.0167
SR 02	0.0425 ± 1.21×10^{-4}	0.7237 ± 4.02×10^{-4}	0.05937	0.0034
SR 03	0.1545 ± 0	0.2840 ± 0	0.01988	0.0108
SR 04	0.1735 ± 7.19×10^{-4}	0.1285 ± 7.26×10^{-4}	0.01179	0.0159
SR 05	0.4021 ± 4.02×10^{-4}	0.1649 ± 0	0.01927	0.0469
Average	0.3012	0. 3453	0.02668	0.0259
Uncertainty	± 14.736 ×10^{-6}	± 0.3850 ×10^{-6}	± 29.616×10^{-6}	± 7519.36×10^{-6}

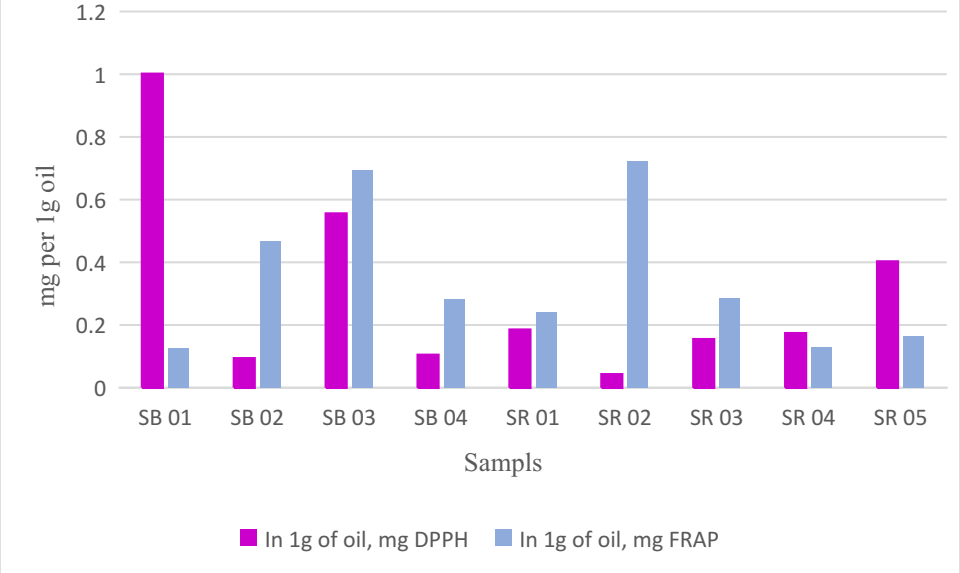


Figure 15: Comparison between DPPH and FRAP of Oil samples

5. Correlation studies:

In order to determine possible existing relations between the yield lipid antioxidant activity (two assays) and α-tocopherols. Correlations between these three groups of variables were determined using Excel's internal statistical function "coefficient. Correlation" (Table 10).

Table Correlations between antioxidant activity (DPPH assay and FRAP test), lipid yield, and α-tocopherols.

Table 9: The Correlations between antioxidant activity (DPPH assay and FRAP test), lipid yield, and α-tocopherols.

	Yield lipid	α-Tocopherol	DPPH	FRAP
Yield lipid	1			
α-Tocopherol	0.23	1		
DPPH	0.0081	0.0026	1	
FRAP	0.1059	0.1114	0.0656	1

The results were gathered in the Table. A positive correlation was found between the FRAP test and α-tocopherol (R^2= 0.1114) and α-tocopherols and lipid yield (R^2= 0.23). The results indicated no meaningful correlation between the DPPH test and α-tocopherols. The antioxidant activity can be attributed to other phenolic components present in the sorghum oil.

Conclusion

IV. Conclusion:

This study aimed to investigate the morphological diversity and bioactive lipid composition of nine local landraces of sorghum (*Sorghum bicolor L*. Moench) from Ain Saleh, Algeria, with a particular focus on antioxidant properties. The agro-morphological evaluation revealed significant variability among genotypes, particularly in terms of panicle weight, panicle branch length, and 100-grain weight, indicating a valuable reservoir of genetic diversity that could support breeding programs for yield improvement under arid conditions.

Lipid extraction showed that white sorghum varieties had generally higher oil yields compared to red sorghum, with SB04 achieving the highest yield (6.014%). The lipid extracts were further characterized for their α-tocopherol (vitamin E) and sterol contents. White sorghum displayed higher average tocopherol concentrations than red sorghum, while some red varieties, such as SR04, showed elevated sterol levels, making them potential candidates for nutraceutical applications.

Antioxidant activity assessed by DPPH and FRAP assays confirmed the presence of bioactive compounds capable of neutralizing free radicals. The antioxidant capacity varied among landraces, with some white sorghum oils (e.g., SB01 and SB03) demonstrating high DPPH activity, and certain red sorghum oils (e.g., SR02) showing strong reducing power in FRAP tests. These results highlight the complexity and specificity of antioxidant mechanisms and suggest that multiple assays are essential for a full assessment.

Overall, the findings validate the nutritional and functional potential of Algerian sorghum lipids. They demonstrate that underutilized varieties, especially those from marginal regions, represent a promising source of bioactive compounds with antioxidant, health-promoting properties. This work supports the valorization of sorghum by-products and offers new perspectives for their integration into functional foods, cosmetics, and pharmaceutical formulations, in line with sustainable and locally adapted bioresource development.

These findings underscore the potential of Algerian sorghum as a valuable source of bioactive lipids, particularly for applications in the development of functional foods, nutraceuticals, and natural antioxidant formulations. The variability among landraces highlights the importance of genetic selection for targeted industrial applications. Future work should focus on detailed profiling of other antioxidant compounds and exploring synergistic effects within the lipid matrix.

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Appendix

The correlation curves:

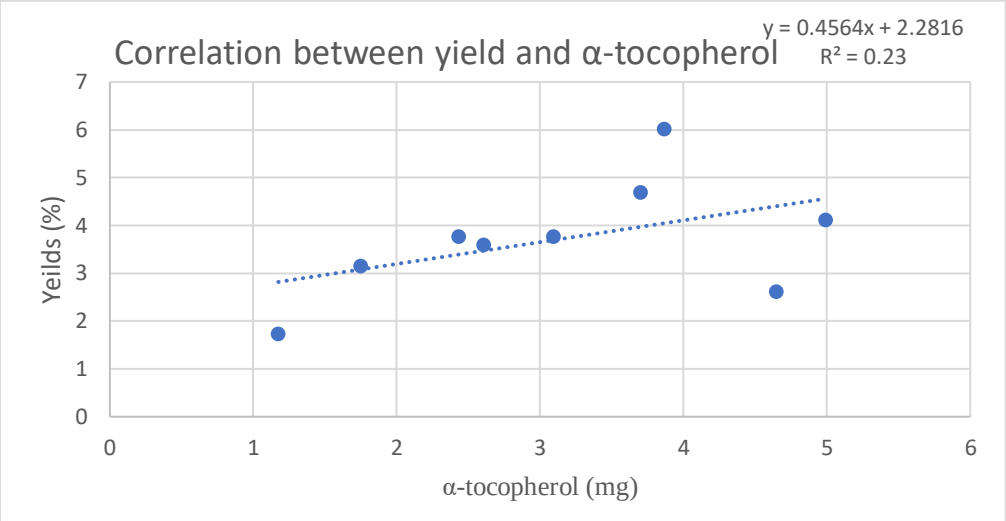

Figure 16: Correlation between yield and α-tocopherol

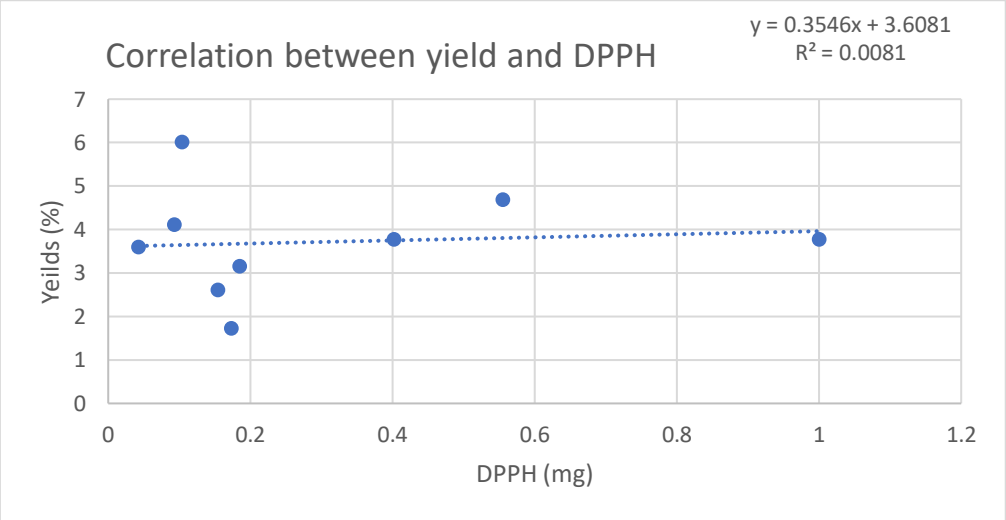

Figure 17: Correlation between yield and DPPH

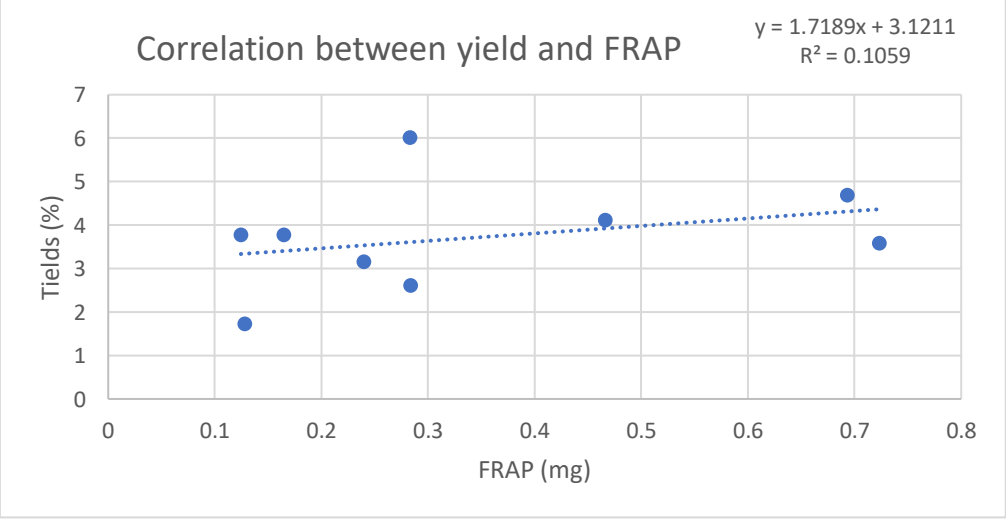

Figure 18: Correlation between yield and FRAP

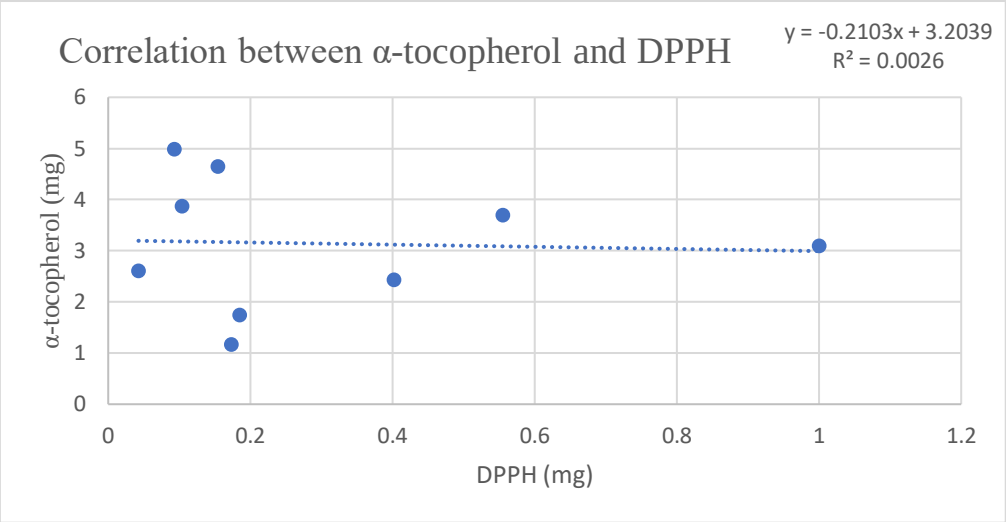

***Figure 19:* Correlation between α-tocopherol and DPPH**

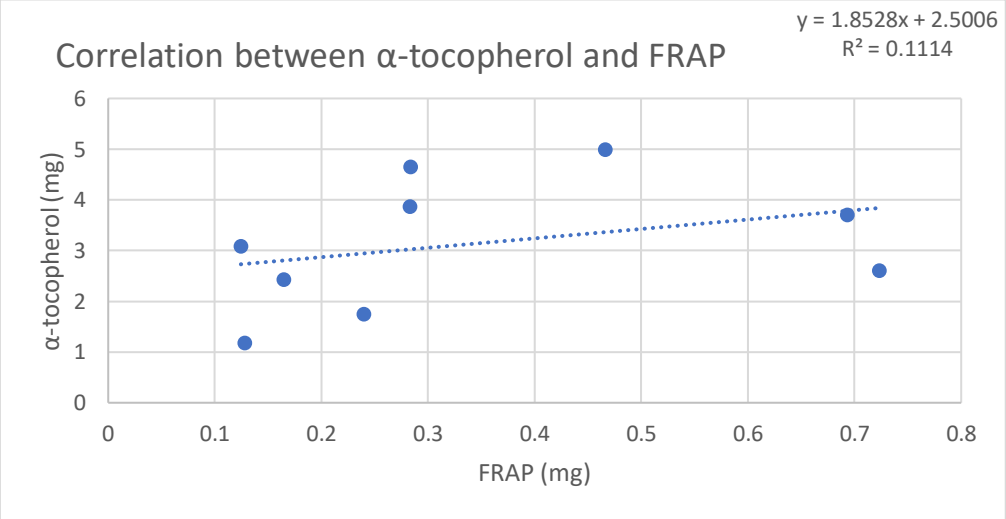

Figure 20: Correlation between α-tocopherol and FRAP

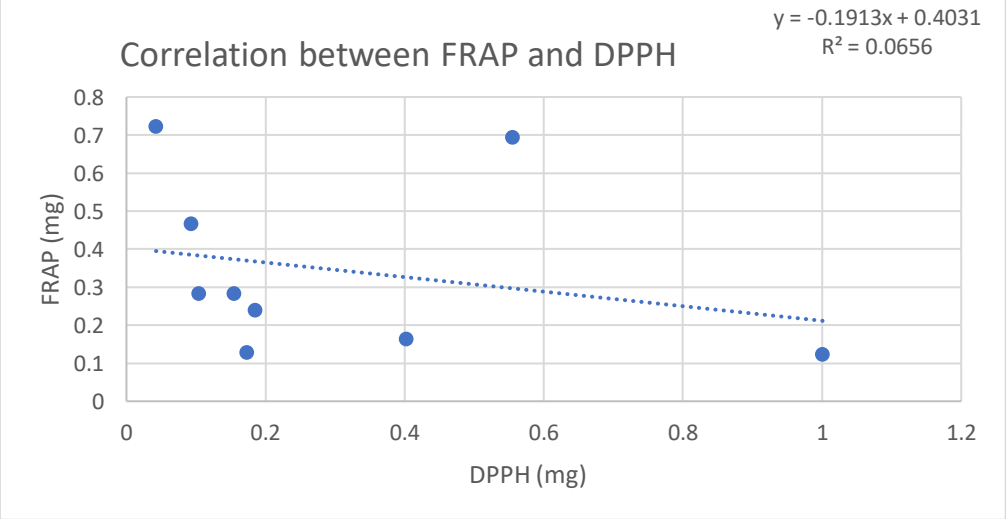

Figure 21: Correlation between FRAP and DPPH

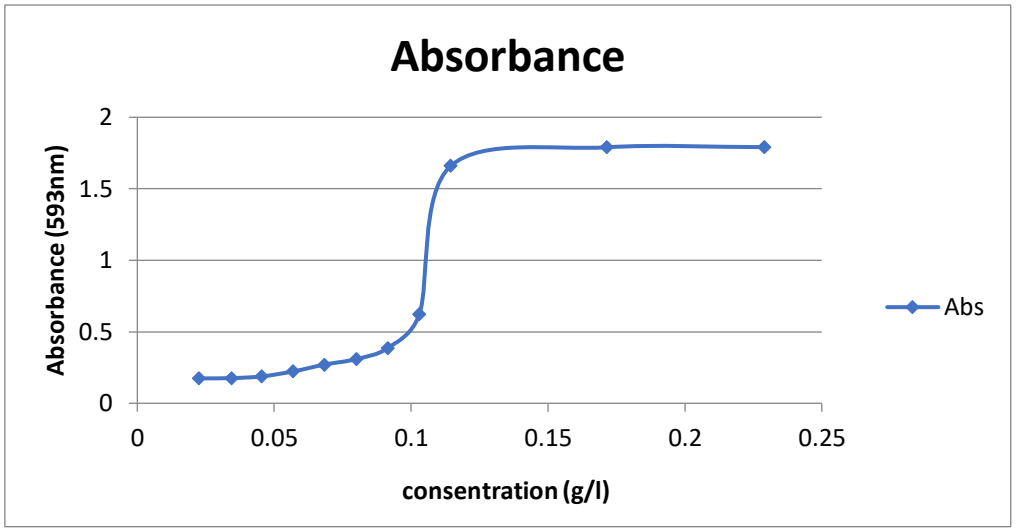

Figure 22: Insaturation curve of FRAP

الملخص :

تبحث هذه الدراسة في التنوع المورفولوجي والقدرة المضادة للأكسدة للدهون المستخلصة من تسعة أصناف محلية من نبات الذرة الرفيعة (Sorghum bicolor L. Moench) تم جمعها من منطقة عين صالح جنوب الجزائر. ونظرًا لقدرة الذرة الرفيعة على التكيف مع البيئات الجافة، وبالنظر إلى القيمة غير المستغلة بشكل كافٍ للجزء الدهني من بذورها، هدفت هذه الدراسة إلى توصيف الخصائص الزراعية والمركبات النشطة بيولوجيًا في الحبوب. أظهرت التحاليل المورفولوجية وجود تباين كبير في حجم السنابل، وكثافة البذور، ووزن الحبة بين الأصناف المدروسة. تم استخلاص الدهون الكلية باستخدام جهاز سوكسليت ومذيب الهكسان، ثم تم تحليل الزيوت لتحديد محتواها من ألفا-توكوفيرول (فيتامين E) والستيرولات باستخدام الطرق الطيفية. كما تم تقييم النشاط المضاد للأكسدة للدهون المستخلصة باستخدام اختبار DPPH(اختزال الجذور الحرة) واختبارFRAP (القدرة الاختزالية الحديدية) أظهرت النتائج أن أصناف الذرة البيضاء أعطت في المجمل نسبًا أعلى من الزيت ومحتوى التوكوفيرول، في حين أظهرت بعض أصناف الذرة الحمراء تركيزات أعلى من الستيرولات. تُبرز هذه النتائج الإمكانات الصحية والغذائية لدهون الذرة الرفيعة، وتدعم فكرة استغلال الأصناف المحلية في الصناعات الغذائية أو التجميلية أو الصيدلانية، خاصة في الزراعة المستدامة في المناطق الجافة.

الكلمات المفتاحية : الذرة (البشنة)، الدهون، الأنشطة المضادة للأكسدة، ألفا توكوفيرول.

Abstract :

This study investigates the morphological variability and lipid-based antioxidant potential of nine local landraces of *Sorghum bicolor L. Moench* collected from In Saleh, southern Algeria. Given sorghum's adaptability to arid environments and the underexplored value of its lipid fraction, the research aimed to characterize both agronomic traits and bioactive compounds present in the seeds. Morphological analysis revealed significant variation in panicle size, seed density, and grain weight. Total lipids were extracted using Soxhlet extraction with hexane, and the oils were analyzed for their α-tocopherol (vitamin E) and sterol contents using spectrophotometric methods. Additionally, antioxidant activities of the lipid extracts were assessed using the DPPH radical scavenging assay and FRAP (ferric reducing antioxidant power) assay. The results showed that white sorghum generally exhibited higher oil yields and tocopherol contents, whereas red sorghum demonstrated higher sterol concentrations in some cases. These findings highlight the nutraceutical potential of sorghum lipids and support the valorization of local landraces for food, cosmetic, or pharmaceutical applications in sustainable and arid-land agriculture.

Keywords: sorghum, lipid, antioxidant activities, α-tocopherol

Résumé :

Cette étude explore la variabilité morphologique et le potentiel antioxydant des lipides de neuf écotypes locaux de Sorghum bicolor L. Moench collectés à Ain Salah, dans le sud de l'Algérie. Étant donné l'adaptabilité du sorgho aux environnements arides et la valeur encore peu exploitée de sa fraction lipidique, cette recherche visait à caractériser à la fois les traits agronomiques et les composés bioactifs présents dans les graines. L'analyse morphologique a révélé une variation significative au niveau de la taille des panicules, de la densité des graines et du poids des grains. Les lipides totaux ont été extraits par Soxhlet à l'hexane, puis analysés pour leur teneur en α-tocophérol (vitamine E) et en stérols à l'aide de méthodes spectrophotométriques. Les activités antioxydantes des extraits lipidiques ont été évaluées à l'aide des tests DPPH (piégeage des radicaux libres) et FRAP (pouvoir antioxydant réducteur du fer). Les résultats ont montré que le sorgho blanc présentait en général des rendements en huile et des teneurs en tocophérol plus élevés, tandis que le sorgho rouge présentait dans certains cas des concentrations plus élevées en stérols. Ces résultats mettent en évidence le potentiel nutraceutique des lipides de sorgho et appuient la valorisation des variétés locales dans des applications alimentaires, cosmétiques ou pharmaceutiques adaptées à une agriculture durable en zones arides.

Mots-clés : sorgho, lipides, activités antioxydantes, α-tocophérol