

Réf :.....

Mémoire de Fin de Cycle
En vue de l'obtention du diplôme

MASTER

Thème

**Effet du salage sur le profil
phénolique et l'activité antioxydant
de certaines variétés d'olives
produites localement**

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Année universitaire : 2021 / 2022

Réf :.....

End of Cycle Memory
For graduation
MASTER

Theme

**Effect of salting on the phenolic
profile and on the antioxidant
activity of some olive varieties
produced locally.**

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Supported the: 15/09/2022

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University year : 2021 / 2022

A decorative border with repeating floral motifs surrounds the text. The top and bottom borders are wider, featuring larger floral designs at the corners. The side borders are narrower, with smaller repeating motifs.

Thanks

Above all, we thank the Almighty God for having given us the courage, the will, the patience and the health during all these years and that thanks to him this work could be carried out.

*Then, we would like to express our great consideration and our deep gratitude to our promoter **Mr. Mokrani** for having accepted to supervise our work here, as well as for his patience, his precious advices, his kindness and availability to guide us, which led us to the culmination of this work.*

*We also thank **Mrs. Ouradi Nabila**, the head of the hygiene laboratory of the wilaya of Bejaia for her benevolence towards us throughout the period of the practical internship and also the entire team of the 3BS laboratory of the University, for their assistance throughout the long months of our internship. Please accept all our feelings of gratitude and respect.*

We also thank the members of the Jury for their patience and for agreeing to review our study and comment on its possible quality.

All our respects are addressed to our dear parents who encouraged and supported us a lot during our studies.

Finally, we address our most sincere thanks to all our family and friends, whom had always encouraged us during the realization of this thesis.

Dedication

May this work show my respects

To my parents

Thanks to their tender encouragement and their great sacrifices, they were able to create the affectionate climate conducive to the pursuit of my studies.

No dedication could express my respect, my consideration and my deep feelings towards them. I pray the good Lord to bless them, to watch over them, hoping that they will always be proud of me. To my two brothers:

Djamel and Billal** and My little sister **Nassima

*They will find here the expression of my feelings of respect and gratitude for the support they have constantly given me. To all my family, especially my cousins and uncles. To my friends **Billal, lyes, lyes, khaled, Koceila, fatah, massi** and **“Hanane”**. They will find here the testimony of loyalty and infinite friendship. To all the Master 2 science of fats promotion, to whom I wish a good professional career. To all my friends from the university whom I met throughout my journey.*

CHAOUCH LOUNES

DEDICATION

To the Almighty God, to whom I owe everything, and above all for having honored and enlightened my path through knowledge.

To my late father "May God keep you in his vast paradise".

*To my dear mother who always knew how to be by my side in joy and sorrow,
Dear mother, "may God keep you for us".*

To my dear sister Sadra and my brother Naim

"I wish you a long life full of joy and success".

To my dear friends Dadi, yanis, yacine, Akli

"They will find here the testimony of an infinite fidelity and friendship".

To all the Master 2 science of fats promotion, to whom I wish a good professional career and all my friends from the university whom I met throughout my journey.

Messaoudene khaled

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Abbreviations list

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AGPI/AGS: **AGPI:** polyunsaturated fatty acid/**AGS:** saturated fatty acid

HT: hydroxytyrosol

(OL & OLE): oleuropein

FA : fatty acid

UFA: unsaturated fatty acid

RNA_m, messenger ribonucleic acid

LDL: Lipoprotein Low Density

COX2: Cyclooxygenase 2, qui fait partie d'une famille d'enzymes inductives par de multiples facteurs pro-inflammatoires

NF- κ B: nuclear factor-kappa B is a transcription factor superfamily protein involved in immune response and cellular stress response

ROS: Reactive Oxygen Species

DNA: deoxyribonucleic acid

Caco-2: is an immortalized cell line of human colorectal adenocarcinoma cells

MMP-2: Matrix metalloproteinase-2 and gelatinase A, is an enzyme encoded by the MMP2 gene in humans. The MMP2 gene than is located on chromosome 16 at position 12.2

MMP-9: Matrix metalloproteinase 9 or gelatinase B

TNF- α : Tumor Necrosis Factor is a cytokine with pro-inflammatory properties and immunology functions

cAMP: cyclic nucleotides cyclic adenosine monophosphate

cGMP: cyclic guanosine monophosphate

PD: Alzheimer Disease.

AD: Parkinson Disease

DW: dry weight

Abbreviations list

TPC: total phenolic compounds

TFC: Total flavonoid content

TFLC: Total flavonols content

TFLC: Total anthocyanin content

DPPH-RSA: 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging activity

ABTS-RSA: 2, 2'-azino-bis(3-éthylbenzothiazoline-6-sulphonique) free radical scavenging activity.

FRP: Ferric reducing power

ICA: Iron chelating activity

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Introduction

The traditional Mediterranean diet (MD) is associated with long life and lower prevalence of cardiovascular disease and cancers. The main components of this diet include high intake of fruit, vegetables, red wine, extra virgin olive oil (EVOO) and fish, low intake of dairy and red meat (Boss et al., 2016).

The olive tree (*Olea europaea* L.) is widely cultivated for the production of both oil and table olives and very significant because of its economic value. Olive and olive oil, a traditional food product with thousands of years of history, are the essential components of the Mediterranean diet and are largely consumed in the world. Beside of their economical contribution to national economy, these are an important food in terms of their nutritional value. Olive and olive oil may have a role in the prevention of coronary heart disease and certain cancers because of their high levels of monosaturated fatty acids and phenolic compounds. In addition, olives (*Olea europaea* L.) and olive oils provide a rich source of natural antioxidants. These make them both fairly stable against auto-oxidation and suitable for human health (Uylaşer and Yildiz, 2014).

According to Food and Agricultural Organisation (FAO), world production of olives was more than 12.76 million tons for 2020, primarily coming from Spain (5.78 million tons), Italy (2.97million tons) and Greece (2.44 million tons) followed by Turkey (1.41 million tons) and Morocco (0.93 million tons). Algeria is one of the major olive producing countries ranking in tenth position with a world production of 0.42 million tons(FAOSTAT., 2020). Olive trees ranked first amongst the fruit trees in Algeria (Algerian Ministry of Agriculture). Table olive production has undergone a remarkable evolution in recent years to reach 293.000 tons (campaign 2016/2017), which represent 10% of the world production (Chabane et al., 2020).

Ripe olives contain high levels of bitter phenolic compounds including oleuropein and ligstroside that make the fruit inedible. In order for olives to be considered suitable for human consumption, the fruit must undergo some form of processing, fermentation, or curing to reduce the concentration of these bitter phenolic compounds(Johnson and Mitchell, 2018). Table olive processing is mainly conducted according to threemethods of a great importance in the international trade and are mainly used on an industrial scale, called Spanish-style for green olives, Californian-style and Greek naturally-style for black olives (Garrido-Fernandez et al., 1997; Sanchez et al., 2006). Each method of debittering produces a different style of table olives with a unique texture and chemical, microbial and sensorial properties (Johnson and Mitchell, 2018). However, there are some traditional preparations that have not attractedmuch attention.

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One of these involves the use of dry salt to eliminate the natural bitterness of the fruits and to make them edible.

Table olives are an important component of the Mediterranean diet, a diet linked with the reduction of certain chronic diseases including cardiovascular disease. Table olives contain a range of biologically active phenolic compounds. The predominant phenolic compound in fresh olive fruit is oleuropein. This phenolic compound is very bitter and must be removed to make olive fruit palatable. This is generally achieved through salt curing or alkaline hydrolysis. The main hydrolysis products of oleuropein include hydroxytyrosol and tyrosol. Many of the health benefits reported for olives are thought to be associated with the levels of hydroxytyrosol.

Several studies have focused on the phenolic content of olive fruits (Esti et al., 1998; Vinha et al., 2005; Ziogas et al., 2010). However, little work was conducted regarding the effect of processing techniques on the phenolic composition of table olives. Thus, the purpose of this study was (i) to determine the phenolic composition (total phenolic content; TPC, total flavonoid content; TFC, total flavonol content; TFLC and total anthocyanin content; TAC) and the antioxidant potential (total antioxidant activity; TAA, DPPH radical-scavenging activity; DPPH-RSA, ABTS radical-scavenging activity; ABTS-RSA, ferric reducing power; FRP and iron chelating activity; ICA) of three fresh black Algerian olives cultivars (Aharoune, Bouchouk and Sigoise), (ii) to study the effect of three solvents extraction (60% acetone, 60% ethanol and water) on the recovery of phenolics and on the antioxidant capacity of the three olives varieties studied in order to find the best solvent extraction to recover the maximum phenols from olives and to correlate their levels with the antioxidant activity of the obtained extracts and (iii) to test the effect of dry salting on the phenolic compounds the contents and the antioxidant capacity of the three Algerian black olives cultivars.

Chapter I

Generalities on olives

1. Botanical description and structure of olives:

1.1. Botanical description

Olive (*Olea europaea* L.) belongs to the botanical order of Ligustrals of the Oleaceae family, which includes the genera Jasminum, Phillyrea, Ligustrum, Syringa, Fraxinus and Olea. Olive trees are cultivated predominantly for their fruit which is used as table olives and also processed into oil. Olive tree comes from a genus of evergreen trees in the family Oleaceae containing 24 genus and 900 species. *Olea europaea* L. is in the same species such as lilacs, jasmine, Forsythia and the true ash trees (Fraxinus) (Şahin and Bilgin, 2018). Olive trees are cultivated predominantly for their fruit which is used as table olives and also processed into oil (komaki et al., 2003). The Taxonomic classification of olive tree is presented in table I (Şahin and Bilgin, 2018).

Table I: Taxonomic classification of olive tree (Şahin and Bilgin, 2018)

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Oleaceae
Genus	Olea
Binomial name	<i>Olea europaea</i>

1.2. Structure of olives

The olive fruit (*Olea europaea* L.) is a drupe, a single-seeded indehiscent fruit with a fleshy outer layer. The unripe fruit is pale green; as the fruit ripens, the colour changes from purple to black. A few varieties are green when ripe, and some turn a shade of copper brown. Olive cultivars vary considerably in size, shape, oil content and flavour. The shapes range from almost round to oval or elongated with pointed ends (Hashim et al., 2005; Morelló et al., 2006). The ripe olive fruit exhibits a typical drupe structure, with a thin protective exocarp, a fleshy mesocarp and a stony endocarp that surrounds the seed (shown in figure 1). Most table olives are harvested in mid-autumn when they are firm and the colour changes from green to yellowish-green. In contrast, oil olives are harvested in late autumn or winter after they have turned black, with a reduction in the chlorophyll

content and an increase in the anthocyanin content, and have attained their maximum oil content (Fedeli and Cortesi, 1993; Haralampidis et al., 1998).

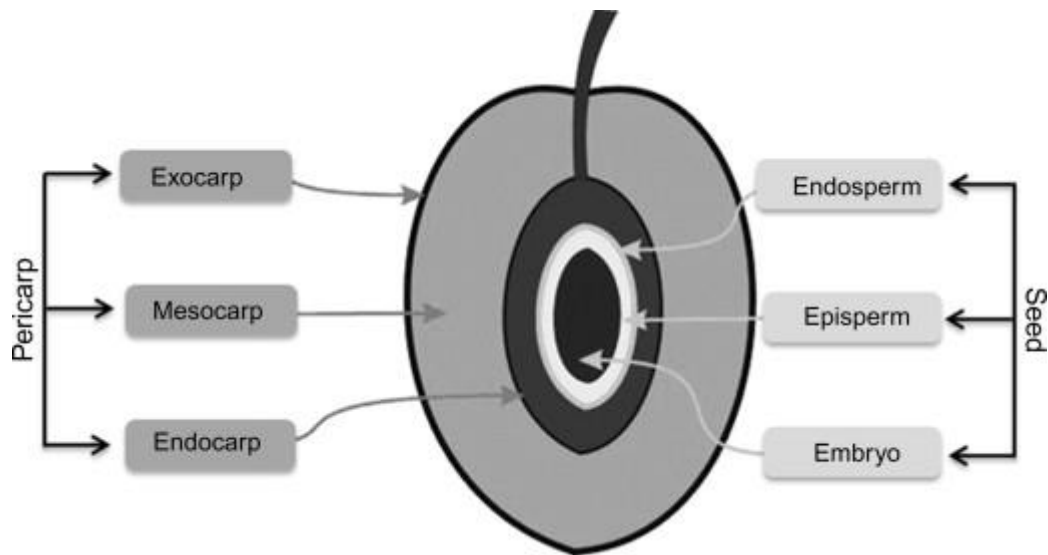


Figure 1: Structure of olive fruit (Calabriso et al., 2015).

1.2.1. Epicarp

This is a protective tissue that accounts for about 1-3% of the drupe weight. The skin itself is covered by a layer of wax, representing 45 to 70% of the skin weight. In the early stages of development the skin is bright green due to the accumulation of chlorophyll, but later it changes to pale-green, straw yellow, pink, purple pink and black. Such marked colour changes are due to unbalanced and varying concentrations of chlorophylls, carotenoids and anthocyanins, the major pigments in olives (Bianchi, 2003).

In table olive processing the structure and composition of the epicarp layers are of paramount importance. The significance lies in the fact, that the cutin and embedded waxes are almost impermeable to water. Other relevant functions of the epicarp include minimising mechanical damage to cells, inhibiting fungal and insect attack. Therefore the outermost layer of skin is most important. It plays a determining role in the processing and the final product quality (Bianchi, 2003).

1.2.2. Mesocarp

This constitutes the major part of the olive. Together with the skin they represent the edible portion of olives, comprising 70-80% of the whole fruit. It is the reserve supply of all the constituents, including water (70-75% of the mesocarp weight) and oil (ranging from 14-15% in green table olives to about 30% in black, mature olives). The textural quality of the table olive, assessed by the consumer at the time of eating, is dependent on cell rupture

and cell separation. These properties are determined by the type of processing to which the olives have been subjected (Bianchi, 2003).

1.2.3. Endocarp

The stone is characteristic of a variety. It represents 18-22% of the olive weight. The enclosed kernels comprise 2-4% of the weight. The kernel contains a relevant amount of oil (22-27%), whilst the woody shell contains, at most, 1%. The size, weight, shell conformation of the stone and its easy separation from the flesh are important parameters that determine the quality of the final product (Bianchi, 2003).

2. Chemical composition of olive

Average content of water, protein and oil in olive fruits are 50%, 1.6% and 22%, respectively. Olive fruit contains 19.1% carbohydrate, 5.8% cellulose and 1.5% inorganic substances. Other important compounds present in olive fruit are pectin, organic acids, pigments and phenols (Boskou, 2006). Organic acids show metabolic activity and are intermediate products resulting from formation and degradation of other compounds (Cunha et al., 2001).

2.1. Lipids

Lipids represent 8 to 24 g/100g of olives; they are ruled by fatty acids unsaturated including mono-unsaturated (oleic acid). The level of triglycerides increases with the ripening of the fruit (17% in green olives and 25% in black olives). The lipid content increases during maturation and reaches its maximum at full maturity. Oleic acid is the main fatty acid in olives (83%). The AGPI/AGS ratio is low and varies during fruit ripening (Owen et al., 2003; Sakouhi et al., 2008).

2.2. Glucids and organic acids

When analyzing the carbohydrate composition of raw olive fruits, the main sugars are glucose, mannitol, fructose and sucrose with concentrations of 29.2, 10.5, 5.2 and 1.5 g/kg, respectively. These sugars represent 3.5-6% of the flesh. Note that sugar content decreases with fruit maturation. The sugar content in olives is the most important fermentative substrate for the growth of the microorganisms which are responsible for fermentation during the natural style elaboration. The soluble sugars are transformed by microorganisms into organic acids as the product of the fermentation and second metabolites responsible for the desirable organoleptic characteristics in the final product (Chabane et al., 2021).

The polysaccharides and pectic substances, major constituents of intercellular lamellae, have a cementing function and determine the texture of the olive flesh. During olive processing and storage the pectic substances are hydrolysed by pectinolytic enzymes, and 'hardness texture of the fruit diminishes (Bianchi, 2003).

Organic acids are one of the minor components of olive fruit and their amount is 1.5% of the fleshy part. Organic acids that play an important role in metabolic activity are the products formed during the formation and degradation of the other components in olive fruit like carbohydrates. Malic and citric acids which affect the colour of the olive are the major organic acids found in it (Ergönül and Nergiz, 2010).

2.3. Proteins

Olive fruit contain also proteins. The protein content varies between 1.5%-2.2% of the fruit weight (Bianchi, 2003). Furthermore, the olive fruit flesh contains free amino acids such as arginine, glutamic and aspartic acid.

2.4. Minerals

The overall composition of minerals varies between olive cultivars. Of the various elements K is the most abundant element in the fruit, followed by Mg, Ca, Na and Fe (Nergiz and Engez, 2000).

2-5. Volatils compounds

Volatile or aromatic compounds are molecules that define the organoleptique characteristic of the olives. They constitute a quality index of the olives produced by controlling their acceptability by the consumer. The aroma of the table olives is made up of a balanced mixture of hydrocarbons, alcohol, aldehydes, ketones and ester (Sabatini and Marsilio, 2008).

2.6. Antioxidants of olives

2.6.1. Phenolic compounds

The olive drupe, in fact, contains high concentration of phenolic compounds that can range between 1 and 3% of the fresh pulp weight (Fernández et al., 1997). Phenolic compounds constitute an important group of naturally occurring compounds in plants. They are secondary plant metabolites, with a great structural diversity and a wide phylogenetic distribution (Harborne, 1989) The most important classes of phenolic compounds in olive fruit include phenolic acids, phenolic alcohols, flavonoids and secoiridoids (Vinha et al., 2005).

a. Secoiridoids

The predominant phenolic compounds in raw olives are certain secoiridoids derived from oleosides, a combination of elenolic acid and glucose residue. Oleuropein, an heterosidic ester of elenolic acid with 3,4-dihydroxyphenethylalcohol (hydroxytyrosol), demethyloleuropein, the acid derivative of oleuropein and ligstroside, an heterosidic ester of elenolic acid with 4-hydroxyphenethylalcohol (tyrosol) are repeatedly reported as the main secoiridoids of the fruit (Gariboldi et al., 1986; Panizzi et al., 1960; Ragazzi et al., 1973). Olive fruit contains also some other oleuropein derivatives, e.g. oleuropein aglycon, hydroxytyrosil elenolate, enololeuropein diastereoisomers (Bianco and Uccella, 2000), hydroxytyrosol and tyrosol glucosides, such as hydroxytyrosol-1-O- β -glucoside, tyrosol-1-O- β -glucoside, hydroxytyrosol-3'-O- β -glucoside and hydroxytyrosol-4'-O- β -glucoside (Bastoni et al., 2001; Bianco and Uccella, 2000) and verbascoside, which is the caffeoyl rhamnosyl glucoside of hydroxytyrosol (Andary et al., 1982).

b. Phenolic acids

Benzoic, cinnamic, phenylacetic and phenylpropionic acid hydroxy derivatives, such as *p*-hydroxybenzoic, protocatechuic, vanillic, syringic, *o*- and *p*-coumaric, caffeic, chlorogenic, ferulic, sinapic, *p*-phenylacetic, 3,4-dihydroxyphenylacetic, homovanillic and dihydrocaffeic acids, are also present in olive at levels depending on fruit variety (Bianco and Uccella, 2000).

c. Flavonoids

Flavonoids present in olive fruit are flavones, mainly luteolin, flavone and flavonol glucosides, mainly rutin and luteolin 7-glucoside, and anthocyanins, mainly cyanidin 3-glycosides (Romani et al., 1999; Vlahov, 1992). Anthocyanins are responsible for the purple colour of natural black olives and a qualitative study of these pigments was carried out by Vázquez and Maestro (Rovellini et al., 1997; Vlahov, 1992), reporting that cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside were the main anthocyanins in natural black olive fruits.

d. Phenolic alcohols

Tyrosol and hydroxytyrosol oleosides and the respective free alcohols have been also identified at considerable amounts (Bianco and Uccella, 2000; Soler-Rivas et al., 2000).

e. Tocopherols

These are present in all oils of plant origin, and also in animal lipids. The α -tocopherol comprises 88.5% of all tocopherols in olive oil. The concentration of α -tocopherol in olive oil is 12–150 ppm. Oils derived from the fruit stone contain higher concentrations of tocopherols, which play the role of antioxidants (Therios, 2009).

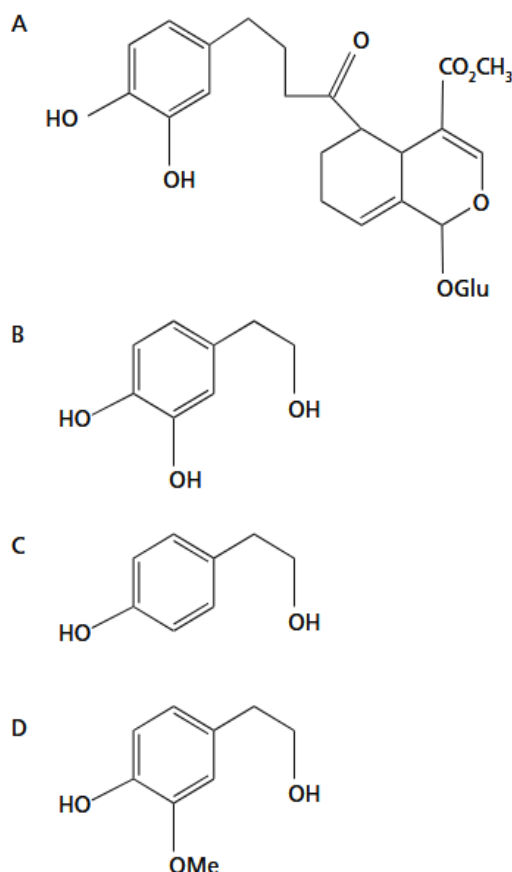


Figure 2: Chemical structures of major bioactive phenolics in olives and olive oil:

(A) oleuropein, (B) hydroxytyrosol, (C) tyrosol, (D) homovanillic alcohol.

f. Chlorophyll and carotenoids

Three chloroplast pigments identified in olives are those of all green plant tissues and do not undergo any change or modification during the stages of ripening. The carotenoids β -carotene, phytofluene and luteoxanthin are present in very small amounts. Both chlorophylls and carotenoids decrease as the season progresses, almost disappearing at the moment of maturity, while anthocyanins begin to appear, little by little, invading the skin and later the whole pulp. The α -chlorophyll is the major component, followed by β -chlorophyll. Carotenoids have been found to be minor components, such as lutein, which is

the major xanthophyll, and β -carotene, the principal carotene. As olive fruit ripens, photosynthetic activity decreases and chlorophyll disappears. As a consequence, the colour of the skin changes from green to yellow, reddish or red. During this period the concentration of carotenoids and chlorophylls diminishes, while the proportion of xanthophylls increases. Chlorophyll degradation is accompanied by the synthesis of other compounds, anthocyanins, because the carotenoids do not produce the final pigmentation of the ripe fruits, i.e. reddish or purple (Therios, 2009).

3. Olive market

The olive tree has been grown for its oil-rich fruit since late prehistoric times. The cultivated variety, *O. europaea* L. var. *europaea*, has become more adaptable to a wider range of climatic and environmental conditions (Carrión et al., 2010). Many cultures have used olive oil primarily as a lamp fuel, however, in the late 19th and 20th centuries, the demand for olive oil decreased after the development of low-cost solvent extraction techniques for seed oils and the use of other sources of light (gas and electricity). Today, the olive fruit and oil provide valuable nutrients for humans, and they play important roles in the diets of the people in the areas of cultivation, in addition to the role in their economy and culture (Blázquez-Martínez, 1996; Civantos, 1998; Uylaser et al., 2008; Vossen, 2007).

3.1. World production of olives

Olive trees possess an amazing ability to survive under unfavourable conditions; however, it is a demanding crop if it is to flourish. Therefore, a suitable environment and proper cultural care are necessary for the full development of the agronomic characteristics and steady production conditions. The tree is cultivated today in many countries, including Spain, Italy, Greece, Tunisia, Turkey, Portugal, Morocco, Syria, Algeria, Egypt, Israel, Libya, Jordan, Lebanon, Cyprus, Croatia, Slovenia, Argentina, Chile, Mexico, Peru, the United States, and Australia (Boskou, 2009).

According to the report of the International Olive Oil Council (IOOC, 2011), Mediterranean countries accounted for approximately 97% of the world's olive cultivation, estimated at approximately 10,000,000 hectares. There are more than 800 million olive trees currently grown throughout the world, of which more than 90% are grown for oil production and the rest for table olives. It is estimated that more than 2,500,000 tons of olive oil are produced annually throughout the world. Approximately 81% of the total olive production comes from the European Community (EC) (Spain, Italy, Greece, Portugal, and France), with the Near East contributing, approximately 7% and

North Africa supplying approximately 11%. The remaining 1% is of American origin, chiefly from Argentina, Mexico, Peru, and the United States.(Figure 3)shows the main countries where olives are reproduced.

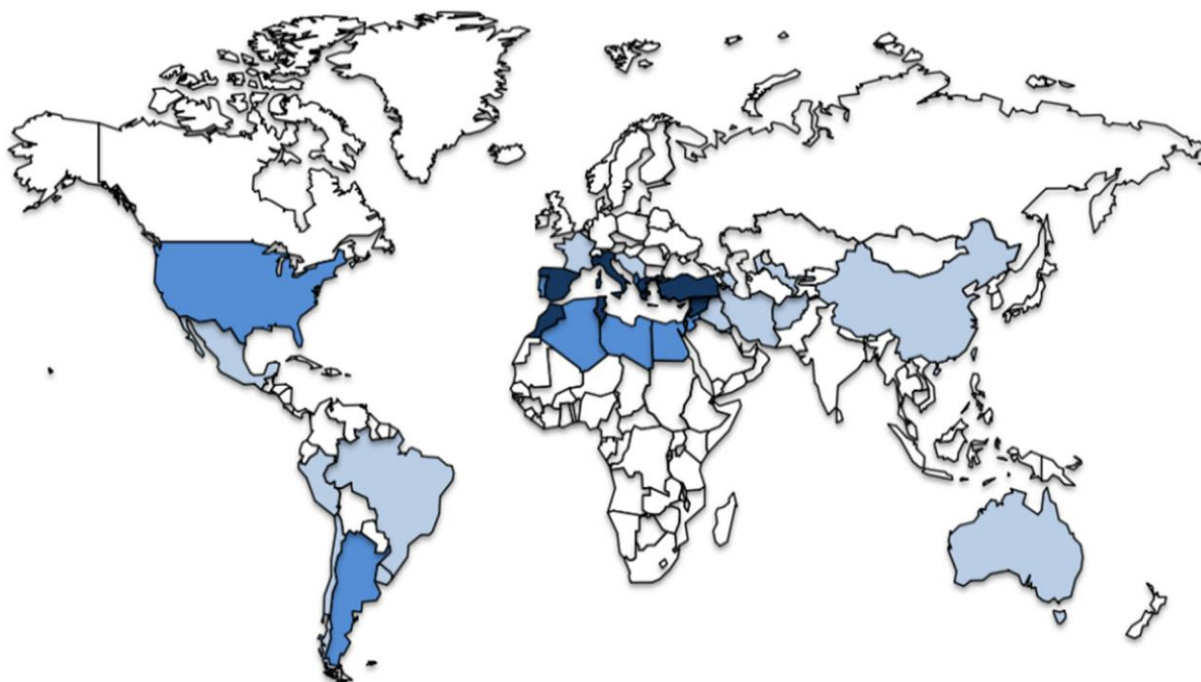


Figure 3: The main countries producing olives, based on production between 2003 and 2013.

- Production more than 1E+7 tonnes.
- Production in the range of 1E+6 to 1E+7 tonnes.
- Production lower than 1E+6 tonnes.(Guo et al., 2018).

3.2 National production of olives

Algeria is one of the major olive producing countries ranking in tenth position with a world production of 0.42 million tons(FAOSTAT., 2020). Olive trees ranked first amongst the fruit trees in Algeria (Algerian Ministry of Agriculture). Table olive production has undergone a remarkable evolution in recent years to reach 293.000 tons (campaign 2016/2017), which represent 10% of the world production (Chabane et al., 2020).

Oliviculture in Algeria has an important and an ancestral place. In 2006, the area covered by olive trees was 263,352 ha (29,995,980 trees) corresponding to 32.5% of total tree growing area except vineyards (810,193 ha). However, the olive oil production is

concentrated mainly in the center of the country, “the Kabylie” with 58.4% of the total oliviculture area (153,708 ha)(Louadj and Giuffrè, 2010)

There are many cultivated and described olive tree cultivars in Algeria; 36 cultivars are homologated by I.T.A.F. (Institut Technique de l’Arboriculture Fruitière et de la Vigne). I.T.A.F. is a national Technical Institute of tree and wine growing created in 1987, the headquarters is situated in Algiers but it has 10 demonstration farms in different regions of Algeria. The most important cultivars of olive are(Louadj and Giuffrè, 2010):

- **Chemlal:** in the Kabylie region, it occupies 40% of the national area for oliviculture, cultivated for olive oil extraction.
- **Sigoise:** in the west of Algeria, it occupies 25% of the national area for oliviculture, it has double destination (olive oil and table olives).
- **Azeradj:** in the Kabylie region (the east center), it occupies 10% of the national area for oliviculture. It has double destination (olive oil and table olives).

Chapter II

Table olives

processing and

biological activities

1. Table olive definition:

According to the Trade Standard Applying to Table Olives (COI/OT/NC no. 1, 2004), table olives are defined as the product “prepared from the sound fruits of varieties of the cultivated olive tree (*Olea europaea* L.) that are chosen for their production of olives whose volume, shape, fleshto-stone ratio, fine flesh, taste, firmness and ease of detachment from the stone make them particularly suitable for processing”. Different kinds of table olives should be classified according to the ripeness stage of the fruit, trade preparation, styles and sizing (Sousa et al., 2008).

2. Olives processing

Most olives are used to produce olive oil. However, many are processed into different types of table olives for direct human consumption (Gandul-Rojas and Gallardo-Guerrero, 2014). Table olives are a chief and traditional fermented food in Mediterranean countries. Contrary to other fermented foods (e.g. carrots, cabbage, pumpkins, beans), table olives have low sugar levels (2–5%), high fat content (20–35%), and a bitter taste caused by oleuropein (Sakouhi et al., 2008). The texture of the edible flesh varies widely, depending on variety, oil content, stage of maturity, soil quality, climate, and other factors that influence the physical and chemical composition of the fruit (Mafra and Coimbra, 2004).

The highly bitter taste caused by oleuropein makes fresh olives hard to eat. Fresh olives must be cured and fermented to make them palatable. Lyetreatment removes the bitterness by converting oleuropein into hydroxytyrosol (HT), elenolic acid glucoside (oleoside-11-methyl ester) and oleuropein aglycone under the circumstance of hydrolytic cleavage of the ester and glycosidic bond (figure 4).

There are three main commercial types of table olives: Spanish-style green olives, Californian style black-ripe olives, and Greek-style natural black olives in brine (Papadaki and Mantzouridou, 2016). Three different processing methods produce the three kinds of table olive (Figure 5) and give them each a different taste.

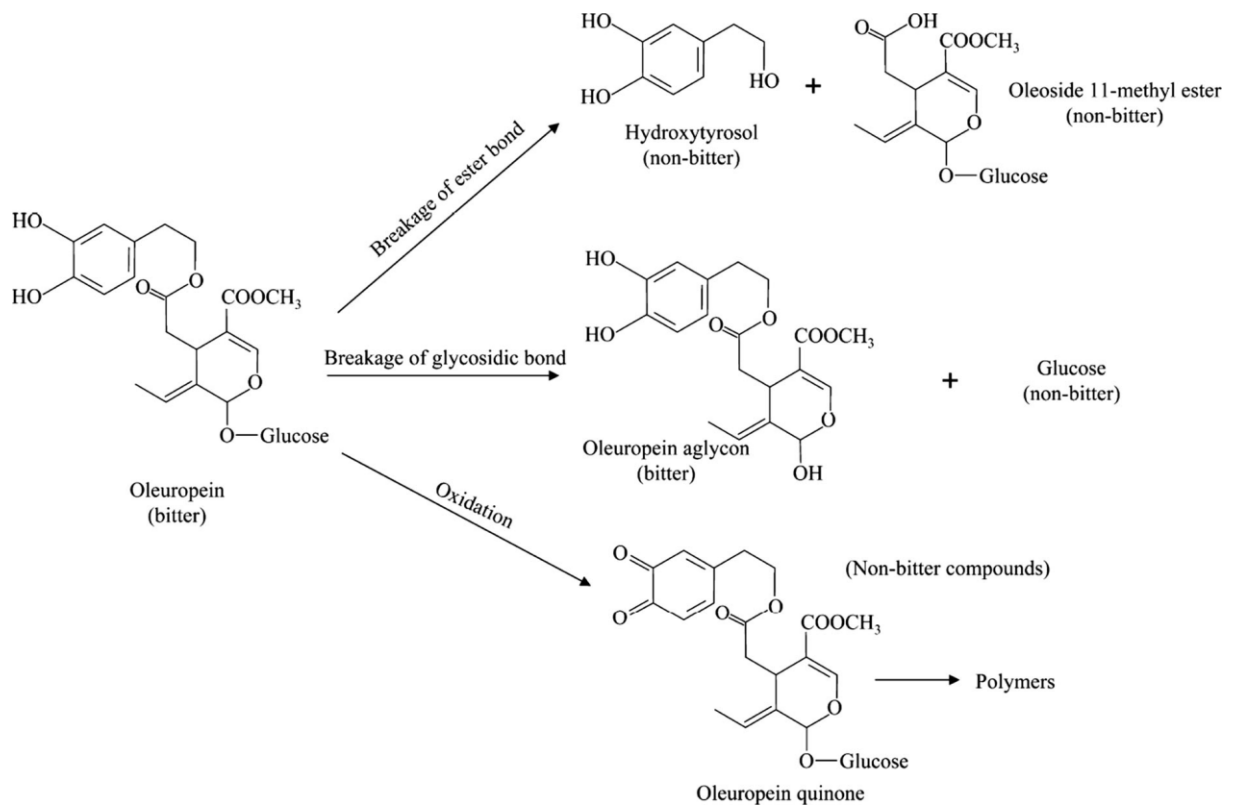


Figure4: Bitterness of oleuropein and products of its chemical transformations (Garcia et al. 2008).

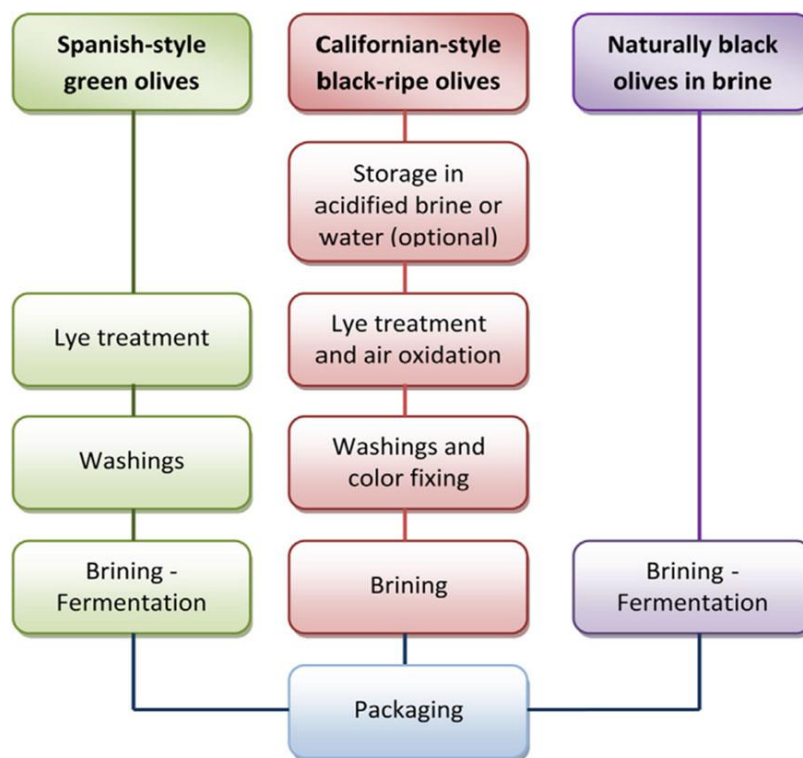


Figure 5: Production process flow charts for Spanish-style green olives, Californian-style black-ripe olives, and naturally black olives in brine (Papadaki and Mantzouridou, 2016).

2.1.Spanish-style green olives

Olive fruits are harvested with colours varying from green to yellow but having reached normal size. Olives are submitted to a sodium hydroxide(NaOH) solution (lye, normally 1.3–2.6 % w/v) until NaOH reaches from two-thirds to three-fourths of the distance between the surface of the olives and the stone(Charoenprasert and Mitchell, 2012). The concentration of NaOH used will depend on the temperature, cv. and degree of the fruit maturity. The lye treatment hydrolyses the OL into non-bitter HT and oleoside-11-methyl ester. Subsequently, olives are washed with water to remove the excess of lye and submitted to a sodium chloride (NaCl) solution (6–8 % w/v) for a mild lactic fermentation(Mateus, 2016). The levels of OL and other phenolics present in the brine can influence the fermentation rate, as they have antimicrobial activity. Finally, the olives are packed in brine (≥ 8 % w/v NaCl) and they can be further processed to prolong shelf-life, through the addition of sorbic acid or its salts, or submitted to pasteurization (62.4°C for 15 min)(Charoenprasert and Mitchell, 2012).

2.2.Californian-style black olives in brine

Olive fruits are harvested before they reach their final maturation stage. First, olives are stored in brine (5–10 % w/v NaCl) for a period which varies from 2 to 6 months, with medium acidification until pH 4 through the addition of lactic and acetic acids and in anaerobic/aerobic conditions to prevent fermentation(Charoenprasert and Mitchell, 2012; Pereira et al., 2006). Posteriorly, fruits undergo a treatment with two to five NaOH solutions (1–2 % w/v), leading to a progressive entry of NaOH into the flesh(Charoenprasert and Mitchell, 2012). In intervals between lye treatments, olives are suspended in water or a weak brine solution in which air is bubbled, leading to oxidation by aeration and polymerization of phenolic compounds, transforming them into different dark compounds, allowing that a rapid darkening of the fruit occurs. Iron salts, such as ferrous gluconate or ferrous lactate, can be used to stabilize and maintain the black color of table olives. The change in colour of olives is also facilitated by the formation of uncoloured ferrous complexes and the following oxidation to dark ferric iron complexes. Normally after, these table olives are canned in brine and submitted to a sterilization treatment(Charoenprasert and Mitchell, 2012).

2.3. Greek-style natural black olives

Olive fruits, when intended to be processed with this method, are harvested in the final stage of maturation with a dark colour. After harvesting, olives are washed and directly immersed in a brine solution (8–10 % w/v NaCl), without any debittering treatment (Boskou et al., 2015; Charoenprasert and Mitchell, 2012). A natural and spontaneous fermentation process starts, driven mainly by yeasts, due to high salt concentration used, and also by lactic acid and gram-negative bacteria. It isn't worthy that fermentation may be carried out in either anaerobic or aerobic conditions. The microbiota is defined by substrate availability, salt level, temperature and pH values, aerobic and anaerobic conditions and antimicrobial compounds present such as phenolic compounds. During fermentation, bitterness of olives is lost because of the diffusion of OL from the fruit to the brine and the posterior acid hydrolysis of this compound (Boskou et al., 2015; Charoenprasert and Mitchell, 2012; Mateus, 2016; Mendes and da Silva Malheiro, 2012).

3. Effect of processing on olive quality

The processing technology greatly influences the chemico-physical composition of the olive and, consequently, the final product. Lye-treatment and fermentation, both commonly used in most table olive preparations, cause chemical and physical changes, affecting the lipid constituents, the phenols, sugars and salts. This usually results in a softening of the olive fruit, which reduces the market value of the final product (Bianchi, 2003).

4. Table olive market

4.1 World production of table olives

According to the International Olive Council (IOC), the world table olive production for the 2013/2014 season accounted for approximately 2.7 million tonnes. The European Union (EU) contributed 30% of the total production. The EU's largest producers are Spain, Greece and Italy (72, 16 and 9% of the total EU production, respectively). Other main producing countries are Egypt (15%) and Turkey (16%). This situation is clearly illustrated in (Figure 6). Also, in (Figure 6), the international trade and consumption of table olives for the same period is presented (based on IOC statistical data). Noticeably, low- or even non-producing countries such as USA, Russia and Brazil consume significant amounts of the product (Papadaki and Mantzouridou, 2016).

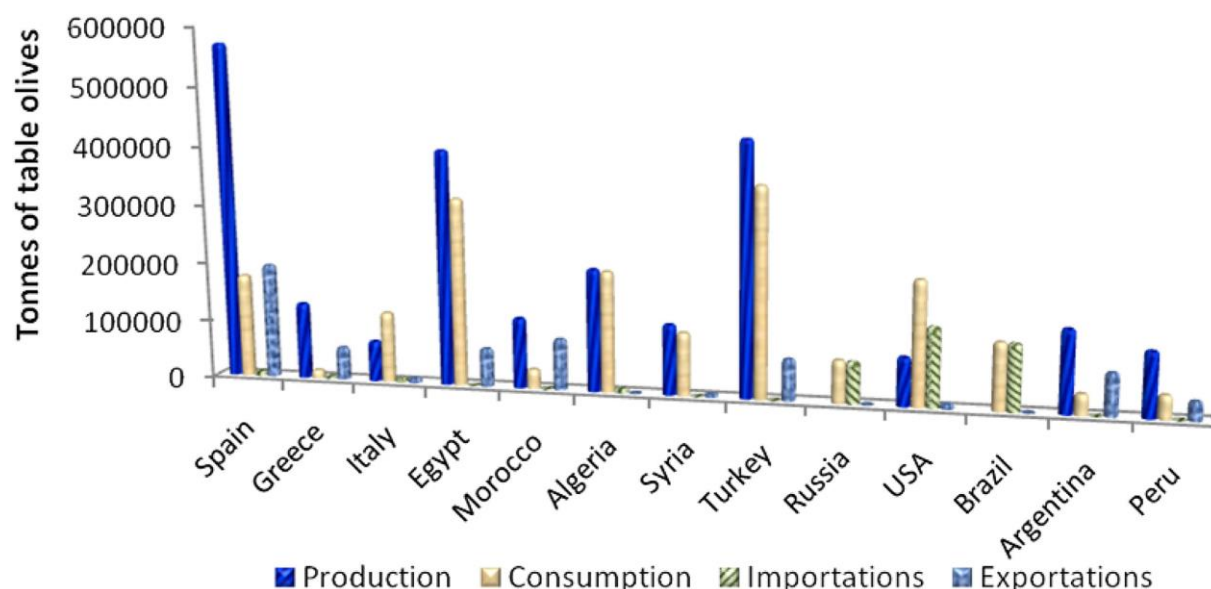


Figure 6: Table olive production, consumption and trade in different countries in 2013/2014 (Papadaki and Mantzouridou, 2016).

4.2. National production of table olives

Algeria is one of the major table olive-producing countries, with an increased production in recent years estimated at 323,000 tons annually, representing more than 10.5% of the global production (International Olive Council [IOC], 2021).

5. Nutritional properties of table olives

Table olive is a very important fermented food of the Mediterranean countries. Olive-fruit is highly appreciated for its good taste, as well as for its nutritional properties. The nutritional benefits are mainly related to α -tocopherol and FA contents (Ribarova et al., 2003). In fact, UFA participate in the regulation of cholesterol level (DELPLANQUE et al., 1999). Monounsaturated fatty acid stimulates transcription of the RNA of LDL-cholesterol receptor (Sorci, Wilson, Johnson, & Rudeell, 1989) and reduces breast cancer risks. Moreover, α -tocopherol defends the body against free radical attacks by protecting polyunsaturated fatty acids (Cheeseman and Slater, 1993; Doelman, 1989; Kamal-Eldin and Andersson, 1997) and preventing the body from cancer and arteriosclerosis (Armstrong et al., 1997; Caruso et al., 1999).

6. Medicinal use of Olives

Olives have multiple medicinal uses, including disorders of the gastrointestinal and cardiovascular systems. The olive fruits and leaves are indicated in arrhythmia,

atherosclerosis, cardiopathy, colic (spasm), diarrhea, fever, gout, headache, hepatitis, hypercholesterolemia, and hypertension. Olive oil is used in traditional medicine as a cardioprotective, gastroprotective, and enteroprotective; it is effective in the treatment of cancer, constipation, diabetes, and rheumatism. Phytochemical studies revealed that olives contain multiple compounds that have been indicated as therapeutics, including aesculin, alpha-tocopherol, apigenin, arabinose, beta-carotene, caffeic acid, catechin, choline, cinchonidine, cinchonine, elenolide, erythrodiol, esculin, estrone, fat, fibre, glucoside, iron, linoleic acid, luteolin, mannitol, myristic acid, oleanolic acid, oleoside, olivine, oleuropeic acid, oleuropein, pectin, palmitic acid, quercetin, quinone, rhamnose, rutin, squalene, tyrosol, verbascoside, tannins, saponins, and secoiridoids (Gilani et al., 2006).

7. Beneficial effects of olives

7.1. Antioxidant effect:

The antioxidant quality of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Ben Othman et al., 2008). The antiradical and antioxidant activities of olive and olive oil phenolic compounds are due mainly to the presence of a 3,4-dihydroxy moiety linked to an aromatic ring, and it was found that the effect depended on the polarity of the phenolic compound (Morelló et al., 2005). Olive secoiridoids can directly protect cells from oxidative stress by acting as free radical scavengers, radical chain breakers, or metal chelators (Boss et al., 2016; Bulotta et al., 2013; Fabiani, 2016; Hassen et al., 2015; Pang and Chin, 2018). Olive secoiridoids also act as oxidative stress defenders through the upregulation of a signaling pathway involving the nuclear factor Nrf2, thus resulting in an increased expression of protective phase II detoxifying enzymes (Calabriso et al., 2018; Calahorra et al., 2018; Menendez et al., 2013; Parzonko et al., 2013; Zhu et al., 2010; Zou et al., 2012). HT protection against oxidative damage can also derive from the induction of mitochondrial biogenesis (Zhu et al., 2010).

7.2. Antibacterial activity

Some researchers have also demonstrated that the phenolic compounds present in olive products, such as oleuropein and hydroxytyrosol (Bisignano et al., 1999; Furneri et al., 2002) (19, 20) and aliphatic aldehydes (Battinelli et al., 2006), inhibit or delay the rate of growth of a range of bacteria and micro-fungi, so that they might be used as alternative food additives or in integrative pest management programs (Pereira et al., 2006).

7.3. Anti-inflammatory effect

Beauchamp et al. (2005) demonstrated that olive secoiridoids (OC) inhibited cyclooxygenase COX1 and COX2 activity in a very similar way to the anti-inflammatory drug ibuprofen (Beauchamp et al., 2005). In particular, hydroxytyrosol (HT) and Oleuropein (Ole) are able to inhibit the inflammatory responses of murine and human monocytes and macrophages *in vitro*, by blocking the expression of COX2 (Bigagli et al., 2017; Maiuri et al., 2005; Rosignoli et al., 2013; Ryu et al., 2015; Scoditti et al., 2014; Zhang et al., 2009). Another key element of chronic inflammation is NF- κ B, which when activated, translocated into the nucleus triggering the expression of genes encoding inflammatory mediators such as cytokines, adhesion molecules and chemokines (Killeen et al., 2014). The attenuating action of Ole and HT on NF- κ B activation has been described in two main areas: i) *in vitro*, both in immune-competent cells stimulated with lipopolysaccharide (Maiuri et al., 2005; Ryu et al., 2015; Scoditti et al., 2014; Zhang et al., 2009), and in human endothelial cells treated with phorbolmyristate acetate (Scoditti et al., 2012); and ii) *in vivo*, both in a mouse model of chronic colitis induced by dextran sodium sulfate (Giner et al., 2011) and in mice with acute renal injury induced by the treatment of cisplatin (Potočnjak et al., 2016).

7.4. Anticancer activity

Olive secoiridoids may work as “redox-active” compounds inducing cancer cell growth arrest or cell death, by either stimulating ROS production or inhibiting antioxidant defense systems, or a combination of both (Acquaviva et al., 2012; Cusimano et al., 2017; Katsoulis, 2016; Rosignoli et al., 2016). Juan *et al.*, reported a positive association between the phenolic components of olive fruits and the reduction of cancer proliferation, in HT-29 human colon cancer cells (Juan et al., 2006). Further animal studies and human intervention trials, have investigated the effects of olive oil phenols on DNA damage and confirmed the ability of these compounds to inhibit the carcinogenesis process at both initiation and promotion/progression phases (Fabiani, 2016). It was observed that HT inhibits the cytotoxicity in human intestinal epithelial Caco-2 and human colon adenocarcinoma HT-29 cancer cell lines through the increment of intracellular capacity to protect against oxidative damage (Terzuoli et al., 2010; Terzuoli et al., 2016).

Due to the similar structure of the olive polyphenols to oestrogens, these have been hypothesized to interact with oestrogen receptors, thereby reducing the prevalence and progression of hormone related cancers (Boss et al., 2016).

7.5. Cardioprotective effect

Prevention of atherosclerotic lesion development are limitation of oxidative injury and prevention of LDL-c oxidation, reversion of angiogenesis through the inhibition of MMP-2 and MMP-9 activity, reduction of inflammatory damage induced by inflammatory markers such as TNF- α , decrease of eicosanoid formation and expression of cell adhesion molecules (Vascular Cell Adhesion Molecule 1 (VCAM-1) and Intercellular Adhesion Molecule 1 (ICAM-1) (Granados-Principal et al., 2010; Ray et al., 2015; Vilaplana-Pérez et al., 2014). It was observed in human aortic endothelial cells treated with physiological concentrations of HT and co-incubated with TNF- α that a significant reduction of E-selectin, P-selectin, ICAM-1 and VCAM-1 secretion occurred, as well as a decrease of markers of endothelial dysfunction (Catalán et al., 2015). HT can also be considered antithrombotic, since it decreases platelet aggregation, eicosanoid synthesis such as thromboxane B₂, leukotriene B₄ and to its capacity to reduce cAMP and cGMP platelet phosphodiesterase and to decrease of cell adhesion molecule expression (Granados-Principal et al., 2010; Manna et al., 2009).

7.6. Neuroprotective effect

Studies demonstrated the antioxidant role of olive oil phenolic compounds in suppressing neurotoxicity, neuroinflammation and amyloid aggregation. The olive polyphenols have modulatory effects on the cellular pro-inflammation NF- κ B pathway, they are involved in the activation of the Nrf2/Phase II genes and are able to prevent the amyloid aggregates and therefore, have potential benefits in the onset and progression of PD and AD. Particular attention is paid to the effects of hydroxytyrosol, the most biologically active and “intriguing” compound of the olive polyphenols’ family, proven to have an ideal pharmacological profile, i.e. a combination of antioxidant and anti-inflammatory activity with an excellent safety profile, high bioavailability, tissue distribution and multiple mechanisms. HT is a product of the hydrolysis of its natural olive ester precursors, oleuropein (OLE), verbascoside and lingstroside, and is generated during the maturation process, preparation and storage of table olives (Calabrese and Crea, 2016).

Materials
and
Methods

Materials and methods

1. Chemicals and Reagents

All chemicals and solvents were of analytical grade. Acetone, ethanol and methanol were obtained from Honeywell (Seelze, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid), ABTS (2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) were purchased from Sigma-Aldrich (Fisher scientific, Fair Lawn, NJ, USA). DPPH (2,2-diphenyl-1-picrylhydrazyl), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), 4-hydroxy-3-methoxybenzaldehyde (vanillin), ferric chloride (FeCl_3), gallic acid, catechin, quercetin and trichloroacetic acid were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Hydrochloric acid (HCl), Sodium carbonate (Na_2CO_3) were purchased from Prolabo (Loire, France). Folin-Ciocalteu's phenol reagent, Potassium ferricyanide ($\text{C}_6\text{N}_6\text{FeK}_3$) and chloride aluminium (AlCl_3) were provided from Biochem-chemopharma (Loire, France).

2. Olive samples

Three black olive cultivars, named Aharoune, Bouchouk and Sigoise, harvested at the fully ripe stage were handpicked from three different regions of the locality of Bejaia on December 2021. Aharoune cultivar was harvested from Tazmalt, Bouchouk variety from Timezrit and Sigoise sample was harvested from Takerietz. The characteristic of every variety was presented in table II.

3. Dry-salting process

The black olives were transported to the laboratory within 48 h. On arrival, the fruits were hand selected, washed thoroughly under tap water and left to dry. Olives were divided into two parts. One part of fresh olives were pitted and the corresponding pulp was lyophilized (Alpha-4 LDplus, Christ, Osterode, Germany) and then ground in an electric blender. The ground pulps were kept at 4°C until extraction. The second part of fresh olives (approximately 1,5 kg), was treated with alternating layers of dry salt into baskets and kept at room temperature for 45–60 days depending on the variety (Panagou, 2006). The dry-salting process caused dehydration and the olives appear shriveled. The obtained (salted) olives were treated as described below.







4. Extract preparation

Freeze dried olive pulps (100 mg) were homogenized with 15 mL of different solvent types (60% acetone, 60% ethanol and distilled water). After stirring for 30 min, the mixture was centrifuged (nive NF 200, Ankara, Turkey) at 5000 rpm for 10 min. The supernatant was

Materials and methods

collected and filtered, whereas the residue was re-extracted with the same conditions. The filtered extracts were combined, washed with hexane (3×10 mL) and then kept at 4°C until analysis (McDonald et al., 2001).

Table II: Origin and characteristic of the three studied black olives varieties.

variety	Origin of the variety	Characteristic	Salting time	Fresh olives	Salted olives
Aharoune	High valley of Soummam	Seasonal variety, hardy and self-fertile. Average kernel pulp ratio: 06.00 The pulp is difficult to separate from the stone. Productivity is high and little alternating.	52 days		
Bouchouk	Valley of Soummam	Seasonal and hardy variety early flowering with low average fruit set rate: 02.60%. Average kernel pulp ratio: 07, 50. The pulp is difficult to separate from the core. Productivity is average and not very alternating	35 days		
Sigoise	Plain of Sig (Mascara)	Seasonal variety, tolerant to salt water, tolerates cold and drought early flowering of medium intensity. Low fruit set rate: 00.07%. Average kernel pulp ratio: 06,44 The pulp detaches easily from the kernel. Productivity is average and alternating variety in extension throughout the national territory	42 days		

5. Moisture determination

The moisture content is determined by measuring the mass of a sample of olive pulp (5g) before and after the water is removed by evaporation(oven-drying). The percentage of moisture is calculated using the following equation:

$$\% \text{ moisture} = \frac{M_{\text{initial}} - M_{\text{dried}}}{M_{\text{initial}}} \times 100$$

M initial and M dried are the mass of the sample before and after drying, respectively.

6. Phenolic compounds analysis

6.1.Determination of total phenolic content (TPC)

The Folin–Ciocalteu method of Singleton et al.(Singleton et al., 1999)was used to determine total phenolic content. Each olive pulp sample extract (0.2 ml) was mixed with 1ml of Folin-Ciocalteu reagent diluted 10 fold for 5 min and 0.8 ml of 75 g/l sodium carbonate (Na₂CO₃) was then added. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 760 nm against a blank (Shimadzu UV mini1240, Suzhou Jiangsu, China). Gallic acid was used as standard to produce the calibration curve. The mean of four readings was used and the total phenolic content was expressed in g of gallic acid equivalents per 100 g dry weight (g GAE/100 g DW).

6.2.Determination of total flavonoid content (TFC)

The total flavonoid content was determined using the method adapted by Amiri(Amiri, 2014). Briefly, 1 ml of 2% aluminiumtrichloride (AlCl₃) in methanol was mixed with the same volume of olive pulp extract. Absorption readings at 430 nm were measured in UV-visible spectrophotometer after 10 min against a blank. The total flavonoid content was determined using a standard curve with quercetin as the standard. The mean of four readings was used and expressed as milligrams of quercetin equivalents per 100 g dry weight (mg QE/100 g DW) of olive pulp.

6.3.Determination of total flavonol content (TFLC)

The total flavonol content was determined using colorimetric method according to (Jimoh et al., 2010). The extract (500 µl) was added to 500 µl of aluminum chloride (2%) and 500 µl of sodium acetate solution (5%). The absorbance at 440 nm was recorded after 2.5 h at room temperature. Quercetin was used to make the standard calibration curve. Results were expressed as milligrams of quercetin equivalents per 100 g (mg RE/100 g DW).

6.4.Determination of total anthocyanin content (TAC)

Total anthocyanin content was determined according to the method of Abdel-Aal and Hucl(Abel-Aal and Hucl, 1999). A ground olive sample (200 mg) was weighted and 12 mL

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of acidified ethanol (ethanol: HCl 1.0N, 85:15, v/v) was added. The solution was mixed and adjusted to pH 1. The resulting solution was shaken for 30 min. The tube was centrifuged at 5000 rpm for 15 min, and the supernatant was poured into a 25-mL volumetric flask and made up to volume with acidified ethanol. Absorbance was measured at 535 nm against a reagent blank. Total anthocyanin content per sample (mg/kg) was calculated as cyanidin 3-glucoside using the following equation:

$$C = (A/\epsilon) \times (\text{vol}/1000) \times \text{MW} \times (1/\text{sample wt}) \times 10^6$$

$C = (A/\epsilon) \times (\text{Vol}/1000) \times \text{MW} \times (1/\text{sample wt}) \times 10^6$ where C is concentration of total anthocyanin (mg/kg), A is absorbance reading, ϵ is molar absorptivity (cyanidin 3-glucoside = 25,965 cm⁻¹ M⁻¹), Vol is total volume of anthocyanin extract, and MW is molecular weight of cyanidin 3-glucoside = 449.

Under test conditions, the equation formula can be simplified to:

$$C = (A/25965) \times (50/1000) \times 449 \times (1/3) \times 10^6$$

6.5. Antioxidant activities

6.5.1. Determination of total antioxidant

activity (TAA) by phosphomolybdenum method

An aliquot of 0.1 mL of olive pulp extract was mixed with 1 mL of the prepared reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). For the blank, 0.1 mL solvent extraction was mixed with 1 mL of the reagent. The tubes were incubated at 95°C in a water bath for 90 min. The samples were cooled to room temperature and their absorbance was recorded at 695 nm. Ascorbic acid was used as standard. The results were reported as mg equivalents of ascorbic acid per 100g dry weight (mg EAA/100 DW).

6.5.2. Determination of DPPH-radical scavenging activity (DPPH-RSA)

The ability of the olive pulp extract to scavenge the 2,2-diphenyl-1-picrylhydrazyl radical was evaluated according to the method of Blois (Blois, 1958). In the presence of antioxidant which is typical for DPPH free radical decays, the change in absorbency at 517 nm is followed spectrophotometrically. Briefly, 0.9 ml of a freshly prepared methanolic solution of DPPH (0.04 mg/ml) was mixed with 0.1 ml of olive pulp extract. After 20 min of incubation in the dark, at room temperature, absorbencies were read at 517 nm. All tests were performed in triplicate. DPPH radical scavenging activity was calculated according to the calibration curve prepared with trolox and results were expressed as milligram equivalents of trolox per 100g dry weight (mg ET/100 DW).

6.5.3. Determination of ABTS-radical scavenging activity (ABTS-RSA)

The radical scavenging capacity of the tested extracts against ABTS (2, 2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid) radical cation was measured according to the method of Re et al. (Re et al., 1999). $ABTS^{\circ+}$ radical cation was produced by reacting 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1, v/v) and allowing the mixture to stand for 12–16 h in the dark before use. The $ABTS^{\circ+}$ radical cation solution was then diluted with methanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. Each extract sample (0.1 ml) was mixed with 1.4 ml of diluted $ABTS^{\circ+}$ radical cation solution. After reaction for 7 min, the absorbance at 734 nm was measured. Trolox was used as standard substance for calibration curve and results were expressed as milligrams equivalents of trolox per 100g dry weight (mg ET/100 DW).

6.5.4. Determination of the ferric reducing power (FRP)

The method of Oyaizu (Oyaizu, 1986) was used to determine the capacity of the olive pulp extracts to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The samples (1 ml) were mixed with sodium phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%, w/v). The tubes were incubated for 20 min at 50°C. After incubation, the mixture was acidified with 2.5 ml of trichloroacetic acid (10%). Finally, a fraction of the reaction mixture (1 ml) was mixed with distilled water (1 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v). Absorbance of all solutions was measured at 700 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as standard to make calibration curve and results are expressed as milligrams equivalents of ascorbic acid per 100g dry weight (mg EAA/100 DW).

6.5.5. Determination of the iron chelating activity (ICA)

Metal ions chelating activity of the olive pulp extracts was determined by the method of Denis et al. (Denis et al., 1994). Into test tubes, 200 μ L of extract was mixed with 400 μ L of distilled water and 200 μ L of 0.2 mM of $FeSO_4$. The reaction was initiated by the addition of 200 μ L of 2 mM of ferrozine. After 10 min at room temperature, the absorbance of the Fe^{2+} -Ferrozine complex was measured at 562 nm. A lower absorbance indicates a stronger chelating power. Solvent extraction was used as positive control instead of sample and distilled water was used as blank instead of ferrozine. Ethylenediaminetetraacetic acid (EDTA) was used as standard to make calibration curve and results were expressed as milligram equivalents of EDTA per 100g dry weight (mg EEDTA/100 DW).

7. Statistical analysis

Descriptive statistical analysis was performed using Microsoft Excel. The data of moisture contents, antioxidant activities and phenolic compounds are expressed as means \pm standard deviations (SD) from four independent assays (n=4). Statistical analysis was performed using analysis of variance (ANOVA) and significant difference between sample means ($P < 0.05$) was calculated using Tukey's post-hoc test. Difference was considered statistically significant at the level of $p < 0.05$. Pearson correlation test was used to determine the correlation among variables. STATISTICA 5.5 (StatSoft Inc., Oklahoma, USA) was used to perform all these analyses.

Results and discussion

1. Moisture

a. Fresh olives

The moisture content of the three black olive varieties is presented in the **figure 7** Results showed that moisture ranged from 59% (Bouchouk) to 66% (Aharoune). The results were in the following order from low to high: Bouchouk < Sigoise < Aharoune.

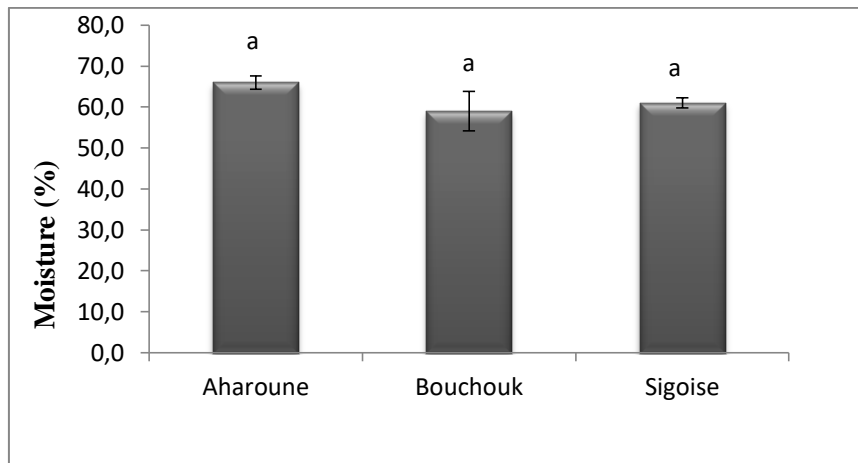


Figure 7: Moisture content of the three fresh olives varieties

The different lowercase letters indicate significant differences ($P < 0.05$) between the varieties studied

Our results were in accordance with those of Uylaser et al., reporting moisture content ranging from 52.81% to 62.19% in Gemlik variety olives grown in Turkey (Uylaser et al., 2008). Kemal Ünal and Cevdet Nergiz found that the moisture content of ripened black olives was 55.37 g/100g when harvested (Ünal and Nergiz, 2003). Usanmaz et al. reported that moisture content of the olives cv. Ayvalik were found to be different for periods and altitudes and ranged from 25.23% (January - 600 m) to 50.53% (October - 300 m) (USANMAZ et al., 2018).

b. Effect of dry salting

The effect of salting on the moisture of the three black olive varieties is presented in table III. Results showed that salting induced decreases in moisture content of olives which ranged from 62.1% (84.7% (Bouchouk)). The moisture content of the salted olives varied from 9% (Bouchouk) to 25% (Aharoune). The moisture was in the following order from low to high: Bouchouk < Sigoise < Aharoune. These decreases in moisture may be due to the dehydration of olives in the presence of salt.

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Table III: Effect of dry salting on the moisture content of the three black olive varieties

Variety	Before Salting (%)	After salting (%)	Decreases (%)
Aharoune	66±1.6 ^a	25±0.9 ^a	-62.1
Bouchouk	59±4.8 ^a	9±1.2 ^c	-84.7
Sigoise	61±1.2 ^a	18± 0.3 ^b	-70.4

Jimenez et al. found that moisture depended on variety and processing method of olives and ranged from 9.6 to 51.5%. They also noted, in Thasos olives, that the storage period of the dry salted olives caused a decrease in the moisture which is in agreement with our results (**Jiménez et al., 2000**).

2. Phenolics

2.1. Total phenolic content (TPC)

a. Fresh olives

The total phenolic content (TPC) of the three black olive varieties is presented in figure 8, Results showed that solvent extraction affect significantly ($P < 0.05$) the TPC which ranged from 2,3 (Bouchouk) to 4,2 g/100g DW (Aharoune) for 60% acetone solvent extraction, from 2,3 (Bouchouk) to 380 g/100g DW (Sigoise) for 60% ethanol solvent extraction and from 1,9 (Bouchouk) to 2,3g/100g DW (Aharoune) for water extraction.

The TPC yields extracted by the selected solvents were in the following order from low to high: Bouchouk < Sigoise < Aharoune for 60% acetone extraction, Bouchouk < Aharoune < Sigoise for 60% ethanol extraction and Bouchouk < Sigoise < Aharoune for water extraction. These results suggest that 60% acetone was the best solvent for extracting TPC from black olives varieties.

Sousa et al. tested tow solvent (methanol and water) on the extraction of TPC from stoned table olives “alcaparras” and found that extraction procedure using water at room temperature was least efficient (very low TPC, 3.48 mg/g dry extract) (**Sousa et al., 2008**), which is in agreement with our results.

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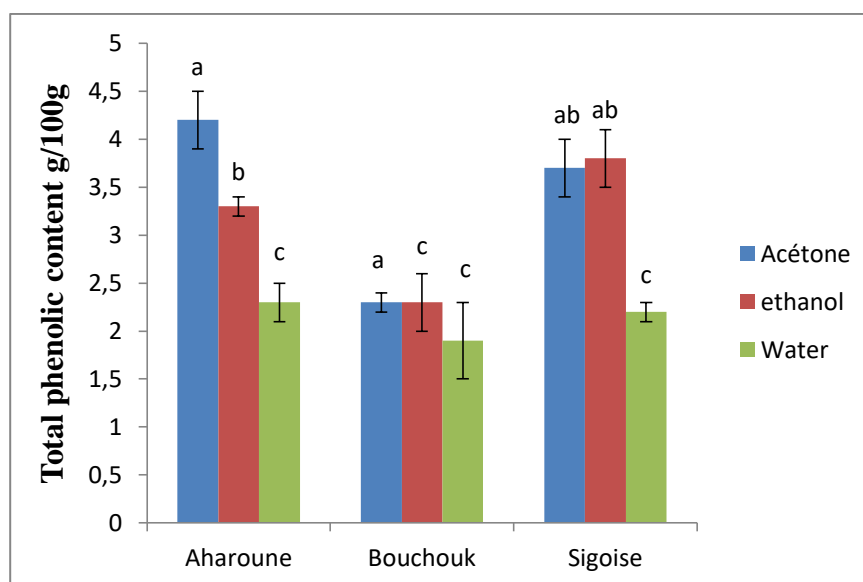


Figure 8:Total phenolic content(TPC) of the three fresh olives varieties

b. Effect of dry salting

The effect of salting on the total phenolic content (TPC) of the three black olive varieties is presented in the table IV. Results showed that the dry salting induces decreases, increases or has no effect according to variety and solvent extraction used. TPC decreases ranged from -18.9% (Sigoise, acetone) to -26% (Bouchouk, ethanol). TPC increases ranged from 9.5% (Aharoune, acetone) to 60.8 (Aharoune water). However, no effect of salting was observed on Bouchouk (water) and Sigoise (ethanol).

The TPC of salted olives varied from 1.7 (Bouchouk, ethanol) to 4.6 g/100g DW (Aharoune, acetone). The TPC yields extracted by the selected solvents for salted olives were in the following order from low to high: Bouchouk < Sigoise < Aharoune for 60% acetone, 60% ethanol and water solvents extraction.

These results demonstrated that the dry salting affects the phenolic compound content of black olives which varied depending on the cultivars. These variations might be related to the dry salting duration which depends on the cultivar. On the other hand, these compounds can be also oxidized when the period of the salting is prolonged.

Our findings are in agreement with those of Soufi et al. (2014) who investigated the effect of dry salting on six Algerian olive cultivars and noted a decrease or an increase, depending on the cultivar, when comparing the ratio *o*-diphenols/phenolics between fresh and salted olives (Soufi et al., 2014). This might be due to the characteristics of each cultivar such as diameter of fruit and/or humidity, since the decrease is related to the diffusion of such

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compounds. The value obtained for Bouchouk cultivar could be explained by its higher fruit diameter among the other studied cultivars. Borzillo et also found that processing black olives by dry salting induced reduction in total phenols (Borzillo et al., 2000).

Previous literature data had reported a decrease in oleuropein content during table olives processing, paralleled to an increase in hydroxytyrosol which derived from hydrolysis of oleuropein. Furthermore, a quantity of verbascoside is hydrolyzed into caffeic acid and hydroxytyrosol (Fernández et al., 1997). However, a decrease in hydroxytyrosol may be done. This decrease might be explained by the oxidation of this compound during the dry salting (Soufi et al., 2014).

Table IV : Effects of dry salting on the total phenolic content (TPC) of the three olives varieties.

Variety	Solvent	Before Salting (g/100gDW)	After salting (g/100gDW)	Decreases or increases (%)
Aharoune	Acetone	4.2 ± 0.3 ^a	4.6 ± 0.3 ^a	+9.5
	Ethanol	3.3 ± 0.1 ^b	4.3 ± 0.1 ^a	+30.3
	Water	2.3 ± 0.2 ^c	3.7 ± 0.1 ^b	+60.8
Bouchouk	Acetone	2.3 ± 0.1 ^a	1.8 ± 0.1 ^d	-21.7
	Ethanol	2.3 ± 0.3 ^c	1.7 ± 0.1 ^d	-26
	Water	1.9 ± 0.4 ^c	1.9 ± 0.2 ^d	No effect
Sigoise	Acetone	3.7 ± 0.3 ^{ab}	3 ± 0.1 ^c	- 18.9
	Ethanol	3.8 ± 0.3 ^{ab}	3.8 ± 0.2 ^b	No effect
	Water	2.2 ± 0.1 ^c	2.6 ± 0.1 ^c	+18.1

Soufi et al. found that TPC in the fresh olives varied from 1,197 (Bouchouk) to 4,355 g GAE/100 g dw (Sigoise from Relizane). The content of these compounds in the salted olives ranged from 1,029 g GAE/100 g dw (Bouchouk) to 2,716 g GAE/100 g dw (Sigoise from Relizane), so decreases of 14.03 and 37.6% reductions. Blekas et al. reported that TPC ranged from 62.3 to 82.9 mg/100g in Thassos black olives cultivar processed in dry salt (Blekas et al., 2002).

2.2. Total flavonoid content (TFC)

a. Fresh olives

The total flavonoid content (TFC) of the three black olive varieties is presented in the figure 9. Results showed that solvent extraction affect significantly ($P < 0.05$) the TFC which ranged from 303.1 (Sigoise) to 513.9 mg/100g DW (Aharoune) for 60% acetone solvent extraction, from 264.6 (Bouchouk) to 587.8 mg/100g DW (Aharoune) for 60% ethanol solvent extraction and from 71.6 (Bouchouk) to 140.7 mg/100g DW (Aharoune) for water extraction.

The TFC yields extracted by the selected solvents were in the following order from low to high: Sigoise < Bouchouk < Aharoune for 60% acetone extraction, Bouchouk < Sigoise < Aharoune for 60% ethanol extraction and Bouchouk < Sigoise < Aharoune for water extraction. These results suggest that 60% ethanol was the best solvent for extracting TFC from black olives varieties.

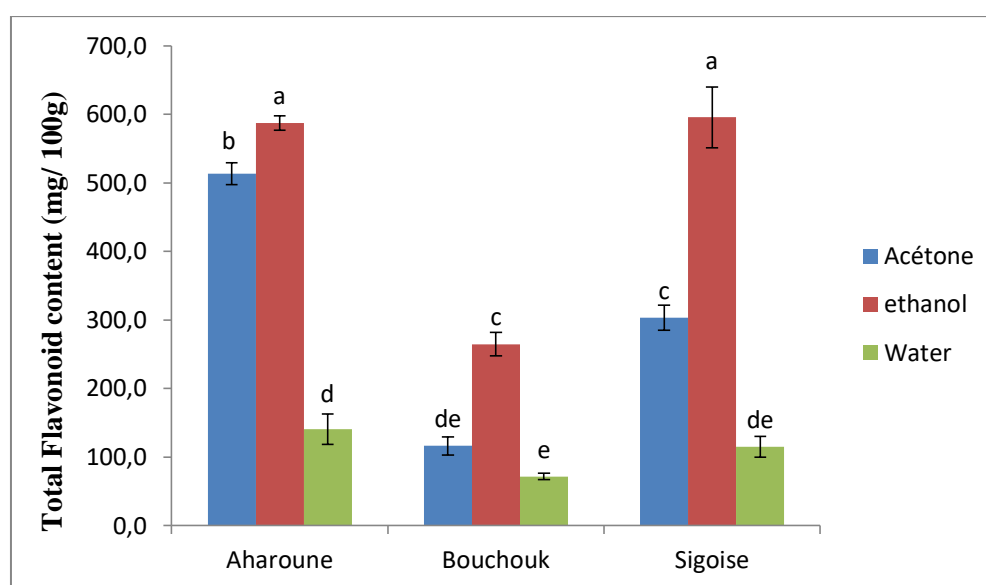


Figure 9: Total flavonoid content (TFC) of the three fresh olives varieties

b. Effect of dry salting

The effect of salting on the total flavonoid content (TFC) of the three black olive varieties is presented in the table V. Results showed that the dry salting induces decreases or increases, according to variety and solvent extraction used. TFC decreases ranged from -25.3% (Aharoune, acetone) to -77.2% (Bouchouk, ethanol). TFC increases ranged from 1.84% (Aharoune, water) to 86.8% (Bouchouk water).

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The TFC of salted olives varied from 60.1 (Bouchouk, ethanol) to 406.2 mg/100g DW (Sigoise, acetone). The TFC yields extracted by the selected solvents for salted olives were in the following order from low to high: Bouchouk < Sigoise < Aharoune for 60% acetone solvent, Bouchouk < Aharoune < Sigoise for 60% ethanol solvent and Sigoise < Aharoune < Bouchouk for water solvent extraction.

Table V: Effects of dry salting on the total flavonoid content (TFC) of the three olives varieties

Variety	Solvent	Before Salting (mg/100g DW)	After salting (mg/100g DW)	Decreases or increase (%)
Aharoune	Acetone	513.9 ± 16 ^b	383.5 ± 6.8 ^a	-25.3
	Ethanol	587.8 ± 10.5 ^a	321.2 ± 13.7 ^b	-45.3
	Water	140.7 ± 22.1 ^d	143.3 ± 14 ^{de}	+1.84
Bouchouk	Acetone	116.2 ± 13.1 ^{de}	70 ± 3.5 ^f	-39.7
	Ethanol	264.6 ± 17.1 ^c	60.1 ± 5.3 ^f	-77.2
	Water	71.6 ± 4.6 ^e	133.8 ± 5.5 ^e	+86.8
Sigoise	Acetone	303.1 ± 18.2 ^c	182.8 ± 3.7 ^c	-39.6
	Ethanol	595.7 ± 44.5 ^a	406.2 ± 26.3 ^a	-31.8
	Water	114.8 ± 15.3 ^{de}	170.1 ± 5.1 ^{cd}	+48.1

Soufi et al. (2016) found total flavonoid contents among Algerian olives cultivars ranged between 872 (Aberkane and Abelout) and 1537 mg CE/100gDW (Azeradj) in fresh olives. These contents are higher than those obtained in this study. However, the flavonoid amounts are comprised only between 394 (Abelout) and 1272 mg CE/100gDW (Azeradj) in salted olives. Consequently, the dry salting caused a decrease in flavonoid contents with a loss rate ranging from 22% (Azeradj) to a mean value of 55% (Abelout and Bouchouk) (Soufi et al., 2016). This decrease can be explained by the diffusion of these compounds under the action of salt and/or their oxidation during salting. Furthermore, the variability of the decrease noted among the studied cultivars can be related to the characteristics of each cultivar such as diameter of fruit, since the decrease is related to the diffusion of such compounds (Bianchi, 2003). In addition, the

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difference of the polarity of each flavonoid compound can also influence their diffusion (Tomas-Barberan and Gil, 2008).

2.3. Total flavonols content (TFLC)

a. Fresh olives

The total flavonols content (TFLC) of the three black olive varieties is presented in figure 10. Results showed that solvent extraction affects significantly ($P < 0.05$) the TFLC, which ranged from 194.6 (Bouchouk) to 316.4 mg/100g DW (Aharoune) for 60% acetone extraction, from 87.6 (Bouchouk) to 187.3 mg/100g DW (Aharoune) for 60% ethanol extraction and from 21.6 (Sigoise) to 53.5 mg/100g DW (Aharoune) for water extraction.

The TFLC yields extracted by the selected solvents were in the following order from low to high: Bouchouk < Sigoise < Aharoune for 60% acetone and 60% ethanol extractions and Sigoise < Bouchouk < Aharoune for water extraction. These results suggest that 60% acetone was the best solvent for extracting TFLC from black olive varieties.

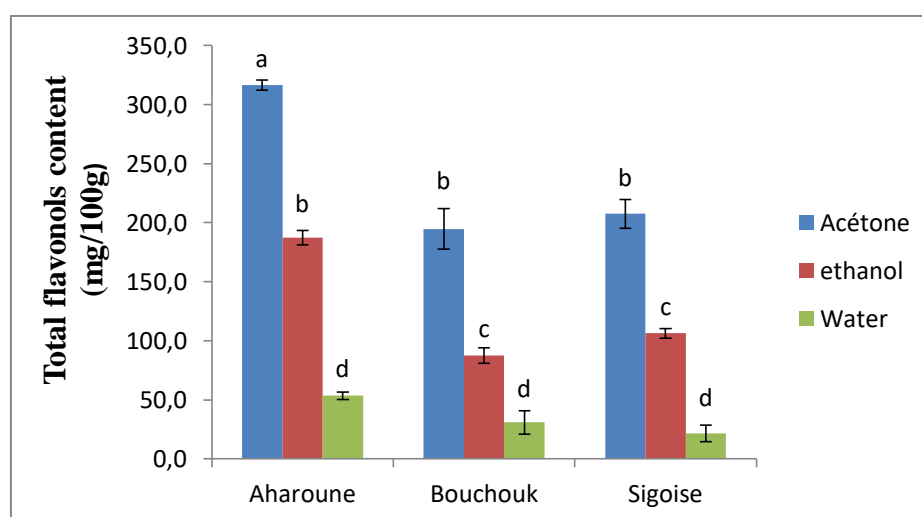


Figure 10: Total flavonols content (TFLC) of the three fresh olive varieties.

b. Effect of dry salting

The effect of salting on the total flavonols content (TFLC) of the three black olive varieties is presented in table VI. Results showed that dry salting induces decreases or increases, according to variety and solvent extraction used. TFLC decreases ranged from -11.1%

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(Aharoune, acetone) to -58.7% (Sigoise, water). TFLC increases ranged from 6% (Sigoise, ethanol) to 189% (Bouchouk water).

The TFLC of salted olives varied from 8.9 (Sigoise water) to 281mg/100g DW (Aharoune, acetone). The TFLC yields extracted by the selected solvents for salted olives were in the following order from low to high: Sigoise<Bouchouk< Aharoune for 60% acetone, 60% ethanol and water solvents. These results suggest that 60% acetone was the best solvent for extracting TFLC from black salted olives varieties.

Table VI: Effects of dry salting on the total flavonols content (TFLC) of the three olives varieties.

Variety	Solvent	Before Salting (mg/100g DW)	After salting (mg/100g DW)	Decreases or increase (%)
Aharoune	Acetone	316.4±4.3 ^a	281±23.7 ^a	-11.1
	Ethanol	187.3±6 ^b	153.8±19 ^b	-17.8
	Water	53.5±3.3 ^d	141.2±6.7 ^{bc}	+163
Bouchouk	Acetone	194.6±17.1 ^b	252.8±5.0 ^a	+29.9
	Ethanol	87.6±6.5 ^c	148.3±14.6 ^{bc}	+69.2
	Water	31±10.0 ^d	89.7±6.4 ^d	+189
Sigoise	Acetone	207.4±12.0 ^b	152.8±16.6 ^b	-26.3
	Ethanol	106.3±4.1 ^c	112.7±26.9 ^{cd}	+6.0
	Water	21.6±6.9 ^d	8.9±3.8 ^e	-58.7

Soufi et al. (2016) noted that the effect of dry salting is dependent on the individual flavonoids; it can induce a decrease (rutin) or an increase (luteolin-7-glucoside). (Soufi et al., 2016). This is in agreement with the data reported by Rice-Evans and Packer (Rice-Evans and Packer, 2003), since salt can generate sodium adducts from flavonol-3-glucoside (rutin), and consequently, the content of the latter decreases. By contrast, these adducts are not obtained from flavone glucoside (luteolin-7-glucoside).

2.4. Total anthocyanin content

a. Fresh olives

The total anthocyanin content(TAC) of the three black olive varieties is presented in the **figure11** Results showed that variety affect significantly ($P < 0.05$) the TAC of olives which ranged from 55.5 (Sigoise) to 152.2 mg/100g DW (Aharoune). The TAC yields were in the following order from low to high:Sigoise<Bouchouk<Aharoune.

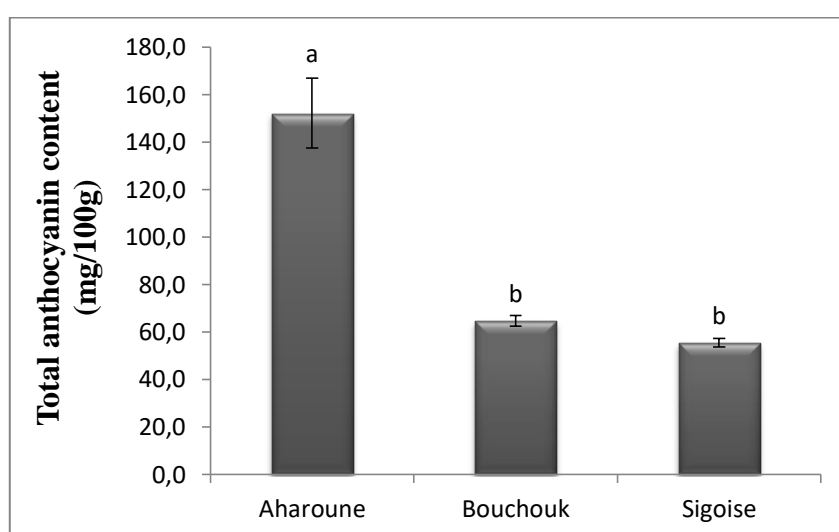


Figure11: Total anthocyanin content (TAC) of the three fresh olives varieties.

b. Effect of dry salting

The effect of salting on the total anthocyanin content(TAC) of the three black olive varieties is presented in tableVII,Results showed that the dry salting induces decreases according to variety. TAC decreases ranged from -26.4% (Aharoune) to -64.6% (Sigoise).The TAC of salted olives varied from 19.6 (Sigoise) to 112 mg/100g DW (Aharoune).

Soufi et al. (2016) observed that dry salting significantly affects ($P<0.05$) the content of olive pigments: the cyanidin-3- glucoside disappeared, but the cyanidin-3-rutinoside is detected only in three cultivars. This can be explained by the fact that anthocyanins are water-soluble compounds which diffused from the olive to the surrounding medium during dry salting. These substances can also be either transformed or degraded during processing (Soufi et al., 2016). According to Garrido-Fernández *et al.* (1997), the anthocyanin contents may be strongly

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influenced by the processing and the cultivar; the total content can decrease to below 50% of its initial value (Garrido-Fernandez et al., 1997), which is in accordance with our results.

Table VII: Effects of dry salting on the total anthocyanin content (TAC) of the three olive varieties.

Variety	Before Salting (mg/100g DW)	After salting (mg/100g DW)	Decreases or increase (%)
Aharoune	152.2±14.7 ^a	112.0±8.5 ^a	-26.4
Bouchouk	64.7±2.2 ^b	31.8±4.6 ^b	-50.8
Sigoise	55.5±1.8 ^b	19.6±2.5 ^b	-64.6

Soufi et al. (2016) observed that dry salting significantly affects ($P < 0.05$) the content of olive pigments: the cyanidin-3- glucoside disappeared, but the cyanidin-3-rutinoside is detected only in three cultivars. This can be explained by the fact that anthocyanins are water-soluble compounds which diffused from the olive to the surrounding medium during dry salting. These substances can also be either transformed or degraded during processing (Soufi et al., 2016). According to Garrido-Fernández *et al.* (1997), the anthocyanin contents may be strongly influenced by the processing and the cultivar; the total content can decrease to below 50% of its initial value, which is in accordance with our results.

2.5. Antioxidant activities

2.5.1. Total antioxidant activity (TAA)

a. Fresh olives

The total antioxidant activity (TAA) of the three black olive varieties is presented in figure 12. Results showed that solvent extraction affects significantly ($P < 0.05$) the TAA which ranged from 3.2 (Bouchouk) to 5.7 g/100g DW (Aharoune) for 60% acetone solvent extraction, from 3.8 (Bouchouk) to 5.4 g/100g DW (For both Aharoune and Sigoise) for 60% ethanol solvent extraction and from 2.8 g/100g DW (Bouchouk) to 6.6 g/100g DW (Aharoune) for water solvent extraction. The TAA values were in the following order from low to high: Sigoise < Bouchouk < Aharoune for 60% acetone extraction, Bouchouk < Sigoise < Aharoune for 60% ethanol and water extraction.

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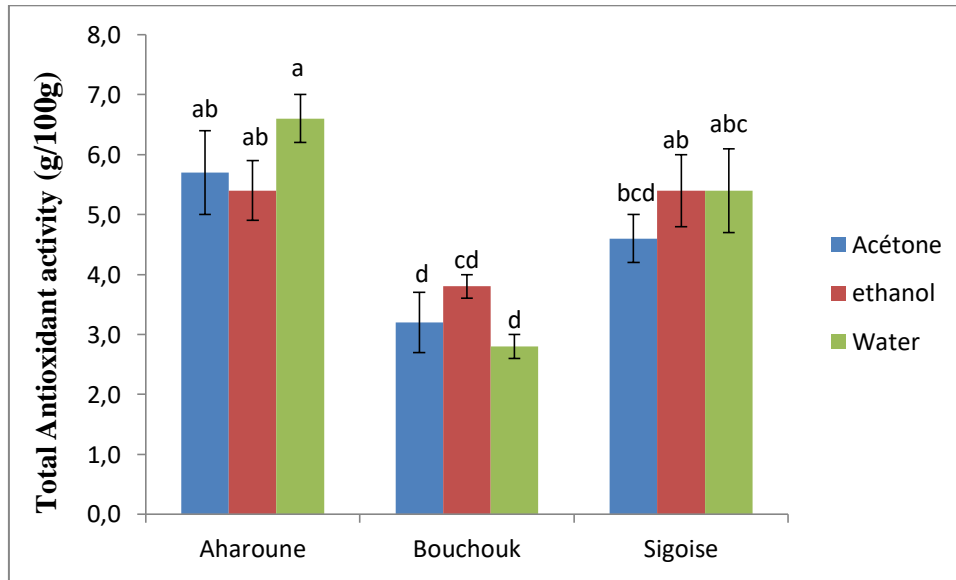


Figure 12: TAA of the three fresh olive varieties

b. Effect of dry salting

The effect of salting on the total antioxidant activity (TAA) of the three black olive varieties is presented in the table VIII. Results showed that the dry salting induces decreases or increases according to variety and solvent extraction used. TAA decreases ranged from -0.3% (Aharoune, water) to -28.9% (Bouchouk, ethanol). TAA increases ranged from 3.7% (Sigoise, ethanol) to 19.2% (Aharoune, acetone).

The TAA of salted olives varied from 2.6 (Bouchouk water) to 6.8g/100g DW (Aharoune, acetone). The TAA of the salted olives were in the following order from low to high: Bouchouk < Sigoise < Aharoune for 60% acetone, Bouchouk < Aharoune < Sigoise for 60% ethanol and Bouchouk < Sigoise < Aharoune for water solvent.

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Table VIII: Effects of dry salting on the total antioxidant activity (TAA) of the three olives varieties.

Variety	Solvent	Before Salting (g/100g DW)	After salting (g/100g DW)	Decreases or increase (%)
Aharoune	Acetone	5.7±0.7 ^{ab}	6.8±0.4 ^a	+19.2
	Ethanol	5.4±0.5 ^{ab}	5.3±0.5 ^{bc}	-1.8
	Water	6.6±0.4 ^a	6.3±0.2 ^{ab}	-0.3
Bouchouk	Acetone	3.2±0.5 ^d	2.8±0.4 ^{de}	-4.54
	Ethanol	3.8±0.2 ^{cd}	2.7±0.4 ^e	-28.9
	Water	2.8±0.2 ^d	2.6±0.4 ^e	-7.14
Sigoise	Acetone	4.6±0.4 ^{bcd}	3.8±0.3 ^d	-17.3
	Ethanol	5.4±0.6 ^{ab}	5.6±0.6 ^{bc}	+3.7
	Water	5.4±0.7 ^{abc}	4.9±0.4 ^c	-9.2

2.5.2. DPPH-radical scavenging activity (DPPH-RSA)

a. Fresh olives

The DPPH-radical scavenging activity (DPPH-RSA) of the three black olive varieties is presented in the figure 13. Results showed that solvent extraction affect significantly ($P < 0.05$) the DPPH-RSA which ranged from 1.1 (Bouchouk) to 2.1 g/100g DW (Aharoune) for 60% acetone solvent extraction, from 1.1 (Bouchouk) to 2.2 g/100g DW (Aharoune) for 60% ethanol solvent extraction and from 0.6 (Bouchouk) to 0.8 g/100g DW (For both Aharoune and Sigoise) for water solvent extraction.

The DPPH-RSA values were in the following order from low to high: Bouchouk < Sigoise < Aharoune for 60% acetone and 60% ethanol extractions and Bouchouk < Aharoune = Sigoise for water solvent extraction.

These results suggest that 60% acetone and 60% ethanol were the best solvent for the DPPH-RSA assay for Aharoune and Bouchouk cultivars since they exhibited the strongest activity. However, for Sigoise variety, 60% acetone seem to be the best solvent for the DPPH-RSA assay. This high DPPH-RSA may be due to the highest content of acetic and ethanolic

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extracts on phenolic compounds having a power antiradical activity comparing to the aqueous extract.

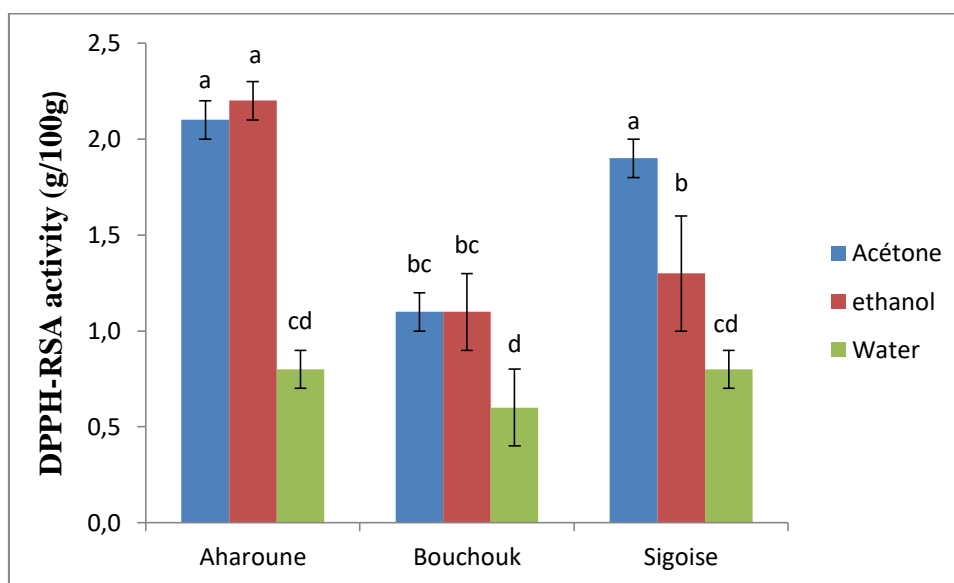


Figure 13: DPPH-RSA of the three fresh olives varieties.

Sousa et al. tested two solvents (methanol and water) on the DPPH-RSA of stoned table olives ‘‘alcaparras’’ and found that aqueous extract obtained at room temperature scavenged DPPH radicals less effectively (61.3% at 5 mg/mL) than extracts obtained using methanol (94.6% at 5 mg/mL) (Sousa et al., 2008), which is in agreement with our results.

b. Effect of dry salting

The effect of salting on the DPPH-RSA of the three black olive varieties is presented in the table IX. Results showed that dry salting induces decreases or increases according to variety and solvent extraction used. DPPH-RSA decreases ranged from -4.54% (Aharoune, ethanol) to -36.3% (Bouchouk, acetone). DPPH-RSA increases ranged from 7.6% (Sigoise, ethanol) to 50% (Aharoune, water).

The DPPH-RSA of the salted olives varied from 0.7 (Bouchouk, both acetone and water) to 2.1 g/100g DW (Aharoune, ethanol). The DPPH-RSA of salted olives were in the following order from low to high: Bouchouk < Sigoise < Aharoune for 60% acetone, 60% ethanol and water solvent. Soufi et al. (2014) reported a significant antiradical activity loss occurred after salting olives with variable values depending on the cultivars. The decrease rate ranged from 29% (Bouchouk and Sigoise) to 58% (Abelout) (Soufi et al., 2014).

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Table IX: Effects of dry salting on the DPPH-RSA of the three olives varieties.

Variety	Solvent	Before Salting (g/100g DW)	After salting (g/100g DW)	Decreases or increase (%)
Aharoune	Acetone	2.1±0.1 ^a	1.7±0.2 ^{ab}	-19
	Ethanol	2.2±0.1 ^a	2.1±0.1 ^a	-4.54
	Water	0.8±0.1 ^{cd}	1.2±0.2 ^{cd}	+50
Bouchouk	Acetone	1.1±0.1 ^{bc}	0.7±0.2 ^e	-36.3
	Ethanol	1.1±0.2 ^{bc}	0.8±0.0 ^e	-27.2
	Water	0.6±0.2 ^d	0.7±0.1 ^e	+16.6
Sigoise	Acetone	1.9±0.1 ^a	1.4±0.2 ^{bc}	-26.3
	Ethanol	1.3±0.3 ^b	1.4±0.1 ^{bc}	+7.6
	Water	0.8±0.1 ^{cd}	0.9±0.1 ^{de}	+12.5

2.5.3. ABTS-radical scavenging activity (ABTS-RSA)

a. Fresh olives

The ABTS-radical scavenging activity (ABTS-RSA) of the three black olives varieties is presented in the figure 14. Results showed that solvent extraction affect significantly ($P < 0.05$) the ABTS-RSA which ranged 2.56 (Bouchouk) to 3.34 g/100g DW (Aharoune) for 60% acetone solvent extraction, from 2.77 (Bouchouk) to 3.37 g/100g DW (Aharoune) for 60% ethanol solvent extraction and from 1.7 (Bouchouk) to 2.48 g/100g DW (Aharoune) for water solvent extraction. The ABTS-RSA values as affected by the solvent extraction were in the following order from low to high: Bouchouk < Sigoise < Aharoune for the three solvents extraction used (60% acetone, 60% ethanol and water).

These results suggest that 60% acetone and 60% ethanol were the best solvent for the ABTS-RSA assay since they exhibited the strongest activity. This high ABTS-RSA may be due to the highest content of acetic and ethanolic extracts on phenolic compounds having a power antiradical activity comparing to the aqueous extract.

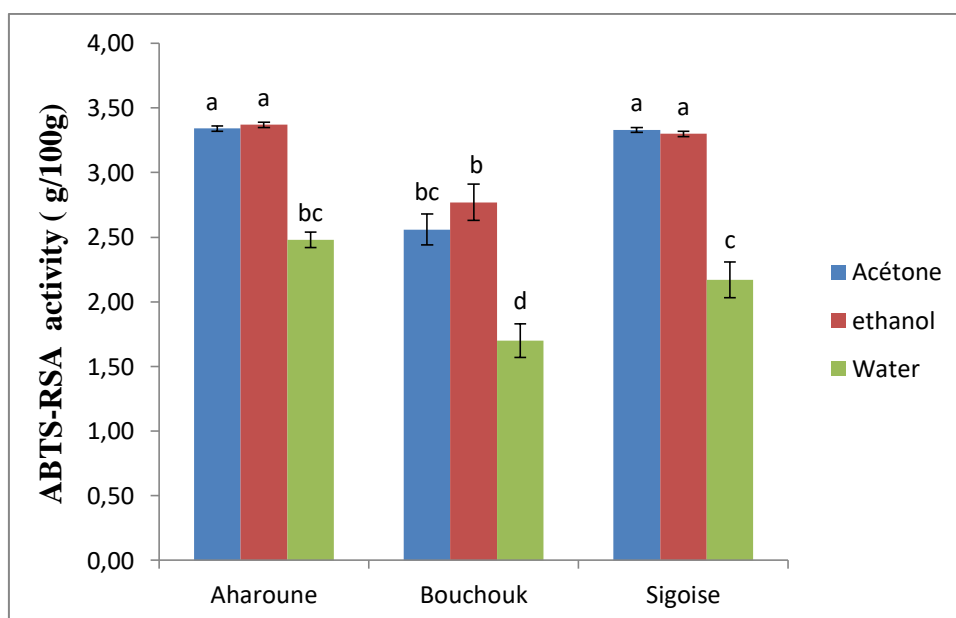


Figure14: ABTS-RSA activity of the three fresh olives varieties.

b. Effect of dry salting

The effect of salting on the ABTS-RSA of the three black olive varieties is presented in the table X. Results showed that dry salting induces decreases or increases according to variety and solvent extraction used. ABTS-RSA decreases ranged from -0.1% (Sigoise, ethanol) to -36.1% (Bouchouk, ethanol). The ABTS-RSA increases ranged from 23% (Sigoise, water) to 33.4% (Aharoune, water).

The ABTS-RSA of the salted olives varied from 1.63 (Bouchouk, water) to 3.3 g/100g DW (Aharoune, acetone and water). The ABTS-RSA values of the salted olives were in the following order from low to high: Bouchouk < Sigoise < Aharoune for 60% acetone, 60% ethanol and water solvent.

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Table X: Effects of dry salting on the ABTS-RSA of the three olives varieties.

Variety	Solvent	Before Salting (g/100g DW)	After salting (g/100g DW)	Decreases or increase (%)
Aharoune	Acetone	3.34±0.02 ^a	3.31±0.01 ^a	-0.89
	Ethanol	3.37±0.02 ^a	3.3±0.003 ^a	-2.07
	Water	2.48±0.06 ^{bc}	3.31±0.03 ^a	+33.4
Bouchouk	Acetone	2.56±0.12 ^{bc}	2.39±0.1 ^b	-6.6
	Ethanol	2.77±0.14 ^b	1.77±0.02 ^c	-36.1
	Water	1.7±0.13 ^d	1.63±0.07 ^c	-4.1
Sigoise	Acetone	3.33±0.02 ^a	3.23±0.02 ^a	-0.1
	Ethanol	3.3±0.02 ^a	3.24±0.15 ^a	-3
	Water	2.17±0.14 ^c	2.68±0.18 ^b	+23.5

2.5.4. Ferric reducing power (FRP)

a. Fresh olives

The ferric reducing power (FRP) of the three black olives varieties is presented in the figure 15. Results showed that solvent extraction affect significantly ($P < 0.05$) the FRP which ranged 1.8 (Bouchouk) to 3.2 g/100g DW (Aharoune) for 60% acetone solvent extraction, from 1.8 (Bouchouk) to 3.2 g/100g DW (Aharoune) for 60% ethanol solvent extraction and from 1.6 (Sigoise) to 1.9 g/100g DW (Aharoune) for water extraction.

The FRP values as affected by the solvent extraction were in the following order from low to high: Bouchouk < Sigoise < Aharoune for 60% acetone and 60% ethanol extractions and Sigoise < Bouchouk < Aharoune for water extraction. These results suggest that 60% acetone and 60% ethanol were the best solvent for the FRP assay since they exhibited the strongest activity. This high FRP may be due to the highest content of acetic and ethanolic extracts on phenolic compounds having a strong ferric reducing power comparing to the aqueous extract

Soufi et al. (2014) observed that lowest reducing power was recorded for Bouchouk cultivar (1.318 g/100g) which is accordance with our results.

Results and discussion

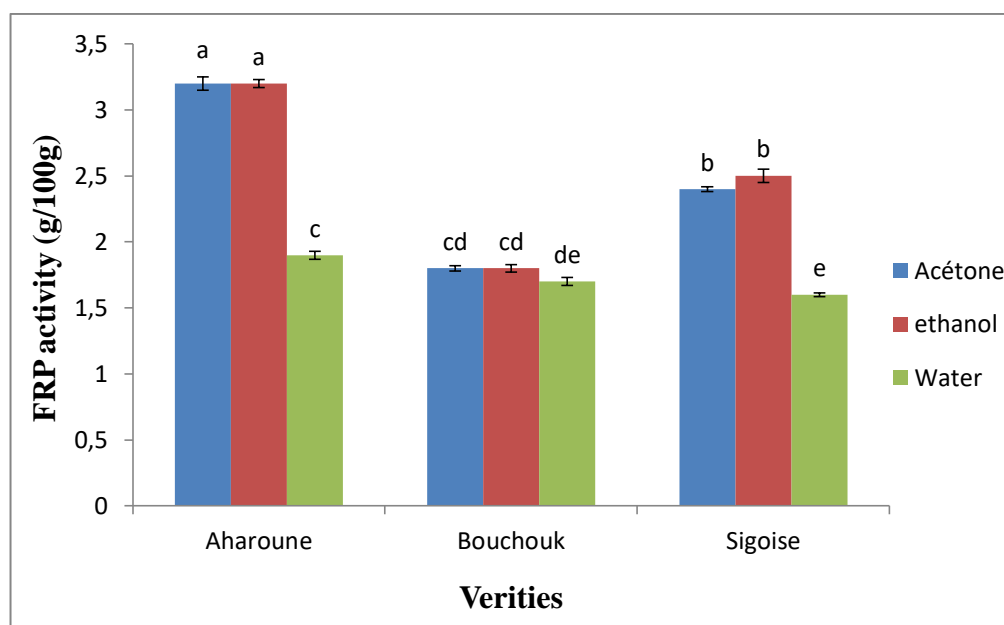


Figure15: FRP of the three fresh olives varieties.

Sousa et al. (Sousa et al., 2008) tested two solvents (methanol and water) on the FRP of stoned table olives “alcaparras” and showed that aqueous extract obtained at room temperature exhibited the lowest reducing power values (0.70 at 5 mg/mL) than extract obtained with methanol (1.5 at 5 mg/mL) which is in agreement with our results.

b. Effect of dry salting

The effect of salting on the FRP of the three black olives varieties is presented in the table XI. Results showed that dry salting induces decreases, increases or has no effect on the FRP, according to the variety and the solvent extraction used. FRP decreases ranged from -5.8% (Bouchouk, water) to -27.7% (Bouchouk, acetone). The FRP increases ranged from 3.1% (Aharoune, acetone) to 47.3% (Aharoune, water). However, salting has no effect on Aharoune (ethanol) and Sigoise (ethanol).

The FRP of the salted olives varied from 1.3 (Bouchouk, acetone) to 3.3 g/100g DW (Aharoune, acetone). The FRP values of the salted olives were in the following order from low to high: Bouchouk < Sigoise < Aharoune for 60% acetone and for water solvents.

Soufi et al. (2014) reported a decrease of the reducing power ranging from 10% (Azeradj) to 35% (Sigoise from Mascara) after processing olives by dry salting (Soufi et al., 2014).

Results and discussion

Table XI: Effects of dry salting on the FRP of the three olives varieties.

Variety	Solvent	Before Salting (g/100g DW)	After salting (g/100g DW)	Decreases or increase (%)
Aharoune	Acetone	3.2±0.05 ^a	3.3±0.15 ^a	+3.1
	Ethanol	3.2±0.03 ^a	3.2±0.06 ^a	No effect
	Water	1.9±0.03 ^c	2.8±0.01 ^b	+47.3
Bouchouk	Acetone	1.8±0.02 ^{cd}	1.3±0.02 ^g	-27.7
	Ethanol	1.8±0.03 ^{cd}	1.4±0.02 ^f	-22.2
	Water	1.7±0.03 ^{de}	1.6±0.05 ^{ef}	-5.8
Sigoise	Acetone	2.4±0.02 ^b	2±0.02 ^d	-16.6
	Ethanol	2.5±0.05 ^b	2.5±0.04 ^c	No effect
	Water	1.6±0.013 ^e	1.7±0.04 ^e	+6.2

Soufi et al. (2016) noted a decrease in ferric reducing capacity except for Bouchouk cultivar, which showed a stable activity. The decrease varied between a mean value of 16% (Abelout and Aberkane) and 41% (Azeradj). This result could be explained by the low contents of flavonoids and/ or other reducing agents in salted olives, since, the antioxidant capacity of flavonoids has been attributed to their electron-donating ability (Morales-Soto et al., 2014).

2.5.5. Iron chelating activity (ICA)

a. Fresh olives

The iron chelating activity (ICA) of the three black olives varieties is presented in the figure 16. Results showed that solvent extraction affect significantly ($P < 0.05$) the ICA which ranged from 2.58 (Sigoise) to 2.66 g/100g DW (Bouchouk) for 60% acetone solvent extraction, from 2.27 (Bouchouk) to 3.32/100g DW (Aharoune) for 60% ethanol solvent extraction and from 0.3 (Aharoune) to 0.45 g/100g DW (Sigoise) for water extraction.

The ICA values of the black fresh olives as affected by the solvent extraction were in the following order from low to high: Sigoise < Aharoune < Bouchouk for 60% acetone extraction Bouchouk < Sigoise < Aharoune for 60% ethanol extraction and Aharoune < Bouchouk < Sigoise for water solvent extraction. These results suggest 60% ethanol were the best solvent for the ICA assay Aharoune variety. However, 60% acetone was the best solvent for the ICA assay

Results and discussion

for Bouchouk and Sigoise cultivars since they exhibited the strongest activity. This powerful ICA may be due to the highest content of acetic and ethanolic extracts on phenolic compounds having a strong iron chelating activity comparing to the aqueous extract.

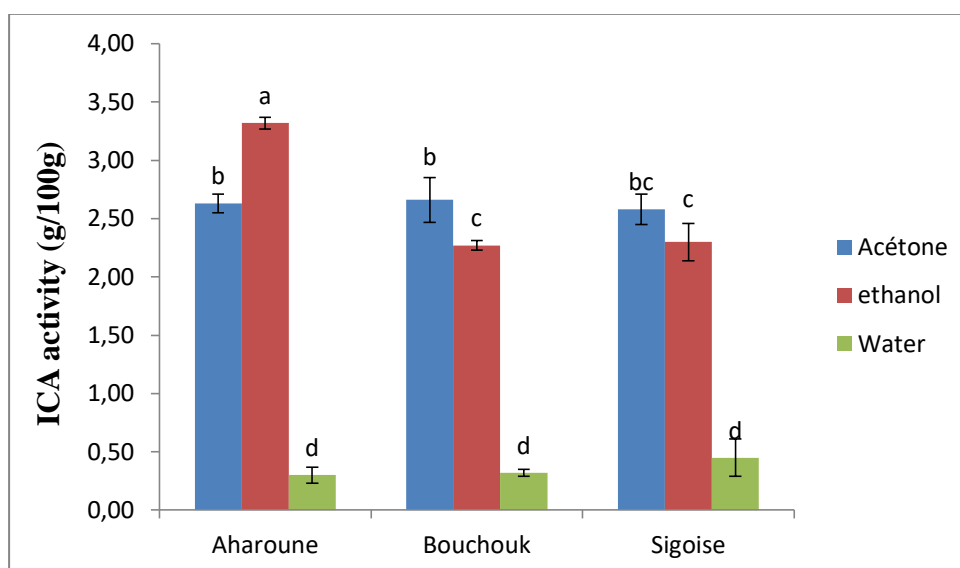


Figure 16:ICA three fresh olives varieties.

b. Effect of dry salting

The effect of salting on the iron chelating activity (ICA) of the three black olives varieties is presented in the tableXII. Results showed that dry salting induces decreases or increases on the ICA, according to the variety and the solvent extraction used. The ICA decreases ranged from -26.6% (Aharoune, ethanol) to -57.5% (Bouchouk, acetone). The ICA increases ranged from 35.5% (Sigoise, water) to 43.3% (Aharoune, water).

The ICA values of the salted olives varied from 0.3 (Aharoune, water) to 3.32 g/100g DW (Aharoune, ethanol). The ICA values of the salted olives were in the following order from low to high: Bouchouk < Sigoise < Aharoune for 60% acetone, 60% ethanol and water solvents.

Soufi et al. (2014) found that processing olives by dry salting induced a decrease in ferrous-chelating activity depending on cultivar and ranging from 10% to 48% (Soufi et al., 2014).

Results and discussion

Table XII: Effects of dry salting on the ICA of the three olives varieties.

Variety	Solvent	Before Salting (g/100g DW)	After salting (g/100g DW)	Decreases or increases (%)
Aharoune	Acetone	2,63±0.08 ^a	1,63±0.11 ^a	-38%
	Ethanol	3,32±0.05 ^b	2,60±0.31 ^a	-26.6%
	Water	0,30±0.07 ^d	0,43±0.10 ^b	+43.3%
Bouchouk	Acetone	2,66±0.19 ^b	1,13±0.01 ^g	-57.5%
	Ethanol	2,27±0.04 ^c	1,41±0.07 ^f	-37.8%
	Water	0,32±0.03 ^d	0,45±0.06 ^{ef}	+40.6%
Sigoise	Acetone	2,58±0.13 ^{bc}	1,56±0.12 ^d	-39.5%
	Ethanol	2,30±0.16 ^c	1,06±0.12 ^c	-53.9%
	Water	0,45±0.16 ^d	0,61±0.19 ^e	+35.5%

3. Pearson correlation analysis

Correlations between different classes of phenolic compounds (TPC, TFC, TFLC, TAC) and the antioxidant activities (TAA, DPPH-RSA, ABTS-RSA, FRP and ICA) were performed using Pearson correlation analysis test (STATISTICA 5.5). (Table XIII)

For fresh olives, high positively Pearson correlation coefficients ($0.41 < r < 0.84$) with p at least <0.05 were found between TPC and antioxidant activities (TAA, DPPH-RSA, ABTS-RSA FRP and ICA). Good linear positive correlations were also observed between antioxidant activities (TAA, DPPH-RSA, ABTS-RSA FRP and ICA) and TFC ($0.47 < r < 0.89$) TFLC ($0.7 < r < 0.82$) and TAC ($0.37 < r < 0.51$). These correlations suggest that fresh olives phenolic compounds, mainly TPC, TFC, TFLC and secondarily TAC, are the main contributors to their antioxidant activities.

For salted olives, strong positive Pearson correlation coefficients ($0.81 < r < 0.95$), with p at least <0.05 , were found between TPC and antioxidant activities (TAA, DPPH-RSA, ABTS-RSA FRP and ICA). Good linear positive correlations ($0.76 < r < 0.99$) were also observed between TFC and antioxidant activities (TAA, DPPH-RSA, ABTS-RSA FRP and ICA). Strong correlation were noted between TAC and TAA ($r = 0.88^{**}$, $p < 0.01$) and FRP ($r = 0.86^*$, $p < 0.05$). However, no correlation was found between TFLC and the different antioxidant activities. These

Results and discussion

correlations suggest that TPC, TFC and TAC are the main contributors to the antioxidant activities of salted olives.

Table XIII : Correlations between TPC, TFC, TFCLC, TAC and the antioxidant activities(TAA, DPPH-RSA, ABTS-RSA, FRP and ICA) of the fresh and salted black olives varieties.

	TFC	TFCLC	TAC	TAA	DPPH-RSA	ABTS-RSA	FRP	ICA
<i>Fresh olives</i>								
TPC	0.84 ^{***}	0.71 ^{***}	0.58 [*]	0.41 [*]	0.77 ^{***}	0.84 ^{***}	0.82 ^{***}	0.59 ^{**}
TFC		0.57 [*]	0.25 ^{ns}	0.47 [*]	0.78 ^{***}	0.86 ^{***}	0.89 ^{***}	0.67 ^{***}
TFCLC			0.79 ^{***}	0.09 ^{ns}	0.82 ^{***}	0.7 ^{***}	0.77 ^{***}	0.80 ^{***}
TAC				0.06 ^{ns}	0.51 ^{**}	0.37 [*]	0.5 ^{**}	0.40 [*]
TAA					0.33 ^{ns}	0.45 [*]	0.45 [*]	0.001 ^{ns}
DPPH-RSA						0.84 ^{***}	0.88 ^{***}	0.77 ^{***}
ABTS-RSA							0.81 ^{***}	0.77 ^{***}
FRP								0.67 ^{***}
ICA								
<i>Salted olives</i>								
TPC	0.98 ^{***}	0.11 ^{ns}	0.83 [*]	0.95 ^{**}	0.81 [*]	0.85 [*]	0.98 ^{***}	0.92 ^{**}
TFC		0.19 ^{ns}	0.87 [*]	0.97 ^{***}	0.76 [*]	0.82 [*]	0.99 ^{***}	0.84 [*]
TFCLC			0.63 ^{ns}	0.26 ^{ns}	-0.3 ^{ns}	-0.35 ^{ns}	0.16 ^{ns}	-0.23 ^{ns}
TAC				0.88 ^{**}	0.40 ^{ns}	0.47 ^{ns}	0.86 [*]	0.57 ^{ns}
TAA					0.73 ^{ns}	0.75 ^{ns}	0.97 ^{***}	0.78 [*]
DPPH-RSA						0.85 [*]	0.76 [*]	0.9 ^{**}
ABTS-RSA							0.84 [*]	0.91 ^{**}
FRP								0.85 [*]
ICA								

*, ** and *** indicate significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Conclusion

Conclusion

Conclusion

This study aimed to investigate the phenolic contents (total phenolic content; TPC, total flavonoid content; TFC, total flavonol content; TFLC and total anthocyanin content; TAC) and the antioxidant activities (total antioxidant activity; TAA, DPPH radical-scavenging activity; DPPH-RSA, ABTS radical-scavenging activity; ABTS-RSA, ferric reducing power; FRP and iron chelating activity; ICA) of three fresh black Algerian olives cultivars (Aharoune, Bouchouk and Sigoise) cultivated in the region of Bejaia, to study the effect of three solvents extraction (60% acetone, 60% ethanol and water) on the extraction of phenolics and on the antioxidant capacity of the three fresh olives varieties in order to find the best solvent extraction to recovery the maximum phenols from olives and to correlate their levels with the antioxidant activity of the obtained extracts and finally to test the effect of a traditional table olive processing (dry salting) on the phenolic compound contents and on the antioxidant activities of the three Algerian black olives cultivars.

Results showed that variety and solvent extraction affected significantly the TPC, TFC, TFLC, TAC and the antioxidant activities (TAA, DPPH-RSA, ABTS-RSA, FRP and ICA). The TPC ranged from 1.9 g/100g (Bouchouk, water) to 4.2 g/100g DW (Aharoun, acetone), TFC from 71.6 mg/100g (Bouchouk, water) to 595.7 mg/100g DW (Sigoise, acetone), TFLC from 21.6 mg/100g (Sigoise, water) to 316.4 mg/100g DW (Aharoune, acetone) on 60% acetone solvent, TAC from 55.5 mg/100g (Sigoise) to 152.2 mg/100g DW (Aharoune), TAA from 2.8 g/100g (Bouchouk, water) to 6.6 g/100g DW (Aharoune, water), DPPH-RSA from 0.6 (Bouchouk, water) to 2.2 g/100g DW (Aharoune, ethanol). ABTS-RSA from 1.7 (Bouchouk, water) to 3.37 g/100g DW (Aharoune, ethanol), FRP from 1.6 g/100g (Sigoise, water) to 3.2 g/100g (Aharoune, acetone and ethanol) and finally, the ICA activity ranged from 0.3 (Aharoune, water) to 3.32 g/100g DW (Aharoune, acetone). These results suggest that 60% acetone was the best solvent for extracting phenolic compounds from fresh black olives, followed by 60% ethanol and water.

Results showed also that dry salting of black olives induces decreases, increases or had no effect on the phenolic compound contents and on the antioxidant activities of black olives depending on the variety and the solvent extraction used.

The cultivar and the solvent extraction also affected the phenolic compound contents and on the antioxidant activities of the salted olives. The TPC ranged from 1.7 (Bouchouk, ethanol) to 4.6 g/100g DW (Aharoune, acetone), TFC from 60.1 (Bouchouk, ethanol) to 406.2 mg/100g DW (Sigoise, acetone), from 8.9 (Sigoise water) to 281 mg/100g DW (Aharoune, acetone), TAC

Conclusion

from 19.6 (Sigoise) to 112 mg/100g DW (Aharoune), TAA from 2.6 (Bouchouk water) to 6.8 g/100g DW (Aharoune, acetone), DPPH-RSA from 0.7 (Bouchouk, both acetone and water) to 2.1 g/100g DW (Aharoune, ethanol), ABTS-RSA from 1.63 (Bouchouk, water) to 3.3 g/100g DW (Aharoune, acetone and water), FRP from 1.3 (Bouchouk, acetone) to 3.3 g/100g DW (Aharoune, acetone) and finally ICA from 0.3 (Aharoune, water) to 3.32 g/100g DW (Aharoune, ethanol). These results suggest that 60% acetone was the best solvent for extracting phenolic compounds from salted olives, followed by 60% ethanol and water.

Pearson correlation analysis showed good linear positive correlations between phenolic compounds and the several antioxidant activities, suggesting that phenolic compounds are the main contributors to the antioxidant potential of black olives.

Finally, we can conclude that black olives (fresh and salted) are a good source of phenolic compounds displaying high antioxidant activities (antiradical scavenger activity, ferric reducing power and iron chelating activity) and therefore, fresh black olives extracts could be used as potential functional ingredients or additives in food industry, cosmetics and medicine. Additionally, the consumption of black salted olives must be encouraged.

In order to complete this work, it would be interesting to:

- Study other olives varieties and other table olives processing method (Spanish-style, Californian-style).
- Use advanced analysis techniques (HPLC, LC/MS, etc.) to identify the phenolic compounds present in black olives varieties responsible of their antioxidant activities.
- Study other biological activities of the black olives extracts such as antibacterial and antifungal activities.
- Evaluate the cytotoxicity effect of black olives extracts.
- Finally, to realize *in vivo* tests of the black olives extracts in order to determine their effects on animal health such anti-inflammatory, anti-diabetic, anticancer activities,...ect.

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Annex

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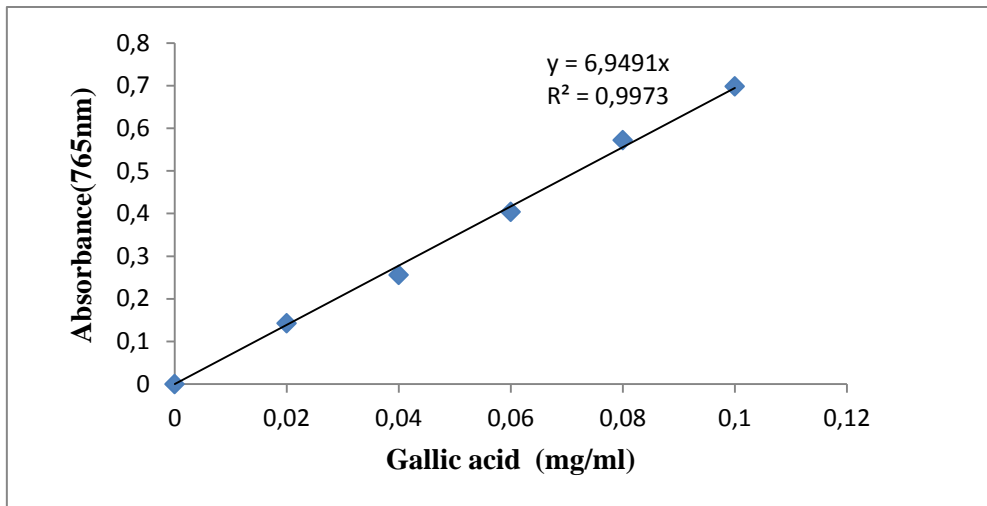


Figure 1: Total Polyphenols calibration curve

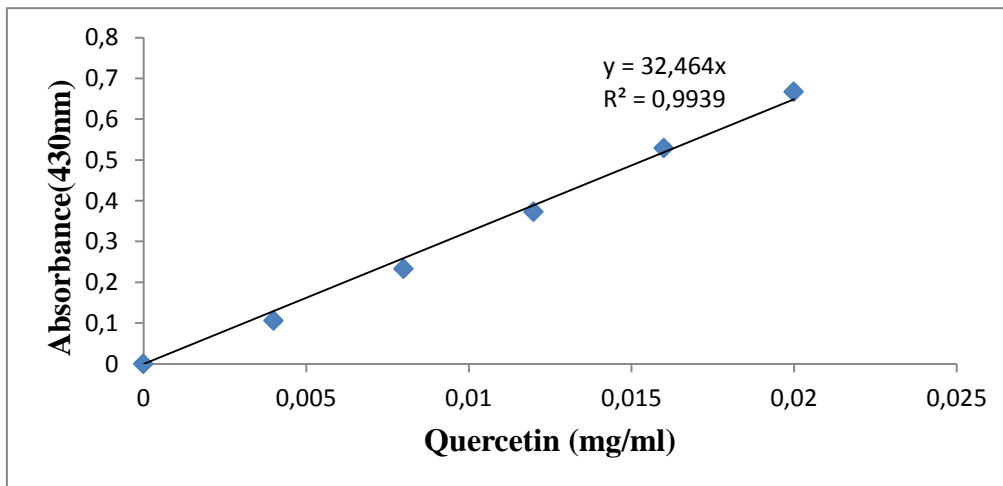


Figure 2: Total Flavonoid calibration curve

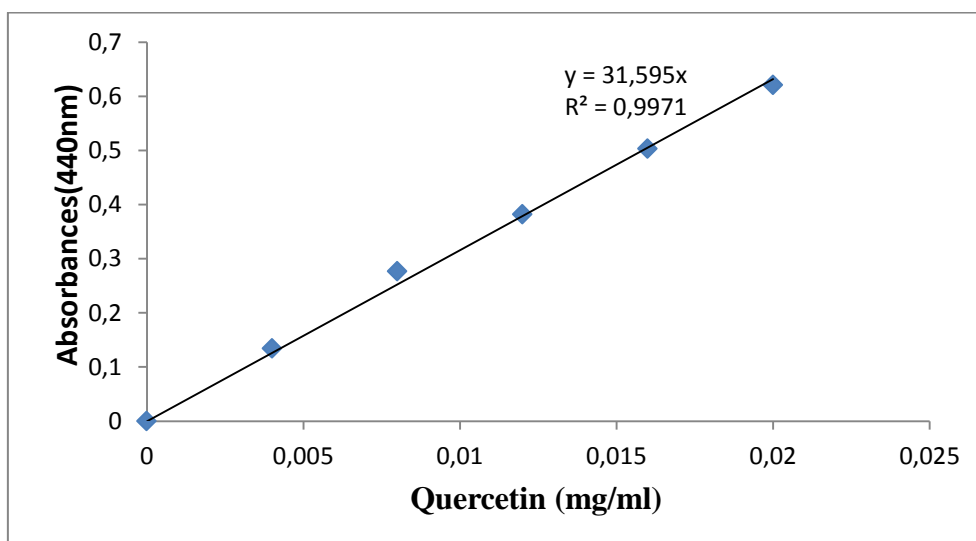


Figure 3: Flavonols calibration curve

Annex

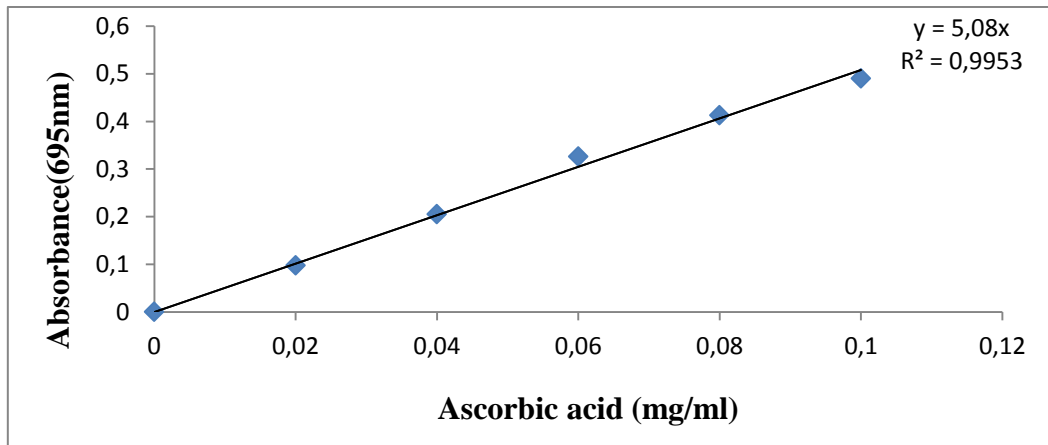


Figure 4: Total antioxidant activity calibration curve

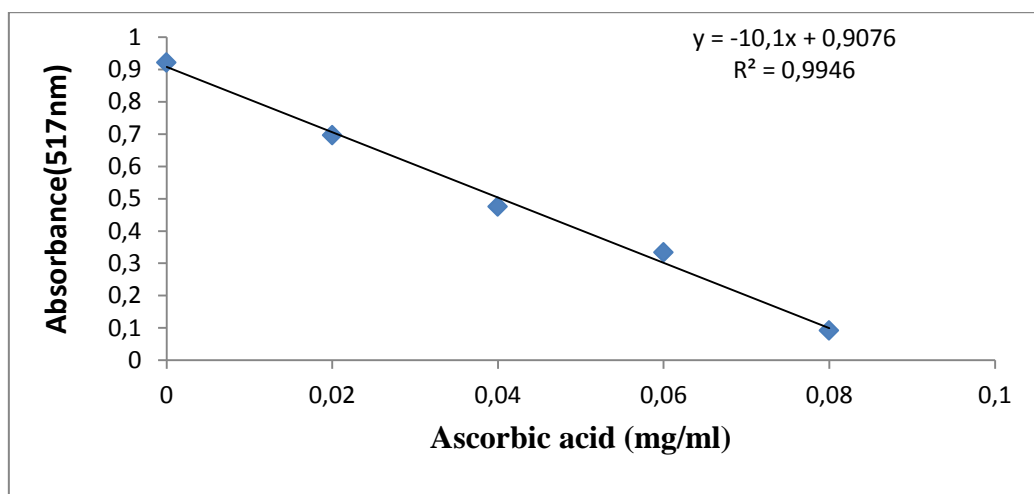


Figure 5: DPPH-RSA activity calibration curve

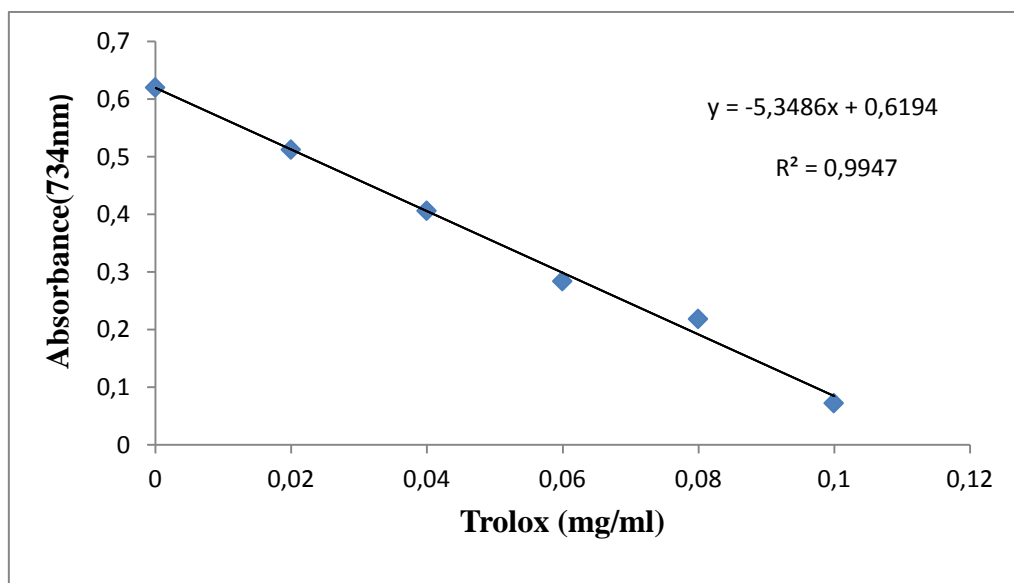


Figure 6: ABTS-RSA activity calibration curve

Annex

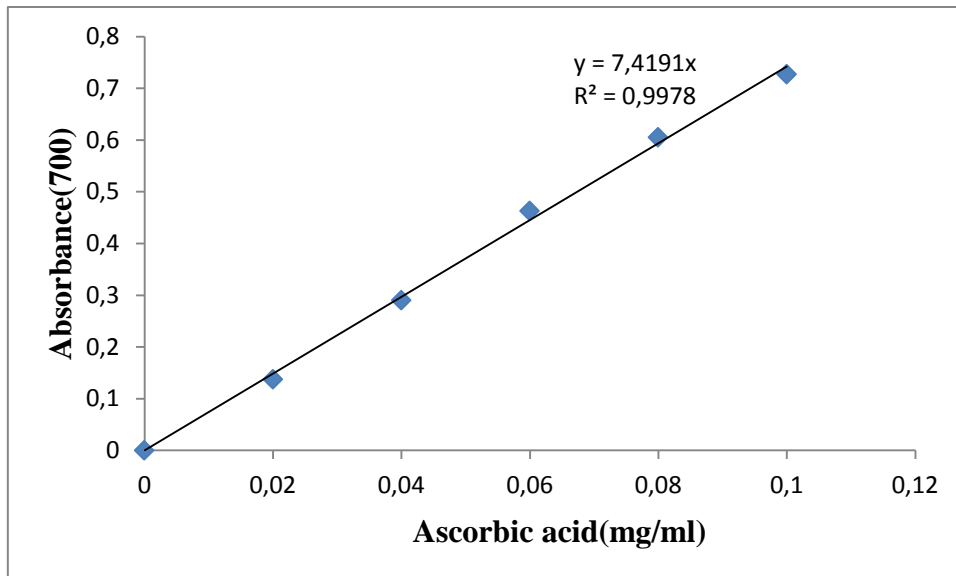


Figure 7: FRP activity calibration curve

Abstract: This study aims to investigate the effects of solvent extraction and dry salting on the phenolic contents (total phenolic content; TPC, total flavonoid content; TFC, total flavonol content; TFLC and total anthocyanin content; TAC) and the antioxidant activities (total antioxidant activity; TAA, DPPH radical-scavenging activity; DPPH-RSA, ABTS radical-scavenging activity; ABTS-RSA, ferric reducing power; FRP and iron chelating activity; ICA) of three fresh black Algerian olives cultivars (Aharoune, Bouchouk and Sigoise) cultivated in the region of Bejaia. Results showed that variety and solvent extraction affected significantly the TPC, TFC, TFLC, TAC and the antioxidant activities (TAA, DPPH-RSA, ABTS-RSA, FRP and ICA). 60% acetone was the best solvent for extracting phenolic compounds from fresh black olives, followed by 60% ethanol and water. Results showed also that dry salting of black olives induces decreases, increases or had no effect on the phenolic compound contents and on the antioxidant activities of black olives depending on the variety and the solvent extraction used. Pearson correlation analysis showed good linear positive correlations between phenolic compounds and the antioxidant activities, suggesting that black olives phenolic compounds are the main contributors to their antioxidant potential. Finally, we can conclude that black olives (fresh and salted) are a good source of phenolic compounds displaying high antioxidant activities (antiradical scavenger activity, ferric reducing power and iron chelating activity) and therefore, fresh black olives extracts could be used as potential functional ingredients or additives in food industry, cosmetics and medicine. Additionally, the consumption of black salted olives must be encouraged.

Keywords: black olives, variety, phenolic compounds, antioxidant activity, dry salting, solvent, increase, decrease.

Abstract: This study aims to investigate the effects of solvent extraction and dry salting on the phenolic contents (total phenolic content; TPC, total flavonoid content; TFC, total flavonol content; TFLC and total anthocyanin content; TAC) and the antioxidant activities (total antioxidant activity; TAA, DPPH radical-scavenging activity; DPPH-RSA, ABTS radical-scavenging activity; ABTS-RSA, ferric reducing power; FRP and iron chelating activity; ICA) of three fresh black Algerian olives cultivars (Aharoune, Bouchouk and Sigoise) cultivated in the region of Bejaia. Results showed that variety and solvent extraction affected significantly the TPC, TFC, TFLC, TAC and the antioxidant activities (TAA, DPPH-RSA, ABTS-RSA, FRP and ICA). 60% acetone was the best solvent for extracting phenolic compounds from fresh black olives, followed by 60% ethanol and water. Results showed also that dry salting of black olives induces decreases, increases or had no effect on the phenolic compound contents and on the antioxidant activities of black olives depending on the variety and the solvent extraction used. Pearson correlation analysis showed good linear positive correlations between phenolic compounds and the antioxidant activities, suggesting that black olives phenolic compounds are the main contributors to their antioxidant potential. Finally, we can conclude that black olives (fresh and salted) are a good source of phenolic compounds displaying high antioxidant activities (antiradical scavenger activity, ferric reducing power and iron chelating activity) and therefore, fresh black olives extracts could be used as potential functional ingredients or additives in food industry, cosmetics and medicine. Additionally, the consumption of black salted olives must be encouraged.

Keywords: black olives, variety, phenolic compounds, antioxidant activity, dry salting, solvent, increase, decrease.

Résumé: Cette étude vise à étudier les effets du solvant d'extraction et du salage à sec sur les teneurs en composés phénoliques (polyphénols totaux ; PT, flavonoïdes totaux; FT, flavonols totaux; FLT et en anthocyanines totales; AT) et sur les activités antioxydantes (activité antioxydante totale ; AAT, activité anti-radicalaire; DPPH-RSA, activité anti-radicalaire; ABTS-RSA, pouvoir réducteur; PR et activité chélatrice du fer ; ACF) de trois variétés d'olives noires algériennes fraîches (Aharoune, Bouchouk et Sigoise) cultivées dans la région de Béjaïa. Les résultats ont montré que la variété et le solvant d'extraction affectaient significativement les PT, FT, FLT, AT et les activités antioxydantes (AAT, DPPH-RSA, ABTS-RSA, PR et ACF). L'acétone 60% était le meilleur solvant pour extraire les composés phénoliques des olives noires fraîches, suivi de l'éthanol 60% et l'eau. Les résultats ont également montré que le salage à sec des olives noires induit des diminutions, des augmentations ou n'avait aucun effet sur les teneurs en composés phénoliques et sur les activités antioxydantes des olives noires selon la variété et le solvant d'extraction utilisé. L'analyse Pearson des corrélations a montré de bonnes corrélations positives linéaires entre les composés phénoliques et les activités antioxydantes, suggérant que les composés phénoliques des olives noires sont les principaux contributeurs à leur potentiel antioxydant. Enfin, nous pouvons conclure que les olives noires (fraîches et salées) sont une bonne source de composés phénoliques présentant des activités antioxydantes élevées (activité antiradicalaire, pouvoir réducteur et activité chélatrice du fer) et par conséquent, les extraits d'olives noires fraîches pourraient être utilisés comme ingrédients fonctionnel ou additifs dans l'industrie alimentaire, cosmétique et médicale. De plus, la consommation d'olives noires salées doit être encouragée.

Mots clés : olives noires, variété, composés phénoliques, activité antioxydante, salage à sec, solvant, augmentation, diminution.