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Réf :.....

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En vue de l'obtention du diplôme

MASTER

Etude phytochimique de la feuille de vigne et formulation d'un aliment fonctionnel

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HIHAT ZINA & OURARI CELIA

DEDICATION

For those which gave me everything without anything in return
There are no words to describe how much my parent has meant
to me throughout all my life.

***Mom**, you have given me so much, thanks for your faith in me,*
and for teaching me that I should never give up. Thanks for
Lending me your ear on countless occasions when I needed to
Vent my frustrations …

***Daddy**, you have always been there for me with encouraging*
words
Thank you for your love and support. Without you, my life
Would fall apart.

To my Brother Brahim and sisters Nassima, Kahina, Célia
To my beloved nephews (Amine, Hidayette, Djibrile, Rachel)

To the person who loved and supported me so much who has been with me in all stages I shall I will be indebted to You all my life Thanks for all
Djallal

A special dedication to our dear co-promoter Landri Kenza for her presence her encouragements and advices without her this work would not be so perfect

To my binomial and friend with which I have division a good
Moments Ourari Célia and her Family

To all my friends and colleagues.

HIHAT ZINA

DEDICATION

With my deepest feelings of gratitude,

I dedicate this modest work

For those which gave me everything without anything in return

There are no words to describe how much my parent has meant to me throughout all my life.

***Mom,** you have given me so much, thanks for your faith in me, and for teaching me that I should never give up. Thanks for lending me your ear on countless occasions when I needed to vent my frustrations …*

***Daddy,** you have always been there for me with encouraging Words .Thank you for your love and support. Without you, my life would fall apart.*

To my lovely sisters Nouara and lamia, My brothers Halim and his wife Souad and my beloved little niece Dacinette , my dear brothers Mahdi and Ghilas .

For the source of my happiness yacine, his support, his encouragement, his presence and for all and all his family you are everything for.

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To my uncles and aunts , cousins and cousins

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Ourari Célia

List of abbreviation

ABTS : Acide 2,2'-azino-bis (3-éthylbenzothiazoline-6-sulphonique)

ANC: Anthocyanins content

°C: Celcius degrees

DPPH: 2, 2-Diphenyl-1-picrylhydrazyl

HSD: High standard deviation

LAB : Lactic acid bacteria

Set: Steamed

CT: Condensed tannins

TFC: Total Flavonoid Content

TPC: Total Phenolic Content

GAE: Gallic Acid Equivalent

EQ : Quercetin equivalent

pH : Hydrogen potential

3 BS: Biomathematics, Biochemistry, Biophysics and Scientometrics

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Summary

Introduction

Many biochemical and physiological processes induce the production of free radicals in the living organism, including their accumulation in the human body, due to an imbalance in the oxidants / antioxidants ratio which causes oxidative stress, this state is involved in most human diseases such as diabetes, cardiovascular diseases and certain types of cancer, which result from the action of these free radicals on biological molecules (proteins, DNA and lipids) **(Poulson, Eppler et al. 1998).**

Epidemiological evidence has shown that an increase in the intake of fruits and vegetables and their derivatives, is a good protective way against oxidative stress. The preventive effect of these foods results from the presence of a range of molecules including polyphenols, flavonoids and some vitamins that oppose the harmful action of these free radicals **(Wang, Cao et al. 1996; Kent, Sugnet et al. 2002; Richard, Melikov et al. 2003**).This could be the reason for which the consumer current demands in the field of food production has changed considerably. These products are not intended to only satisfy hunger and to provide necessary nutrients for humans but also to prevent nutrition-related diseases and improve physical and mental wellbeing of the consumers, who more and more believe that foods contribute directly to their health.

People have always thought that the source of antioxidants is only done by consuming fruits and vegetables but most of them are unaware that the tree that gives the fruit is richer than the fruit itself.

Often neglected and considered waste, the vine leaf including *Vitis vinifera L.* the most widely cultivated and economically important fruit crop in the world. Many grapevine varieties are also grown for their food products, not only for table grapes, but also for the consumption of their leaves. Due to their astringent and hemostatic properties and phenolic composition, grape leaves are considered as a healthy food and are consumed in several countries, including Saudi Arabia, Lebanon, Turkey and Greece **(Koşar, Küpeli et al. 2007).**The biochemical composition of both grapes and leaves is determinant for their nutritional value and taste **(Maia, Monteiro et al. 2016**).

On the other hand the fermented dairy products already have a positive image for the health because of the beneficial action of its viable bacteria **(Gimeno Sendra 2010)**. The

yogurt appears a food of choice within the frame work of a balanced diet. Indeed, it is rich in proteins and assures a contribution of vitamins and trace elements, particularly calcium, while having a moderate energy contribution **(Yahia, Hamadou et al. 2012**). However, the presence of antioxidants in this fermented milk is weak and can contain artificial additives.

This study was carried out, in the view of the health promoting properties and high nutritional benefits of *Vitis Vinifera L.* leaves, from one hand to promote this neglected by-product and on the other hand to prepare a steamed yogurt at laboratory scale enriched with dried powder of grape leaves. After physicochemical and microbiological analysis of yogurts the antioxidant capacity and the total phenolic compounds were determined. Finally a sensory evaluation of yogurts was performed.

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I. Overview of vine

The vine is one of the oldest plants; it has been present for millennia in many parts of the world **(Demelin 1986)**.The vine is native to the Near East, but cultivated since antiquity in all the Mediterranean basin. Today, its culture is widespread worldwide in the region where climate is similar to the Mediterranean one (California, Australia) **(Ozenda 2000)**.The cultivated forms are classified in *Vitis vinifera sp. Vinifera* subspecies. They are mainly grow in the Mediterranean region and have a lifespan of about fifty year **(Demelin 1986)**.The medical and nutritional value of grapes has been indicated for thousands of years. The Egyptians consumed this fruit at least 6000 years ago and several Greek philosophers praised the healing power of grapes **(Busserolles, Gueux et al. 2006)**.

Vernacular names

French: vigne rouge, **English**: red leaf vine, Grape Vine, red vine, **German**: RoterWeinlaub, Rebeblätter, **Italian**: Quick rossa , **Spanish**: Hojas de vidroja, **Portuguese**: videira , **Greek**: ΚόκκινοΑ μπέλι, **Arabic**: Elkarama Nabthia Elhamraa.

1.1. Morphological description

Vitis vinifera L. (Vitaceae) is a perennial, woody vine, usually climbing by tendrils.The different partsare: the vine or trunk, the vine shoot (climbing stem), the leaves, the flowers, the stalks, and the fruits or grapes. The vine can measure up to a meter high. The leaves of vines are deciduous, alternate, palmatilobed or palmate or pinnate and are opposed to tendrils or grapes (Fig.01). They are equipped with Stipules and have a long petiole.The webbed vine leaves have five main lobes more or less cut, and are heart-shaped at the base. The leaves are green of variable intensity, and are generally pubescent on the reverse **(Botineau and Pelt 2002)**.

1.2. Classification of grapes leaves

Kingdom: Plantae

Deputy règne:Tracheobionta

Division: Magnoliophyta

Class :Magnoliopsida

Subclass: Rosidae

Order: Vital

Family: Vitaceae

Gender: Vitis

Species: *Vitis vinifera*

Figure 01: Photography of ***Vitis vinifera L.*** **(Bremer, Bremer et al. 2009).**

1.3. Distribution of grape cultivation

1.3.1. In the world

Grapes from the genus *Vitis* are the fruit of the highest fruit production in the world in terms of quantity and economic importance **(Vivier and Pretorius 2002)**. Table grapes are the third most-consumed fruit in the world behind bananas and apples **(OIV 2017, Statista 2017)**. The size of the world vineyard in 2016 amounts to 7.5 million hectares (Fig.02). China is the largest producer of grapes with 12.6 million tones followed by Italy (8.6 million tons), the United States (7.0 million tons) and France (6.3 million tons) **(OIV, 2017)**.

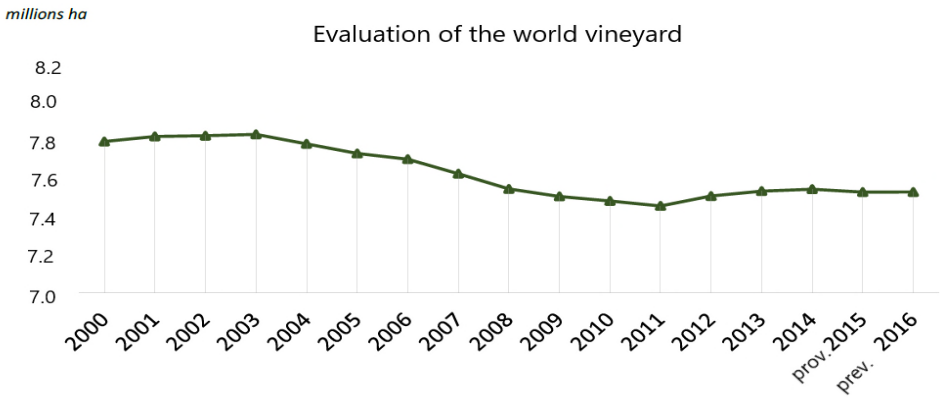


Figure 02: Evaluation of the world vineyard **(OIV, 2017).**

1.3.2. In Algeria

Algerian viticulture has undergone significant fluctuations during the last centuries. During the French colonization, it is characterized by a very strong development in the production of wine grapes (1854000 tons produced in 1961) **(FAO, 2017)**.

However, our country experienced a regression during the 1980s during which the vines were voluntarily torn off to be replaced by table grape, from a vineyard area of 345714 ha (1965) to 105640ha (1989). with productions respectively of 1902000 and 270426 tonnes according to **(FAO, 2017)**.

Over the last two decades, increases in table grape production have been recorded, rising from 141294 (1995) to 518035 (2014) tonnes **(FAO, 2017)**.

In Algeria, grape cultivation comes in 12th position after olives **(FAO, 2017)**. According to the Ministry of Agriculture, the Bejaia city comes in 14th position at the national level. for the area planted with vineyards in the Bejaia city are 460.66 ha (hectares). with a related area of 450.75 ha(hectares).. The total grape production obtained is estimated at 29195 qx (quintaux) (Fig. 03) **(DSA, 2015)**.

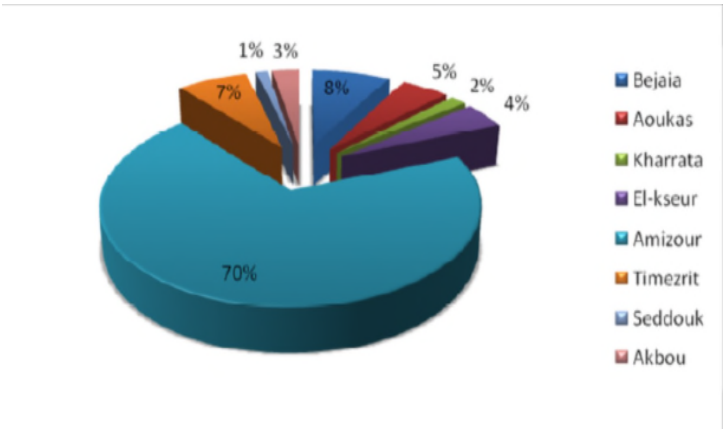


Figure 03: Total production as a percentage of grapes at Bejaia city **(DSA, 2015)**.

1.4. Biochemical composition of grape leaves

The biochemical composition of the grape leaves is decisive for their nutritious value and their taste **(Maia, Monteiro et al. 2016)**.The leaves of this plant are rich in tannins, flavonoids and procyanidins. Additionally, the leaves also contain organic acids, lipids, enzymes and vitamins **(Bombardelli and Morazzoni 1995; Felicio, Santos et al. 2001)** including anthocyanins and proanthocyanidins, sugars, sterols, amino acids and minerals **(Handoussa, Hanafi et al. 2013)**.Tartrate, inositol,choline **(Anderson, Druzgal et al. 2010)**. The energy value of the vine leaves is 0,60 UF / kg MS **(Magnier 1991)**.

The main chemical constituents of *Vitis vinifera L*. leaves are shown in **Table I**:

Table I: Main chemical constituents of *Vitis vinifera L*. leaves **(Jonadet, Meunier et al. 1983; Bruneton 2002; Raynaud, Barnola et al. 2005)**.

Families of chemical constituents	Chemical constituents
Anthocyanosides	O-glucosides of cyanidol and peonidol.
Phenolic acids	Mono-tartaric acid, phenylpropanoic acids.
Tannins	Hydrolyzable tannins (esters of glucose and gallic acids and dehydrohexahydroxydiphenic.
Flavonol glycosides	Glucosides of flavonols: quercetin-3-O-β-D-glucuronide, isoquercitrin (quercetin-3-O-β-glucoside), and kaempferol-3-glucoside

1.5. General information on phenolic compounds

Phenolic compounds include a wide range of chemicals comprising at least one aromatic ring and one or more hydroxyl groups, in addition to other constituents **(Salunkhe 1990)**. Natural polyphenols range from simple molecules to highly polymerized compounds, the most important are: phenolic acids, anthocyanins, flavonoids and tannins **(Hmid 2013)**.

- **Phenolic acids**

There are two main classes of phenolic acid; the derivatives of benzoic acid (C1-C6) andderivatives of cinnamic acid (C3-C6) **(Tsao 2010)**. The concentration of the hydroxybenzoicacid is generally very low in edible vegetable. These derivatives are quite rare in the human diet comparing with hydroxycinnamic acids which are very present **(Macheix, Fleuriet et al. 2005)**.

- **Flavonoids**

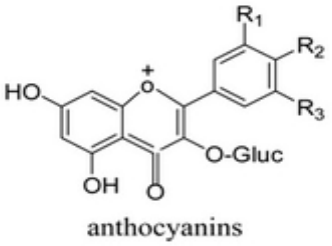
Flavonoids have the C6–C3–C6 general structural backbone in which the two C6 units (RingA and Ring B) are of phenolic nature. Due to the hydroxylation pattern and variations in thechromane ring (Ring C), flavonoids can be further divided into different sub-groups such asflavones, flavonols and anthocyanins **(Tsao 2010)**.

- **Tannins**

Tannins are complex phenolic compounds obtained from the condensation of simplephenols. They are divided into two groups: hydrolysable tannins (carbohydrate ester and phenolic acids) and condensed tannins (dimers, oligomers and/or polymers of flavannes-3-ols or flavannes -3, 4-diols) **(Makkar 2003; Macheix, Fleuriet et al. 2005)**.

- **Anthocyanin**

They are flavonoids due to the C6-C3-C6 carbon skeleton in their molecules,derivatives of the flavylium cation found in the oxo or carbonium forms, their huge diversity resulting from the many potential attachment sites for functional-methoxy and hydroxylgroups in the cation ring (Fig.04). In food, they are mainly found as anthocyanidin mono- diandtriglycosides **(Joshi and Goyal 2011)**. Anthocyanins are responsible for colors ranging from pale pink to red to purple and deep blue **(Dahmoune, Madani et al. 2013)**.



anthocyanins

	R_1	R_2	R_3
peonidin-3-*O*-glucoside	OCH_3	OH	-
petunidin-3-*O*-glucoside	OH	OH	OCH_3
malvidin-3-*O*-glucoside	OCH_3	OH	OCH_3
cyaniding-3-*O*-glucoside	OH	OH	-
delphinidin-3-*O*-glucoside	OH	OH	OH

Figure 04: Chemical structures of the anthocyanins of grape leaves **(Xia, Deng et al. 2010)**.

1.6. Biological activities

Since ancient times, the different parts of vine tree plant have been used because of many biological activities in folk medicine, grape leaves have been used to stop bleeding, inflammation, and pain, such as the kind brought on by hemorrhoids in the traditional medicine **(Bombardelli and Morazzoni 1995; Baytop 1999)**. The leaves of vine have astringent and haemostatic properties, they are used in the treatment of diarrhea, hemorrhage, varicose veins, hemorrhoids, inflammatory disorder, pain, hepatitis, and free radical related diseases and externally for centuries in Anatolia to heal wounds and drain furuncles **(Davis 1965; Baytop 1985)**. Furthermore, in recent years, the leaves are used in the formulation of dietary antioxidant supplements. A number of in vivo and in vitro studies were conducted on the plant material and have revealed that *V. vinifera* leaves exert various biological activities including hepatoprotective, spasmolytic, hypoglycemic,

and vasorelaxant effects **(Fleming 1998)**. Table II summarizes some of biological activities of grape leaves.

Table II: Effect of grape leaves on biological targets involved in some diseases.

Treatments	**Results**	**References**
Diabete disease		
The dried extract of grape leaves was suspended in 0.5% aqueous carboxymethylcellulose (CMC) suspension in distilled water prior to oral administration to animals (10 ml/kg, b.w)	The extract of grape leaves of *Vitis vinifera* are antioxidant and antidiabetic (action of procyanidols) and interfere On adipogenesis.	**(Şendoğdu, Aslan et al. 2006)**.
inflammatory disease		
A group of rats was pretreated with aqueous ethanol extract of *V. vinifera* (250 mg/kg**).**	The ethanolic extract from grape leaves has anti-inflammatory effect in rats.	**(Handoussa, Hanafi et al. 2013)**.
Allergy disease		
Skin prick tests with a standard aeroallergen battery and V *vinifera* pollen extract were performed on all patients.	*Vitis vinifera. L* leaves have antiallergic properties in allergy caused by pollen.	**(Brito, Gimeno et al. 2008)**.
Antimicrobial activity		
In vitro antimicrobial activity was examined for aqueous and ethanol extracts from *Vitis vinifera* leave.	The ethanolic as well as the aqueous extract of *Vitis vinifera L*. was active against more than 85% and 65%of the studied bacterial strains	**(Parekh and Chanda 2006)**.

II.Yogurt

2.1. Definition and Classification

Yogurt is a product made from heat treated milk that may be homogenized prior to the addition of lactic acid bacteria (LAB) cultures containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* whose load must be between respectively $\geq 10^5$; $\geq 10^8$ **(Estrada Andino 2011)**.Yogurt is one of the most popular fermented milk products worldwide and has gained widespread consumer acceptance as a healthy food **(Mckinley 2005)**. The general composition of yogurt (Table III) is more or less similar to that of milk. Therefore, yogurt is a rich source of milk proteins, carbohydrate and minerals such as calcium and phosphorous **(Weerathilake, Rasika et al. 2014)**.

Table III: Chemical composition of typical yoghurt **(Kumar and Mishra 2004)**.

Constituent (per 100 g)	Full-fat yogurt	Low-fatyogurt	Fruit yogurt
Water (g)	81.9	84.9	77.0
Total solids (g)	18.1	15.1	23.0
Fat (g)	3.0	0.8	0.7
Protein (g)	5.7	5.1	4.1
Lactose (g)	7.8	7.5	-
Calcium (mg)	200	190	150
Phosphorus (mg)	170	160	120
Sodium (mg)	80	83	64
Potassium (mg)	280	250	210
Zinc (mg)	0.7	0.6	0.5

Industrially, Yogurts can be largely divided into two types. A steamed yogurt is made in retail containers giving a continuous undisturbed gel structure as the final product. On the other hand, stirred yogurt has a delicate protein gel structure that develops during fermentation.

In stirred yogurt manufacture, the gel is disrupted by stirring before mixing with fruit before packaging. Stirred yogurts should have a smooth and viscous texture. In terms of rheology, stirred yogurt is a viscoelastic and pseudo plastic product.Yogurt come in a variety of textures (e.g. liquid, set, and smooth), fat contents (e.g. luxury, low-liquid, virtually fat-free) and flavors (e.g. natural, fruit, cereals), can be consumed as a snack or

part of a meal, as a sweet or savory food, and are available all year round. This versatility, together with their acceptance as a healthy and nutritious food, has led to their widespread popularity across all population sub groups **(Mckinley 2005).**

2.2. Production diagram

The production steps in the manufacture of stirred and adjusted yogurt are illustrated in the following (Fig. 05). The type of milk depends on the variety or type of Yogurt to be prepared **(Weerathilake, Rasika et al. 2014).**

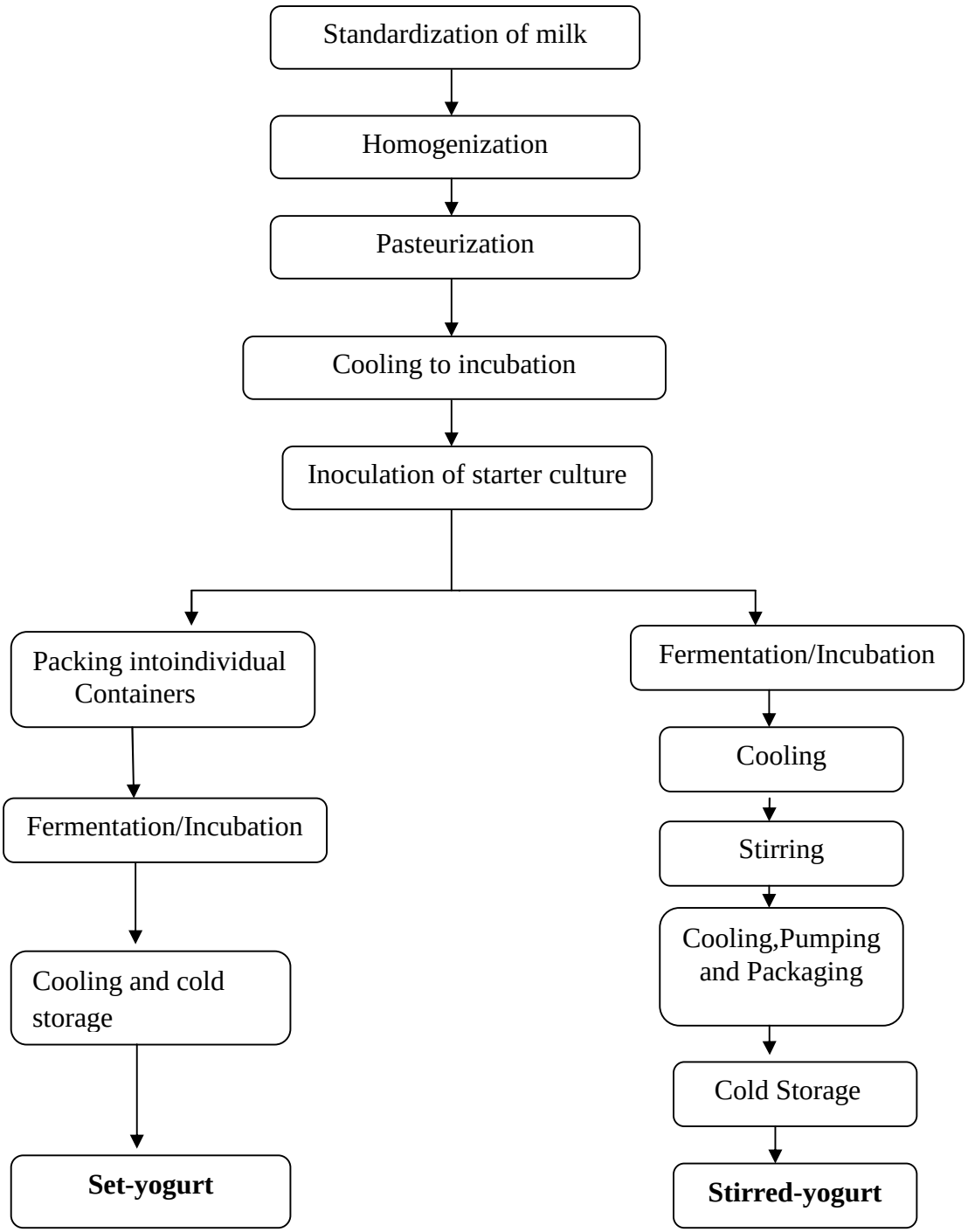


Figure 05: Manufacturing process of set- and stirred-yogurt.

2.3. Nutritional and therapeutic effects of Yogurt

In addition to the appreciation of yogurt for its taste and texture, it is also appreciated for its important nutritional value, however it is richer than milk in essential amino acids, proteins, calcium, vitamin D, B6, B12 and riboflavin and in lactose **(Ayar and Gurlin 2014; Nikkhah 2014)**.The variable fat content in yogurt offers flexibility of consumption by healthy and sick people **(Nikkhah 2014)**.

A number of studies have shown that yogurt consumption for 15 to60 days has a cholesterol-lowering effect, with a decrease in serum total cholesterol levels of 4% and serum LDL of 5% **(Nikkhah 2014)**. On the other hand, tests in humans have shown that the presence of live lactic acid bacteria in yogurt allows a better assimilation of lactose in lactase deficient individuals(Clement, Schauman et al. 2015; Teo, Muniandy et al. 2016)Yogurt is more digestible than unfermented milk because of its high content of free amino acids due to the proteolytic activity of lactic acid bacteria **(Mahaut-Smith, Ennion et al. 2000)**.

The consumption of yogurt, by activating the metabolism of galactose, would be one of the factors that may reduce the risk of developing cataracts **(de Syndifrais 1997).** Yogurt also has a preventive role against gastrointestinal infections. Old studies have shown the interest of yogurt in the treatment of childhood diarrhea **(Mahaut-Smith, Ennion et al. 2000; Roberfroid, Huybregts et al. 2008)**. Similarly, the immunomodulatory effect of yogurt has been demonstrated. Its role in increasing the production of interferons and immunoglobulins and in B cell activation is attributed to *L. bulgaricus* **(Mahaut-Smith, Ennion et al. 2000; Clement, Schauman et al. 2015)**. Yogurt also increases the bioavailability of calcium **(Luquet and Watanabe 1986),**and can prevent osteoporosis and high blood pressure **(Nikkhah 2014; Clement, Schauman et al. 2015).**

2.4. Yogurt and bioactive compounds

Due to a growing demand for functional fermented dairy foods with improved nutritional qualities, the food processing industry has prompted to cut down on ingredients such as fat, sugar and additives, by necessitating some important changes in sensory qualities that influence consumer acceptance of fermented dairy products **(Stijepić,**

Đurđević-Milošević et al. 2012). Polyphenols are chemical compounds that act as antioxidants which are known for their potential beneficial effects on health. Although some bioactive substances have been added to yogurt in order to enhance the health outcomes of conventional yogurt **(Manthou, Georgakouli et al. 2016)**.

I. Material and methods

1.1. Plant material

The grape leaves used in this work are from of the genus *Vitis vinifera L* of the variety Ahmar Bou-Amar identified by the botanists of the university Abderaheman Mira of bejaia . They are deciduous, with webbed veins and have five main lobes more or less cut. The limb has five main veins that start from the petiolar point. The leaves have a greenish color at the center and a dark red color on the margins of the lobes.

Figure 06: Photography of the grape leaf used in this study.

The samples were harvested in September 2017 in Taazibth, Kendira, Bejaia province of Algeria (North East of Algeria-Bejaia; latitude 36.540°; longitude 5.0268). Sampling was done randomly on several domestic plants. The harvested samples were cleaned with tap water, to rid them of foreign particles and dust, drained and then put in a ventilated space in the shade for 15 days.

Once the drying is done, the leaves were crushed using an electric grinder, in order to obtain a very fine powder. The later was sieved using a down porosity electric sieve system (Retsch AS200), and the resulting powders $\Phi \leq 500\mu m$, $\Phi \leq 250\mu m$ and $\Phi \leq 125\mu m$ are stored at room temperature, protected from light and moisture in an airtight and opaque container.

1.2. Evaluation of moisture content

The determination of moisture content was carried out according to the method described by **(Doymaz 2004).** A test sample (1g) is dried in an oven (Ecocelle, Schutzart DIN EN

60529-IP 20, Germany) at 103°±2°C until a constant weight, the moisture content is calculated according to the following formula:

$$H\% = \frac{Wi - Wo}{Wi} \times 100$$

Where:

- **H (%)**: is the percentage of moisture content.
- $\mathbf{W_0}$: corresponds to the loss in weight (g) on drying .
- $\mathbf{W_I}$: is the initial weight of sample (g).

1.3. Extractions of polyphenols

Phenolic compounds were extracted using a stirred conventional solvent extraction method described by **(Katalinić, Možina et al. 2010)**. Briefly,10g of grape leaves powder ($\Phi \leq 250$ µm) were extracted with 100 ml of alcoholic solvent (ethanol/water 80/20, v/v) at 60°C. Contact time was 60 min. After extraction, samples were filtered with Whatman N°.1 to remove residual particles and the residual tissue was washed with 3 x 25 mL of solvent. Extracts obtained from the plant material were gathered together in total extract. The total extract was concentrated *in vacuo* (< 40 °C) to 150 mL. Thus obtained extracts were centrifuged at 5000 rpm for 10 min. The extract was stored at 4 °C until further analysis.

1.4. Phytochemical analysis

1.4.1. Determination of total phenolic content (TPC)

The total phenolic content of the extract was determined using Folin-Ciocalteu methode scribed by **(George and Bennett 2005).** This method is based on reducing, in the alkaline medium, phosphotungstic mixture H3PW12O40 and H3PMo12O40 of Folin reagent, by the oxidizable group of phenolic compounds, leading to the formation of reduction products of blue color **(Enneb, Belkadhi et al. 2015)**.

Thus, a 2.5 mL sample of water- diluted Folin-Ciolcateu reagent (1/10) was added to the extract. The mixturewas incubated for 2 min at room temperature, and 2 mL of sodium

carbonate (75 g/L) was added. The mixture was incubated for 15 min at 50°C. The specific absorbance at 760 nm was immediately measured, using UV-Visible light spectrophotometer (UV-VIS Spectrophotometer UV-9200, Biothech Engineering Management CO.,Ltd. UK). TPC concentration was calculated from a calibration curve, using gallic acid as a standard and the results were expressed as mg gallic acid equivalents per gramme of dry weight (mg GAE/g of DM **(Appendix 04).**

1.4.2. Determination of flavonoids content (TFC)

The flavonoids content in the extract were determined according to the mostly applied colorimetry method of **(Bahorun, Gressier et al. 1996)**, based on the formation of aluminium-flavonoid complexes. Flavonoids form complexes with aluminum in the form of Al^{+3} ions after decomposition of aluminum chloride. The complexes are responsible for the absorption of light in the visible (Fig.07) **(Ribéreau-Gayon 1968).**

One mL of 2% (w/v) aluminium chloride ($AlCl_3$) was added to 1 mL of diluted extract and then mixed using vortex mixer (EV-102, tehtnicazelezniki, Germany) for approximately 10 s. The mixture was allowed to stand for 15 min. Absorbance of the mixture was determined at 430 nm versus the prepared blank using Uv-vis light spectrophotometer (UV-VIS Spectrophotometer UV-9200, Biothech Engineering Management CO, Ltd. UK). TFC was expressed as mg quercitin equivalent per gramme of dry matter (mg QE/g of DM). is determined by reference to a calibration curve made under the same conditions with quercetin **(Appendix 05).**

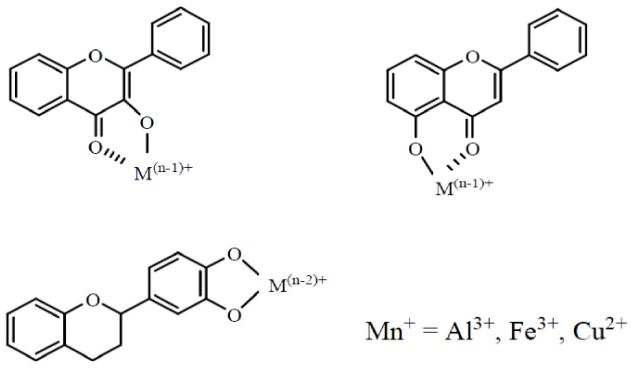


Figure 07: The chelation of metal ions by flavonoids **(Dangles 2006)**.

1.4.3. Determination of total anthocyanins content (ANC)

Total monomeric anthocyanins content of extract samples was monitored by the pH differential method as outlined by **(Lee, Durst et al. 2005).** Monomeric anthocyanin pigments reversibly change color with a change in pH; the colored oxonium form exists at pH 1.0, and the colorless hemiketal form predominates at pH 4.5. The difference in the absorbance of the pigments at 520 nm is proportional to the pigment concentration **(Lee, Durst et al. 2005).**

After dilution of the extract samples with potassium chloride buffer (0.025 M, pH =1.0) and sodium acetate buffer (0.040 M; pH = 4.5) and allowed to equilibrate for 20 to 50min. The absorbance of equilibrated samples was measured versus a blank cell (filled with distilled water) for pH 1.0 and 4.5 at maximum absorbance wavelengths ($\lambda_{visile\ max}$ =520 nm) and at 700 nm to correct for haze. Measurements were performed in triplicates using UV-visible spectrophotometer (UV-VIS Spectrophotometer UV-9200, Biothech Engineering Management CO, Ltd, and UK). Results are expressed as cyanidin-3-glucoside basis. Degraded anthocyanins in the polymeric form are resistant to color change regardless of pH and are not Included in the measurements because they absorb at pH 4.5 as well as pH 1.0. Anthocyanin Pigment concentration is calculated as follow:

$$\mathbf{ANC} = (\mathbf{A} \times \mathbf{MW} \times \mathbf{DF} \times \mathbf{10}^{3})/(\mathrm{\varepsilon})$$

Where:

- $A = (A_{520nm} - A_{700nm})_{pH=1} - (A_{520\,nm} - A_{700nm})_{pH=4.5}$
- **MW** (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu);
- **DF** = dilution factor;
- **l** = pathlength in cm = 1;
- **Ɛ**= molar extinction coefficient = 26 900 L.mol–1 .cm–1, for cyd-3-glu;
- $\mathbf{10^3}$= factor for conversion from g to mg.

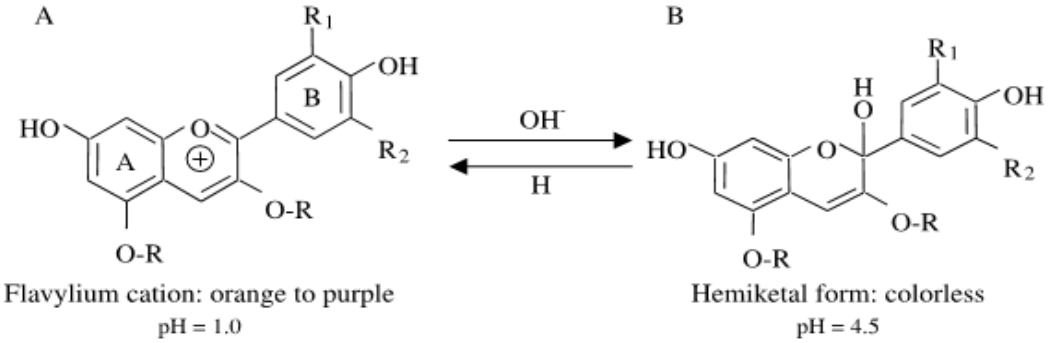


Figure 08: Sturcturs of anthocyanins in pH 1.0 and 4.5 buffers, and the structures of the flavylium cation (A) and hemiketal forms (B). Glycosidic substituent.

1.4.4 Determination of condensed tannin content (CT)

The method is based on butanol/HCl dosage described by **(Wilfred and Nicholson 2006)** with small modifications. This method is based on the ability of monomer and condensed 3–4, flavandiols to oxidise in acid and alcoholic medium at high temperature to give colored procyanidins **(Glories 1978).**

Seventy seven milligrams of ferric ammonium sulphate ($fe_2(SO_4)$ dissolved in 500 ml of the HCl / butanol) mixture was prepared, then 2.5ml of acidic solution was added to 250 µl of the extract.After mixing and incubating at 95 °C for 50 min, absorbance at 530 nm was measured against a blank.The condensed tannins were expressed as mg of cyaniding equivalent/100 g dry matter (DM) and calculated as follow :

$$X = (Absorbance \times DF \times MW) / (\text{Ɛ})$$

Where:

- **DF:** dilution factor.
- **MW:** molecular weight of cyanidin=287g/mol.
- **Ɛ :** molar extinction coefficient= 34700l/mol/cm.

1.5. Determination of antioxidant and antiradical activity

The antioxidant activity of the extract was determined by two methods. The first is the estimation of the reducing power which measures the ability of extracts to reduce metal ions (ferric iron to ferrous iron). The second evaluates the antiradical power by measuring

the percentage of neutralization of a radical (DPPH • and ABTS • +) by the antioxidants present in our samples.

1.5. 1.Reducing power

1.5.1.1. Reduction of ferric chloride $FeCl_3$

The ferric reduction antioxidant power of the extract was determined using potassium ferricyanide-ferric chloride method **(Ozsoy, Can et al. 2008)**. Reducing power analysis is based on the reduction of ferric ironFe3+ferricyanide-complex to ferrous iron Fe2 + in the presence of reducing antioxidants. The reduced form gives a green color that is proportional to the reducing power of the extract **(Gülçin 2012)**.

One ml of extract was added to 2.5 ml of phosphate buffer (0.2 M pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50 ° C. for 20 minutes.After 2.5 ml of trichloroacetic acid (10%) was added. Two and one-half milliliters of the mixture were taken and mixed with 2.5 ml of water and 0.5 ml of 1% FeCl 3. The absorbance at 700 nm was measured by a spectrophotometer (UV-VIS). After allowing the solution to stand for 30 minutes. The evaluation of the percentage reduction of ferric chloride relative to the concentrations of standard (vitamin C) is carried out under the same conditions **(Appendix 06)**.

1.5.1.2. Reduction of ferrosine

The principle of this test is the complexation of ferrous ions with a ligand compound: sodium 3- (2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4"-disulfonate (ferrosine) . The ferrosine-iron II complex of violet color has an absorption maximum at 562 nm according to the method cited by **(Bourgou, Ksouri et al. 2008).**

To 50 µl of $FeCl_2$ (2 mM) are added 100 µl of extract. The reaction is initiated with100µl of ferrosine (5mM) and 2.75ml of distilled water. The mixture is homogenized and incubated for 10 min at room temperature and the absorbance is read at 562 nm.

A positive control with EDTA is achieved in the same conditions. The percentage of inhibition is calculated according to the following formula:

I% = [Abs control-Abs test performed]/ Abs control] x100

1.5.2. Neutralization of free radical

1. 5.2.1.Inhibition of the radical DPPH·

DPPH· (2,2-diphenyl-1-picrylhydrazyl) is a stable highly colored free radical **(Fig.09)** ,that can abstract labile hydrogen atoms from phenolic antioxidants (ArOH) with concomitan formation of a colorless hydrazine (DPPH-H) **(Malien-Aubert, Dangles et al. 2001)**.

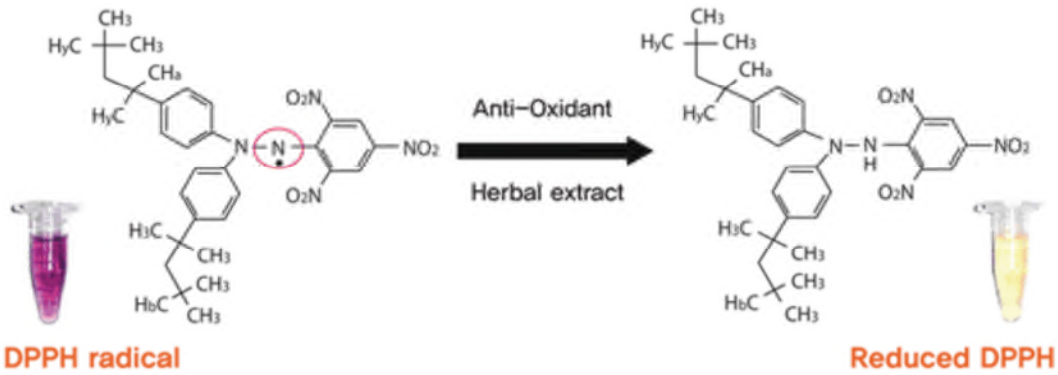


Figure 09: DPPH· Radical reduction **(Molyneux, Cekirge et al. 2004).**

The antiradical potency, by neutralizing the DPPH radical, of the extract is evaluated according to the method described by **(Brand-Williams, Cuvelier et al. 1995)**.One ml of the extract was added to 2 ml of DPPH· solution (2.10^{-4}mol/ L) and the mixture was left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. The antioxidant capacity of the extract was expressed as the percentage of DPPH• reduced and was calculated by the following equation:

PI%= [(Abs sample – Abs Extract) / Abs sample] ×100

Where:

- **Abs $_{control}$:** Absorbance of the Control after 30 minutes at 517 nm.
- **Abs $_{extract}$:** Absorbance of the extract after 30 minutes at 517 nm.

1.5.2.2. Inhibition of the radical ABTS$^{\cdot +}$

The method that determines the scavenger activity of the ABTS radical is based on the ability of an antioxidant to trap the greenish blue staining of the ABTS • + (2. 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic) cationic radical. Transforming it into colorless ABTS-H +, by a hydrogen donation **(Antolovich, Prenzler et al. 2002)**.

The decrease in absorbance caused by the antioxidant reflects the capture capacity of the free radical.

The percentage inhibition of the ABTS • + radical is evaluated by the method of **(Re, Pellegrini et al. 1999)**, which is based on the ability of antioxidants to interact with the radical ABTS • +, reducing its absorbance at 734 nm. A free radical solution (7 mM ABTS and 2.45 mM potassium persulfate) was prepared and incubated in the dark at room temperature for 12-16 h before use. This solution was then diluted with 50% ethanol to an absorbance of 0.705 ± 0.02 at 734 nm and equilibrated at 30 ° C. Control, draft and extract samples were prepared, respectively; 2 ml of radical solution, 2 ml of radical solution mixed with 20 µl of extraction solvent and 2 ml of radical solution mixed with 20 µl of extract. Absorbance was read at 734 nm after 6 min of incubation at room temperature in the dark and antioxidant activity was calculated according to the equation:

$$PI\% = [(Abs\ blanc - Abs\ sample) / Abs\ control] \times 100$$

Where:

- **Abs $_{sample}$:** Absorbance of the extracts, after 30 minutes, at 734 nm.
- **Abs $_{blanc}$:** Blanc absorbance at 734nm.
- **Abs $_{control}$:** Absorbance of the control after 30minutes, at 734nm.

1.6. Formulation of a steamed yogurt at laboratory scale

1.6.1. Manufacture of yogurt

The preparation of yogurt was made in the laboratory 3BS (University of Bejaia) with addition of grapes leaves powder. Milk was homogenized and heated to 95°C for 5min then cooled to 40°C. After then, traditional starter culture was added and the mixture was incubated until the gel was formed, and stored at refrigerator (6°C ±2).

Table IV: Recipe of standard yogurt and yogurt flavored with grape leaves powder.

Recipe	Milk (L)	Sugar (g)	Lactic Ferment (%)
Standard Yogurt	1	80 – 100	0.02
Steamed yogurt (grape leaves powder)	1	80 – 100	0.02

1.6.2. Physico-chemical analysis of yogurt

Physico-chemical properties of the manufactured yogurts (standard yogurt, yogurt enriched with *Vitis vinifera L* leaves powder) were determined namely, pH, dornic acidity, viscosity, dry extract and fat content (Table V). These tests were carried out at the laboratory "LAITERIE SOUMMAM".

Table V: Physico-chemical analysis of manufactured yoghourts.

Measure	Method
pH	The pH value of yoghurt was measured at fixed temperature (9.5 -10.5 °C) with a calibrated pH electrode **(JORA standard)**.
Total dry extract	3 grams of Sample were weighed in a cup then lower the hood of the desiccators the analysis will start automatically **(JORA standard)**.
Dornic acidity (°D)	Titrate with sodium hydroxide (NAOH, 0.9 N) to pH = 8.30 and read the burette drop **(JORA standard)**.
Fat content (%)	Add sulfuric acid (H2SO4) (10 ml) and add 11 ml of yoghurt to the butylimeter, then add 1 ml of homo-isoamyl alcohol (d = 0.811) to the surface of the product and place in a centrifuge. Express. results by reading the achievement value corresponds to the lowest point of butylimeter **(JORA standard).**

1.6.3. Microbiological analysis

Microbiological quality of the prepared yogurts was evaluated by enumerating total viable microorganisms. The enumerated microorganisms include total flora, yeast, molds, total coliforms, salmonella and specific bacteria of yogurt.

Table VI: Microbiological analysis of manufactured yogourt.

Micro_organisms	Selective Mediums	Incubation temperature	Incubation Time	Method
Total Coliforms	VRBL	30°C	24h	3g of the Yoghurt samples was spread plated in triplicates into prepared and dried petri-plates of suitable media for the enumeration of different organisms
Total Flora	PCA	30°C	72 h	
Yeasts, moulds	YGC	25°C	5 days	
Salmonella	Hektoen	37°C	24 to 48 hours	
Streptococcus Thermophilus	M17	c37°C	48h	
Lactobacillus bulgaricus	MRS	37°C	72 h	

VRBL: Violet Red Bile Agar
YGC: Yeast extract glucose chloramphenicol agar
M17: M17 agar
MRS: Rogoza and Sharpe agar

1.6.4. Antioxydant activity and TPC content of yogurt

The Radical scavenging capacity in manufactured yogurts was measured by the DPPH• assay(Section I. 5.2).The TPC content in fermented milk products were also determined by using Colorimetric methods (SectionI.4.1).

Sample preparation (Yogurt water extract)

Yogurt sample (10g) was mixed with 2.5ml distilled water and the yogurt pH was adjusted to 4.0using 1M Hcl. The yogurt was then incubated at 45°C for 10 minutes, followed by centrifugation (10000 rpm, 20 minutes, 4 °C). The supernatant was collected and the pH was adjusted to 7.0 using NaOH. The neutralized supernatant was recentrifuged (10000 rpm, 20 minutes, 4^{o}C) and the supernatant was used in analysis**(Zainoldin and Baba 2009).**

1.7. Sensory analysis

Evaluation of sensory properties of yogurts (standard yogurt, yogurt fortified with grape leaves) was studied. The panel was constituted by ten trained panellists from the staff members of the Life and Nature Sciences (University of Bejaia). Panel lists evaluated

the color, taste, texture, flavor and odor of each sample, using a numerical scale 1 - 5 (1 = not acceptable, 5 = extremely good).

1.8. Statistical analysis

All experiments were conducted in triplicate and results are expressed as mean ± standard deviation (SD). The analysis of variance (*ANOVA*) was performed using XLSTAT Release 10 (Addinsoft, Paris, France). Tukey's multiple range test (HSD) was used to compare means of the determined parameters. Evaluations were based on the $p< 0.05$ significance level.

II. Results and discussion

2.1. Moisture content

Humidity, being the water content of our matrix, is important in order to estimate the drying yield. The high rate of humidity is a source of degradation of antioxidants by the oxidation phenomenon. Indeed, water is a source of degradation whose disadvantage can be eliminated by a quick drying, immediately after harvest **(Ribéreau-Gayon 1968).**

Thus, the moisture of the fresh grape leaves and residual moisture of the studied powder is determined using the weighted method, which consists in determining the water loss by drying in an oven, which will make it possible to standardize the water content and so to ensure better grinding and homogeneity. The result obtained is shown in figure 10 and 11.

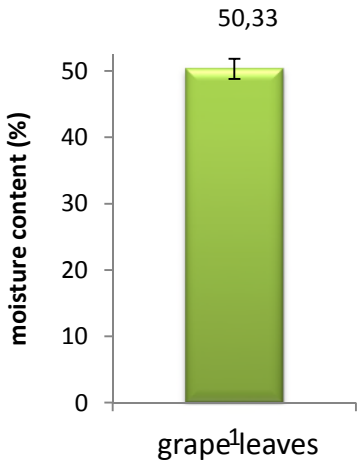


Figure 10: Histogram of moisture content of the studied leaves.

The vertical bar represents the standard deviation
Values are the means of three determinations± standard deviation

The moisture content obtained for fresh grape leaves is 50.33% .These results show the water richness of fresh grape leaves. This parameter is of great importance for the extraction of polyphenols because its presence is a troublesome element of the extraction yield.

According to **Koussa, Dubos et al. (2002)** , the average water content of fresh grape leaves is of 77%.These results are superior to that found in this study.

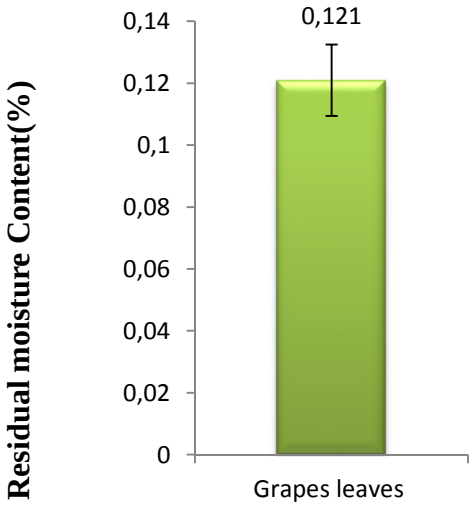


Figure 11: Histogram of residual moisture content of the studied powder

The vertical bar represents the standard deviation
Values are the means of three determinations± standard deviation

The results show 0.121% of moisture content in the powder of the grape leaves. This low humidity rate makes it better to preserve the antioxidant properties of the phenolic compounds of the powder.

2.2. Determination of polyphenols

2.2.1. Total phenolic content

The method using the Folin-Ciocalteu reagent is one of the methods designated for the determination of the content of phenolic compounds in foods or medicinal plants. The polyphenol content of the grape leaves powder is reported in milligram equivalents of gallic acid per 1 gram of sample (mg EAG / g), with reference to a calibration curve carried out under the same conditions . The results obtained are shown in figure 12.

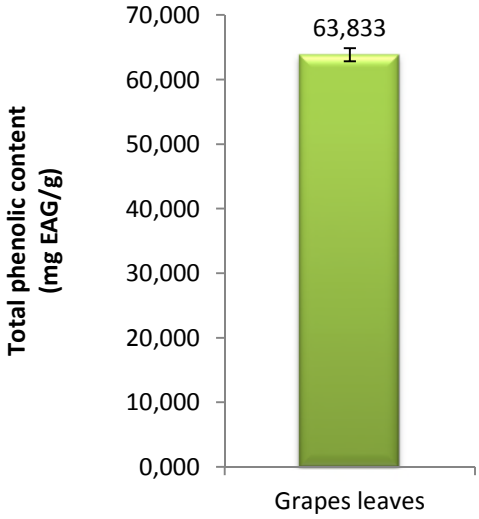


Figure 12: Histogram of the total phenolic content of grape leaves.

The vertical bar represents the standard deviation

Values are the means of three determinations ± standard deviation

The result of the present study shows that the content of phenolic compounds in the powder of the grape leaves leaves is on average 63.83 mg EAG / g.

Handoussa, Hanafi et al. (2013) have reported a total polyphenol content in *Vitis vinifera L* leaves of 289.33 ± 13.02 mg EAG / g DW. These results are superior to those obtained in the present work.

The results found in this study are in the interval given by **Pantelić, Zagorac et al. (2017)** in a study conducted on 22 samples of grape leaves harvested in different regions of Serbia which ranged from 27.5 to 76 mg EAG / g DW.

Total phenolic contents in five aqueous extracts of grape leaves tested by **Orhan, Orhan et al. (2007)** ranged from 11.63 to 19.85 mg EAG / g DM. These results are much lower than those found in the present study.

Jaradat, Zaid et al. (2017) in their study on the influence of the means of conservation on the total polyphenol content, found that in fresh grape leaves this content was 125.45 ± 0.66 mg EAG / g, 103.33 ± 0.82 mg EAG / g in frozen leaves and 87.35 ± 0.32 EAG / g extract in canned leaves.

The levels found by **Hebash, Fadel et al. (1991)** were of the order of 16.2 and 22.9 mg EAG / g DW are low compared to our results; which allows us to conclude the richness of our extracts in total polyphenols. Our results are in agreement with**(Monagas, Gómez-**

Cordovés et al. 2006; Orhan, Aslan et al. 2006; Pari and Suresh 2008; Oliboni, Dani et al. 2011) who reported that grape leaves are rich in phenolic compounds.

The differences observed between the results of this study and those reported in the literature can be attributed to various factors such as genetic factors ,the degree of maturity, climatic and geographical conditions **(Veberic, Colaric et al. 2008)**.

2.2.2. Total flavonoid content

Flavonoids are a large class of polyphenols that possess a free hydroxyl group (OH) in position 5, capable of giving presence of aluminum chloride a yellowish complex, by chelation of the aluminum ion (Al3 +), which is proportional to the amount of flavonoids present in the extract **(Djeridane, Yousfi et al. 2006)**.

The flavonoid content of the studied powder, expressed per mg of Quercetin equivalent (EQ) / g of the sample with reference to a calibration curve produced under the same conditions, is shown in figure 13.

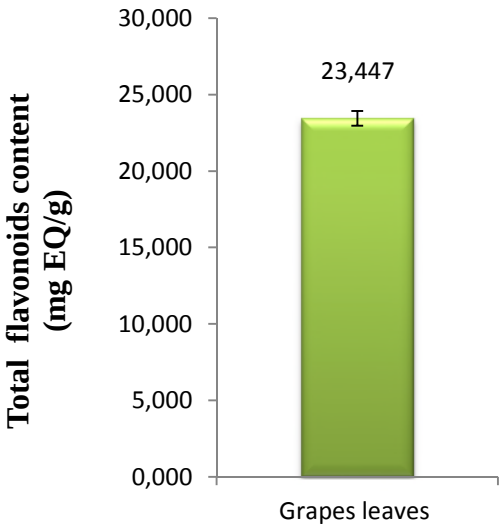


Figure 13: Histogram of total flavonoids content of grape leaves**.**

The vertical bar represents the standard deviation

Values are the means of three determinations ± standard deviation

The results of the present study show that the content of flavonoid compounds in the powder of grape leaves is 23.47 mg EQ / g.

Dani, Oliboni et al. (2010) work on the grape leaves (*Vitis labruscavar Bordo*) of South America, found that the content of flavonoids are of the order of 8.95 mg rutin /g) these results are lower than those found in the present study.

The discrepancy found between the results obtained and the data of various authors is entirely justified. Indeed, the extraction conditions as well as the origins of the samples and the season of collection govern these variations **(Ranalli, Contento et al. 2006).** Exposure to light and the technique of conservation of the plants which can also affect the content of flavonoids **(Rawel, Meidtner et al. 2005)** and the temperature that influences the extraction of a given compound **(Sarikurkcu, Tepe et al. 2008)**. Differences can also be attributed to geographical origin or climatic factors.

2.2.3. Total anthocyanin content

The determination of the anthocyanin content is based on their properties to exist in an acid medium, in a colored form and in a colorless form in equilibrium; the position of equilibrium depends on the pH. Therefore the variation of the color intensity between two pH values is proportional to the pigment content **(Ribéreau-Gayon 1968).**

The results of the anthocyanins of the grape leaves powder are shown in figure 14.

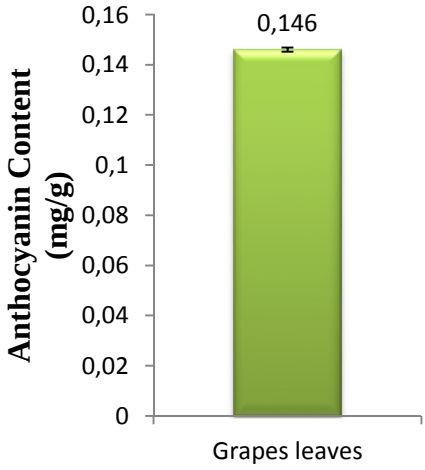


Figure 14: Histogram of anthocyanin content of the studied powder

The vertical bar represents the standard deviation
Values are the means of three determinations± standard deviation

The result of the present study shows that the content of anthocyanin compounds in the powder of grape leaves is on average 0.146mg / g.

Hogan, Zhang et al. (2009) reported that The Norton grape was detected to have the highest TAC (0.93 mg/g), followed by the Cabernet Franc clone1 (0.64 mg/g) and the Cabernet Franc clone313 (0.17 mg/g). The value obtained in this study is in this range.

Anthocyanins are very sensitive compounds, several factors (high temperature, pH, light, structure, and concentration of anthocyanins) which can destabilize them **(Laleh, Frydoonfar et al. 2006).**The degradation of anthocyanins during storage is due to enzymatic and non-enzymatic browning reactions **(Chaovanalikit and Wrolstad 2004).**

2.2.4 Total tannin content

After an incubation time of 50 min at a temperature of 95 ° C, a white precipitate was formed corresponding to the proteins precipitated by the tannins. The added HCL / butanol mixture reacts with the tannins and forms colorful complex . The results obtained are shown in Figure 15.

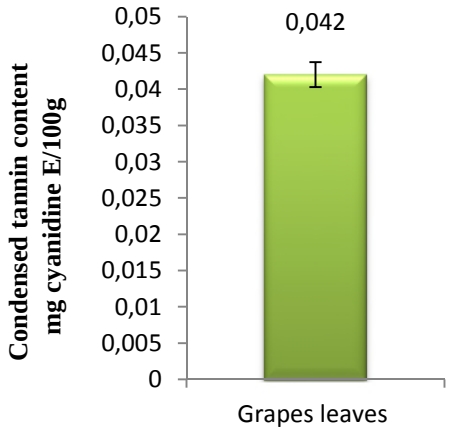


Figure 15: Historgamm of condensed tannin content of the studied powder

The vertical bar represents the standard deviation
Values are the means of three determinations± standard deviation

Based on the results obtained in this study, the tannin content of *Vitis vinifera L.* leaves is 0.042 mg cyaniding E / 100g.

According to **Marouf and Reynaud (2007)**, the tannins have an adverse effect on the digestibil ity of nutrients, especially dietary nitrogen, which is explained by the ability of the later to combine with dietary proteins. This makes them unassailable by proteolytic enzymes.

There are many factors that affect the tannin content, such as harvest time and temperature **(Elsheikh and Elzidany 1997)**.

2.3. Antioxidant and anti-radical activities

There are different types of antioxidants in fruits and it is very difficult to measure each antioxidant separately. As a result, several methods have been developed to evaluate the total antioxidant activity of fruits or other plant tissues**(Guo, Yang et al. 2003).**

2.3.1. Reducing power

2.3.1.1 Reduction of ferric chloride

The increase in absorbance indicates an increase in reducing power **(Ribéreau-Gayon 1968; Ozsoy, Can et al. 2008)**. The result of the evaluation of the reducing power of the studied mixture is shown in figure16.

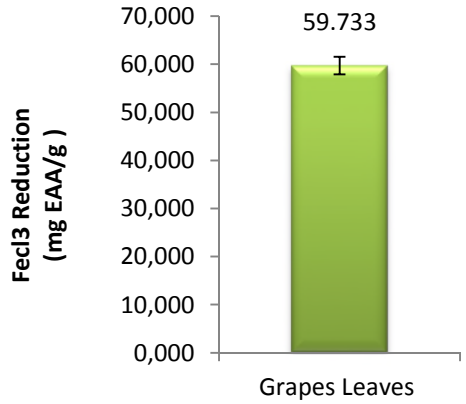


Figure 16: Reducing power of the studied powder studie.

The vertical bar represents the standard deviation
Values are the means of three determinations± standard deviation

According to the obtained result, the reducing power of extracts of grapes vine leaves powder is 59.73 mg EAA / g at a concentration of 2.14µg/ml.

According to the work of **Katalinic, Mozina et al. (2013),** the reducing power of the extract of *Vitis vinifera* leaves was about 63.6 to 183.5 % of extract. These authors noted that the reductive power test is a good indicator of antioxidant activity.

Aouey, Samet et al. (2016) reported that chemical composition and organic fruit and grape seeds have been thoroughly investigated. While studies of quantitative analysis of compounds found in *V. vinifera* leaves and its biological effects are poorly studied.

The reduction of ferric chloride is often used as an indicator of electron donator activity which is an important mechanism for the antioxidant action of polyphenols **(Yang, Yu et al. 2009).**

2.3.1.2 Reduction of ferrosine

Ionization of $FeCl_2$ releases ferrous ions (Fe^{2+}) which complexes with ferrosine to form a colored complex. Phenolic compounds compete with ferrosine to bind with ferrous (Fe^{2+}) ions, their high chelation power reduces the free ferrous concentration to the ion, thereby decreasing the intensity of the reaction between ferrous ions (Fe^{2+}) and ferrosine which limits the formation of Ferrosine complex**(Süntar, Akkol et al. 2011).**

The result of the reducing power, expressed as percentage inhibition of ferrosine, is represented in figure17.

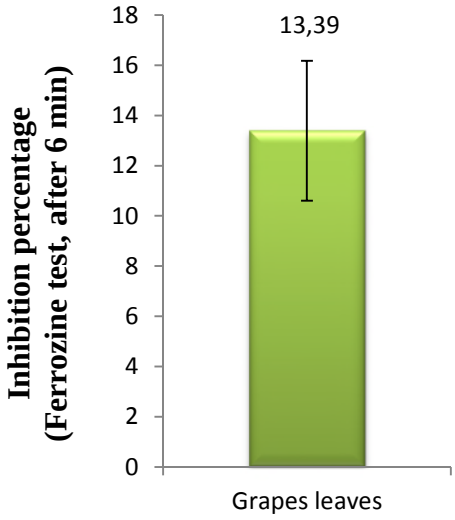


Figure 17: Histogram of iron chelation of the studied powder.

The vertical bar represents the standard deviation
Values are the means of three determinations± standard deviation.

According to the obtained results, the reduction capacity is 13.39% for a concentration of 2.14µg / ml.

Luther III, Shellenbarger et al. (1996) have reported that the potential problem in the classical ferrosine method is the incomplete reduction of organically complexed Fe^{3+}, which may explain the low percentage obtained in this study.

2.3.2. Radical-scavenging

2.3.2.1. Anti-Radical Power DPPH •

2,2-Diphenyl-1-picrylhydrazyl (DPPH•) is a stable free radical used to evaluate the ability of a natural antioxidant to purify a free radical (free radical-scavenging). The percentage inhibition of the DPPH° radical by the studied mixture is shown in figure18.

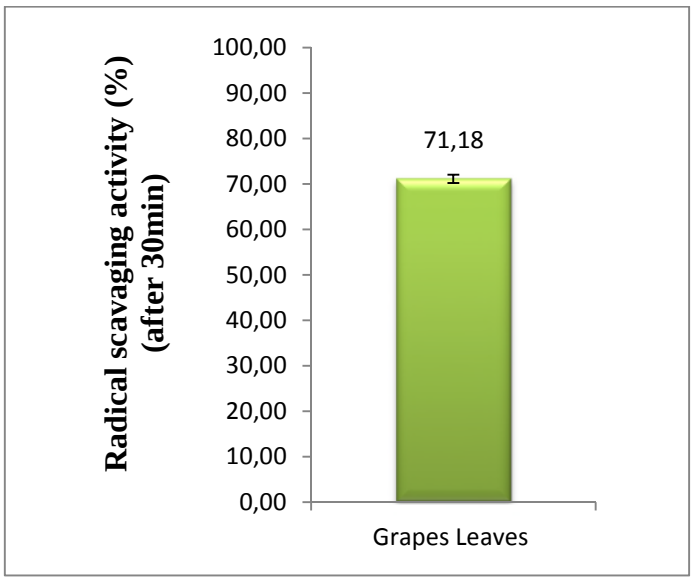


Figure 18: Antiradical power against the radical DPPH° of the studied powder

The vertical bar represents the standard deviation
Values are the means of three determinations± standard deviation

The results of the present study, shows that the percentage of inhibition of the DPPH° is 71.18% for a corresponding concentration of 2.14 µg / ml of grape leaves extract.

Orhan, Orhan et al. (2009) have reported an inhibition percentages of DPPH° by *Vitis vinifera L.* leaves, harvested in turkey, ranging from 41.4 ± 2.4 % to 67.6 ±0.9 % at a concentration of (50 µg/ mL^{-1}) .The value obtained in our study is higher.

Several studies have shown that the antiradical power is influenced by the extraction method, the solvent (nature and concentration), the temperature and the extraction time **(Stanisavljević, Stojičević et al. 2009; Jalili, Alipour et al. 2011; Lim, Choi et al. 2011).** These differences in results can be attributed not only to the extraction method used but also to the composition of our sample.

The reduction of the DPPH° by plant extracts has been attributed to severalfactors such us the presence of phenolic compounds which easily give rise to protons to reduce it**(Tepe, Eminagaoglu et al. 2007; Li, Wang et al. 2009).**

Neutralization of free radicals inhibits lipid oxidation which may be detrimental to cellular components and functions, so the consumption of natural antioxidants may contribute to protection against degenerative diseases induced by oxidative stress **(Lim, Choi et al. 2011)**.

2.3.2.2. Anti-radical cation ABTS $^{\bullet+}$

ABTS "2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)" is used as a free radical to evaluate the antioxidant activity of the samples. The method is based on the ability of antioxidant molecules to quench the long life of the radical cation ABTS • + **(Kumaraswamy and Satish 2008).**

The radical cation of theacid"2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)" is stable under its free elm. It is formed by the administration of colorless ABTS with potassium persulfate **(Re, Pellegrini et al. 1999).**

The percentage of inhibition of the cationic radical ABTS + by the studied mixture is shown in figure19.

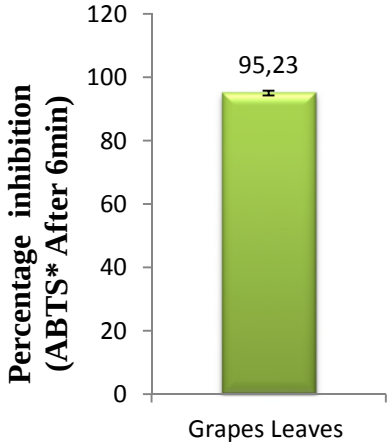


Figure 19: Percentage inhibition of the ABTS*+ radical of the studied powder.

The vertical bar represents the standard deviation
Values are the means of three determinations± standard deviation

According to the obtained results the inhibition capacity of the ABTS + radical by the studied powder extracts, after six minutes of incubation is 95.23 % at a concentration of 2.14 µg/ml.

Several studies have shown that anti-radical activity against the $ABTS^{*+}$ radical is very influenced by the extraction solvent, which can be explained by the variation in quantity and quality of antioxidants depending on the nature of the used solvent **(Floegel, Kim et al. 2011).**

According to **Manian, Anusuya et al. (2008)**, phenolic compounds of high molecular weight have more capacity to capture free radicals (ABTS +) and their effectiveness depends on the molecular weight, the number of aromatic nuclei and the nature of substitution of the hydroxyl groups.

2.4. Analysis of prepared steamed yogurts

2.4.1. Physico-chemical analysis

Physico-chemical properties of the manufactured steamed yogurts (standard yogurt, yogurt with grape leaves powder) are shown in table (09).

Table VII: Physicochemical analysis of the prepared steamed yogurts.

	pH	Dornic acidity(D°)	Total dry extract	Fat contents(%)
Standard yogurt	4.59	90	22.41	1.6
Yogurwithgrape leaves powder	4.57	88	22.86	1.8
Standard (JORA)	4.3-4.8	78-100	23.9- 25.15	2.75-3.15

Results of this analysis revealed that pH, total dry extract content, acidity, and fat content determination were conform to norms. However a decrease in pH and acidity were observed in yogurt made from grape leaves powder this is probably due to the enrichment of yogurt by this powder. On the other hand an increase in total dry extract, fat content were observed after addition of grape leaves powder to standard yogurt. This may be due to its impact on the aggregation of casein network in yogurts via electrostatic interaction.

And on the resistance of the yogurt matrix to flow. Indeed the addition of plant extracts generally decreased the consistency of the products owning to reduced water-binding capacity of proteins **(El-Said, Haggag et al. 2014).**

2.4.2. Microbiological analysis

Microbial quality of the manufactured steamed yogurts is given in table (10).

Table VIII: Microbiological analysis of formulated yoghurts

	Total coliforms at 37°C	Yeasts and moulds	Salmonella at 37°c	*Streptococcus thermophilus*	*Lactobacillus bulgaricus*
Standard yogurt	2.10^2	Absent	Absent	1.6×10^8	1.2×10^5
Yogurt with grape leaves powder	3.10^2	Absent	Absent	1.5×10^8	1.3×10^5
Standard (JORA	$<10^2$	Absent	Absent	$\geq 10^8$	$\geq 10^5$

Molds, yeast and coliforms, are the primary contaminants in yogurt **(Amakura, Okada et al. 2000)**,.The results of the microbiological analyzes of the too yogurts clearly show their perfect compliance with the standards. We note from these results a complete absence of all pathogenic germs and a negligible number of total germs. This is linked to the good conditions of manufacture, storage and compliance with aseptic rules during the manufacture of samples and their analyzes.

Yogurt enriched with grape leaves powder did not influence the viability of lactic acid bacteria flora (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) when compared with control yogurt, which could be related with the composition of proliferation media (sugar and lipid) of samples after addition of powder. The total viable numbers of lactic flora is an important parameter which contributes in the shelf life of yoghurt**(Georgakouli, Mpesios et al. 2016).**

2.4.3. Antioxidant activity and TPC content

2.4.4. Polyphenol content

The total phenolic compounds of the prepared yogurts are given in Figure 20. The results suggest that the addition of grape leaves powder statistically ($p < 0.05$) influences the amount of polyphenols.

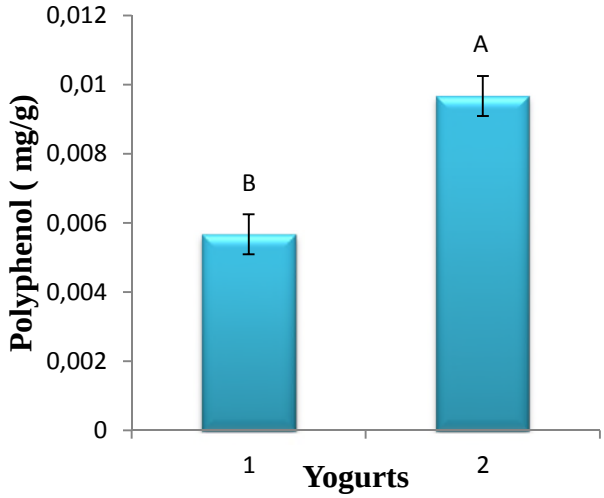


Figure 20: Histogram of the total phenolic content in prepard yogurts**.**

The vertical bar represents the standard deviation

Values are the means of three determinations± standard deviation

These results shows that there is a significant difference in both standard and enriched yogurts.The highest level of TPC has been detected in yogurt with grape leaves powder(A), followed by standard yogurt(B) (0.009 and 0.005 mg GAE / g. respectively). These data provide a confirmation of avaluable supplementation of yogurt with bioactive components of grape leaves powder.

2.4.5. Radical scavenging activity

The antioxidant activity of the two stirred yoghurts is depicted in figure 21

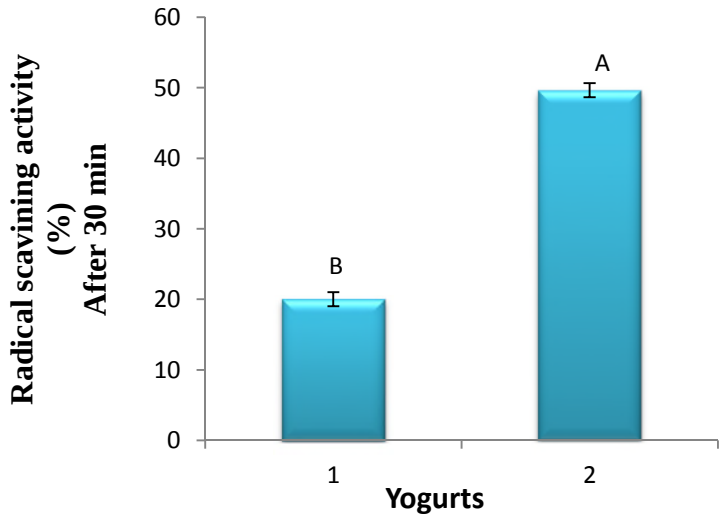


Figure 21: Antiradical power against the radical DPPH* of prepard yogurt

The vertical bar represents the standard deviation

Values are the means of three determinations± standard deviation

It was noticed from this figure that the antioxidant activity of different steamed yogurts, revealed that the addition of grape leaves powder (A), increased significantly the inhibitory activity against DPPH° radical compared with standard yogurt, being pronounced in yogurt with grape leaves powder (49.66 %) compared with standard (B) (20 %).Therefore the grape leaves were well correlated and dominantly responsible for the antioxidant activity. The studied powder proton donating ability and in association with a number of hydroxyl groups in the polyphenols structures to stabilize free radicals it could due to their ability to quench hydroxyl radicals by transferring hydrogen atom to free radical **(El-Said, Haggag et al. 2014).** It is clear that addition of this powder gave the highest value, this difference was statistically significant when p <0.05, in the antiradical capacity providing additional evidence of its antioxidant activity.

These results are in accordance with those recently reported, where it was found that the increase of the antioxidant activity of yogurt enriched with polyphenols, is related directly to the phenolic content **(Georgakouli, Mpesios et al. 2016).**

2.5. Sensory analysis

The samples of different manufactured yogurts with grape leaves powder and standard yogurt were sensory evaluated, and scores were recorded.

- **Design of experiment**

Designing an experiment is a fundamental step in order to verify if the collected data will be statistically valid **(Périnel and Pagès 2004)**. In our study, an optimal plan was validated.

Design evaluation

A-Efficacity	1.000
D-Efficacity	1.000

- **Discussion**

After the generation of the experimental plan for sensory analysis, we notice that the two criteria A-Efficiency and D-Efficiency are equal, because all the eigen values are equal. These results indicate that our plan is validated and allows us to set up a sensory study conducted with 8 expert subjects evaluating two products.

Product characterization identifies which descriptors arebest discriminate products and what are the important characteristics of these products in the sensory analysis **(Azib, Boukandoul et al. 2013).**

- **Product characterization**

This test displays the ordered descriptors of the one with the strongest power discriminating on the products to the one with the weakest**(Azib, Boukandoul et al. 2013)**.

The figure 22 represents the characteristics ordered from the one having the highest discriminating power to the one that has the lowest discriminating power on the prepared steamed yoghurts

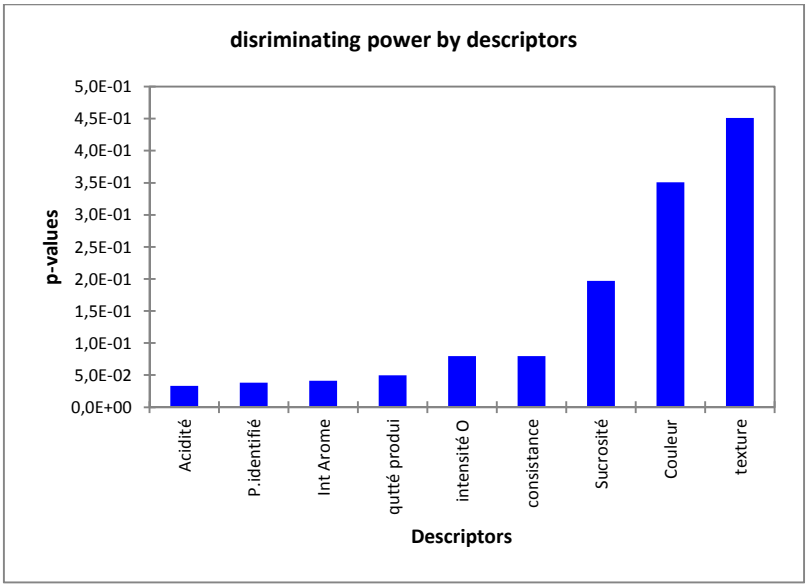


Figure 22: Discriminating power by descriptor.

- **Discussion**

The figure 22 gathers the ordered descriptors of more discriminating at least discriminating on the two samples. It allows to visualize that the acidity is the most discriminating descriptor. All associated *p*-values show a significant effect on the descriptor. The descriptors sweetness, consistency, intensity of odor, intensity of the aroma, product to identify, amount of added product have low discriminating power. This explains why the experts did not find discrepancies in the descriptors listed above for the two tested samples. On the other hand, the least discriminated descriptor is texture and color. This proves that the experts found divergences of this descriptor for the two samples. Which means the success of the adopted manufacturing process.

- **Model coefficients**

The purpose of this test is to treat for each product-descriptor combination, the coefficient, the estimated mean, the *p*-value and a confidence interval on the coefficient **(Næs and Risvik 1996).** The results of the model coefficients are shown in the figures below.

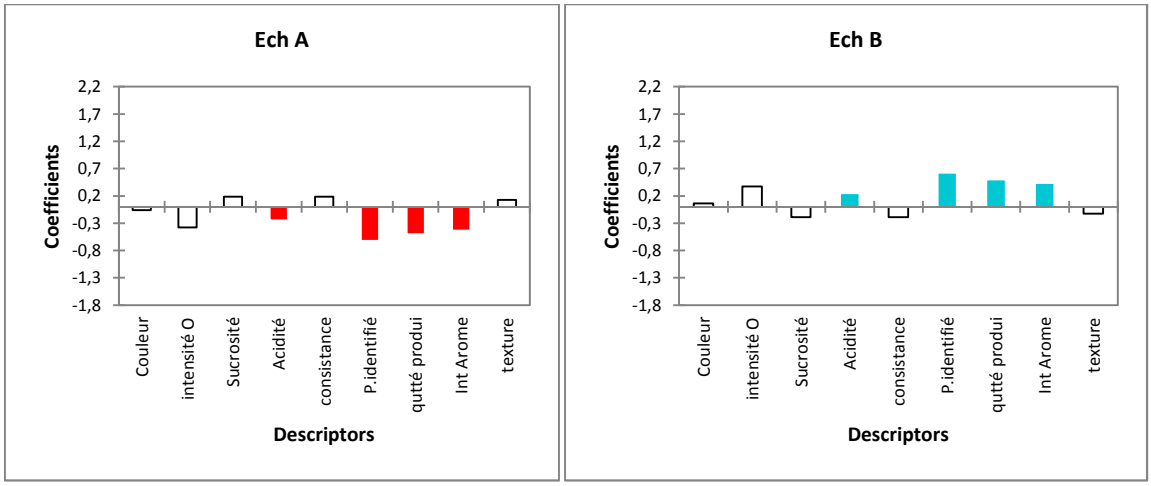


Figure 23: Model coefficients of yoghurts.

➢ **Discussion**

The graphs in the figure 23 allow to define the appreciation or the non-appreciation of the descriptors of the two samples A, B by the expert subjects .The analysis of each chart defines each product :The blue color is associated to a coefficient that has a significant positive value and the red color is associated to a coefficient that has a significant negative value. White color is associated to an insignificant value.

✓ Sample A: The figure shows that the aroma intensity and quantity of product added , the identified product and acidity characteristics presented in red were not appreciated by all the panels, and in white those that the jurors did not arrive to detect them.

✓ Sample B: The figure illustrates that the aroma intensity, amount of product added and the identified product and the acidity presented in blue were detected, these were appreciated by expert juries.In blue, are displayed the characteristics of the product which are not detected by the juries.

- **Mapping preferences**

We did the mapping preferably in order to have an idea on the preference of the judges but these results do not necessarily reflect the preferences of the consumer because the

number of naive subjects questioned is only 8 people which is not representative of population:

In this contour are displayed for each product the percentage of the judges. Thus we find that sample A has a satisfaction percentage of 100%. Sample B has a similar appreciation by the jury. The following figure defines the contour line:

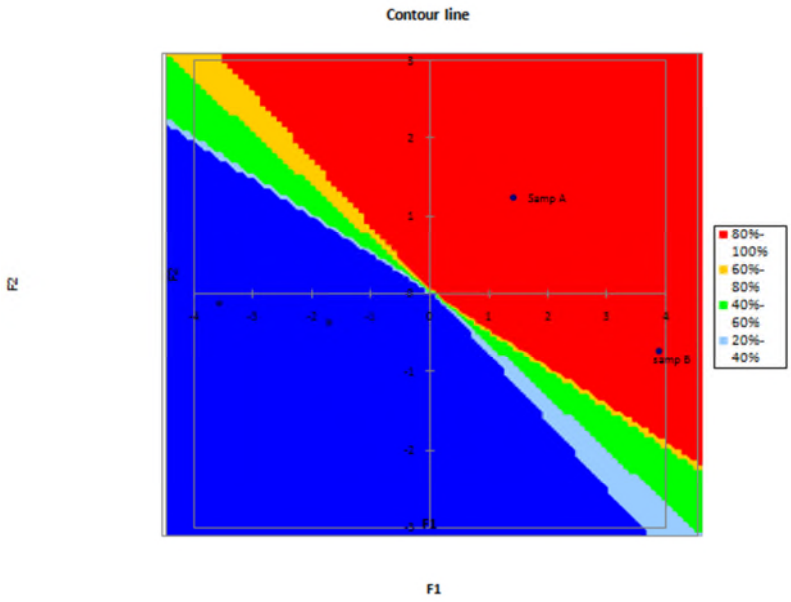


Figure 24: the contour line.

Conclusion and perspectives

This work was carried out in order to valorize the grape leaves after extraction of the phenolic compounds contained therein. Subsequently production of steamed yoghurt enriched with this by-product was performed.

In this purpose, phytochemical analyzes were carried out (determination of total polyphenols and flavonoids, anthocyanins, tannins) of ethanolic extract. The antioxidant activity was then evaluated, followed by further analyzes carried out on the elaborated yogurts.

The results of the phytochemical assays showed that the ethanolic extract of grape leaves contained 63.83 mg EAG / g total polyphenols and 23.44 mg EQ / g flavonoids, and 3.65 mg / g anthocyanins and 0.042 mg cyanidins / 100 g in tannins.

The evaluation of antioxidant properties by the reducing power to ferric chloride reduction and ferozine reduction and the DPPH and ABTS free radical test revealed that the ethanolic extract of vine leaves showed strong antioxidant activity.

The incorporation of *Vitis vinifera L* powder allowed a significant enrichment in phenolic compounds of the prepared yoghurt because of the richness of *Vitis vinifera leaves L*in terms of polyphenols 0.009mg / EAG g (enriched yoghurt) and 0.005 mg EAG / g and (control yogurt)). It was also shown a greater antioxidant activity with the incorporation of *Vitis vinifira* L. (20% of control yoghurt against of 40% enriched yoghurt).

The results of the physicochemical and microbiological analyzes showed that the two steamed yoghurts are appropriate for consumption and have a satisfactory quality and comply to standards.

In addition, the results of the sensory analysis considered by a panel of 8 expert juries, showed the same preference of the standard yogurt and yoghurt enriched with the powder of vine leaf.

As a by-product, leaves of *Vitis vinifera L*. can constitute an interesting potential matrix, to enrich foods in terms of bioactive substances namely polyphenols.

Finally, the obtained results are incomplete characterization, with perspectives, it would be interesting to:

- Determine other types of activities such as anti-inflammatory, antibacterial, etc ;
- Study the modifications of the compositions induced by the presence of powder of vine leaf on preservation time;
- Conduct a complete physico-chemical study, from the raw material to the finished product, including powders;
- Reproduce the formulations of this pilot scale study in an agri-food industry.

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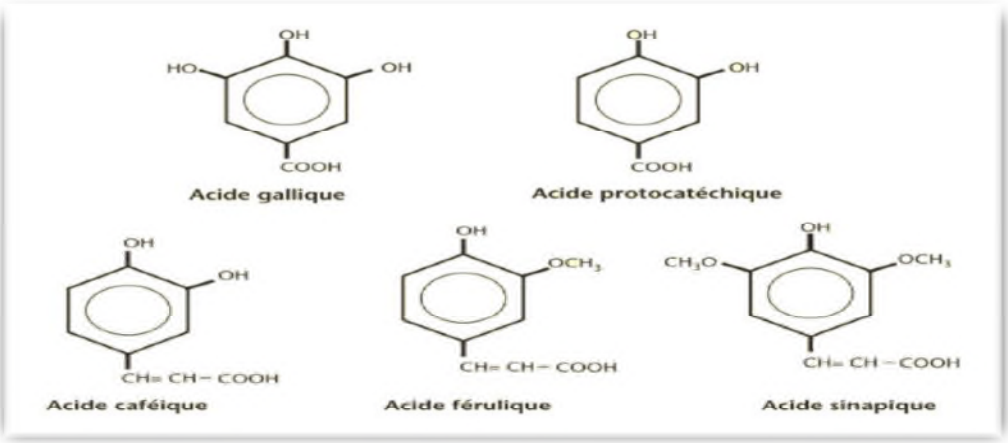
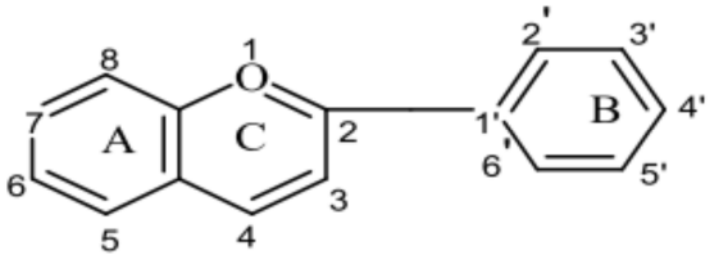
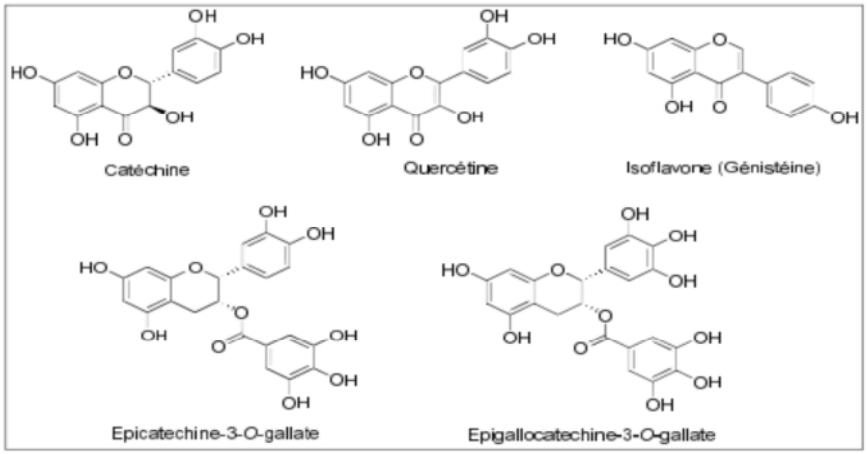


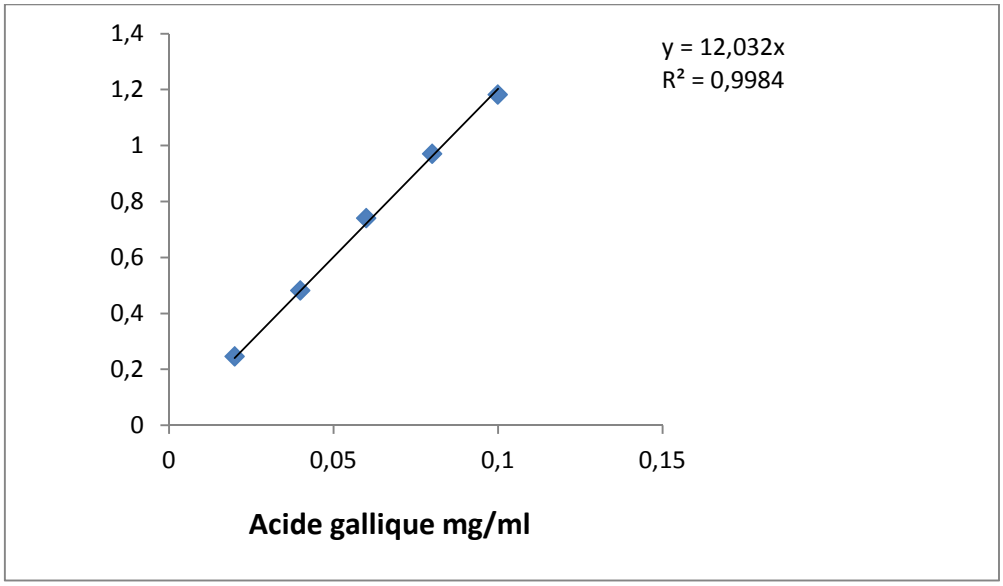
Appendix 01: Basic structure of some phenolic acids **(Léger 2006)**



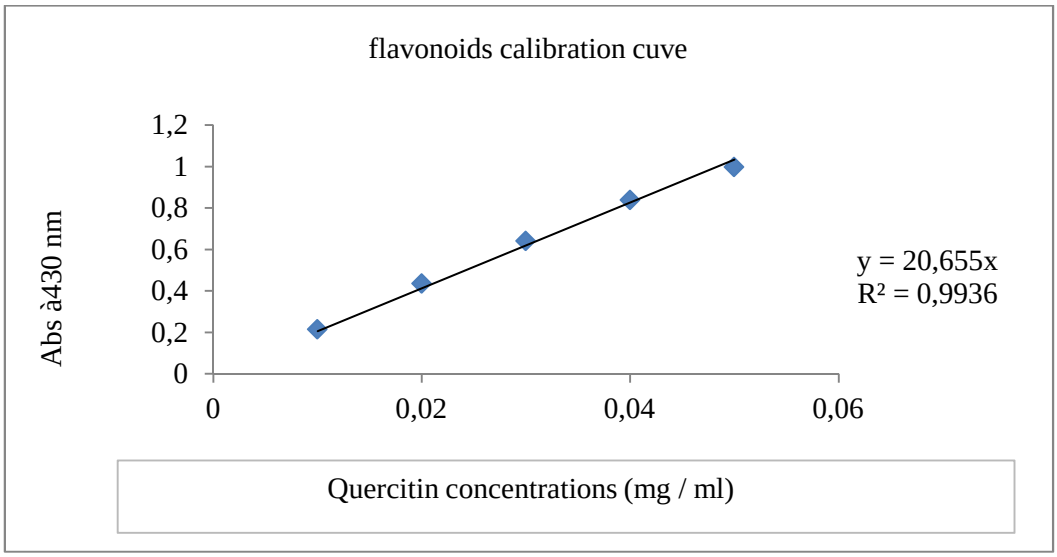
Appendix02: Basic structure of flavonoids **(Léger 2006)**



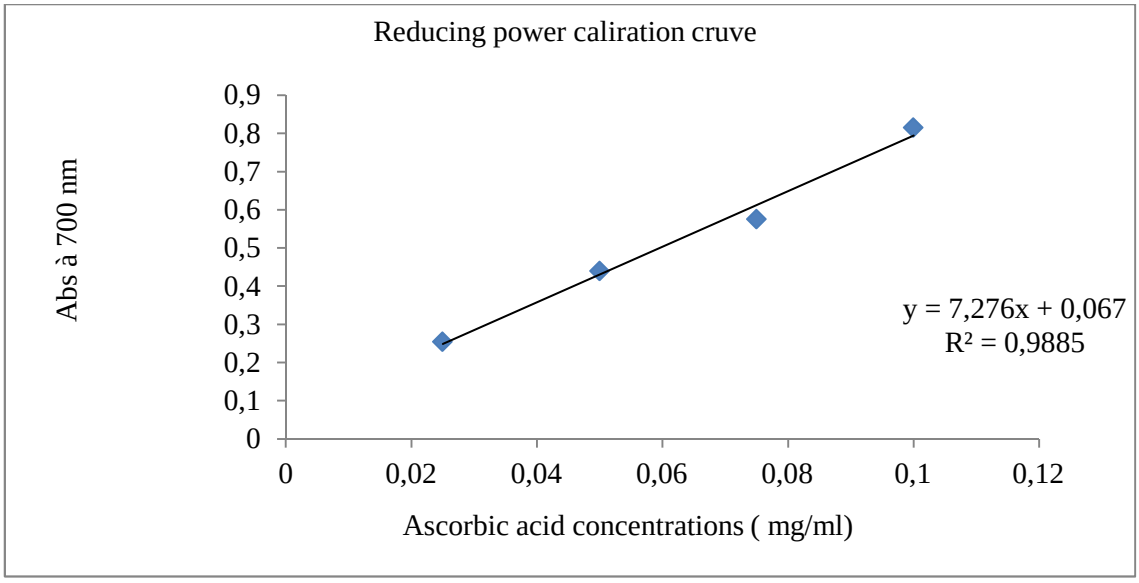
Appendix 03: Structure of some flavonoids **(Belkheiri 2010).**



Appendix 04: calibration curve of total polyphenols



Appendix 05: Flavonoids calibration curve



Appendix 06: Reducing power calibration curve.

∫Questionnaire d'évaluation sensorielle de deux échantillons du yaourt∫

Age :…..... **Sexe** : F ou H **Profession**…….. **Date** :……

Trois échantillons de yaourt étuvé codés **A et B** vous sont présentés, il vous est demandé d'évaluer différentes caractéristiques et d'attribuer une note de 1 à 5 pour chaque échantillon sur l'échelle suivante :

1. Couleur :

1 : Pas appréciée
2 : Peu appréciée
3 : Moyennement appréciée
4 : Bien appréciée
5 : Très appréciée.

Echantillon A	Echantillon B

Attribuez une note de **1** à **9** pour chaque échantillon selon votre préférence par rapport à la couleur :

A ☐ **B** ☐

2. Odeur :

1 : Très faiblement intense
2 : Faiblement intense
3 : Moyennement intense
4 : Fortement intense
5 : Très fortement intense.

Echantillon A	**Echantillon B**

Attribuez une note de **1à 9** pour chaque échantillon selon votre préférence par rapport à l'odeur :

A ☐ **B** ☐

3. Consistance :
1 : Trop liquide
2 : Liquide
3 : Faiblement mou
4 : Moyennement mou
5 : Mou

Echantillon A	**Echantillon B**

Attribuez une note de **1à 9** pour chaque échantillon selon votre préférence par rapport à la consistance :

A ☐ **B** ☐

4. Sensation en bouche :

A. Saveur

- **Saveur sucré :**

1 : Très fort
2 : Fort
3 : Absent
4 : Faible
5 : Moyen

Echantillon A	Echantillon B

Attribuez une note de **1à 9** pour chaque échantillon selon votre préférence par rapport à la saveur :

A ☐ **B** ☐

- **Saveur acide :**

1 : Très forte
2 : Forte
3 : Absente
4: Moyenne
5 : Faible

Echantillon A	Echantillon B

Attribuez une note de **1à 9** pour chaque échantillon selon votre préférence par rapport à la saveur :

A ☐ **B** ☐

- **Attribution de la saveur :**

1. Aucune
2.Feuille de lentisque
3.Romarin

4. Feuille de vigne

5. Feuille de myrte

Attribuez une note de **1à 9** pour chaque échantillon selon votre préférence par rapport à la saveur :

A ☐ **B** ☐

B. Arôme :

1 : Absent
2 : Faible

3 : Moyen
4 : Fort
5 : Très fort

Echantillon A	Echantillon B	Echantillon C

Attribuez une note de **1à 9** pour chaque échantillon selon votre préférence par rapport à l'arome:

A ☐ **B** ☐

C. Texture

1 : Très granuleuse
2 : Granuleuse
3 : Moyenne
4 : Lisse
5 : Très lisse

Echantillon A	Echantillon B

Attribuez une note de 1à 9 pour chaque échantillon selon votre préférence par rapport à l'odeur :

A ☐ **B** ☐

5. Classez selon d'ordre de préférence les échantillons (A, B) en leur attribuant une note de 1 à 9 :

	Echantillon A	Echantillon B
Classement		
Note		

6. Quels sont les caractéristiques qui ont motivé votre préférence ?

1 : La couleur
2 : L'odeur
3 : La texture
4 : Le gout
5 : La consistance

Autre……
…..

* Merci pour votre coopération *

Appendix 07 : Questionnaire of sensory analysis

Product Name	Mark	Country of manufacturer
Gallic acid monohydrate	BIOCHEM Chemopharma	Quebec
Quercetin acid	Riedel-de Haën	France
methanol	GPR RECTAPUR	Germany
Ethanol	SIGMA-ALDRICH	Germany
Acetone	SIGMA-ALDRICH	Germany
Folin-Ciocalteu 1/10	BIOCHEM Chemopharma	Germany
Sodium carbonate 7.5% decahydric	SIGMA-ALDRICH	France
Aluminum chloride to 2%	BIOCHEM Chemopharma	USA
potassium ferricyanide (1%)	BIOCHEM Chemopharma	Quebec
Trichloroacetic acid (10%).	BIOCHEM Chemopharma	Quebec
Iron chloride	BIOCHEM Chemopharma	Quebec
di-hydrogen phosphate potassium at M / 15	BIOCHEM Chemopharma	Quebec
di-sodium hydrogen	BIOCHEM Chemopharma	Quebec
0.6 M sulfuric acid	BIOCHEM Chemopharma	Quebec
sodium phosphate 28 mM	BIOCHEM Chemopharma	Quebec
ammonium molybdate 4 mM	SIGMA-ALDRICH	Germany
DPPH (2,2-Diphemyl-1-picrylhydrazyl)	SIGMA-ALDRICH	Germany
Potassium persulfate 2.45 mM	SIGMA-ALDRICH	Germany

Appendix 08: Solvent and reagents used during work.

Equipment	Apparatus
✓ Beakers ✓ Graduated burette ✓ Quartz tank ✓ Graduated cylinders ✓ Graduated pipettes ✓ Test tubes ✓ Erlenmeyer ✓ Funnels ✓ Vials	✓ Bain-marie (MEMMERT) ✓ Precision balance (SARTORIUS) ✓ Micropipettes ✓ Magnetic stirring plate ✓ (VELP SCIENTIFICA) ✓ Refrigerator (SAMSUNG) ✓ Spectrophotometer (RAYLEIGH) ✓ Vortex (VELP SCIENTIFICA) ✓ Ventilated oven (MEMMERT) ✓ Electric sieve (RETSCH)

Appendix 09: Equipment and apparatus used during the work.

Résumé

Dans certains pays, les feuilles de *Vitis vinifera L* .ont été utilisées pour l'alimentation et pour traiter les troubles. Le but de ce présent travail est l'analyse phytochimique et évaluation de l'activité antioxydante des feuilles de *Vitis vinifera. L*. L'extraction des polyphénols a été réalisée par la méthode conventionnelle. Un yaourt étuvé a été enrichi avec la poudre des feuilles de raisins , les produits finaux ont été soumis à des analyses physicochimiques, microbiologiques et sensorielles.

Les résultats des dosages phytochimiques montre que l'extrait éthanolique des feuilles de vigne contient une teneur de 63.83 mg EAG/g en polyphénols totaux et 23.44 mg EQ/g en flavonoïdes, et 3.65 mg/g en anthocyanines et 0.042 mg cyanidines / 100 g en tannins.

Les analyses physico-chimiques et microbiologiques du yaourt ont montré qu'il est conforme aux normes, De plus que le dosage des polyphénols et l'activité antioxydante de ce dernier montre que la feuille de vigne avait un effet favorable sur la qualité nutritionnelle du yaourt élaboré comme suit : 0.009mg/EAG g (yaourt enrichi) et 0.005 mg EAG/g et (yaourt témoins) .Il manifeste également une plus grande activité antioxydante avec l'incorporation de la poudre de *Vitis vinifira* .L 20% (yaourt témoins) et 40% (yaourt enrichi). Ainsi l'analyse sensorielle a révélé une meilleure qualité organoleptique.

Mots clés : Feuilles de *Vitis vinifera.L,* polyphénols, yaourt, analyse phytochimique, activité antioxydante.

Abstract

In some countries, the leaves of *Vitis vinifera L*. have been used for feeding and to treat disorders. The aim of this work is the phytochemical analysis and evaluation of the antioxidant activity of Vitis vinifera leaves. L. The extraction of the polyphenols was carried out by the conventional method. Steamed yogurt was enriched with the grape leaf powder and the final products were subjected to physicochemical, microbiological and sensory analyzes.

The results of the phytochemical assays show that the ethanolic extract of grape leaves contains 63.83 mg EAG / g total polyphenols and 23.44 mg EQ / g flavonoids, and 3.65 mg / g anthocyanins and 0.042 mg cyanidines / 100 g in tannins.

The physicochemical and microbiological analyzes of yoghurt have shown that it complies with the standards. Moreover, the polyphenol dosage and the antioxidant activity of the latter show that the grape leaves has a favorable effect on the nutritional quality of the yogurt. prepared as follows: 0.009mg / EAG g (enriched yogurt) and 0.005 mg EAG / g and (control yoghurt) .It also exhibits greater antioxidant activity with the incorporation of *Vitis vinifira L* powder .20% (control yogurt)) and 40% (enriched yogurt). So the sensory analysis revealed a better organoleptic quality.

Keywords: *Vitis vinifera.L* leaves, polyphenols, yogurt, phytochemical analysis, antioxidant activity.