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MASTER

Thème

**The effect of *Myrtus Communis* fruits on
physicochemical and antioxidant activity of
yogurt.**

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With those which very gave me without anything in return has those which encouraged me and supported in the most difficult moments

And those with which I must so much

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

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All the food promotion of science: 2015/2016

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List of abbreviation

Abs:	Absorbance
ABTS:	2, 2' –azynobis-[3- ethylbenzothiazoline- 6- sulfonic acid].
AT:	tritatable acidity
Aw:	Activity of water
CFU:	unit format colony
DPPH:	2, 2-Diphenyl-picrylhydrazyl
GAE:	Gallic Acid Equivalent
Kcal:	Kilo calorie
MG :	Matière grasse
pH:	Hydrogen potential
RP:	Reducing power
TPC:	Total Phenolic Compounds
V/v:	volume/ volume
W:	Watt
MAE:	Microwave

Introduction

Introduction

Dietary polyphenols have received tremendous attention among nutritionists, food scientists and consumers due to their roles in human health. Research in recent years strongly supports a role for polyphenols in the prevention of degenerative diseases, particularly cancers, cardiovascular diseases and neurodegenerative diseases (Tsao, 2010). Most of the drugs from plants which have become important in modern medicine had a folklore origin and are traditionally used in some systems of medicine (Bakhtaoui *et al.*, 2014).

Myrtle (*Myrtus Communis L.*) belongs to the family of Myrtaceae; it is shrub typical representative of the Mediterranean flora. Myrtle is an evergreen shrub, which grows as wild in several regions all over the world (Aydın and Özcan, 2007). Different parts of the plant have found various uses in the food industry, such as for flavoring meat and sauces, in the cosmetic and pharmaceutical industries (Messaoud and Boussaid, 2011). And it has been also used in folk medicine because of its astringent and balsamic property (Flamini *et al.*, 2004; Oddo *et al.*, 2004).

In recent years consumers have an increased awareness of the relationship between diet and health and are demanding the natural products, fresh like nutritional characteristics and attractive sensory properties. The industry searches for market opportunities and tends to diversify their products by the preparation of new foods, enhancing sensorial, nutritional and functional attributes (Andrés *et al.*, 2015).

Yoghurt with added antioxidants from natural sources appears to be a convenient food format to satisfy consumer interest in original yoghurt nutrients, beneficial effects of starter cultures, and health benefits of added antioxidants. For this reason, several attempts to produce yoghurts fortified with natural antioxidant-rich extracts have been undertaken, including supplementation with polyphenols-rich extract (Chouchouli *et al.*, 2013).

Introduction

In the present studies, the changes in physicochemical, phenolic content, organoleptic properties and antioxidant activity of yogurt upon the addition of *Myrtus Communis* fruits were investigated.

Bibliography

Part

I. 1. Myrtle (*Myrtus communis L.*)

Myrtus communis L. (myrtle) (Myrtaceae) is an evergreen shrub which grows mainly in Mediterranean climates and has long been used by locals for its culinary and medicinal properties. (Ghasemi *et al.*, 2014). It belongs to the Myrtaceae family with some 145 genus and over 5500 species (Berka *et al.*, 2012).

In Algeria, the wild plant known as —Al-Rihan‖ or —el-halmouchel‖ grows very well in many areas, on mounds or hills, in coastal or in more remote areas. Different parts of the plant have found various uses in the food industry, such as for flavoring meat and sauces, in the cosmetic and pharmaceutical industries (Aidi Wannes *et al.*, 2010; Messaoud *et al.*, 2011). Myrtle flowers, leaves and berries are used for external applications to heal wounds, for skin diseases (psoriasis, herpes, bruises etc.) and for internal functions to treat many diseases such as dysentery, urinary tract infections, hemorrhoids, and even hair loss. In some areas, its use is recommended to lower blood sugar as well as to improve digestion. However its main use is for the treatment of respiratory problems (Berka *et al.*, 2012). Additionally a source of essential oil content in its leaf, flower and fruit glands (Ghasmi *et al.*, 2014).

I.2. Morphological description

The common myrtle, *Myrtus communis L.*, are a small wild shrub of 1 à 3m from height to sheets persistent and dense, ovoid lancéolées, with nervation pinnate. These flowers are large (10 - 15mm), white, hermaphrodites. Flowering this fact in summer (June at July). These fruits are spherical bays dark crimsons (diameter: 5mm) with many seeds, appears as from November at December. It is a species resistant to hot and cold time and the stone ground with cabbages and/or silica (Quizel and santa, 1963; Govaerts and Lucas, 2008).



Figure 1: Branches and fruit of *Myrtus Communis* (chokri *et al.*, 2010)

I.3. Chemical composition

The chemical composition and the mineral contribution of fruit of myrtle were indicated in **table (I) and (II)**. This fruit is rich in fibers and contains considerable quantities out of proteins, reducing sugars and essential oils (Aydin and Ozcan, 2007).

Table I: The chemical composition of fruit of the myrtle (Aydin and Ozcan, 2007)

components	Contents (%)
Moisture	74.44
Proteins	4.17
Fibers	17.41
Energy (Kal/g)	11.21
Reducing sugars	8.64
Essential oils	6.56

Table II: The Mineral composition of the fruit of the myrtle (p. p. m) (Traveset *et al.*, 2001)

Minerals	Contents
Nitrogenize	0.310
Phosphorus	0.043
Potassium	0.750
Calcium	0.274
Magnesium	0.131
Sodium	0.192
Copper	3.5
Iron	32
manganese	9
zinc	7

I.4. Phenolic composition

Polyphenols are secondary compounds widely distributed in the plant kingdom. They are cyclic derivatives of benzene with one or more hydroxyl groups associated to the aromatic ring. The main classes of polyphenols are defined according to the nature of their carbon skeleton: phenolic acid, flavonoid (Andjelković *et al.*, 2006).

Fruits are rich of volatile compounds, tannins, sugars, anthocyanins, fatty acids and organic acids such as citric and malic acids. Several studies have focused the antioxidant, antimicrobial and anticancer features of various myrtle extracts. (jose Antonio curel *et al.*, 2015).

I.4.1. Biological activity of phenolic composed

The different biological activities of phenolic compounds myrtle were showed in table (III).

Table III: Biological activity of phenolic composed of myrtle (Hadi, 2004; Murphy, 1999; Sedat serce *et al.*, 2010).

Activity	Role
Antioxidant	<ul style="list-style-type: none"> • The radicals free could into chelating metals of transmission such as copper and iron. • Can protect itself from the peroxidation from lipid and can clean the radicals free. • Among these antioxidants more drawing seem to be the flavonoids which can react the lipidic peroxidation effectively since it can react with the free majority of the radicals
Antimicrobial	<ul style="list-style-type: none"> • The deprivation of substrate. • The interruption of the membrane function. • Destruction of the cellular wall and deactivation of the enzymes. • Inhibition of transcriptase reverses of the HIV.
pest-destroying effect	<ul style="list-style-type: none"> • the consumption of plants with tannins could affect the biology of certain species of intestinal nematodes by decreasing the production of eggs

I.5. Antioxidant activity

The myrtle is employed like remedy to treat the diseases related to the oxydative stress for its capacity in antioxidant compounds such as myrtu-commulone and semimyrtucommulone which can stop the formation of the oxygenated reagents and of the peroxides which have a relationship with the initiation and the maintenance of the inflammatory activity. The essential oil of this plant presents also an antioxidant character (Feibt *et al.*, 2005; Rotstein *et al.*, 1974; Montoro *et al.*, 2006; Yadegarinia *et al.*, 2006).

I-6- Antidiabetic activity

Experiment were carried out on rabbits reaches diabetes: in their administrating amounts of 50 Mg/kg of the extract of *Myrtus communis* each day during one weeks, a fall of 51% of the rate of glucose in blood was observed, without affecting the rate of insulin, as well as a fall of the rate of blood triglyceride of 14%. That would be explained by the inhibiting activity of the extract of the myrtle on alpha glucosidase and stimulation of the glucokinase which is a key enzyme of glycolysis (Sepici *et al.*, 2004).

I.7. Antibacterial activity

The richness of the myrtle in phenolic compounds (flavonoids and tannins) and out of essential oil is at the origin of its activity antibacterial, *Escherichia coli* and *Staphylococcus aureus* is the germs most sensitive; Myrtucommulone has and B and semimyrtucommulone are polyphenols oligomeric present in the sheets of the myrtle, having a antibacterial activity comparable with that of penicillin and streptomycine in a pure state (Feibt *et al.*, 2005; Rotstein *et al.*, 1974; Montoro *et al.*, 2006; Yadegarinia *and al.*, 2006). The plant extract can inhibit the growth of bacteria like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Mansoureh Masoudi *et al.*, 2016).

I-8- Pharmacological activity

Many authors announced that the myrtle and its essential oils have a great potential like plants medicinal, with properties: hypoglycemic (**El fellah *et al.*, 2002; Appendino *et al.*, 2006; Gholamhoseinian-Najar *et al.*, 2009**), anti-inflammatory drugs (**Rossi *et al.*, 2009; Amira *et al.*, 2012**), anti-ulcerous (**Sumbul *et al.*, 2010**), anti-mutagen (**Hayder *et al.*, 2008; Mimica-Dukic *et al.*, 2010**) and antioxidant (**Montoro *et al.*, 2006; Aidi wannes *et al.*, 2010; Tuberoso *et al.*, 2010**).

I-9- Activation of immune system:

Inflammation is a feature of nearly every disease where the immune system is called to respond. Perhaps the most beneficial effect essential oils have on immune modulation is their ability to inhibit the multitude of inflammatory processes that contribute to almost every immune response in illness and disease. We will look closely at these processes and how essential oils inhibit their activities (**Michael *et al.*, 2001**).

II. 1. Definition and Classification

Yogurt which is a product of Lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus*; is one of the most traditional cultured milk. (Amakoromo *et al.*, 2012). Typical composition of yoghurt is shown in Table IV.

Table IV: Chemical composition of typical yogurt

Constituent (per 100 g)	Standard yogurt	Fruit yogurt
Water (g)	81.5	77.0
Total solids (g)	18.1	23.0
Fat (g)	3.0	0.7
Protein (g)	5.7	4.1
Lactose (g)	7.8	
Calcium (mg)	200	150
Phosphorus (mg)	170	120
Sodium (mg)	80	64
Potassium (mg)	280	210
Zinc (mg)	0.7	0.5

- Industrially, yoghurts can be largely divided into two types. A set-style yogurt is made in retail containers giving a continuous undisturbed gel structure in 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 and then it is packaged. Stirred yogurts should have a smooth and viscous texture. In terms of rheology, stirred yogurt is a viscoelastic and pseudoplastic product. Yoghurts 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, cereal), 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 subgroups (Mckinley, 2005).

II.2. Health Benefits

Better growth and increased nutrient utilization are associated with yoghurt consumption, due to increased protein digestibility. Milk fat also becomes easily digestible due to certain pre-digestion reactions during fermentation. Yoghurt provides higher levels of protein, carbohydrate, calcium and certain B vitamins than milk (**Kumar and Mishra, 2004**). Yoghurt is not just seen as a diet food but also a health food because of its therapeutic value and it is consumed as both as a food and a thirst quenching beverage. Increased yoghurt consumption enhances the intestinal environment and immune system due to the presence of yoghurt starter and probiotic bacteria which should be present at recommended concentration of log 6 to 8 CFU /g at the time of consumption (**Amakoromo et al., 2012**).

Health benefits of yoghurt are correlated with the presence of living microorganisms like lactic acid bacteria, streptococci, bifidobacteria or their combinations, which originate from the starter cultures and are recognized as functional ingredients. Yoghurt with added antioxidants from natural sources appears to be a convenient food format to satisfy consumer interest in original yoghurt nutrients, beneficial effects of starter cultures, and health benefits of added antioxidants. For this reason, several attempts to produce yoghurts fortified with natural antioxidant-rich extracts have been undertaken, including supplementation with polyphenol-rich wine extract (**Chouchouli et al., 2013**).

The diagram of the manufacture of simple and fruits enriched yogurt was showed in figure n°2

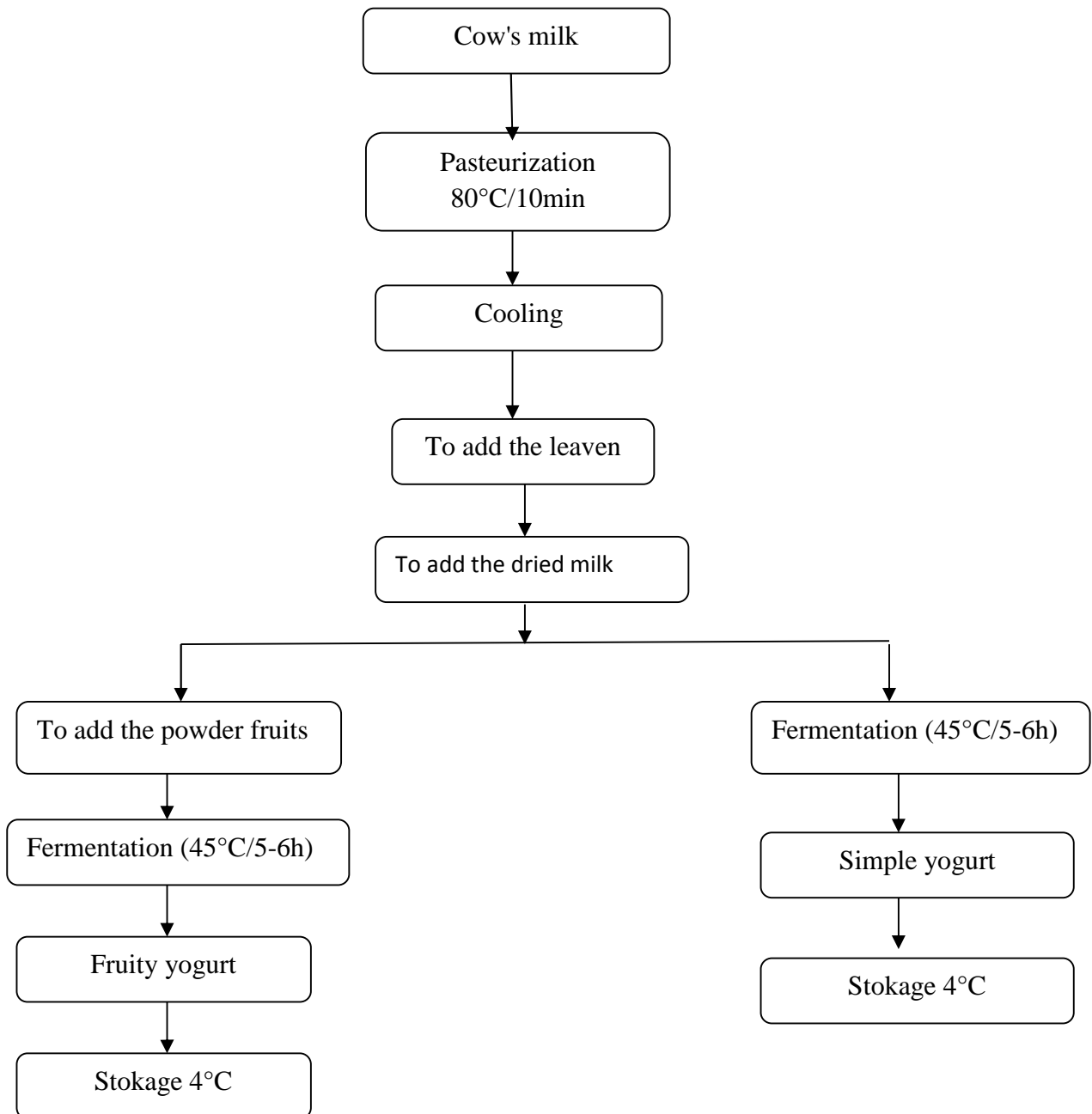


Figure 2: The diagram of the manufacture of simple and fruits enriched yogurt (Zainoldin and Baba, 2009).

*Material and
methods*

III. 1. Chemicals

All solvents and reagents used were of analytical grade. Sodium carbonate (Na_2CO_3) was supplied from prolabo(CE).folin-ciocalteau's phenol reagent, 1,1-diphenol-2-picryl-hydrazil(DPPH) were purchased from sigma- aldrich (germany). gallic acid and rutin were supplied from biochem-chmopharma(UK).

III.2. Plant Material

Myrtus communis L plant was collected during the month of January 2016 on the level of the area of El-kseur of the wilaya of Bejaia. The harvested plant materials were washed with running tap water to remove surface contaminants then with water distilled. The fruits part was dried in the microwave at 700 w during 14 minutes until constant weight. It's peeled manually and seeds are recovered. The fruits were ground with an electric vertical grinder (IKA model A11 Basic, staufen, Germany), the obtained powder was passed through standard 250 μm sieve and only the fraction with particle size $<250\mu\text{m}$ was used. The powder was stored in airtight bags until use.

III.3. Evaluation of moisture content:

Thermal drying method was used in the determination of moisture content of the sample. 10 g of sample the echantillon was placed in drying oven at $103^\circ\text{C} \pm$, until constant weight The moisture content (MC) was calculated by the following formula (Doymaz *et al.*, 2004).

$$H\% = \frac{W_i - W_0}{W_i} \times 100$$

- Where W_0 correspond to the loss in weight (g) on drying and W_i correspond to the initial weight of sample (g).

III.4. Formulation of fruity stirred yogurt at laboratory scale

III.4.1. Preparation of starter culture

The preparation of the culture is considered followed according to the protocol (Zainoldin and Baba, 2009) with modification. Full-cream milk pasteurized at heated summer with 41°C. A mixture which contains *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* was mixed thoroughly with the preheated milk followed by incubation for 24 hours at 41°C. The yogurt formed formed was stored at 4°C and used as starter culture within 14 days.

III.4.2. Preparation of fruit-yogurt

The preparation of yogurt was made in laboratory “BBBS” (Biomathématique biochimie biophysique et scientométrie) respecting the diagram to make standard yogurt. Yogurt was made by mixing 10ml of starter culture with 90ml of preheated milk. Myrtle fruits-yogurt with varying composition (0.2%, 0.4%, 0.6%, 0.8%) was made by adding 0.18g, 0.36g, 0.54g, 0.72g of gently mashed fruit into 90ml of preheated milk. Total milk solid content for the yogurt was corrected by adding 18g of milk powder for every 10ml mashed fruit used. After (5- 6) hours incubation at 41°C, the yogurt formed was stored at 4°C (Zainoldin and Baba, 2009).

III.4.3. Sample (yogurt water extract) preparations

Yogurt sample (10g) was mixed with 2.5ml distilled water and the yogurt pH was adjusted to 4.0 using 1M HCL. The yogurt was then incubated at 45°C for 10 minutes followed by centrifugation (10000rpm, 20 minutes, 4°C). The supernatant was harvested and the pH was adjusted to 7.0 using NaOH. The neutralized supernatant was recentrifuged (10000rpm, 20 minutes, 4°C) and the supernatant was used in analysis (Zainoldin and Baba, 2009). Figure n °3

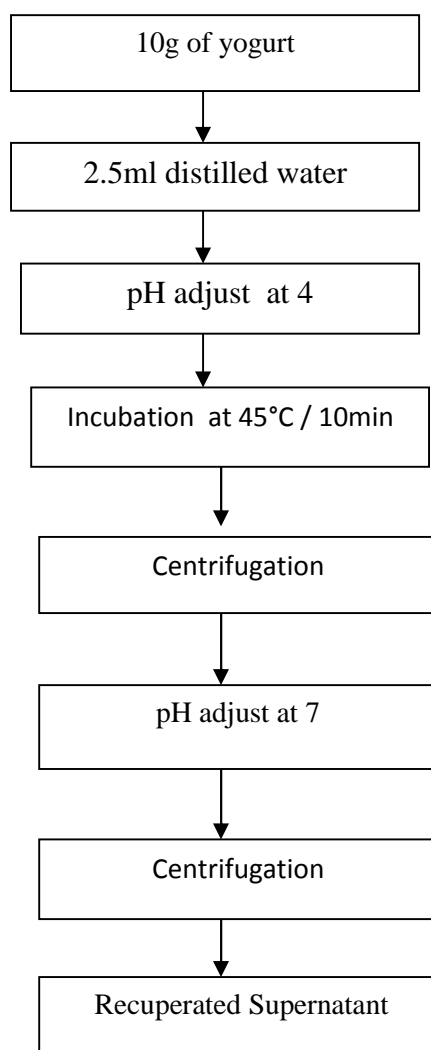


Figure 3: Sample (yogurt water extract) preparations

III.4.4. pH measurement

The pH of yogurt was measured by mixing 1ml of yogurt in 3ml of distilled water. The pH reading was read using Microprocessor pH Meter (pH 211). This procedure was performed in triplicate (Zainoldin and Baba, 2009).

III.4.5. Total tritatable acidity

Yogurt sample (1ml) was mixed thoroughly with 9 ml of distilled water phenolphthalein solution (0.1%, 3 drops) was added and the yogurt suspension was titrated

using 0.1M NaOH. The mixture was stirred continuously and titrated was continued until the indicator changed to a definite pink colour lasting for 30 seconds. The volume of NaOH required to neutralize the yogurt acid was recorded and used to calculate the content of titratable acids (lactic acid percentage equivalent) using the following formula: (Zainoldin and Baba, 2009).

$$LA\% = \frac{10 \times V_{\text{NaOH}} \times 0,009 \times 0,1}{W} \times 100$$

Where:

10 = Dilution factor

W = weight of sample for titration

V_{NaOH} = Volume of NaOH used to neutralize the lactic acid

0.1 = Normality of NaOH

III.4.6. Syneresis measurement

Yogurt syneresis (the released of whey) was determined by the centrifugation method with some modifications. Yogurt (20g) was centrifuged (640g, 20 min, 4°C) and the clear supernatant was harvested and weighed. Syneresis was calculated according to the following equation (Zainoldin and Baba, 2009).

$$\text{syneresis}\% = \frac{\text{weith of supernatant(g)}}{\text{weith of yogurt (g)}} \times 100$$

III.4.7. Total phenolic content

The total phenolic content was determined by an assay modified from. Homogenized yogurt water extract (1ml) was transferred into a test tube and mixed with 1 ml of 95% ethanol and 5ml of distilled water (Zainoldin and Baba, 2009). A volume of 250µL of diluted extract with distilled water was added to 1.25 mL of 10-fold diluted Folin–Ciocalteu reagent. The solution was mixed and incubated at room temperature for 2min. After 2 min, 1 mL of 7.5% sodium carbonate (Na₂CO₃) (v/v) were added. After incubation at 50°C for 15 min, the absorbance of the sample was measured at 760 nm

against a blank (made as reported for the sample) by using a UV–VIS Spectrophotometer (SpectroScan 50, Nkesia, Cyprus). The assay was performed in triplicate. For quantification, a calibration curve was generated with the standard solution of gallic acid, ($R^2 = 0.998$). The TPC were expressed as mg of gallic acid equivalent (GAE) per gram of powder and dry weight (AW) basis (George *et al.*, 2005).

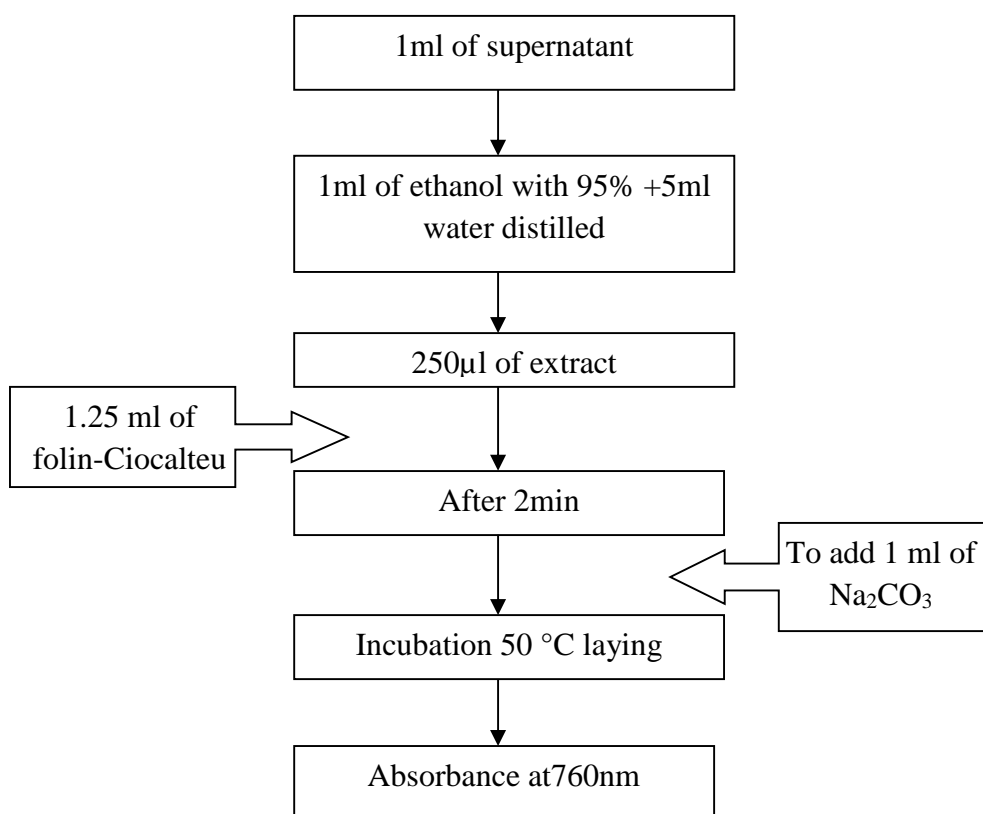


Figure 4: Phenolic content protocol

III. 4.8. Antioxidant activity

III. 4.8.1. 1, 1-diphenyl-2-picrylhydrazyl radical inhibition assay (DPPH)

Antioxidant activity of yogurt samples by 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition homogenized yogurt water extract (250 µl) was added into 3 ml of 60 µM DPPH in ethanol. The decrease in absorbance was monitored at 517 nm until a constant reading was obtained. The reading was compared with the controls which contained distilled water (250 µL) instead of yogurt water extract. The inhibition percentage was calculated follows: (Zainoldin and Baba, 2009).

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \times 100$$

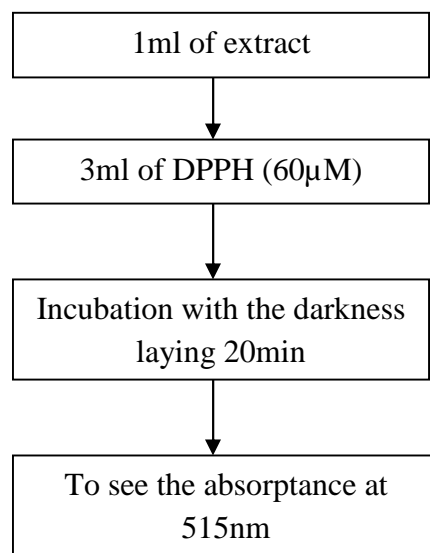


Figure 5: Test of DPPH

III.4.8.2. Iron reducing power

In this study, the yellow color of the test solution changes to green depending on the reducing power of test specimen. The presence of reductants in the solution causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. One mL of desired dilution with distilled water of fruits extracts was mixed with 2.5 mL of a 0.2 M sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% Potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$). The mixture was incubated in a water bath at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid were added. At the end, 1mL of the obtained solution was added to 5 mL of distilled water and 1mL of 0.1% ferric chloride (FeCl_3), the intensity of the blue green color was measured at 700 nm. Tests were carried out in triplicate (**Gulcin *et al.*, 2005**).

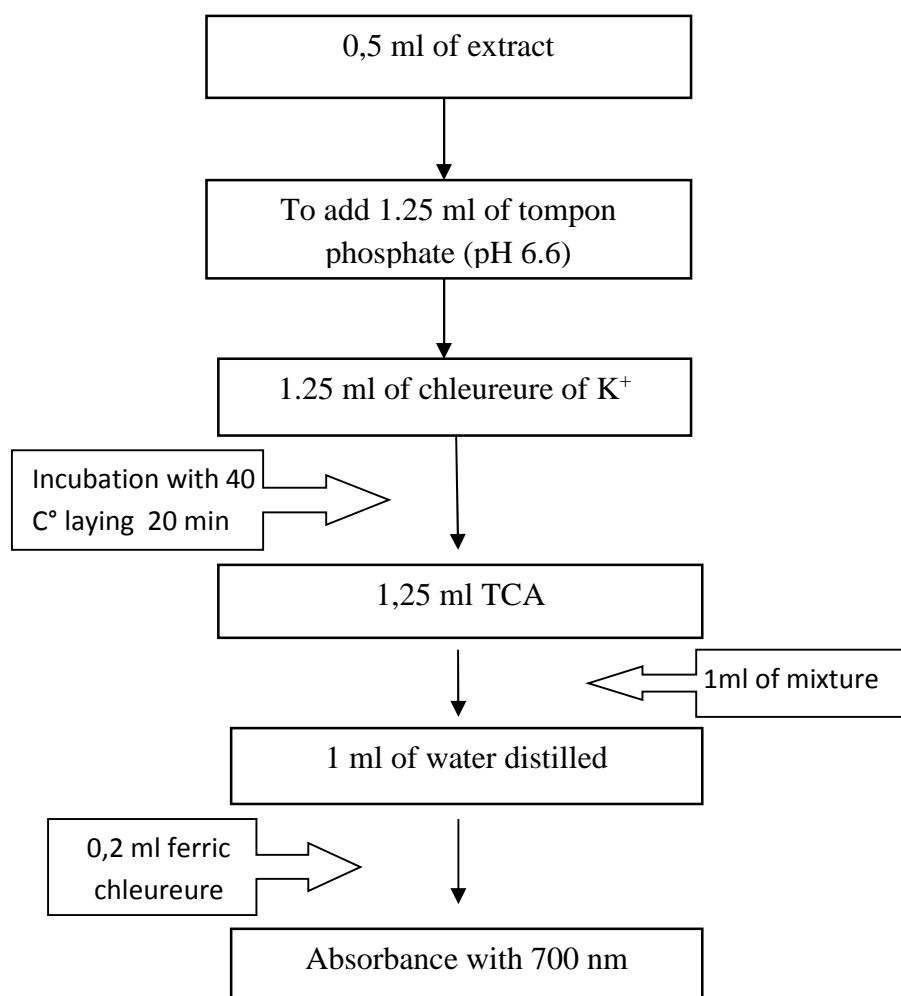


Figure 6: Iron reducing power

III.4.8.3. ABTS method (2, 2' -azynobis-[3- ethylbenzothiazoline- 6- sulfonic acid])

The ABTS method used was previously described by (Erel, 2004). The ABTS⁺ radical cation was produced by mixing a volume of 8 mM ABTS with the same volume of 3 mM potassium persulfate and incubating for 16 h at room temperature in the dark. The working solution was obtained by diluting with 50% methanol to an absorbance of 0.70 ± 0.02 at 734 nm. Quercetin was used as a standard and the results were expressed as Quercetin mg equivalents per gram of dry weight (mg QE/ g DW). The percentage inhibition was calculated according to the following formula:

$$\%Inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where A is the absorbance of the control or of the sample.

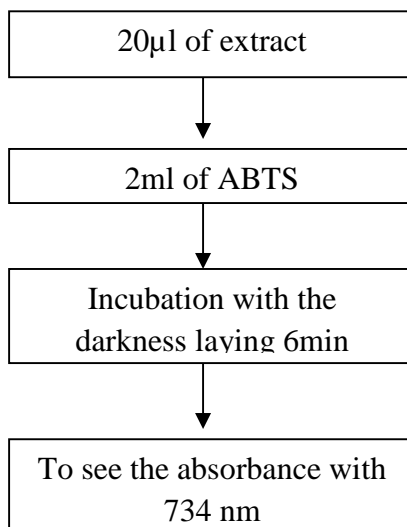


Figure 7: the ABTS test

Results and discussion

IV. Sample preparation

IV.1. Water content

The water content (% of moisture) of the *Myrtus Communis* fruit is represented in table V.

Table V: water content of the *Myrtus Communis*

Fruit	
Moisture %	56.7%

The content water of the myrtus communis fruits is 56.7%, this parameter has a great importance because it presents water is regarded as a source of oxidation (**Ribéreau-gayon, 1968**).

IV.2. Analysis of prepared stirred yogurt

IV.2.1. Physico-chemical analysis of yogurt

IV.2.1.1. pH and total titratable acidity

Table VI: pH and lactic acid percentage

Powder concentration	pH	AT
0,2	5,046 ± 0,049	0,89 ± 0,005
0,4	4,57 ± 0,043	1,2 ± 0,51
0,6	4,02 ± 0,12	1,83 ± 0,057
0,8	4,76 ± 0,145	0,91 ± 0,017
Standart	3 ± 0,101	4.3 ± 0,011

For the pH measurement during fermentation, it showed that myrtle fruit helps to enhance the milk fermentation rate. After 7hours fermentation, all fruit concentration enriched yogurt showed a best pH reading since for yogurt at 0, 6g/ml compared to plain yogurt.

In terms of lactic acid percentage (TTA), it showed that all fruit enriched yogurt has significantly different acid lactic percentage compared to plain yogurt. Since at concentration to 0, 6 g /ml compared to plain yogurt.

pH measures free H⁺ ion whereas the total titratable acidity measure total organic acid that present in yogurt. Both measurements are important because acidification is the key mechanism during yogurt fermentation was due to the proto-cooperative action of two strain of bacteria *S.thermophilus* and *L.bulgaricus* (**Zainoldin and Baba, 2009**).

The presence milk sugar (carbon source) and milk protein (nitrogen source) in the rich medium of milk and optimum incubation environment (pH 7 and 41C°) encourage the bacterial strain (*S. thermophilus*) to grow rapidly. They transform lactose acid into lactic acid, acetaldehyde, diacetyl, and formic acid. The accumulation of all these fermentation products corresponds to the increasing of acid production during fermentation. The liberation of lactic acids reflects the high metabolic activity of the lactic acid bacteria (**Zainoldin and Baba, 2009**).

The value of pH decreased to theof $4,02 \pm 0,12$ to $3 \pm 0,101$, respectively after 24 and 48 h of fermentation with significant difference of $p < 0.05$. Enterobacteria were not detectable by plate count in 1 ml of homogenate. All further experiments referred to myrtle homogenate, which was supplemented with yeast extract and fermented for 48 h (optimal culture conditions). (**Jose Antonio Curiel et al., 2015**). The milk initially had a pH of 7.0, once inoculated, the pH gradually decreased to a pH of 4.5 (**Agil and Hosseinian, 2012**).

IV.1.2.2. Syneresis measurement

Yogurts with different concentrations of myrtle pericarp showed a higher syneresis percentage compared to plain yogurt (figure n°8). Yogurt with 0.6g/ml pericarp fruit showed the highest syneresis (83, 55%). This increasing in syneresis is probably due to decreasing in water holding capacity that leads to more releases of whey. The introduction of fruits did not increase the fiber contents in yogurt, which otherwise would hold the water and thus increase the syneresis. The watery structure of the fruits themselves may lead to more releases of whey in the pericarp-yogurt. Our results was highest than that obtained by **Zainoldin and Baba, (2009)** about the effect of *Hylocereus polyrhizus* and *Hylocereus undatus* on Physicochemical, Proteolysis, and Antioxidant Activity in Yogurt it was about (70, 32%).

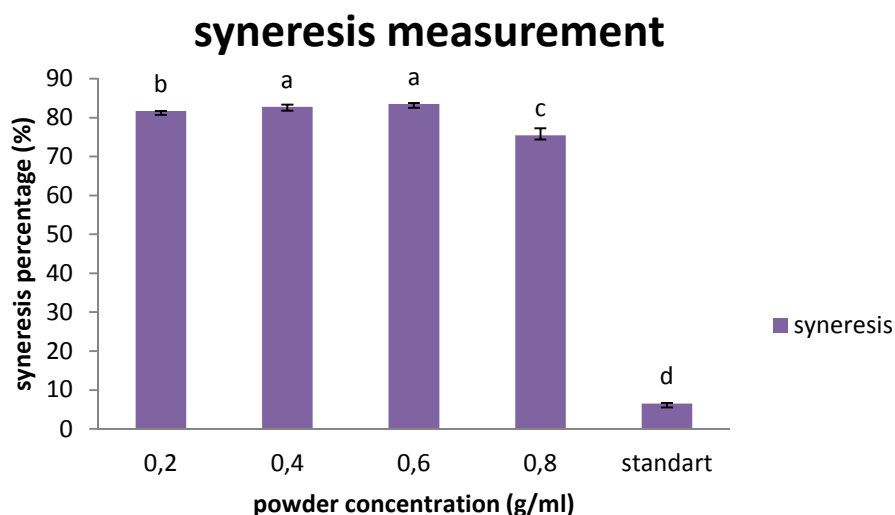


Figure 8: syneresis meurement in yogurt

IV.2. Total phenolic content assay

After addition of the reagent of folin-ciocalteu and sodium carbonate, a blue color was noted in the reactional medium whose intensity varies according to the total polyphenol concentration.

A large number of plants were screened to be sources of novel phenolic compounds for alimentary, cosmetic and pharmaceutical uses. First, this study reported the capacity of a lactic acid bacterium to enhance the antioxidant properties and the phenol profile of myrtle berries (Curiel *et al.*, 2015).

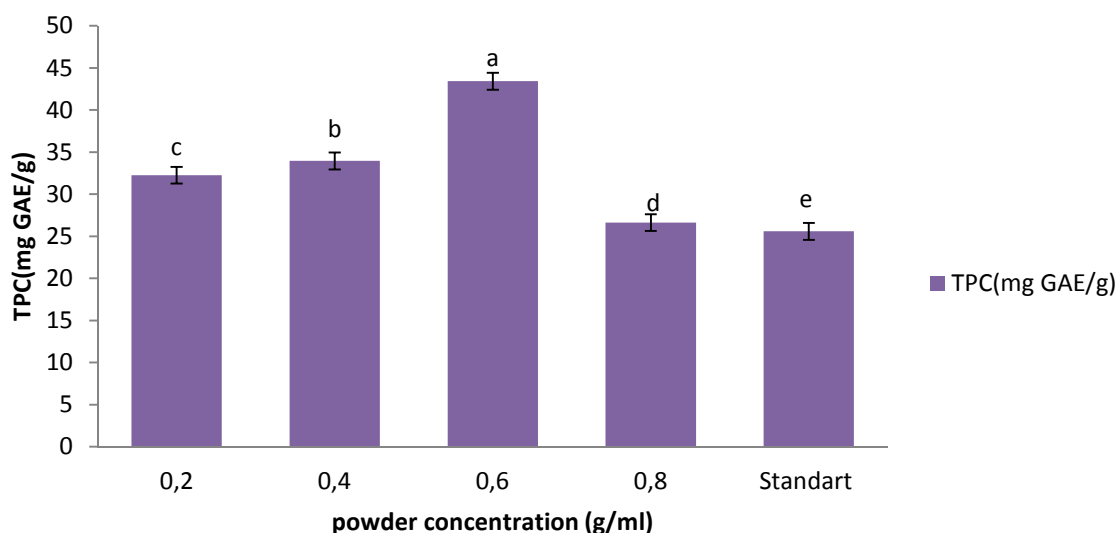


Figure 9: Total phenolic content in yogurt enriched by *Myrtus Communis* fruits

All fruit enriched yogurt showed increment in total phenol content compared to plain yogurt. The highest TPC content was attributed to enriched yogurt at concentration 0,6g/ml fruit powder ($P < 0.05$) at 43.42 ± 0.169 mg GAE/g.

The variation of the contents for different yogurt was attributed with the present polyphenol polarity in the myrtle fruit. While referring to literature, our results obtained was lower than that obtained by Zainoldin and baba (2009). The content of total phenolic recorded in their study is estimated at 64.43 mg/ml. That also proved that there is appreciably different in the contents from phenolic compounds between yogurt enriched by fruit and simple yogurt (Chouchouli *et al.*, 2013). This result can be showed that the fermentation caused an increase of the concentration of total phenols (Curiel *et al.*, 2015).

In addition the phenolic composition of the fruits is influenced by the intrinsic factors (species, variety) and extrinsic (conditions of culture) but can be also modified by the drying of the fruit which can destroy or convert polyphenols into a nonantioxydant form (Manach *et al.*, 2004; Vinson *et al.*, 2005).

IV.3. Antioxidant activity

IV.3.1. DPPH inhibition assay (2, 2-Diphenyl-picrylhydrazyl)

The neutralization of free radical DPPH, is a method fast and largely used, compared with other methods, to evaluate the antioxidant activity in food and the complex biological systems (Kuda *et al.*, 2005; Mokbel and Hashinaga, 2006). This reaction leads to a turn of the color violet of the reagent to the pink and the absorbance will decrease. A reduction in the absorbance indicates an important capacity antiradicalaire extract (Molyneux, 2004). The DPPH radical scavenging assay measures the reduction of DPPH by antioxidants, which is recorded as a change in color. The results of DPPH essay was represented in figure n°10.

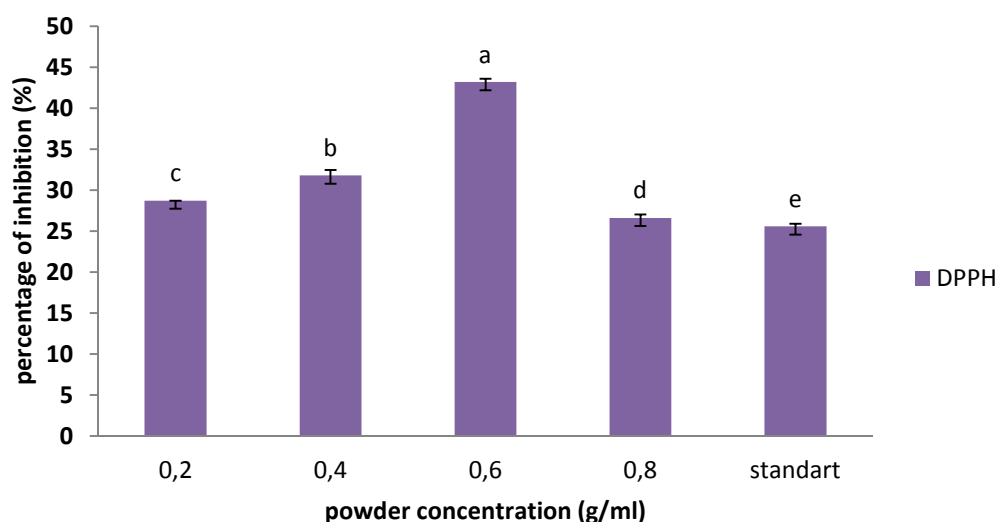


Figure 10: Percentage of antioxidant inhibition in yogurt

Using the DPPH radical scavenging method, it result that the fruit enriched yogurts at 0.6g/ml showed the highest percentage of inhibition ($43.21 \pm 0.425\%$) compared to the others at 0.2, 0.4, 0.8 g/ml with ($28.72 \pm 0.671\%$), ($31.79 \pm 0.379\%$) (26.62 ± 0.323) respectively. It also shown that all fruit enriched yogurt showed a significant different

($p < 0.05$) in the percentage of inhibition, compared to plain yogurt and it is suggested that addition of myrtle fruit into yogurt may change or enhance the percentage of inhibition.

The higher antioxidant activity of myrtle fruit yogurt is a desirable characteristic that may enhance the therapeutic values of yogurt. According to the data of the literature on the contribution of flavonoids to the activity antioxidant, measurements of the activities of these compounds like our result, that can be allotted to the richness of the myrtle in anthocyanes, precisely with the cyanidine-3-O-glucoside which is the majority anthocyanidine myrtle (Serraino *et al.*, 2003).

II.3.2. Iron reducing power

The reducing power was an analysis of the antioxidant activity which is fast and reproducible and easy has to carry out. It is based on the capacity of the phenolic compounds has to reduce the ferri-iron Fe^{+3} in ferro-iron Fe^{+2} , the power of reduction is one of the antioxidant mechanisms (Karagozler *et al.*, 2008).

The results represented in the following figure, show the evolution of the reduction power with different fruit powder concentration of *Myrtus Communis* pericarps and the simple yoghurt without fruit. There is also significant different between yoghurt with the fruits and simple yoghurt ($P < 0.05$).

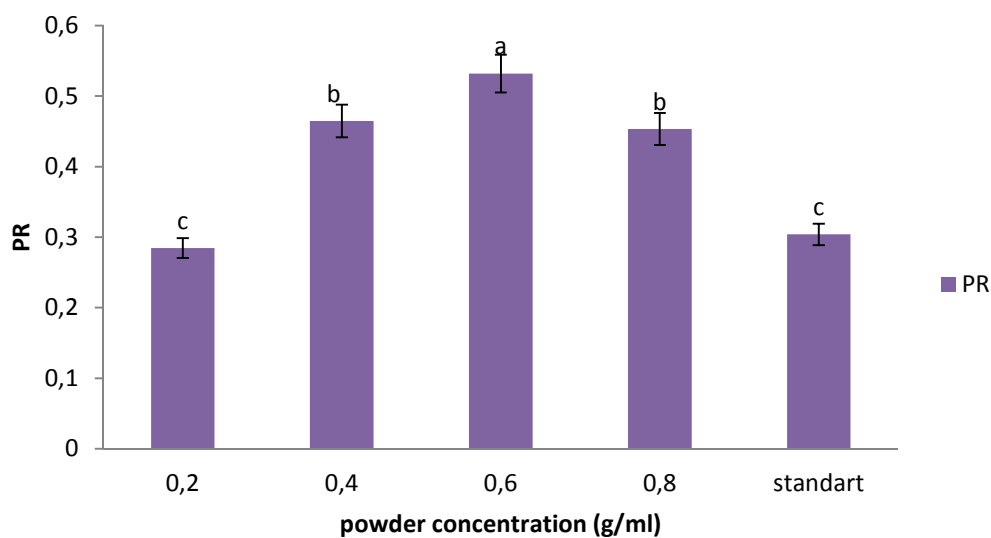


Figure 11: Reducing power activity with the potassium ferrocyanide of yogurt

Concerning the reducing power assay, the presence of reductants (antioxidants) in the samples would result in the reduction of the Fe^{3+} -ferricyanide complex to its ferrous form (Fe^{2+}) by donating an electron. Hence, the Fe^{2+} can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Higher absorbance value indicates higher reducing power. It the same for DPPH test; the highest value of reducing test was observed in fruit enriched yogurts at 0,6g/ml with significantly. This result suggests that the content of phenolic compounds can play a major role in the antioxidant activity of all extracts. Phenolic contents are the antioxidants that contribute to the high antioxidant capacity observed in different parts of plants. (Ajila *et al.*, 2007).

IV.3.3. ABTS essay (2, 2' -azynobis-[3- ethylbenzothiazoline- 6- sulfonic acid])

The antiradicalaire activity of *Myrtus Communis* fruits enriched yogurt was represented in figure n°12

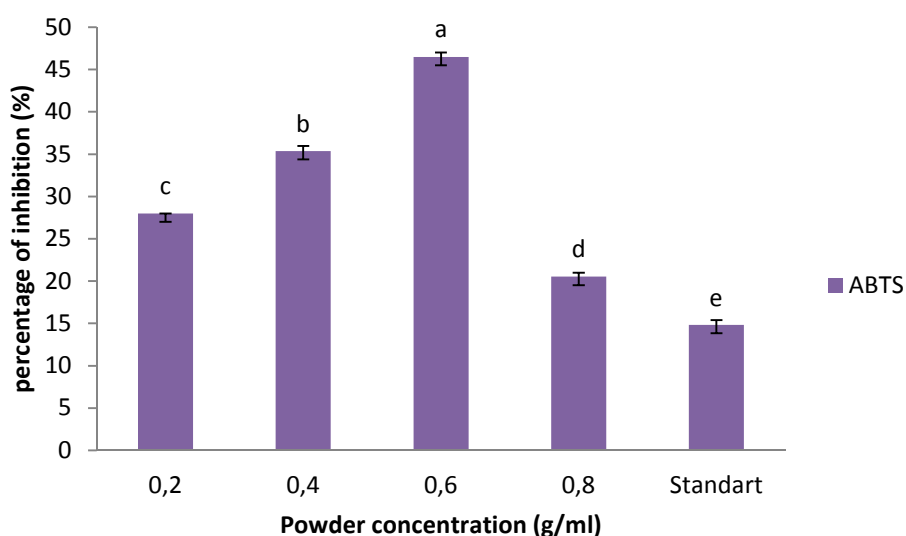


Figure 12: Percentage of antioxidant inhibition in yogurt

The $\text{ABTS}^{+\bullet}$ scavenging activity was based on the ability of antioxidants to quench the long-lived cation radical, in comparison to that of BHA a synthetic antioxidant.

The $\text{ABTS}^{+\bullet}$ assay allowed to determine the electron-donating capacity of antioxidant compounds and showed that the yogurt with 0.6g/ml showed the higher antioxidant activity at $46.5 \pm 0.472\%$ than that obtained with other concentrations. In addition, the significant different ($P < 0.05$) was found between fruits enriched yogurt and

simple yogurt at $14.83 \pm 2.570\%$. Our results were in accordance with a previous work on Moroccan myrtle leaves showing the ability of myrtle extract to scavenge ABTS+• radical (Amenour *et al.*, 2010).

Conclusion

Conclusion

In this present study, the changes in physicochemical, phenolic content, organoleptic properties and antioxidant activities of yogurt upon the addition of *Myrtus communis* fruits were investigated. The effects of lactic acid bacteria, syneresis, titratable acidity, pH changes, phenolics content, antioxidant activity were also measured. These aspects are important in evaluating the overall benefits of the addition of fruits into yogurt.

According to the results obtained the MAE Myrtle fruits powder is very rich in phenolic compounds and present the higher antioxidant activity. Effectively, the *Myrtus communis* fruits enriched yogurt with concentration of 0.6g/ml showed higher TPC content ($43,42 \pm 0,169$ mg GAE/g) and antioxidant activity for DPPH, PR and ABTS tests ($43,21 \pm 0,425$, $0,532 \pm 0,008$ and $46,5 \pm 0,472$ % respectively).

Our study indicated that myrtle fruits addition has positively influenced the increase of the lactic acid L(+) content and decrease the pH. These preliminary data indicate that the use of cow milk, a selected indigenous starter culture and fruits myrtle may be suitable for setting up a new yogurt with balanced nutritional characteristics and rich in live Lactic Acid Bacteria, though further research is certainly needed.

In perspective, this study can be completed by:

- Characterization of phenolic compounds present in fruits enriched yogurt.
- The realization of other food products containing myrtle or other bodies.
- Possibilities of combination with various fruits.

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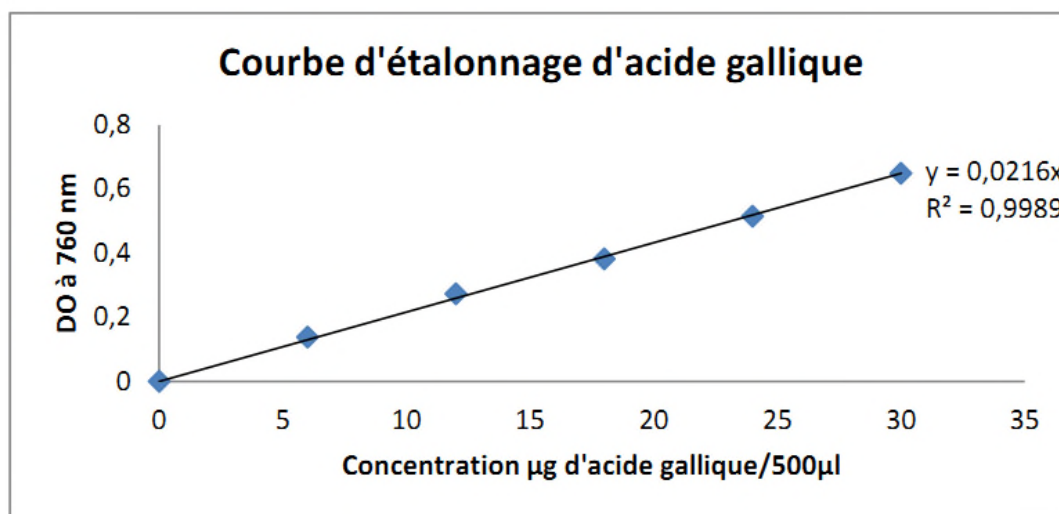
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Appendix

Appendix I:



Courbe d'étalonnage de l'acide gallique.

Annexe II : Matériels et méthodes

1. Appareillage

- ❖ Balance de précision RADWAG WAS 600/C
- ❖ Broyeur électrique (ENIEM)
- ❖ Dessiccateur RADWAG MAC 50/NP
- ❖ Etuve ventilée (Mettler)
- ❖ Microonde SAMSUNG model ME 8123ST
- ❖ Four à moufle NABERTHERM
- ❖ Spectrophotomètre UV-Vis SRECTROSCAN50
- ❖ Vortex classic Advanced
- ❖ PH meter (microprocessor ph meter)
- ❖ Tamiseur automatique RETSH AS 200 central.

2. Produits chimiques

- ❖ Ethanol
- ❖ Carbonate de sodium (Na_2CO_3) (SIGMA-ALDRICH)
- ❖ Folin-Ciocalteu (PROLABO)

- ❖ Chlorure d'aluminium (AlCl_3) (SIGMA-ALDRICH)
- ❖ Méthanol (PROLABO)
- ❖ Acide chlorhydrique (HCl) (SIGMA-ALDRICH)
- ❖ Chlorure de potassium (KCl).
- ❖ Acétate de sodium ($\text{CH}_3\text{CO}_2\text{Na}$) (BIOCHEM Chemopharma)
- ❖ Na_2HPO_4 et NaH_2PO_4 (GPR RECTAPUR)
- ❖ Ferricyanide de potassium (K^+) (SIGMA-ALDRICH)
- ❖ Chlorure de fer (FeCl_3) (BIOCHEM Chemopharma)
- ❖ Vanilline ($\text{C}_8\text{H}_8\text{O}_3$) (BIOCHEM Chemopharma)
- ❖ DPPH (SIGMA-ALDRICH)
- ❖ TCA ($\text{C}_2\text{HCl}_3\text{O}_2$) (SIGMA-ALDRICH)
- ❖ phenolphthalein

Abstract

Yogurt is a coagulated milk product obtained from the lactic acid fermentation by the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The additions of fruits in to milk may enhance the taste and the therapeutical values of milk products. However fruits also may change the fermentation behaviour. In this present study, the changes in physicochemical, total phenolics content and the antioxidant potential of yogurt upon the addition of *Myrtus communis* fruits were investigated. Fruits enriched yogurt (0.2%, 0.4%, 0.6% and 0.8 % w/w) were prepared and the pH, TTA, syneresis measurement, total phenolic content DPPH antioxidant inhibition percentage, iron reducing power and ABT test were determined. *Myrtuscommunis* fruit enriched yogurts generally showed lower pH readings (pH 4.02–5.04) compared to plain yogurt (pH 3.01). Fruit yogurt showed a higher lactic acid percentage (1.83%). Significantly higher syneresis percentage (83, 55%) compared to plain yogurt (6.48%) were seen in all fruit enriched yogurts. The DPPH antioxidant activity of plain yogurt (25.60%) was enhanced by the presence of myrtle fruit (28.72-43.21). fruit enriched yogurt showed an increment in total phenolic content (32.25 - 43.42mg/ml) compared to plain yogurt (25.59mg/ml). Therefore, it could be concluded that the addition of *Myrtus communis* fruit in to yogurt enhanced syneresis percentage, total phenolics content and antioxidant activity in yogurt.

Keywords: Antioxidant activity, *Myrtus communis*, yogurt, fruits .

Résumé

Le yaourt est un produit laitier coagulé obtenu a partir de la fermentation lactique par l'action de *Lactobacille bulgaricus* et *Streptocoque thermophilus*. L'addition de fruits dans le lait peut améliorer le goût et les valeurs thérapeutiques de produit laitier. Cependant les fruits peuvent aussi changer le comportement de fermentation. Dans ce présent travail, les changements de physicochimique, le contenu de composés phenoliques et l'activité antioxydante du yaourt enrichi par le fruit de *Myrtus communis* ont été étudiés. Le yaourt enrichi par les fruits (0.2 %, 0.4 %, 0.6 % et 0.8 % w/w) ont été préparés et le pH, TTA, mesure de syneresis, le contenu en composés phénoliques, le pourcentage d'inhibition DPPH, le pouvoir reducteur et le test ABTS ont été déterminés. Le yaourt enrichi par le fruit de *Myrtus communis* a montré généralement des lectures pH inférieures (le pH 4.02-5.04) comparé au yaourt simple (le pH 3.01) et un pourcentage élevé en acide lactique haut (1.83 %). Une augmentation Significative de syneresis (83, 55 %) comparé au yaourt simple (6.48 %) a été observée. L'activité antioxydante de yaourt simple obtenue par test DPPH est de (25.60 %) améliorée par la suite par la présence de fruit de myrte (28.72-43.21). le yaourt enrichi a montré un incrément dans le contenu de composé phénolique (32.25 - 43.42mg/ml) comparé au yaourt simple (25.59mg/ml). Donc, on pourrait conclure que l'addition de fruit de *Myrtus communis* au yaourt fait amélioré le pourcentage syneresis, le contenu en copposés phénoliques et l'activité d'antioxydant dans le yaourt.

Mots-clés : *MyrtusCommunis*, activités antioxydants, yaourt, fruits.