

EFFECTS OF DIFFERENT DRYING METHODS ON PHENOLS CONTENTS AND ANTIOXIDANT ACTIVITY OF AZAROLES (*Crataegus azarolus*L.)

Soussene Boudraa^{1*}, Djamel Fahloul¹, Driss Elothmani², Sara Zidani¹, Moni Saadoudi¹

¹Food Science Laboratory (LSA), Department of Food Engineering, Veterinary and Agriculture Institute, University HadjLakhdarBatna, Algeria

²Unite GRAPPE, École Supérieure d'Agricultures Angers Loire (ESA), INRA, 55, rue Rabelais BP 30748, 49007 Angers Cedex 01, France

*E-mail: b_sawsen82@yahoo.fr

Abstract

The effects of two different drying methods (freeze-drying and microwave) on phenols contents and antioxidant activity through different *in vitro* tests (Diphenylpicrylhydrazyl Assay (DPPH), the ferric reducing antioxidant power (FRAP) and the oxygen radical absorbance capacity (ORAC)) of Azaroles (*Crataegus azarolus* L.) were determined. The total polyphenols of freeze-dried Azaroles (438.87 mg GAE/100 g) are higher (309.5 mg GAE/100g) compared to the fruits dried in the microwaves with a power at 600 W. With high powers superior to 600 W drying negatively influences the polyphenols of Azaroles. Freeze-drying resulted in Azaroles with higher antioxidant activity. Microwave-dried at 180 W Azaroles had a higher content of ascorbic acid, α -tocopherol and β -carotene. Results of the present study confirmed that freeze-drying is the best method of retention of polyphenols and antioxidant properties of fresh fruit of Azaroles compared drying by the microwave. It was found that the Azaroles fruit contains relatively high amounts of antioxidant. The selection of a particular drying method is important for hawthorn fruits desired characteristics of the dried products, as well as on the operating conditions and cost. This work demonstrates that Azaroles (indigenous cultivars) can be a good of different nutrients for the local population.

Keywords: *Crataegus azarolus* L., Freeze-drying, Microwave drying, Polyphenols, Antioxidant activity.

Submitted: 26.11.2016

Reviewed: 16.02.2017

Accepted: 22.02.2017

1. INTRODUCTION

Crataegus azarolus is a species of hawthorn known by the common names Azaroles. It is native to the Mediterranean Basin and is a common plant there, growing on sites comparable to those the European common hawthorn grows on. The main *Crataegus* species in Algeria, Azaroles (*Crataegus azarolus* L.), commonly called "Zaaroura", grows spontaneously and is considered as a typical tree of the wooded areas of North Algeria (Constantine, Guelma, Souk Ahras and Setif). *Crataegus* L. species (Rosaceae) is consumed fresh or dried and used to produce jam, marmalade syrup (Bignami et al. 2000; Vivar-Vera et al. 2005). Known as Hawthorn, it is used in medicinal for the treatment of mild heart diseases. Flavonoids and procyanidins are the main constituents responsible for the observed biological activities. Generally, the fruit of the plant are used. The most important feature of *Crataegus* extracts is their positive

isotropic effect. They increase the activation of the heart muscle cells, provide them a feeding well, regulate the blood flow, and are coronary dilators (Ammon&Handel, 1981; Kaul, 1998). Drying is the oldest and the most popular preservation method for food and agricultural products. The fundamental concept of drying is to trim down moisture of products to a level, which will stop microbiological growth and keep the product's nutritive value and bioactive compounds in considerably higher levels (Kwok et al. 2004; Changrue, 2006). Several drying methods have been developed in order to preserve different kinds of food materials because of myriad environmental, energy efficiency and economic concerns. Besides, all methods have something in common; the heat is applied by conduction, convection, radiation. However, freeze-drying is an existing technology that is able to retain product quality, yet providing all the benefits of dried foods in terms of the shelf life, transport, and storage costs. However, the major disadvantage

of freeze-drying is its relatively high cost (Chan et al. 2009).

In order to prevent quality damage due to long drying time, microwave drying has been introduced. Microwave heating is a sort of dielectric heating, which uses electromagnetic radiation in the frequency ranging from 300 MHz to 300 GHz. According to Changrue(2006), the decrement of drying time due to volumetric heating of dielectric material increase the use of the microwave as a source of thermal energy.

Although studies have focused on the drying kinetics of *Crataegus azarolus* L., the lack of published work on the effect of freeze-drying and microwave drying at levels power on antioxidant properties including phenolic compound, ascorbic acid and carotenoid contents of Azaroles explains the interest for the present work.

2. MATERIALS AND METHODS

2.1. Fruit collections

Healthy mature hawthorn (*Crataegus azarolus* L.) fruits were harvested by October-November (2014) in North-West Algeria. Azaroles had an initial moisture content of percentage-wet basis, which was determined by drying in a convective oven (Memmert DO 6836, Germany) at 103 ± 1 °C for 24 h (Anon, 1995). The fruit was conserved at -20 °C until used. Azaroles were sorted. After that, the total quantity was divided in three batches, one for each process Microwave drying and freeze-drying.

2.2. Drying Methods

2.2.1. Freeze-drying

Freeze-drying was carried out in a laboratory freeze-dryer (4KB TXL-75; Virtis Benchtop K, New York) at a plate temperature, which was adjusted to -80 °C. Sublimation occurred at a pressure of 5 μ bar for 68 h. The Freeze Drying process was performed in three independent repetitions. The fruit was conserved at -20 °C until further analysis.

2.2.1. Microwave drying

The drying apparatus used consisted of a laboratory microwave oven (GE107Y,

SAMSUNG Electronics) with technical features of 230 V, 50 Hz with a frequency of 2,450 MHz. The dimension of the microwave cavity was 335 mm \times 330 mm \times 195 mm. Drying trials were carried out at different microwave generation powers 100,180, 360, 450, 600 and 900W. Drying was performer per cycle (30 sec ON / 30 sec OFF); each cycle corresponds to the application of microwaves for a given 30 sec power and 30 sec power off. At the end of each cycle, the products are weighed on a scale of precession model: GL-300. The drying kinetics was thus determined by the evolution of the mass of the products after each cycle.

Drying was run until moisture content of about 10 % w.b. water was attained; the Mass of the material was recorded continuously during drying with the accuracy ± 0.1 g. The Drying process was performed in three independent repetitions. The fruit was kept at -20 °C and ready for further analysis.

2.3. Proximate analysis

2.3.1. Determination of total phenols content

The total phenols contents (TPC) of Azaroles were determined by using the Folin-Ciocalteu method by Singleton and al.(1965). 300 μ L of diluted azaroles juice in the ratio of 1:100 with methanol: water (6:4) which was mixed with 1.5 ml of 10 fold-diluted of Folin-Ciocalteu reagent and 1.2 ml of 7.5 % sodium carbonate. The mixture was allowed to stand for 90 min at room temperature before the absorbance was measured by a Safas UV-Visible spectrophotometer at 760 nm. Gallic acid was used as a standard. The results were expressed as mg Gallic acid equivalent in a 100 g of fruit extract (mg GAE/100 g of fruits).

2.3.2. Determination of total flavonoids content

An aliquot (1.5 ml) of each extract was added to an equal volume of a solution of 2% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (2 g in 100 ml of methanol) and thoroughly mixed. The mixture was vigorously shaken and the absorbance was read at 367.5 nm after 10 min of incubation. Results were expressed in mg quercetin/L of dry weight (Bahorunet al. 1996).

2.3.3. Determination of total flavanols content

The total flavanols content of the Azaroles

samples were estimated using a modified p-dimethylaminocinnamaldehyde (DMACA) method by Arnous and al. (2001). The concentration of flavanols was calculated from a calibration curve, using catechin as standard solutions (2, 4, 8, 10, 12 mg/L). Azaroles' extracts (1 ml) were introduced into a test tube and 5 ml DMACA solution (0.1 % in 1 N HCl in MeOH) were added. The mixture was vortexed and allowed to react at room temperature for 10 min. Following this, the absorbance at 640 nm was read using a Spectrophotometer UV MC2 SAFAS. The results were expressed as milligrams of catechin equivalents per 100 g \pm SD fresh Azaroles components for the triplicate extracts.

2.3.4. Determination of anthocyanidins

The anthocyanidins were determined by using the pH-differential official method (Lee, 2005). The extracts (20 μ L) were mixed with 180 μ L of the pH 1.0 and 4.5 buffers and absorbance was measured at 520 and 700 nm by a Spectrophotometer UV MC2 SAFAS. The anthocyanidins were expressed as cyanidin-3-glucoside.

2.3.5. Determination of proanthocyanidins

Modified vanillin assay of (Price et al. 1978; Deshpande & Chetyan, 1985) was adopted for examination of the proanthocyanidin contents. Briefly, 1 ml of methanolic solution of condensed tannins, 5 ml of freshly prepared 0.5% vanillin solution in methanol containing 4% concentrated HCl (sample) or 5 ml of 4% concentrated HCl in methanol (blank) are added and mixed well. The absorbance of the sample or the blank is then read at 500 nm, after 20 min standing at 30 °C. The condensed tannins are expressed as milligrams of catechin equivalents per 100 g of samples.

2.3.6. Determination of ascorbic acid

The ascorbic acid was estimated following the method of Keller & Schwager (1977). In brief, 0.5 g of dried fruit sample was homogenized with 20 ml of extracting solution (5 g oxalic acid 0.75 g EDTA in 1000 ml of distilled water). It was centrifuged for 15 min at 6,000 x g: 8,000 rpm and the supernatant collected. The supernatant (1 ml) was added to 2,6-dichlorophenol indophenol (DCPIP) (5 ml

of 20 μ g/ml), the solution turned pink. The optical density of the mixture was taken at 520 nm (E_s). After taking the optical density (OD) of the mixture, one drop of ascorbic acid was added to bleach the pink color and again the OD was taken at the same wavelength (E_t). The OD of DCPIP solution was also taken at 520 nm (E_o). The standard curve was prepared by using different concentrations of ascorbic acid by following the same method.

2.3.7. Determination of β -Carotene

Total β -carotene was determined to use the method described by Speek and al. (1988). The method is based on saponification of the sample, followed by organic extraction where after total β -carotene in the extract is determined to use spectrophotometry.

2.3.8. Determination of α -tocopherol

The α -tocopherol content in the extracts was calculated from the regression equation of the standard curve. The content was determined spectrophotometrically according to the method of Kivçak & Akay (2005).

2.4. Determination of antioxidant capacity

2.4.1. ORAC Assay

The oxygen radical absorbance capacity (ORAC) procedure used Spectrofluorometer Jenway model 6270. Analyses were conducted in phosphate buffer pH 7.4 at 37 °C. Peroxyl radical was generated using 2,2'-azobis-(2-amidino-propane) dihydrochloride which was prepared fresh for each run. The fluorescein was used as substrate. Fluorescence conditions were as follows: excitation at 485 nm and emission at 535 nm. The standard curve was linear between 0 and 100 μ M Trolox. Results were expressed as μ M TE/g dry matter (Prior et al. 2003).

2.4.2. DPPH assay

The antioxidant capacity of the fruit was studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazine (DPPH) radical. The determination was based on the method proposed by Brand-Williams et al. (1995). Briefly, 100 μ L of Azaroles juice were diluted in the ratio of 1:100 with methanol: water (6:4) was mixed with 2 ml of 0.1 mM DPPH in methanol. The mixtures were incubated in the

dark for 30 min. The absorbance of the resulting solution was measured at 517 nm by a spectrophotometer UV MC2 SAFAS.

2.4.3. FRAP Assay

Total antioxidant capacity is measured by Ferric Reducing Antioxidant Power (FRAP) assay of Benzie&Strain(1996). FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. Briefly, 40 μ L of diluted juice in the ratio of 1:20 with methanol: water (6:4) sample was mixed with 0.2 ml of distilled water and 1.8 ml of FRAP reagent. After incubation at 37 °C for 10 min, the absorbance of the mixture was measured by a UV-Vis spectrophotometer at 593 nm. FRAP reagent should be pre-warmed at 37 °C and should always be freshly prepared by mixing 2.5 ml of a 10 mM 2,4,6-tri-(1-pyridyl)-5-triazine solution in 40 mM HCl with 2.5 ml of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 25 ml of 0.3 M acetate buffer pH 3.6. A calibration curve was prepared, using an aqueous solution of ferrous sulfate $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (200, 400, 600, 800 and 1000 μ M/L). FRAP values were expressed on a dry weight basis as μ M of Fe^{2+} /g.

2.5. Statistical analysis

The experimental data were expressed as means \pm standard deviations. All determinations were carried out in triplicates. A statistical analysis of the results was performed using the 2009 XLStat software. An equal average hypothesis was tested by analysis of variance (ANOVA). The medium was significantly different when compared with the method of Newman-Keuls ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.2. Proximate Compositions of azaroles

3.2.1. Total phenols content

The total phenolic content (TPC) of the raw material and microwave dried Azaroles are shown in the Table 1. Total phenolic contents for fresh Azaroles fruits is 45.16 mg QEA per 100 g (Dry weight) sample. It was shown that the degradation of total phenolic significantly varied according to the drying methods at different microwave drying powers (100 – 900 W). It was noted that extracts of dried pulp always

showed lower concentration of total phenolic than those from fresh fruits. The loss of phenolic during drying might be due to the process conditions, in particular the temperatures and the duration used (Shieber et al.2001).we can explained that this was ascribed to microwave energy causing breakdown of cellular constituents, resulting in higher release of polyphenols from the matrices. Many researchers have found that TPC in various plant species have irregular change under different drying processes (Dewanto et al.2002;Chanet al. 2008).

Freeze-dried samples had the highest phenolic contents (438.87 mg GAE/100 g dw). TPC in microwave dried sample was as low as 237mg GAE /100g dw at power 100 W and was comparable to that of freeze-dried samples $p > 0.05$.

3.2.2. Total flavonoids content

The total flavonoids content of Azaroles powders prepared by two drying methods ranged from 56 to 175 mg Eq/L dw (Table 1). The increase in microwave output power not significantly increased the total flavonoids contents of dried Azaroles. The formation of these compounds at high temperatures might be because of the availability of precursors of these molecules by non-enzymatic inter-conversion between molecules (Que et al. 2008).

The greatest flavonoid loss (68 ,22%) was noted for the powder prepared by microwave drying at 100 W.This result conflicted with a previous statement that freeze-drying was a less damaging method for flavonoid retention.

Microwave drying at 180 W seems to be very interesting to improve and to preserve the highest flavonoids contents respectively. Consequently, antioxidant activity of these flavonoids compounds could be improved and preserved.

Drying processes led to lower the loss of flavonoids in fresh Azaroles than that of total polyphenols and anthocyanins. This phenomenon could be attributed to the stronger heat stability of flavonoids compared with anthocyanins and other polyphenols.

Table 1. Effect of microwave power and freeze drying on polyphenols, flavonoids, flavan-3-ols, anthocyanins, proanthocyanins, acid ascorbic, β -Carotene and α -Tocopherol of azarole

<i>Fruit Crataegus azarolus</i> L. (azarole)								
Drying methods	Polyphenols (mg GAE /100g dw)	Flavonoids (mg EQ/L dw)	Flavan-3-ols (mg catechin/g extract dw)	Anthocyanins (mg of cyanidin-3-glucoside / 100 g extract dw)	Proanthocyanins (mg catechin equivalents per 100 g extract dw)	Acidascorbic mg.100 g ⁻¹ dw	β -Carotene mg.100 g ⁻¹ dw	α -Tocopherol mg.100 g ⁻¹ dw
Fresh	445.16±14.2 ₄ ^a	177±8.48 ^a	6.19±1.22 ^b	2.40±0.70 ^a	259.87±6.75 ^a	7.12±0.50 ^a	3.40±0.28 ^c	1.95±0.21 _a
Freeze drying	438.87±17.5 ₅ ^a	74.10±5.68 _{bc}	16.87±4.61 _a	0.49±0.08 ^b	237.75±149.4 ₅ ^b	1.80±0.60 ^f	1.05±0.40 ^d	0.92±0.05 _{cd}
Microwave drying								
100W	237±60.35 ^d	56.25±8.83 _c	6.58±0.74 ^b	0.17±0.03 ^d	139.50±16.22 _c ^b	3.92±0.20 ^c	3.08±0.06 ^e	1.26±0.13 _b
180W	295.25±14.4 ₁ ^{bc}	175±1.26 ^a	2.65±0.23 ^c	0.21±0.02 ^{cd}	256.50±45.16 ^a	5.01±0.14 ^b	5.47±0.33 ^a	0.93±0.03 _{cd}
300W	288±16.17 ^{bc}	81.69±1.89 _b	8.58±0.54 ^b	0.10±0.01 ^d	152.25±58.73 _b ^a	3.22±0.31 ^d	3.51±0.37 ^c	0.82±0.14 _d
450W	268.25±9.28 _{bc}	72.76±3.15 _{bc}	6.19±0.32 ^c	0.41±0.04 ^{bc}	111.00±35.31 _c ^b	2.87±0.26 ^{de}	4.26±0.18 ^b	1.00±0.24 _c
600W	309.5±41.24 _b	71.42±3.78 _{bc}	7.62±1.44 ^b	0.21±0.02 ^{cd}	72.00±14.05 ^c	3.16±0.14 ^d	1.15±0.10 ^d	0.91±0.26 _{cd}
900W	243.25±23.2 ₉ ^{bc}	69.19±0.63 _{bc}	16.00±3.63 _a	0.16±0.02 ^d	192.00±76.11 _b ^a	2.56±0.34 ^e	4.15±0.04 ^b	0.98±0.03 _c

a, b, c, d: In each column, means followed by a different letter are significantly different at the threshold of P < 0.05 (Method of Newman and Keuls).

3.2.3. Total flavanols content

Flavan-3-ols seem to be more stable with the microwave treatment and its contents in microwave-dried at 900 W were higher than in the fresh fruits. Statistically higher content of flavan-3-ols were measured in Azaroles dried in the freeze-drying; Fast freezing determines ice crystals to grow inside cells with very little cell separation and much less damage.

Flavonoids, especially the flavan-3-ols are more thermostable (Bravo, 1998). Therefore, they can be added to food products, representing a valuable resource. They may act as a functional ingredient or nutraceutical to terminate free radical chain reactions in biological systems, and therefore may play an important role in alleviating risk in development of chronic diseases.

3.2.4. Total anthocyanidins

The total anthocyanidins concentration for Azaroles berries, freeze-dried berries, microwave-dried berries, was determined to

investigate the effect of drying techniques on the retention of these phenolic pigments. The results of total anthocyanins contents in the samples are shown in the Table 1. Azaroles berries contain the highest anthocyanin content of 2.40 mg of cyanidin-3-glucoside/100 g dry fruit and freeze-drying shows the higher anthocyanin retention (0.497 ± 1.00 mg of cyanidin-3-glucoside/100 g dry fruit) among the samples.

The smallest loss was observed for anthocyanins after freeze-drying at (80% loss), and microwave drying at the power of 450 W (82 % loss). A slightly greater loss was observed of microwave drying at the power of 900 W and 100 W (> 96 %).

It is still very important to prevent losses in anthocyanins contents after the drying process, because they have the strongest antioxidant properties of all biologically active compounds of Azaroles.

Freeze-drying showed the highest potential for retaining anthocyanidins content in the samples

tested in comparison to the other drying techniques. The anthocyanin contents of microwave-dried fruit followed the freeze-drying. Drying at higher powers levels can lead to the loss of anthocyanins because these pigments are heat sensitive and are unstable during thermal treatment.

The vanillin assay that was used in this study is based on the reaction between an aromatic aldehyde and catechin. The standard monomer of condensed tannins also reacts in the assay and results in the formation of a red-colored adduct. Methanol is commonly used as solvent in the vanillin assay as in methanol. Vanillin reaction is more receptive toward polymeric condensed proanthocyanindins compared to monomeric flavanols (Price et al. 1978).

3.2.5. Proanthocyanidins

The proanthocyanindins content was 259.87 mg CE/100g in the fresh sample and was significantly higher ($p < 0.05$) in the microwave drying at 180 W and freeze-dried (Table 1). Comparing the dried plant samples, the microwave-dried ones at 180 W had slightly higher total condensed proanthocyanindins content than freeze-dried samples. Microwave dried samples at 180 W is a good method to preserve large molecular weight condensed tannins.

The total tannin contents of dried Azaroles just after drying were higher. This decrease in total tannins in dried only might have been due to the deactivation of the enzyme polyphenol oxidase which might have converted tannins into other products.

3.2.6. Ascorbic acid

The ascorbic acid content of the fresh and dried Azaroles at various microwave-powers and freeze-drying (presented in Table 1). The ascorbic acid content significantly decreased from an initial mean value of 7.12 mg/100 g dry matter of the fresh Azaroles to a least value of 1.80 mg/100 g dry matter after freeze-drying. The best ascorbic acid values in the microwave drying at 180 W a value 5.01 mg/100 g. The lower result was found at power 450 W with a value of 2.87mg/100g. The lowest ascorbic acid value was 2.56 mg/100 g at 900 W. The reduction of ascorbic acid

contents was observed under different microwave power levels-drying may be due to the destruction of vitamin C by the electromagnetic waves of the microwave power as the samples were dried. This is because thermal damage and irreversible oxidative reactions are the two main causes of ascorbic acid degradation during drying because of long drying times. In this study, the drying times were relatively short compared with freeze-drying.

3.2.7. β -Carotene

To examine the influence of the microwave on the carotene content, the dried Azaroles content was compared with that of the fresh ones (Table 1). Provitamin A carotenoid is sensitive to heat, light and prolonged processing. The β -Carotene levels of the dried Azaroles significantly ($p < 0.05$) increased from an initial value of 3.40 mg/100g dry matter to a maximum value of 5.47 mg/100 g dry matter after microwave 180 W it has been reported that in Azaroles, carotene content degradation depends on many factors including processing temperature.

The β -carotene is thermolabile pigment and that microwave heating induces a decrease in their content, causing higher damages in carotenoids content. Whether these observations are more dependent on the heat exposure time or the temperatures achieved within the process is an interesting issue that deserves more study.

β -Carotene is the most important provitamin A, mainly because of its prevalence in plant foods consumed by humans, and it is provitamin A that has the greatest activity. However, when taken as a separate supplement, it can have harmful effects. Hence, microwave drying at 180 W of Azaroles fruit is an excellent source of β -carotene.

3.2.8. α -Tocopherol

The results of the α -tocopherol analysis are reported in Table 1. The range for alpha-tocopherol content was from 1.95mg/100 g in fresh Azaroles to 0.92 mg/100 g in the totally freeze dried samples. A small amount of α -tocopherol was detected in the fresh fruits, as well as the microwave drying and freeze-dried Azaroles samples.

These changes were probably caused by the partial evaporation of water during the defrosting and heating of fruits, which had the effect of concentrating constituents in fresh mass and increasing dry weight (Table 1). It should, however, be added that such products underwent only one heat treatment in water. Although tocopherols are fat soluble, they are susceptible to thermal treatment in water and losses due to rising chemical extractability of lipid molecules. Their degradation is additionally accelerated by the presence of oxygen and exposure to light during processing.

3.3. Antioxidant capacity

Different researchers have employed different approaches to quantitatively determine the activity of antioxidants in Azaroles fruit. The antioxidant activity of the fresh and dehydrated Azaroles, the ORAC, DPPH and FRAP methods were used. The greatest antioxidant activity was observed for the freeze-drying and microwave drying at 450 W drying samples.

3.3.1. ORAC assay

The mean ORAC in raw Azaroles was $55.75 \mu\text{mol TE/g}$ sample. The mean ORAC values of Azaroles pomace using different drying methods are shown in Table 2. The highest mean ORAC value of $6637.43 \mu\text{mol TE/g}$ (DRY basis) was observed in freeze-dried Azaroles and the lowest in the microwave dryer at 180 Watts sample (Table 2). The highest content observed in freeze-dried samples might be due to a lack of any heat used in other methods. The freeze-drying method showed significantly higher ORAC values as compared to the other drying methods used.

3.3.2. DPPH assay

The results of DPPH assay are presented in Table 2. The high DPPH scavenging activity of microwave drying can be explained owing to the presence of a higher quantity of total polyphenols, which might have been released due to the disruption of the cell wall, from the insoluble portion of the Azaroles or the formation of novel compounds having powerful donating ability (Cho et al. 2006).

The ability of reduction in synthetically generated radical DPPH in fresh fruits was

$52.70 \mu\text{g Trolox/g}$, and it was higher in samples dehydrated by other drying methods by microwave drying at 450 W ($33.66 \mu\text{g Trolox/g}$).

The freeze-drying process involves drying frozen berries by sublimation. The low temperature and solid state of water during freeze-drying protect primary structure of the berries and retain the color of the berries. Ratti (2001) reported a close relationship between antioxidant content, vitamin content and color of the products. In addition, due to the absence of liquid water during the freeze-drying, there was a minimal loss of anthocyanins and other polyphenols; hence, this drying technique retained the highest antioxidant activities in dried Azaroles berries.

Microwave drying involves volumetric heating where by the berries absorb the microwave energy directly and convert it into heat internally. The microwave drying improved the color attribute and reduced the loss of anthocyanins and polyphenols because of the high temperature and short period of drying time.

3.3.3. FRAP assay

The FRAP of Azaroles extracts are shown in Table 2. The ability of the Azaroles fruits extracts to reduce ferric ions was determined using the FRAP (Ferric ion reducing ability of plasma) assay developed by Benzie & Strain (1996). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability of freeze-drying was $104.27 \mu\text{M of Fe (II)/g}$ while that of the extracts of other power levels was decreased with a decrease in power and increase in the drying time.

For the FRAP assay, we observed more differences between fresh and dried samples, a higher FRAP value was obtained by the freeze-drying method than by microwave drying at 300 and 450 W. It was observed that Azaroles fruits dehydrated by the microwave drying method contained greater antioxidant activity and reduced power. This behavior could be the result of two factors: (i) it is known that polyphenols in an intermediate stage of oxidation have greater antioxidant power than

initially even though this is temporary; and (ii) high-temperature stabilization procedures may lead to the formation of new compounds with higher antioxidant activity. This is essentially the case of the Maillard reaction, which creates various products that are the Maillard reaction products.

4. CONCLUSIONS

The results in this study are essential in order to obtain the optimum benefits of antioxidant properties present in Azaroles fruits during drying.

According to its high antioxidant capacity, Azaroles is considered a potential dried product. All studied drying methods (microwave power levels 100, 180, 360, 450, 600 and 900 W, freeze-drying) had adverse effects on acid ascorbic, α -tocopherol, β -carotene, total phenolic content, flavonoids, anthocyanins, proanthocyanidins, flavan-3-ols total phenolic and antioxidant capacity of Azaroles fruit. Freeze-drying had the lowest negative effects. Microwave drying had a clear negative effect on the antioxidant capacity of Azaroles fruits. High amounts of carotenoids, tocopherol, ascorbic acid and antioxidant potential were recorded in fresh fruit of Azaroles, can be recommended preserving these phytoconstituents and antioxidants potential, with a minor loss of constituents in dehydrated fruits of Azaroles place of lyophilization. For household purposes, it may be preferable to use microwave drying was not appropriate to preserve nutrients and

antioxidants.

Microwave dried Azaroles with applied 450 W had higher levels of antioxidant activity than other Azaroles dried at other microwave drying powers and with the rest of the drying methods. Thus, this fruit could be considered as an important source of biologically active components with a high antioxidant activity to assess the requirements of today's consumers, who are very interested in the potential role of functional or nutraceutical foods. Among all studied methods of drying, the best and therefore the one keeping the content of bioactive compounds and antioxidant activity, is the one freeze-drying.

Drying is an important process to prepare starting materials for further processing steps, it is important to compare different drying methods to identify the most suitable method with low costs and less effect on material quality. The results showed that inappropriate drying conditions resulted in big loss of bioactive compounds and antioxidant capacity.

5. REFERENCES

- [1] Ammon, H.P. & Händel, M. (1981). *Crataegus*, Toxicology and Pharmakologie, Teil II Pharmakodynamik. *Planta Medica*, **43** : 209-239.
- [2] Anon (1995). *Contrôle de qualité des produits alimentaires, méthodes d'analyses officielles*, AFNOR-DGCCRF, Paris, Fr., 416 p.
- [3] Arnous, A., Makris, D. & Kefalas, D. (2000). Effect of Principal Polyphenolic Components in Relation to Antioxidant Characteristics of Aged Red Wines. *Journal of Agricultural and Food Chemistry*, **4** : 5736-5742.

Drying methods	ORAC ($\mu\text{M TE/g dw}$)	DPPH ($\mu\text{g TE / g dw}$)	FRAP ($\mu\text{M of Fe(II)/g dw}$)
Fresh	55.75 \pm 0.32 ^d	52.70 \pm 14.75 ^a	49.86 \pm 0.85 ^e
Freeze drying	66.37 \pm 0.50 ^a	45.53 \pm 14.59 ^{ab}	104.27 \pm 1.42 ^a
Microwave drying			
100W	55.26 \pm 0.59 ^e	38.57 \pm 11.66 ^{ab}	57.4.02 \pm 3.77 ^d
180W	51.36 \pm 0.55 ^h	45.98 \pm 9.80 ^{ab}	67.21 \pm 2.34 ^c
300W	52.92 \pm 0.05 ^f	43.42 \pm 19.28 ^{ab}	79.841 \pm 5.87 ^b
450W	58.28 \pm 0.62 ^b	33.66 \pm 7.47 ^b	83.485 \pm 2.08 ^b
600W	56.92 \pm 0.61 ^c	45.795 \pm 12.68 ^{ab}	72.97 \pm 3.22 ^c
900W	51.66 \pm 0.29 ^g	45.964 \pm 9.25 ^{ab}	69.98 \pm 2.83 ^c

- [4] Bahorun, T., Gressier, B., Trotin, F., Brunete, C., Dine, T., Vasseur, J., Gazin, J.C., Pinkas, M., Luycky, M., & Gazi, M. (1996). Oxygen species scavenging activity of phenolic extract from hawthorn fresh plant organs and pharmaceutical preparation, *Arzeimittel-Forschung*, **46** :1086-1089.
- [5] Benzie, I.F.F. & Strain, J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay *Analytical Biochemistry*, **239** : 70–76.
- [6] Bignami, C., Poalocci, A., Scossa, A. & Bertazza, G. (2000). Preliminary evaluation of nutritional and medicinal components of *Crataegus azarolus* fruits. *Journal of Food Engineering*, **5** : 241-247.
- [7] Brand-Williams, W., Cuvelier, M. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant capacity. *Journal Food Science and Technology*, **28** : 25–30.
- [8] Bravo, L. (1998). Polyphenol: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, **56** : 317–33.
- [9] Chan, E.W.C., Lim, Y.Y., Wong, L.F., Lianto, F.S., Wong, S.K., Lim, K.K. & Lim, T.Y. (2008). Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. *Food Chemistry*, **109** : 477–483.
- [10] Chan, E.W.C., Lim, Y.Y., Wong, S.K., Lim, K.K., Tan, F. S. & Lianto, M.Y.Y. (2009). Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species *Food Chemistry*, **113** : 166–172.
- [11] Changrue, V. (2006). Hybrid (osmotic, microwave-vacuum) drying of strawberries and carrots. A thesis submitted to McGill University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Montreal, Quebec, Canada.
- [12] Cho, J., Sullards, M.C. & Olzmann, J.A. (2006). Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer disease. *Journal of Biological Chemistry*, **10** :816–24.
- [13] Deshpande, S.S. & Chetyan, M. (1985). Evaluation of vanillin assay for tannin analysis of dry beans. *Journal of Food Science*, **50** : 905-916.
- [14] Dewanto, V., Wu, X., Ado, K. & Liu, R.H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*, **50** : 3010–3014.
- [15] Kaul, R. (1998). *Der Weissdorn*, Stuttgart Wissenschaftliche Verlagsgesellschaft. 35-154.
- [16] Keller, T. & Schwager, H. (1977). Air pollution and ascorbic acid. *European Journal of Forest Pathology*, **7** : 338-350.
- [17] Kivçak, B. & Akay S. (2005). Quantitative determination of α -tocopherol in *Pistacia lentiscus*, *Pistacia lentiscus* var. *chia*, and *Pistacia terebinthus* by TLC-densitometry and colorimetry. *Fitoterapia*, **76** :62–66.
- [18] Kwok, B.H.L., Hu, C., Durance, T. & Kitts, D.D. (2004). Dehydration Techniques Affect Phytochemical Contents and Free Radical Scavenging Activities of Saskatoon berries (*Amelanchier alnifolia* Nutt). *Journal Food Science*, **69** : 122-126.
- [19] Lee, J. (2005). Total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants and wines by the pH Differential Method: Collaborative Study. *Journal of AOAC International*, **88**(5) : 1269-1278.
- [20] Price, M.L., Scoyoc, S.V. & Butler, L.G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry*, **26** : 1214-1218.
- [21] Prior, R.L., Hoang, H., Gu, L., Wu X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B. & Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (Oxygen Radical Absorbance Capacity (ORAC)) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry*, **51** : 3273–3279.
- [22] Que, F., Mao, L., Fang, X. & Wu, T. (2008). Comparison of hot air-drying and freeze-drying on the physicochemical properties and antioxidant activities of pumpkin (*Cucurbita Moschata* Duch) flours. *Journal of Food Science and Technology*, **43** : 1195-1201.
- [23] Ratti, C. (2001). Hot air and freeze-drying of high value foods. *Food Engineering Reviews*, **49** : 311–319.
- [24] Schieber, A., Stintzing, F.C. & Carle R. (2001). By-products of plant food processing as a source of functional compounds recent developments. *Trends food science. technology*, **12** : 401-413.
- [25] Singleton, V.L., Joseph, A. & Rossi, J.R. (1965). Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, **16** : 144–153.
- [26] Speek, A.J., Speek-Saichua, S. & Schreurs W.H.P. (1988). Total carotenoid and beta-carotene contents of Thai vegetables and the effect of processing. *Food Chemistry*, **27** : 245-257.
- [27] Vivar-Vera, M., Salazar-Montoya, J., Calva-Calva, G. & Ramos-Ramírez, E. (2005). Extraction, thermal stability and kinetic behavior of pectin methylesterase from hawthorn (*Crataegus pubescens*) fruit. *LWT- Food Science and Technology*, **40** : 278-284.