Adv. Hort. Sci., 2019 33(4): 567-580 DOI: 10.13128/ahsc-8214

AHS
Advances in Horticultural Science

Optimization of phenolic compounds recovery and *in vitro* antioxidant activity of Algerian eggplant (*Solanum melongena* L.)

L. Arkoub-Djermoune [1,2 (*)], F. Benmeziane [3], K. Madani [1,4], L. Boulekbache-Makhlouf [1]

[1] *Laboratoire de Biomathématiques, Biophysique, Biochimie et Scientométrie, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algeria.*

[2] *Université de Tizi Ouzou, Faculté des Sciences Biologiques et des Sciences Agronomiques, Département d'Agronomie, Tizi Ouzou (15000), Algeria.*

[3] *Université Chadli Bendjedid d'El-Tarf, Faculté des Sciences de la Nature et de la Vie, Département d'Agronomie, BP 73 (36000), El-Tarf, Algeria.*

[4] *Centre de Recherche en Technologies Agro-Alimentaires, Route de Tergua-Ouzemour, 06000 Bejaia, Algeria.*

OPEN ACCESS

(*) **Corresponding author:**
dlynda2002@yahoo.fr

Citation:
ARKOUB-DJERMOUNE L., BENMEZIANE F., MADANI K., BOULEKBACHE-MAKHLOUF L., 2019 - *Optimization of phenolic compounds recovery and in vitro antioxidant activity of Algerian eggplant (Solanum melongena L.).* - Adv. Hort. Sci., 33(4): 567-580.

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 21 June 2019
Accepted for publication 30 August 2019

Key words: antioxidant activity, eggplant, extraction condition, optimization, phenolic compound.

Abstract: The optimum conditions for extraction of total phenolic contents (TPC) and maintaining the highest antioxidant activity from eggplant were determined. Extraction experiments were carried out by investigating the effects of the solvent nature (acetone, ethanol, methanol, or water), solvent concentration (30-90%), extraction temperature (30-100°C), extraction time (30-120 min), solid to solvent ratio (1/25-1/100 g/mL), and number of extractions (1, 2 and 3) on the recovery of phenolic compounds and antioxidant activity of the extracts. The TPC was assessed to determine the polyphenolic component while free radical scavenging activity (FRSA) and ferric-reducing power (FRP) were used to evaluate the antioxidant activity of eggplant extracts. All extraction parameters had significant effects ($p<0.05$) on the TPC extraction and the antioxidant activities. The best conditions were obtained using three extraction steps with aqueous acetone 70% (v/v) at 25°C for 60 min and with 1 g/50 mL solid to solvent ratio. The optimum extraction conditions exhibit the TPC concentrations of 794.94 mg GAE/100 g and antioxidant activities of 737.86 mg TE/g (FRSA) and 28.00 mg TE/g (FRP). The free radical scavenging and ferric-reducing potentials were found to be positively significantly correlated with phenolic content under the influence of all extraction parameters.

1. Introduction

Eggplant (*Solanum melongena* L.) commonly known as brinjal, is an economically important common vegetable grown and consumed

throughout the world. It is a good source of phenolic compounds (Jung *et al.,* 2011; Salerno *et al.,* 2014; Arkoub-Djermoune *et al.,* 2016; Dranca and Oroian, 2017; Sharma *et al.,* 2019), vitamins and minerals, especially iron, compared to other commonly consumed vegetables, and it is nutritionally comparable to tomato (Kalloo, 1993). It is ranked amongst the top ten vegetables in terms of oxygen radical absorbance capacity due to the fruit phenolic constituents (Cao *et al.,* 1996) which are powerful antioxidants (Jung *et al.,* 2011; Piao *et al.,* 2014; Scorsatto *et al.,* 2017; Sharma *et al.,* 2019).

Studies have shown that eggplant extracts suppress the development of blood vessels required for tumor growth and metastasis (Matsubara *et al.,* 2005), and inhibit inflammation that can lead to atherosclerosis (Han *et al.,* 2003). Extracts from eggplant fruit skin have been demonstrated to possess high capacity in scavenging of superoxide free radicals and inhibition of hydroxyl radical generation by chelating ferrous iron (Kaneyuki *et al.,* 1999; Noda *et al.,* 2000; Boulekbache-Makhlouf *et al.,* 2013). Superoxide radicals generated in vivo are usually converted into hydrogen peroxide, and like other free radicals, can damage lipids, proteins, and DNA (Halliwell *et al.,* 1995). From the 120 vegetable species evaluated for antioxidant activity using four different assays (2,20-azinobis-[3-ethylbenzthiazoline-6-sulphonic acid), 2, 2-diphenyl-1-picrylhydrazyl radical, inhibition of lipid peroxidation, and Superoxide scavenging), eggplant ranked among the top 10 species for superoxide scavenging (SOS) activity (Hanson *et al.,* 2006). Nasunin, an anthocyanin isolated from the skin of purple eggplant fruit, is one phenolic compound implicated in both inhibition of hydroxyl radical generation and SOS activity (Kaneyuki *et al.,* 1999; Noda *et al.,* 2000).

Extraction is the first step in isolation of phenolic compounds from plant materials. Considering the compositional diversity of the natural sources of polyphenols, as well as the structure and physicochemical properties of these compounds, specific processes must be designed and optimized for each phenolic source (Santos-Buelga and Williamson, 2003; Pinelo *et al.,* 2005). The extraction protocol must enable complete extraction of phenolics, as well as minimization of oxidation, degradation, and polymerization of desired products (Zuo *et al.,* 2002). Many factors, such as type of solvent (water, methanol, ethanol, ethyl acetate, acetone, and hexane), pH, temperature, time, solid/solvent ratio, and extraction number, can affect the efficiency of the extraction process (Zuo *et al.,* 2002; Mo *et al.,* 2011).

There are several efficient extraction methods for determination of phenolic compounds in solid samples. These include supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), solid-phase microextraction (SPME), ultrasound-assisted extraction (UAE) (Dranca and Oroian, 2017; Ferarsa *et al.,* 2018; Nipornram *et al.,* 2018), and microwave-assisted extraction (MAE) (Mahugo Santana *et al.,* 2009; Dahmoune *et al.,* 2013; Koyu *et al.,* 2018). Solid-liquid extraction (SLE), which was used in this investigation, has been the method of choice of numerous researchers for extraction of phenolics from many sources (Liyana-Pathirana and Shahidi, 2005; Durling *et al.,* 2007; Bachir Bey *et al.,* 2013; Benmeziane *et al.,* 2014; Mokrani and Madani, 2016; Caldas *et al.,* 2018; Mohd Hazli *et al.,* 2019). Effect of extraction process can be generally evaluated based on a one factor one time approach, also known as single experiment, in which only one factor is variable at one time while all others are kept constant. The availability of phenolic compounds in eggplant as an antioxidant source is documented. However, no optimal protocols have been established so far for phenolic extraction from these vegetable. To the best of our knowledge, no data was reported about the effect of extracting parameters on the survey of phenolics from eggplant.

Hence, the objective of this study was to optimize the extraction of total phenolics compounds (TPC) maintaining the highest antioxidant capacity (DPPH free radical-scavenging activity; FRSA and ferric reducing power; FRP) from eggplant (*Solanum melongena* L.) using single factor experiments approach under conditions compatible with food use. The objective in extracting phytochemicals from their plant sources is to liberate these compounds from the vacuolar structures where they are found, either through rupturing plant tissue or through a process of diffusion. The factors that contribute to the efficiency of extraction are, in particular, solvent type (ethanol, methanol, acetone and water), solvent concentration (30%, 50%, 70% and 90%, v/v), temperature (25, 50, 75 and 100°C), time (30, 60, 90 and 120 min), solid to solvent ratio 1/25, 1/50, 1/75 and 1/100 g/mL), and number of extraction (1, 2 and 3). The stability of antioxidant activities (FRSA and FRP) in extracts was also investigated.

2. Materials and Methods

Chemicals

Folin-Ciocalteu reagent was provided from

Biochem, Chemopharma (Montreal, Quebec). Sodium carbonate (Na_2CO_3), acetone, ethanol and methanol were obtained from Prolabo (made in CE). Potassium ferricyanide ($C_6N_6FeK_3$), ferric chloride ($FeCl_3 6H_2O$), trichloroacetic acid, gallic acid and trolox from Biochem-chemopharma (UK). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (Steinheim, Germany).

Plant material

The fresh eggplant (*Solanum melongena L.*) used in this study was purchased from local market of Bejaia city (Algeria) at February 2010. After transferring at the laboratory, the sample was washed with distilled water and wiped then some tests were done on fruits in order to determine their physico-chemical characteristics. Eggplant fruits presented a pH of 4.15, water content of 92.8 g/100 g FW, titratable acidity of 1.24 g/100 g DW, total sugar content of 20.55 g/100 g DW, total soluble solids content (Brix) of 31.48 g/100 g DW and ash content of 0.55 g/100 g DW (Arkoub-Djermoune *et al.,* 2016). The sample was frozen at -20°C before analysis and a quantity of eggplant were mixed and used for each extraction step.

Plant material extraction

Approximately 0.1 g of crushed eggplant was weighed in a glass vial and extracted with 10 mL of the extracting solvent. The mixture was shaken at a constant rate using a water bath shaker, centrifuged for 20 min at 4000 g (nüve NF 200, Ankara, Turkey), and filtered through a Watman filter paper. The extraction process was carried out in duplicate. The obtained filtrates extracts were subsequently used for the determination of total phenolic compounds (TPC) and antioxidant activities: DPPH-free radical scavenging activity (FRSA) and ferric reducing power (FRP) measurements.

Experimental design

In the present study, single factor experiments was used to determine the optimum conditions for extracting phenolic compounds from eggplant. A total of six parameters namely extraction solvent (ethanol, methanol, acetone and water), solvent concentration (30-90%; v/v), extraction temperature (30-100°C), extraction time (30-120 min) solid to solvent ratio (1/25-1/100 g/mL), and number of extractions (1, 2 and 3) were studied in which one parameter was varied at a while the other parameters were fixed. The optimal extracting conditions were selected on the basis of the TPC, FRSA and FRP measurements.

Solvent nature and concentration

By setting extraction time (30 min) and temperature (25°C) and sample/solvent ratio (0.1 g/10 mL), samples were extracted with acetone (30%, 50%, 70%, and 90%; v/v), ethanol (30%, 50%, 70%, and 90%; v/v), methanol (30%, 50%, 70%, and 90%; v/v) and water.

Extraction temperature

Using the best solvent type and solvent concentration, eggplant samples were extracted at temperatures ranging from 25°C to 100°C (25, 50, 75 and 100°C). The best extraction conditions studied were selected according to the value of three responses (TPC, FRSA and FRP).

Extraction time

Eggplant samples were extracted using the best solvent concentration and extraction temperature determined previously. The extracts were prepared by varying the extraction time from 30 min to 120 min (30, 60, 90 and 120 min). The best extraction conditions studied were selected according to the value of three responses (TPC, FRP and FRSA).

Solid to solvent ratio

Eggplant samples were extracted using the best solvent concentration. The extraction procedure was repeated by varying the sample/solvent ratio 1/25, 1/ 50, 1/75, and 1/100 g/mL, while fixing the extraction time and temperature at 60 min and 25°C as determined previously. TPC and antioxidant activity values for consecutive extractions were added for determination of this parameter.

Number of extractions

The final step of this experiment was to determine the effect of the extractions number. In order to determine this effect, the extraction setting with the optimal conditions selected previously: solvent (acetone 70%; v/v), temperature (25°C), time (60 min) and the solid to solvent ratio (1/50 g/mL), the extraction was repeated three times on the solid residue after centrifugation of the mixture at 4000 rpm during 20 min.

Total phenolic compound determination (TPC)

The amount of TPC in eggplant extract was determined using the Folin-Ciocalteu reagent and gallic acid as standard as described by Velioglu *et al.* (1998). In brief, 200 µL of each extract were introduced into test tubes then added with 1.5 mL of Folin-Ciocalteu reagent (previously diluted ten times). After 5 min, 1.5 mL of sodium carbonate (60 g/L) were added. The tubes were mixed and allowed

to stand in darkness at room temperature for 30 min. Absorption at 760 nm against a blank was measured using a Shimadzu UV-Vis spectrophotometer (Kyoto, Japan). The concentration of phenolic compounds extracts is determined by reference to the calibration curve obtained under the same conditions using the Gallic Acid as the standard, expressed as milligram Gallic Acid Equivalent per one hundred gram of the Fresh Weight (mg GAE/100 g FW). All measurements were carried out in triplicate.

Antioxidant activity

Free radical scavenging activity of DPPH· (FRSA). In the 1,1-diphenyl-2-picrylhydrazyl (DPPH·) assay, antioxidants were capable to reduce the stable radical DPPH· to the yellow colored diphenylpicrylhydrazine (DPPH-H). The test is based on the reduction of an alcoholic solution of DPPH· in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H (Gülçin, 2007).

The DPPH· radical-scavenging activity of eggplant extracts was estimated as described by Milardović *et al.* (2006). Briefly, 100 µL of samples extract were mixed with 3 mL of DPPH in methanol (6.10^{-5}M). The mixtures were left for 30 min at room temperature and its absorbance then measured at 517 nm against a blank. All measurements were carried out in triplicate. The percentage scavenging was calculated using the following equation:

$$\text{DPPH-FRSA (\%)} = [(A_{contr} - A_{extr})/A_{contr}] \times 100$$

Where A_{contr} is the absorbance of the control (without extract) after 30 min and A_{extr} is the absorbance of extract. The inhibition percentage were expressed as milligram Trolox Equivalent per gram of the Fresh Weight (mg TE/g FW).

Ferric reducing power (FRP)

The ferric reducing power of eggplant extract was performed by using the potassium ferricyanide-ferric chloride method. Substances, with reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which subsequently reacts with ferric chloride to form ferric ferrous complex who has an absorption maximum at 700 nm (Jayanthi and Lalitha 2011). The reducing power of the extracts was evaluated according to the protocol of Oyaizu (1986). One millitre of different concentrations of the samples was mixed with phosphate buffer (1 mL, 0.2 M, pH = 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1 mL, 1 g/100 mL). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (TCA) (1 mL, 10 g/100 mL) was added to the solution which was then centrifuged for 10 min at 3000 g. The supernatant was gathered and mixed with distilled water (1.5 mL) and $FeCl_3$ (150 µL, 0.1 g/100 mL), and the absorbance was measured at 700 nm, increased absorbance of the reaction mixture indicate an increase in reducing power. Results are expressed as milligram Trolox Equivalent per gram of Fresh Weight (mg TE/g FW). The values are presented as the means of triplicate analyses.

Statistical analysis

Results were analyzed using Statistica software (version 5.5.fr; StatSoft, Inc, Tulsa, USA). All values are expressed as mean ± standard deviation (SD) of duplicate extractions and triplicate assays. One-way analysis of variance (ANOVA) with the LSD (Least Significant Difference) test was used to determine significant differences ($p<0.05$) among the means.

3. Results and Discussion

Solvent nature and concentration

The selection of extraction solvent is critical for the complex food samples as it will determine the amount and type of phenolic compounds being extracted. Aqueous alcohols particularly acetone, ethanol and methanol are the most commonly employed in phenolic extraction from botanical materials (Chan *et al.,* 2009).

In this study, diverse solvents, in mixtures with water, were used to extract antioxidant phenolic compounds from eggplant. All solvents used in the present work at different concentrations (30%, 50%, 70%, and 90%) under the same extraction conditions (30 min at 25°C) were capable of extracting phenolic compounds (Table 1). Pure organic solvents were not used because obtained extracts were cloudy. Therefore, dilution with water to 90% was necessary. Solvent type had a significant influence ($p<0.05$) on TPC and antioxidant activities (FRSA and FRP).

The aqueous acetone (70%**;** v/v) extracts showed the highest yield of TPC (1032.16 mg GAE/100 g). Similar results were reported in our previous study (Boulekbache-Makhlouf *et al.,* 2013) that 70% acetone was better for phenolic extraction from eggplant byproduct (peel) than 70% ethanol, and 70% methanol. Kallithraka *et al.* (1995) found that 70% acetone was the best solvent for the extraction of grape seed phenolics especially proanthcynidins.

Similar to the present study, acetone:water mix-

Table 1 - Effect of the solvent type and concentration on the extraction efficiency for TPC and antioxidant activities (FRSA and FRP) of eggplant

Solvent	Concentration	TPC (mg GAE/100 g FM)	FRSA (mg TE/g FM)	FRP (mg TE/g FM)
Acetone	30%	809.06 ± 29.65 c	410.23 ± 19.78 e	18.61 ± 0.59 d
	50%	785.06 ± 31.22 c	200.11 ± 10.01 h	18.78 ± 1.44 d
	70%	1032.16 ± 40.04 a	648.59 ± 14.57 a	26.06 ± 0.42 a
	90%	775.18 ± 26.45 c	365.85 ± 28.37 f	21.33 ± 1.09 c
Ethanol	30%	871.19 ± 32.90 b	360.30 ± 12.01 f	15.44 ± 1.58 ef
	50%	641.04 ± 25.53 de	458.18 ± 34.53 d	13.78 ± 1.44 f
	70%	539.38 ± 37.65 f	298.12 ± 29.32 g	17.23 ± 0.29 de
	90%	512.18 ± 21.32 f	537.80 ± 25.99 bc	24.78 ± 1.13 ab
Methanol	30%	615.62 ± 38.20 e	288.55 ± 18.88 g	16.67 ± 0.44 e
	50%	532.32 ± 47.23 f	305.20 ± 25.32 g	15.50 ± 1.36 ef
	70%	669.28 ± 13.62 de	518.87 ± 19.36 c	22.33 ± 0.50 c
	90%	708.81 ± 33.89 d	638.89 ± 19.85 a	14.00 ± 1.26 f
Water	-	660.81 ± 23.58 de	574.80 ± 17.96 b	23.39 ± 0.48 bc

Values are presented as means ± SD of six measurements. Values with different letters are significantly different ($p<0.05$).
n= 2.

tures have been reported to be one of the most effective solvents for extracting phenolics from different natural sources. Meneses *et al.* (2013) and Mokrani and Madani (2016) demonstrated that 60% acetone was the best solvent for extracting antioxidant phenolic compounds from brewer's spent grains and peach fruit, respectively. Acetone:water mixture is capable to break polyphenol-protein complexes. This fact would explain the high efficiency of this solvent to extract phenolic compounds. Downey and Hanlin (2016) demonstrated that mixtures of acetone ranging from 50% to 70% are more effective in extracting condensed tannins from grape skin.

In the other hand, it has been also demonstrated that acetone is more effective than other organic solvents for extracting phenolics from different raw materials such as berries and apples (Kähkönen *et al.,* 2001), star fruits (Shui and Leong, 2006), onions (Curcic *et al.,* 2012), barley seeds (Liu and Yao, 2007), beach peas (Chavan and Amarowicz, 2013), pistachio byproducts (Mokhtarpour *et al.,* 2014), fenugreek (Mashkor, 2014) and soybean (Lien *et al.,* 2015).

The result also showed that there were no significant difference between aqueous ethanol 50%, methanol (30%, 70%, and 90%), and water extracts. However, the use of water as single solvent provides a cloudy extracts with a high content of impurities (Chirinos *et al.,* 2007).

Generally, acetone is the best solvent for extracting proanthocyanidins and tannins; ethanol efficiently extracts flavonoids and their glycosides, catechols and tannins; whereas phenolic acids and catechin were better extracted with methanol. These facts are in agreement with polarity of the solvent used for the extraction and solubility of phenolics in them since the polarity of acetone, ethanol and methanol is 0.355, 0.654 and 0.762, respectively (Tan *et al.,* 2013). Therefore, there is no single solvent able to extract all of the classes of phenolic compounds from a sample, simultaneously.

Acetone is a more efficient solvent for extracting phenolic compounds with a high molecular weight such as condensed tannins. It is strongly believed that the higher molecular weight of the solvent, the lower the polarity which enable other substances of about the same molecular weight to be easily extracted. This can be associated to ''like dissolves like" or ''polarity versus polarity" principle as both acetone and tannins are of high molecular weight. Acetone has the lowest polarity but contains the highest total phenolic compounds value (Alasalvar *et al.,* 2006; Uma *et al.,* 2010). This would explain why acetone was found to be more efficient for extracting phenolics from eggplant. In addition, the lowest value of TPC was obtained with 70% ethanol, 90% ethanol and 50% methanol. This fact is due to a polarity of the solvent used for the extraction and solubility of phenolic compounds in them because these solvents due to their polarity are more effective for extracting polyphenols linked to polar fibrous matrices (Tabart *et al.,* 2007) and their antioxidant activity depends not only on the concentration of polyphenols but also on their chemical structure (the number and position of hydroxyl groups) (Sroka and Cisowski, 2003). In addition, the effectiveness of phenolic compounds as antioxidants does not only

depend on their composition but also influenced by the degree of polymerization, concentration and interaction of their various chemical structures with colorimetric analysis substances (Moure *et al.,* 2001). However, the acetone:water mixtures are more useful for extracting polyphenol from protein matrices, since they appear to degrade the polyphenol protein complexes (Tabart *et al.,* 2007). According to Grujic *et al.* (2012), mixtures of organic solvent and water have been revealed to be more efficient in extracting phenolic compounds than mono-component solvents. Inevitably, total phenolic content is also influenced by the solubility of phenolic compounds in the solvent used, as their diverse chemical structures might alter their solubility (Chaalal *et al.*, 2012).

The antioxidant activity of the eggplant phenolic extracts was determined by two methods, namely the 1,1-diphenyl-2- picrylhydrazyl free radical scavenging activity (FRSA) and the ferric reducing power (FRP) assays, which have been widely used for the assessment of antioxidant capacity of various plant extracts and natural products.

Determination of scavenging stable DPPH· free radical is a very quick way to evaluate the antioxidant activity of the extracts in a very short time. With this method, it was possible to assess the antiradical ability of an antioxidant by measuring the reduce in the absorbance of DPPH· at 517 nm. As a result of a color change from violet to yellow, the absorbance diminishes when the DPPH· radical is scavenged by an antioxidant through hydrogen donation to form a stable DPPH-H molecule. In the radical form this molecule had an absorbance at 515 nm which disappeared by receiving an electron or hydrogen from an antioxidant to become a stable diamagnetic molecule.

Aqueous acetone 70% was also observed to be the solvent presenting the highest antioxidant activity (Table 1). The percentage of the DPPH· radical-scavenging activity (FRSA) of acetone was 648.59 mg TE/g FM, more three times than acetone 50% (200.11 mg TE/g FM), which represents the lowest value. However, there were no significant difference between 90% acetone and 30% ethanol; 70% ethanol, methanol (30%, 50%) and water extracts with methanol (70%) and ethanol (90%) extract. Our results are in accordance of those reported by Mokrani and Madani (2016), Chaalal *et al.* (2012) and González-Montelongo *et al.* (2010) who shown that aqueous acetone were more efficient for extracting peach, prickly pear seeds and banana peel phenolics, respectively and therefore, produced extracts with higher antioxidant activity. This can be explained by the solubility of phenolic compounds which depends widely on the nature of the solvent (polarity) used, their degree of polymerization, as well as their interaction with other food components and the formation of insoluble complexes (Naczk and Shahidi, 2004). Since acetone was the solvent presenting the highest yield of TPC and antioxidant activity simultaneously, it is believed that phenolics contributing efficiently to the total antioxidant activity of eggplant extract are phenolic compounds with higher molecular weight and lower polarity, according to ''like dissolves like" principle.

Ferric reducing power (FRP) of eggplant extracts was measured by the direct reduction of $Fe^{3+}(CN^{-})_6$ to $Fe^{2+}(CN^{-})_6$ and was determined by measuring absorbance of the resulting Perl's Prussian blue complex formed after the addition of ferric ions (Fe^{3+}). In this method, the yellow color of the test solution changes to various shades of green and blue depending on the content of reductants (antioxidants) in the sample. These reducing agents reduce the Fe^{3+}/ferricyanide complex to the ferrous form. Thus, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Gülçin *et al.,* 2006; Gülçin, 2011). The same thing as FRSA, 70% acetone extract was observed to have significantly the highest FRP (26.06 mg TE/g FM), two times approxymatively more than 50% ethanol (13.78 mg TE/g FM), which represents the lowest value. Some extract have showed no significant differences at $p<0.05$ (Table 1). A similar result has been reported by Mokrani and Madani (2016) and Chaalal *et al.* (2012) in peach and prickly pear seeds extracts, respectively. The differences observed in the antioxidant activity (FRSA and FRP) of eggplant extracts could be due the variation of the quantity and quality of phenolic compounds present in the different extracts. Thus, by compromising between the yield of TPC and antioxidant activities (FRAS and FRP), 70% acetone was chosen as the best solvent to optimize the following extraction conditions.

Extraction temperature

As depicted in figure 1, extraction temperature demonstrated a significant effect ($p<0.05$) on phenolic content and antioxidant activities of eggplant extracts. The efficiency of TPC extraction and the antioxidant activities were influenced by the temperature. The TPC dropped when the temperature

increased from 25°C to 100°C. With the increase of temperature from 25 to 100°C, TPC, FRSA and FRP decrease from 1032.16 to 886.72 mg GAE/100 g FM (Fig. 1A), from 648.59 to 203.05 mg TE/g FM (Fig. 1B) and from 26.06 to 21.17 mg TE/g FM (Fig. 1C), respectively. This decrease may be attributing to a degradation of phenolic compound by increasing the temperature which has a great effect on the antioxidant activity, in particular the antiradical activity.

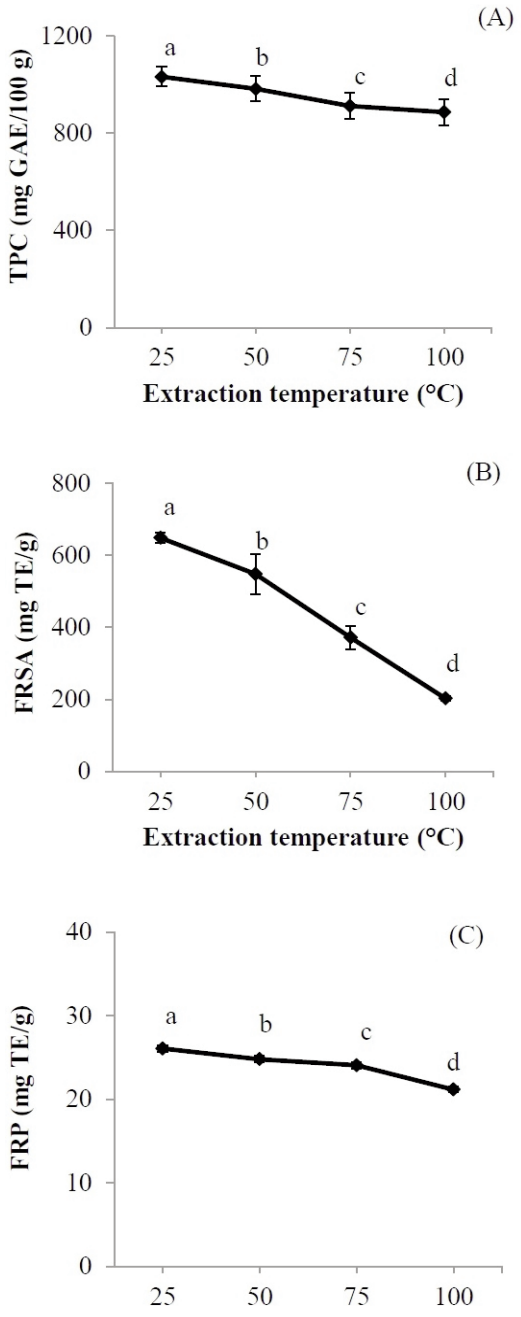

Fig. 1 - Impact of temperature on the extraction efficiency for total phenolic compounds (A), DPPH free radical scanenging activity (B) and ferric reducing power (C) of eggplant (n= 2). Values with different letters are significantly different ($p<0.05$).

In this study, the optimal temperature for antioxidant extraction from eggplant was 25°C. Similar to the result reported by Naczk and Shahidi (2004) and Mokrani and Madani (2016) who found that heating cannot increase the phenolic extraction indefinitely and at temperature above 50°C, the stability of these compounds decreases with dramatic effects with the antioxidant activity. It should be noted that increasing temperature beyond a certain value can lead to decomposition of some phenolic compounds. Rostagno *et al.* (2007) reported a decomposition of isoflavones in soybean during heat treatments. Malonyl isoflavones also degrade when extraction is performed between 75 and 100°C. Extraction between 100-125°C affects acetyl isoflavones and higher temperatures sharply reduced the glucosides concentrations. It is not surprising to find out that the antioxidant activities results showed a similar trend to the total phenolic concentration. This could be due to the fact that each assay measures different kind of phenolics, and each phenolic compound shows different antioxidant properties, which depends on the chemical structure and substitution position (Pokorny, 2003).

According to Liyana-Pathirana and Shahidi (2005) and Hismath *et al.* (2011), heating mobilizes certain antioxidants while promoting concurrent decomposition of antioxidants, which are already mobilized at lower temperatures. When an acetone:water solvent is used for extraction, the evaporation of acetone will change the acetone-water ratio because acetone, with a boiling point of 56.2°C, becomes volatile (Al-Farsi and Lee, 2008). Moreover, Santos-Buelga and Williamson (2003) have reported that some phenolics are thermosensitive, particularly certain flavonoids, such as anthocyanin and flavan-3-ol derivatives which are the most prevalent polyphenols in eggplant. Furthermore, Cacace and Mazza (2003) reported that temperature affected the extraction of anthocyanins and increasing the temperature over 30-35°C resulted in the decomposition of anthocyanins. This could be explained by a higher vulnerability of anthocyanins to high temperature.

Nevertheless, several other studies reported that heat enhanced TPC recovery Ju and Howard (2003), Pinelo *et al.* (2005), Al-Farsi and Lee (2008) and Benmeziane *et al.* (2014). This was probably due to the increased phenolic solubility, faster diffusion rate, better mass transfer, extraction yield, reduced solvent viscosity and surface tension (Richter *et al.,* 1996). According to Wissam *et al.* (2012) an increase

in temperature increases the efficiency of the extraction since heat render the cell permeable, increase solubility and diffusion coefficients of the compounds to be extracted and decreases the viscosity of the solvent, thus facilitating its passage through the solid substrate mass but the use of temperatures higher than 50°C decreases the total polyphenols yield which is probably due to their degradation. The improvement of the antioxidant extraction with temperature was probably due to the increasing diffusivity of the solvent in the solid matrix and the solubility of the phenolic compounds in the solvent, which favour the extraction (Herrero *et al.,* 2005; Juntachote *et al.,* 2006). In fact, the polarity of water (dielectric constant) is decreased when the temperature is increased, due to the breakdown of hydrogen bonds when water is subjected to high temperatures, changing the water properties. Under these conditions, the water becomes less polar and acts like an organic solvent such as methanol or ethanol, increasing the solubility of the organic materials in it (Ballesteros *et al.,* 2017). The effect of extraction temperature is very variable from plant material to another; it depends especially on their composition on phytochemicals and to their resistance to heat. Based on the results obtained with the effect of extraction temperature, we deduced that the phenolic compounds present in eggplant extracts were thermally unstable.

It is well known that the use of water in infusion is very common and easy process used for preparing tea, which extracts the polyphenols from tea with hot water. Which can't be applied in our case to extract phenolics from eggplant because they are very sensitive to heat treatment. While, the use of pure water as single solvent for extraction presents some problems and provides an extract with a high content of impurities (organic acids, sugars, soluble proteins) particularly at high temperatures, that could interfere in the phenolic identification and quantification (Chirinos *et al.,* 2007). When membrane filtration was used, the presence of protein and polysaccharide reduced the filterability. Moreover, the cumulative cost of the concentration operation increases since water is more difficult to remove than acetone and other organic solvents (Bachir Bey *et al.,* 2013). Furthermore, water dissolves many nutrients like sugar and protein. Therefore, aqueous extracts are more susceptible to microorganism invasion during storage. Several studies have reported that acetone:water mixtures are good solvent systems for extraction of polar antioxidants and are more useful for phenolic extraction from protein matrices since phenolic-protein complexes dissolve more easily (Kallithraka *et al.,* 1995; Sun *et al.,* 2002).

Since the highest TPC value was extracted at the temperature of 25°C with an extraction yield of 1032.16 mg GAE/100 g FM, this temperature was chosen as the best temperature for extracting phenolic compounds from eggplant and the extracts provide high phenolic content and maintaining a highest antioxidant activity with health benefits and could be great interest for application in pharmaceutical products.

Extraction time

Extraction time is essential in economizing energy and cost of the extraction process. The extraction time only slightly influenced the TPC and antioxidant activities of eggplant (Fig. 2). The TPC, FRSA and FRP increased when extraction time was increased from 30 to 60 min. After 60 min, further increase of the

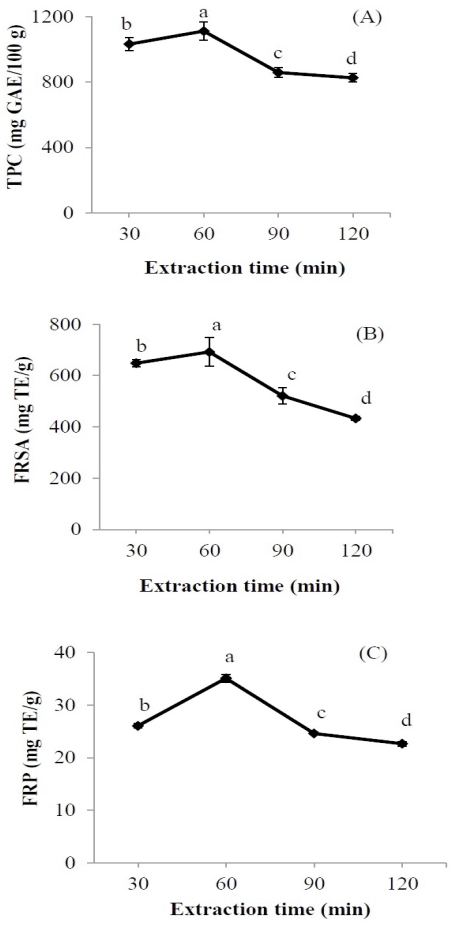

Fig. 2 - Impact of the extraction time on the extraction efficiency for total phenolic compounds (A), DPPH free radical scanenging activity (B) and ferric reducing power (C) of eggplant (n= 2). Values with different letters are significantly different ($p<0.05$).

processing time significantly decreased ($p<0.05$) the rate of phenolics and antioxidant activity. A time of 60 min was selected as the optimal time for extraction. This duration allowed extraction of 1111.23 mg GAE/100 g FM of phenolics, which corresponded to FRSA and FRP of 692.47 and 35.11 mg TE/g FM, respectively.

The optimal time for phenol extraction changes depending upon the material used for extraction but it is an important factor that influence TPC extraction and, hence, antioxidant activity. Moreover, the type of phenolic compounds extracted and the temperature of extraction were also important factors. A similar trend was shown by Liyana-Pathirana and Shahidi (2005) on whole grains, bran of both soft and hard wheat and Al-Farsi and Lee (2008) on date seeds. This observation was well explained by Fick's second law of diffusion, which predicts a final equilibrium between the solute concentrations in the solid matrix (plant matrix) and in the bulk solution (solvent) after a certain time (Silva *et al.,* 2007).

In addition, Liyana-Pathirana and Shahidi (2005) showed that the total antioxidant activity increased with an increase in extraction time from 15 to 60 min. Beyond 70 min, the total antioxidant activity decreased sharply and reached a minimum at 105 min, probably due to decomposition of the active compounds during the prolonged extraction time. In this investigation, 1 h for TPC extraction was optimal, and prolonged extraction up to 2 h decrease the TPC or the antioxidant activity at 25°C. Our results revealed that an excessive time beyond 60 min is not useful to extract more phenolic compounds from eggplant.

Taking into account these facts, an extraction time of 60 min was selected as the best extraction time for extracting phenolic compounds and antioxidant activities of eggplant.

Solid to solvent ratio

The choice of the solid to solvent ratio was investigated because it influences phenolic recovery and the antioxidant activity. The total phenolic content and antioxidant activity of eggplant extracted by 70% acetone at 25°C for 60 min using four solid/solvent ratios: 1/100, 1/75, 1/50 and 1/25 g/mL are shown in figure 3. Sample/solvent ratio had a significant effect ($p<0.05$) on TPC and the antioxidant activities (FRSA and FRP).

The TPC, FRSA and FRP radicals scavenging capacity increased from 1111.23 to 1258.07 mg GAE/100 g, from 692.47 to 855.50 mg TE/g and from 35.11 to 39.33 mg TE/g, respectively, with the increase of solid/solvent ratio from 1/25 to 1/50 g/mL. The TPC recovery using a ratio of 1/25 was poor. Extraction of antioxidants reached a maximum with a ratio of 1/50, which produced phenolic concentrations of 1258.07 mg GAE/100 g (Fig. 3A). The antioxidant activity changes with the same patherns with phenolic concentrations with a changing ratio from 1/25 to 1/50 g/mL, but with ratio of 1/75 and 1/100 g/mL, the FRSA and the FRP decreased (Fig. 3 B, C). Ćujić *et al.* (2016) have noted that the higher solid to solvent ratio generate a decrease in the consumption of plant material and decrease in the cost of extraction. Additionally, Bucić-Kojić *et al.* (2007) reported a significant difference of the polyphenols recovery yield from grape seeds depending on liquid-to-solid ratio, with the highest polyphenols concentration obtained using a ratio of 40:1. Furthermore, Prasad *et al.* (2012) employed a factorial design approach to identify the significant factors contributing to high extraction yield, antioxidant capacity and phenolic content in the extracts from *Mangifera pajang* peri-

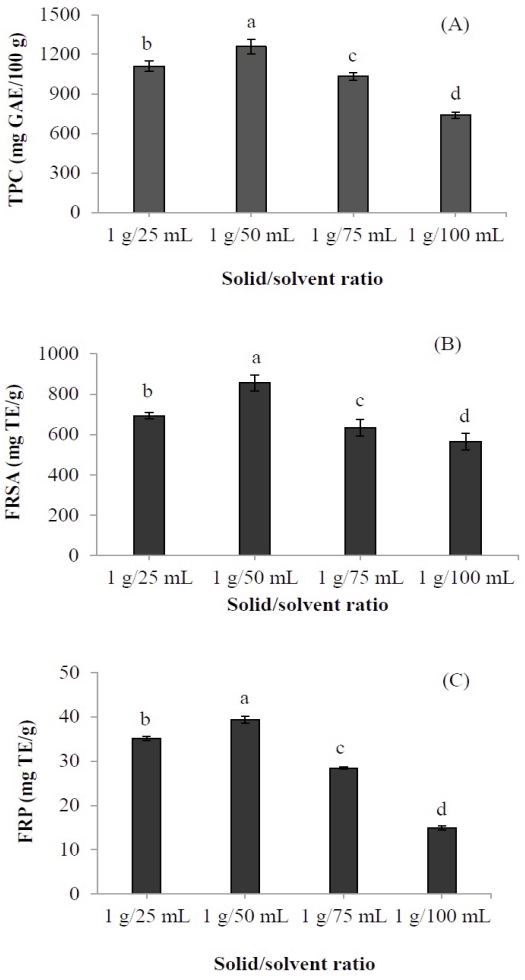

Fig. 3 - Impact of the solid to solvent ratio on the extraction efficiency for total phenolic compounds (A), DPPH free radical scanenging activity (B) and ferric reducing power (C) of eggplant (n= 2). Values with different letters are significantly different ($p<0.05$).

carp. liquid-to-solid ratio was reported as highly significant contributor.

Since the solid to solvent ratio of 1/50 g/mL was selected as the best ratio for phenolics extraction and antioxidant activities of eggplant.

Number of extractions

A series of successive extractions were performed under operating conditions favoring the best extraction: 70% acetone as solvent, a temperature of 25°C, extraction time of 60 minutes and solid/solvent ratio 1/50 g/mL. Three sequential extractions appear sufficient; the first extract contain approximatively 60% of total extractable polyphenols (1258.07 mg GAE/100 g) and exhibits an antioxidant activity of 855.61 mg TE/g (FRSA) and 39.33 mg TE/g (FRP) (Fig. 4). This rate relatively was completed by the second (27%) with a tenor of 558.44 mg GAE/100 g and gives an antioxidant activity of 415.30 mg TE/g (FRSA) and 18.44 mg TE/g (FRP) and third (13%) extraction with a concentration of 264.98 mg GAE/100 g which exhibits an antioxidant activity of 245.95 mg TE/g (FRSA) and 9.33 mg TE/g (FRP). A similar result to that found in the present study was obtained by Benmeziane *et al.* (2014) on the table grape (*Vitis Vinifera* L.) with percentages of 60%, 22%, 13% in the three fractions respectively. According to Bonnaillie *et al.* (2012), depletion of the raw material and the concentration of the extraction medium are conducted mainly in the first three stages, the last stage, rather dilutes the volumes retained in the solid. Consequently, the number of extractions can be limited to three successive contacts with fresh solvent, these authors for their part, found in a series of three successive extractions of dandruff peanut following results: 60%, 35% and 5% respectively.

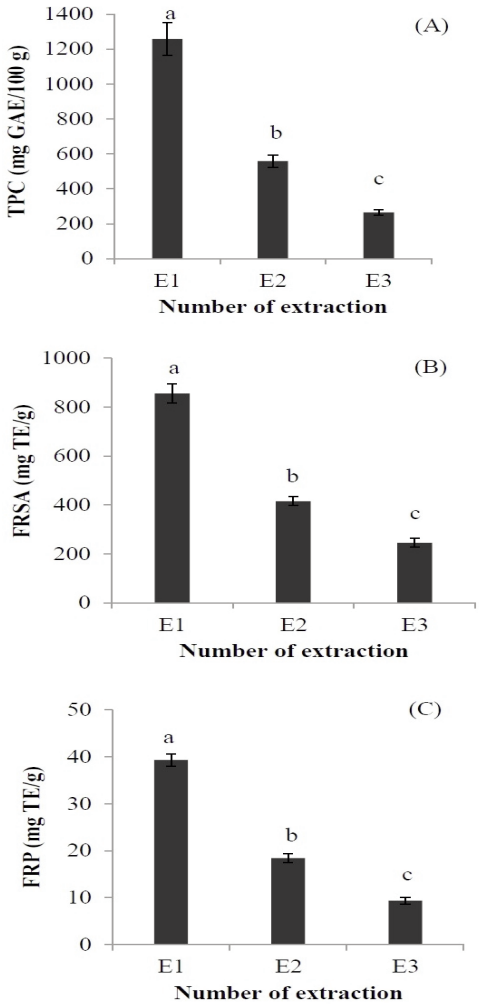

Fig. 4 - Impact of the number of extraction on the extraction efficiency for total phenolic compounds (A), DPPH free radical scanenging activity (B) and ferric reducing power (C) of eggplant (n= 2). Values with different letters are significantly different (p<0.05).

The maximum amount of extractable total phenols in the extract prepared with the mixture of the three successive extractions was assessed to 794.94 mg GAE/100 g FW and maintaining a highest antioxidant activity of 737.86 mg TE/g (FRSA) and 28.00 mg TE/g (FRP) which can be probably attributed to the dilution factor in the three mixture extracts.

Pearson correlation analysis

In order to more appreciate the relationships between antioxidant capacities and phenolic content of eggplant extracts, correlations between assays under different extracting conditions were analyzed.

Under the parameter of solvent type (Table 2), no significant correlations were found between the TPC and the antioxidant activities (FRSA and FRP). This can be explained by a synergism of eggplant phenolics present in the extract which may contribute to the overall observed antioxidant capacity.

Under the influence of extraction temperature (Table 2), TPC was correlated positively with FRSA assay (r= 0.82) at *p<0.05* and FRP assay (r= 0.66) at *p<0.001*.

Concerning the influence of extraction time condition (Table 2), The TPC was observed to be correlated positively with FRSA (r= 0.95) and FRP (r= 0.83) at *p<0.001*. From this correlation, we can believe that eggplant phenolic compounds extracted at different times display antioxidant capacities.

About the parameter solid to solvent ratio, TPC were observed to be positive significantly (*p<0.001*) correlated with FRSA assay with Pearson correlation coefficients of 0.90 and 0.90, respectively (Table 2). Previous studies showed that antioxidant activity of

Table 2 - Pearson correlations coefficient between different assays under influence of extraction conditions

	Total phenolic compounds				
	Solvent type	Extraction temperature	Extraction time	Solid to solvent ratio	Number of extraction
Free radical scavenging activity against DPPH radical	0.23 NS	0.82 ***	0.95 ***	0.90 ***	0.99***
Ferric reducing power	0.26 NS	0.66*	0.83 ***	0.90 ***	0.99***

NS = Not significant.
* = Significant at p<0.05.
*** = Significant at p<0.001.

phenolic compounds depends widely on their structure. Therefore, the solid to solvent ratio affect positively the antioxidant capacity of eggplant phenolics.

Under the influence of number of extraction, correlations between TPC and antioxidant assays (FRSA and FRP) were positively high (0.99, *p<0.001*). We can suggest that the hydrogen electron donating abilities of eggplant extracts were directly proportional to the concentration of total phenolics. This relationship suggested that the phenolic compounds of eggplant extracts might be the major contributors to the analyzed antioxidant activities.

4. Conclusions

In the present study, single factor experiments approach was used to determine the optimization of the extraction process of eggplant phenolics, investigating some variables which such as effect of solvent extraction (solvent type and solvent/water mixture concentration), extraction temperature, extraction time, solid to solvent ratio and number of extractions. To the best of our knowledge, no data was reported about the effect of extraction parameters on the recovery of phenolic compounds from eggplant. The results of the present investigation demonstrated that all the extraction parameters exhibited significant effects (*p<0.05*) on the extraction efficiency of TPC and the antioxidant activity (FRSA and FRP) of eggplant extracts. The optimal extraction conditions, selected by compromising between the rate of total phenolic compounds (TPC) and their antioxidant activities (FRSA and FRP), were extraction with 70% aqueous acetone at 25°C for 60 min using a 1g/50 mL solid to solvent ratio and three successive extractions seem necessary for the depletion of plant material. Therefore the maximum extractions of polyphenols present in eggplant were the optimum conditions for TPC recovery and maintaining the highest antioxidant activity for eggplant vegetable. These conditions allowed recovery of 794.94 mg GAE/100 g FM and produced DPPH free radical scavenging activity (FRSA), ferric reducing power (FRP) of 737.86 mg TE/g FM and 28.00 mg TE/g FM, respectively. A significant Pearson correlation coefficients were found between TPC and FRSA and FRP of eggplant extracts under the influence of extraction parameters. The results obtained in this study indicate that eggplant can be considered as a natural source of phenolics compounds known for their good antioxidant capacity. Since antioxidant compounds provide health benefits, eggplant extracts could be of great interest for application in pharmaceutical products. This study will provide bases for future investigations on the optimization of the extraction of phenolic compounds from eggplant using other models such as response surface methodology. However, it is interesting to test other extraction methods such as microwave and ultrasound extractions, ultrafiltration, supercritical fluid and subcritical water extractions on extracting phenolic compounds from eggplant. These alternative new technologies use less solvent and energy and may increase the safety and the quality of products.

Acknowledgements

The authors gratefully acknowledge all participants enrolled in the study and express their appreciation to Algerian Ministry of Higher Education and Scientific Research for the financial support.

References

AL-FARSI M.A., LEE C.Y., 2008 - *Optimization of phenolics and dietary fibre extraction from date seeds*. - Food Chem., 108(3): 977-985.

ALASALVAR C., MAGDALENA K., RYSZARD A., FEREIDOON S., 2006 - *Antioxidant and antiradical activities in extracts of hazelnut kernel (*Corylus avellana *L.) and*

hazelnut green leafy cover. - J. Agric. Food Chem., 54(13): 4826-4832.

ARKOUB-DJERMOUNE L., BOULEKBACHE-MAKHLOUF L., ZEGHICHI-HAMRI S., BELLILI S., BOUKHALFA F., MADANI K., 2016 - *Influence of the thermal processing on the physico-chemical properties and the antioxidant activity of a solanaceae vegetable: eggplant*. - J. Food Qual., 39(3): 181-191.

BACHIR BEY M., LOUAILECHE H., ZEMOURI S., 2013 - *Optimization of phenolic compound recovery and antioxidant activity of light and dark dried fig (*Ficus carica *L.) varieties.* - Food Sci. Biotechnol., 22(6): 613-619.

BALLESTEROS L.F., RAMIREZ M.J., ORREGO C.E., TEIXEIRA J.A., MUSSATTO S.I., 2017 - *Optimization of autohydrolysis conditions to extract antioxidant phenolic compounds from spent coffee grounds*. - J. Food Eng., 199: 1-8.

BENMEZIANE F., DJAMAI R., CADOT Y., SERIDI R., 2014 - *Optimization of extraction parameters of phenolic compounds from Algerian fresh table grapes (*Vitis Vinifera*)*. - Int. Food Res. J., 21(3): 1061-1065.

BONNAILLIE C., SALACS M., VASSILIOVA E., SAYKOVA I., 2012 - *Etude de l'extraction de composés phénoliques à partir de pellicules d'arachide (*Arachis hypogaea *L.)*. - Rev. Génie Ind., 7: 35-45.

BOULEKBACHE-MAKHLOUF L., MEDOUNI L., MEDOUNI-ADRAR S., ARKOUB L., MADANI K., 2013 - *Effect of solvents extraction on phenolic content and antioxidant activity of the byproduct of eggplant*. - Ind. Crops Prod., 49: 668-674.

BUCIĆ-KOJIĆ A., PLANINIĆ M., TOMAS S., BILIĆ M., VELIĆ D., 2007 - *Study of solid-liquid extraction kinetics of total polyphenols from grape seeds*. - J. Food Eng., 81(1): 236-242.

CACACE J.E., MAZZA G., 2003 - *Optimization of extraction of anthocyanins from black currants with aqueous ethanol*. - J. Food Sci., 68(1): 240-248.

CHAALAL M., TOUATI N., LOUAILECHE H., 2012 - *Extraction of phenolic compounds and* in vitro *antioxidant capacity of prickly pear seeds*. - Acta Bot. Gallica., 159(4): 467-475.

CALDAS T.W., EL MAZZA K., TELES A.S., MATTOS G.N., BRÍGIDA A.I.S., CONTE-JUNIOR C.A., BORGUINI R.G., GODOY R.L., CABRAL L.M.C., TONON R.V., 2018 - *Phenolic compounds recovery from grape skin using conventional and non-conventional extraction methods*. - Ind. Crops Prod., 111: 86-91.

CAO G., SOFIC E., PRIOR R. L., 1996 - *Antioxidant capacity of tea and common vegetables*. - J. Agric. Food Chem., 44(11): 3426-3431.

CHAN S.W., LEE C.Y., YAP C.F., AIDA W.M.W., HO C.W., 2009 - *Optimisation of extraction conditions for phenolic compounds from limau purut (*Citrus hystrix*) peels*. - Int. Food Res. J., 16: 203-213.

CHAVAN U.D., AMAROWICZ R., 2013 - *Effect of various solvent systems on extraction of phenolics, tannins and sugars from beach pea (*Lathyrus maritimus *L.)*. - Int. Food Res. J., 20(3): 1139-1144.

CHIRINOS R., ROGEZ H., CAMPOS D., PEDRESCHI R., LARONDELLE Y., 2007 - *Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum *Ruíz & Pavón) tubers*. - Sep. Purif. Technol., 55(2): 217-225.

ĆUJIĆ N., ŠAVIKIN K., JANKOVIĆ T., PLJEVLJAKUŠIĆ D., ZDUNIĆ G., IBRIĆ S., 2016 - *Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique*. - Food Chem., 194: 135-142.

CURCIC G., STANKOVIC M.M.S., RADOJEVIC I.D., STEFANOVIC O.D., COMIC L.R., TOPUZOVIC M.D., DJACIC D.S., MARKOVIC S.D., 2012 - *Biological effects, total phenolic content and flavonoid concentrations of fragrant yellow onion (*Allium flavum *L.)*. - Med. Chem., 8(1): 46-51.

DAHMOUNE F., BOULEKBACHE L., MOUSSI K., AOUN O., SPIGNO G., MADANI, K., 2013 - *Valorization of* Citrus limon *residues for the recovery of antioxidants: Evaluation and optimization of microwave and ultrasound application to solvent extraction*. - Ind. Crops Prod., 50: 77-87.

DOWNEY M.O., HANLIN R.L., 2016 - *Comparison of ethanol and acetone mixtures for extraction of condensed tannin from grape skin*. - S. Afr. J. Enol. Vitic*.,* 31(2): 154-159.

DRANCA F., OROIAN M., 2017 - *Total monomeric anthocyanin, total phenolic content and antioxidant activity of extracts from eggplant (*Solanum melongena *L.) peel using ultrasonic treatments*. - J. Food Process Eng., 40(1): 12312-12321.

DURLING N.E., CATCHPOLE O.J., GREY J.B., WEBBY R.F., MITCHELL K.A., FOO L.Y., PERRY N.B., 2007 - *Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol-water mixtures*. - Food Chem., 101(4): 1417-1424.

FERARSA S., ZHANG W., MOULAI-MOSTEFA N., DING L., JAFFRIN M.Y., GRIMI N., 2018 - *Recovery of anthocyanins and other phenolic compounds from purple eggplant peels and pulps using ultrasonic-assisted extraction*. - Food Bioprod. Process., 109: 19-28.

GONZÁLEZ-MONTELONGO R., GLORIA LOBO M., GONZÁLEZ M., 2010 - *Antioxidant activity in banana peel extracts: testing extraction conditions and related bioactive compounds*. - Food Chem., 119: 1030-1039.

GRUJIC N., LEPOJEVIC Z., SRDJENOVIC B., VLADIC J., SUDJI J., 2012 - *Effects of different extraction methods and conditions on the phenolic composition of mate tea extracts*. - Molecules, 17(3): 2518-2528.

GÜLÇIN I., 2007 - *Comparison of* in vitro *antioxidant and antiradical activities of L-tyrosine and L-Dopa*. - Amino Acids, 32(3): 431-438.

GÜLÇIN İ., 2011 - *Antioxidant activity of eugenol: A structure-activity relationship study*. - J. Med. Food., 14(9): 975-985.

GÜLÇIN İ., ELIAS R., GEPDIREMEN A., BOYER L., 2006 -

*Antioxidant activity of lignans from fringe tree (*Chionanthus virginicus *L.)*. - Eur. Food Res. Technol., 223(6): 759-767.

HALLIWELL B., AESCHBACH R., LÖLIGER J., ARUOMA O., 1995 - *The characterization of antioxidants*. - Food Chem. Toxicol., 33(7): 601-617.

HAN S.W., TAE J., KIM J.A., KIM D.K., SEO G.S., YUN K.J., CHOI S.C., KIM T.H., NAH Y.H., LEE Y.M., 2003 - *The aqueous extract of* Solanum melongena *inhibits PAR2 agonist-induced inflammation*. - Clin. Chim. Acta., 328(1): 39-44.

HANSON P.M., YANG R.Y., TSOU S., LEDESMA D., ENGLE L., LEE T.C., 2006 - *Diversity in eggplant (*Solanum melongena*) for superoxide scavenging activity, total phenolics, and ascorbic acid*. - J. Food Compos. Anal., 19(6): 594-600.

HERRERO M., MARTIN-ALVAREZ P.J., SEÑORÁNS F.J., CIFUENTES A., IBÁÑEZ E. 2005. - *Optimization of accelerated solvent extraction of antioxidants from* Spirulina plantesis *microalga*. - Food Chem., 93: 417-423.

HISMATH I., AIDA W.M.W., HO C.W., 2011 - *Optimization of extraction conditions for phenolic compounds from neem (*Azadirachta indica*) leaves*. - Int. Food Res. J., 18: 897-905.

JAYANTHI P., LALITHA P., 2011 - *Reducing power of the solvent extracts of* Eichhornia crassipes *(Mart.) Solms*. - Int. J. Pharm. Pharm. Sci., 3(3): 126-128.

JU Z.Y., HOWARD L.R., 2003 - *Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin*. - J. Agric. Food Chem., 51(18): 5207-5213.

JUNG E.J., BAE M.S., JO E.K., JO Y.H., LEE S.C., 2011 - *Antioxidant activity of different parts of eggplant*. - J. Med. Plant Res., 5(18): 4610-4615.

JUNTACHOTE T., BERGHOFER E., BAUER F., SIEBENHANDL S., 2006 - *The application of response surface methodology to the production of phenolic extracts of lemon grass, galangal, holy basil and rosemary*. - Int. J. Food Sci. Technol., 41: 121-133.

KÄHKÖNEN M.P., HOPIA A.I., HEINONEN, M., 2001 - *Berry phenolics and their antioxidant activity*. - J. Agric. Food Chem., 49(8): 4076-4082.

KALLITHRAKA S., GARCIA-VIGUERA C., BRIDLE P., BAKKER J., 1995 - *Survey of solvents for the extraction of grape seed phenolics*. - Phytochem. Anal., 6(5): 265-267.

KALLOO G., 1993 - *Eggplant:* Solanum melongena *L.,* pp. 587-604. - In: KALLOO G., and B.O. BERGH (eds.) - *Genetic improvement of vegetable crops.* Pergamon Press Ltd, Oxford, UK, pp. 893.

KANEYUKI T., NODA Y., TRABER M.G., MORI A., PACKER L., 1999 - *Superoxide anion and hydroxyl radical scavenging activities of vegetable extracts measured using electron spin resonance*. - IUBMB Life, 47(6): 979-989.

KOYU H., KAZAN A., DEMIR S., HAZNEDAROGLU M.Z., YESIL-CELIKTAS O., 2018 - *Optimization of microwave assisted extraction of* Morus nigra *L. fruits maximizing tyrosinase inhibitory activity with isolation of bioactive constituents*. - Food Chem., 248: 183-191.

LIEN D.T.P., TRAM P.T.B., TOAN H.T., 2015 - *Effects of extraction process on phenolic content and antioxidant activity of soybean*. - J. Food Nut. Sci., 3(1-2): 33-38.

LIU Q., YAO H., 2007 - *Antioxidant activities of barley seeds extracts*. - Food Chem., 102(3): 732-737.

LIYANA-PATHIRANA C., SHAHIDI F., 2005 - *Optimization of extraction of phenolic compounds from wheat using response surface methodology*. - Food Chem., 93(1): 47-56.

MAHUGO SANTANA C., FERRERA Z.S., PADRÓN M.E.T., RODRÍGUEZ J.J.S., 2009 - *Methodologies for the extraction of phenolic compounds from environmental samples: new approaches*. - Molecules, 14(1): 298-320.

MASHKOR I.M., 2014 - *Phenolic content and antioxidant activity of fenugreek seeds extract*. - Int. J. Pharmacogn. Phytochem. Res., 6(4): 841-844.

MATSUBARA K., KANEYUKI T., MIYAKE T., MORI M., 2005 - *Antiangiogenic activity of nasunin, an antioxidant anthocyanin, in eggplant peels*. - J. Agric. Food Chem., 53(16): 6272-6275.

MENESES N.G.T., MARTINS S., TEIXEIRA J.A., MUSSATTO S.I., 2013 - *Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains*. - Sep. Purif. Technol., 108: 152-158.

MILARDOVIĆ S., IVEKOVIĆ D., GRABARIĆ B.S., 2006 - *A novel amperometric method for antioxidant activity determination using DPPH free radical*. - Bioelectrochemistry, 68(2): 175-180.

MO E.K., KIM S.M., YANG S.A., OH C.J., SUNG C.K., 2011 - *Assessment of antioxidant capacity of sedum (*Sedum sarmentosum*) as a valuable natural antioxidant source*. - Food Sci. Biotechnol., 20(4): 1061-1067.

MOHD HAZLI U.H.A., ABDUL-AZIZ A., MAT-JUNIT S., CHEE C.F., KONG K.W., 2019 - *Solid-liquid extraction of bioactive compounds with antioxidant potential from* Alternanthera sesillis *(red) and identification of the polyphenols using UHPLC-QqQ-MS/MS*. - Food Res. Int., 115: 241-250.

MOKHTARPOUR A., NASERIAN A.A., VALIZADEH R., MESGARAN M.D., 2014 - *Extraction of phenolic compounds and tannins from pistachio by-products*. - Annu. Rev. Res. Biol., 148: 208-213.

MOKRANI A., MADANI K., 2016 - *Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica *L.) fruit*. - Sep. Purif. Technol., 162: 68-76.

MOURE A., CRUZ J.M., FRANCO D., DOMÍNGUEZ J.M., SINEIRO J., DOMÌNGUEZ H., NÚÑEZ M.A.J., PARAJÓ J.C., 2001 - *Natural antioxidants from residual sources*. - Food Chem., 72: 145-171.

NACZK M., SHAHIDI F., 2004 - *Extraction and analysis of phenolics in food*. - J. Chromatogr. A., 1054(1): 95-111.

NIPORNRAM S., TOCHAMPA W., RATTANATRAIWONG P., SINGANUSONG R., 2018 - *Optimization of low power ultrasound-assisted extraction of phenolic compounds*

*from mandarin (*Citrus reticulata *Blanco cv. Sainampueng) peel*. - Food Chem., 241: 338-345.

NODA Y., KNEYUKI T., IGARASHI K., MORI A., PACKER L., 2000 - *Antioxidant activity of nasunin, an anthocyanin in eggplant peels*. - Toxicology, 148(2): 119-123.

OYAIZU M., 1986 - *Studies on products of browning reaction-antioxidative activities of products of browning reaction prepared from glucosamine*. - Jpn. J. Nutr. Diet., 44: 307-315.

PIAO X.M., CHUNG J.W., LEE G.A., LEE J.R., CHO G.T., LEE H.S., MA K.H., GUO J., KIM H.S., LEE S.Y., 2014 - *Variation in antioxidant activity and flavonoid aglycones in eggplant (*Solanum melongena *L.) germplasm*. - Korean J. Breed. Sci., 2(4): 396-403.

PINELO M., FABBRO P.D., MANZOCCO L., NUÑEZ M.J., NICOLI M.C., 2005 - *Optimization of continuous phenol extraction from* Vitis vinifera *byproducts*. - Food Chem., 92(1): 109-117.

POKORNY J., 2003 - *Natural antioxidants*, pp. 31-48. - In: ZEUTHEN P., and L. BØGH-SØRENSEN (eds.) *Food preservation techniques*. Woodhead Publishing Ltd, Cambridge, UK, pp. 580.

PRASAD K.N., KONG K.W., RAMANAN R.N., AZLAN A., ISMAIL A. 2012 - *Selection of experimental domain using two-Level factorial design to determine extract yield, antioxidant capacity, phenolics, and flavonoids from* Mangifera pajang *Kosterm*. - Sep. Sci. Technol., 47(16): 2417-2423.

RICHTER B.E., JONES B.A., EZZELL J.L., PORTER N.L., AVDALOVIC N., POHL, C., 1996 - *Accelerated solvent extraction: a technique for sample preparation.* - Anal. Chem., 68(6): 1033-1039.

ROSTAGNO M.A., PALMA M., BARROSO C.G., 2007 - *Microwave assisted extraction of soy isoflavones*. - Anal. Chim. Acta, 588: 274-282.

SALERNO L., MODICA M.N., PITTALÀ V., ROMEO G., SIRACUSA M.A., GIACOMO C.D., SORRENTI V., ACQUAVIVA R., 2014 - *Antioxidant activity and phenolic content of microwave-assisted* Solanum melongena *extracts*. - Sci. World J., 2014: 719486-719493.

SANTOS-BUELGA C., WILLIAMSON, G., 2003 - *Methods in polyphenol analysis*. - Royal Society of Chemistry. Cambridge, UK, pp. 398.

SCORSATTO M., PIMENTEL A.C., RIBEIRO DA SILVA A.J., SABALLY K., ROSA G., MORAES DE OLIVEIRA G.M., 2017 - *Assessment of bioactive compounds, physicochemical composition, and i*n vitro *antioxidant activity of eggplant flour*. - Int. J. Cardiovasc. Sci., 30(3): 235-242.

SHARMA H., CHAWLA N., DHATT A.S., 2019 - *Nutraceutical content and free radical scavenging capacity of brinjal (*Solanum melongena *L.) genotypes*. - Sci. Hortic., 244: 294-303.

SHUI G., LEONG L.P., 2006 - *Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals*. - Food Chem., 97(2): 277-284.

SILVA E.M., ROGEZ H., LARONDELLE Y., 2007 - *Optimization of extraction of phenolics from* Inga edulis *leaves using response surface methodology*. - Sep. Purif. Technol., 55(3): 381-387.

SROKA Z., CISOWSKI W., 2003. - *Hydrogen peroxide scavenging, antioxidant and antiradical activity of some phenolic acids*. - Food Chem. Toxicol., 41: 753-758.

SUN J., CHU Y.F., WU X., LIU R.H., 2002. - *Antioxidant and antiproliferative activities of common fruits*. - J. Agric. Food Chem., 50(25): 7449-7454.

TABART J., KEVERS C., SIPEL A., PINCEMAIL J., DEFRAIGNE J.O., DOMMES J., 2007 - *Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage*. - Food Chem., 105(3): 1268-1275.

TAN M.C., TAN C.P., HO C.W., 2013 - *Effects of extraction solvent system, time and temperature on total phenolic content of henna (*Lawsonia inermis*) stems*. - Int. Food Res. J., 20(6): 3117-3123.

UMA D.B., HO C.W., AIDA W.M.W., 2010 - *Optimization of extraction parameters of total phenolic compounds from henna (*Lawsonia inermis*) leaves*. - Sains Malays., 39 (1): 119-128.

VELIOGLU Y.S., MAZZA G., GAO L., OOMAH B.D., 1998 - *Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products*. - J. Agric. Food Chem., 46(10): 4113-4117.

WISSAM Z., GHADA B., WASSIM A., WARID K., 2012 - *Effective extraction of polyphenols and proanthocyanidins from pomegranate's peel*. - Int. J. Pharm Pharm. Sci., 4(Supplement 3): 675-682.

ZUO Y., CHEN H., DENG Y., 2002 - *Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector*. - Talanta, 57(2): 307-316.