Algerian Journal of Environmental Science and Technology
December edition. Vol.8. N°4. (2022)
ISSN : 2437-1114
www.aljest.org

Optimization of antioxidant phenolic compounds extraction from *Thomson* orange peels and their incorporation in yogurt

Y. Benchikh[1, 2*], M. Bachir bey[2], H. Dahnoun[1], N. Mohellebi[3]

[1] Département de Biotechnologie, Institut de la Nutrition, de l'Alimentation et des Technologies Agro-Alimentaires (INATAA), Université Frères Mentouri-Constantine 1, 25000 Constantine, Algeria,
[2] Laboratoire de Biochimie Appliquée, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algeria,
[3] Laboratoire de Microbiologie Appliquée, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algeria,

*Corresponding author: yassine.benchikh@umc.edu.dz / benchikh.yassine@hotmail.com; Tel.: +213 7 93 39 00 91

ARTICLE INFO

Article History:

Received ***: 24/03/2020***
Accepted ***: 29/11/2020***

***Key Words*:**

Orange peels;
Optimization;
Phenolic compounds;
Antioxidant activity;
Enriched yogurt.

ABSTRACT/RESUME

Abstract: *The present work was carried out to valorize phenolic compounds of Thomson orange peels which considered as great industrial waste all over the world. Extraction conditions of the phenolic compounds from orange peels and incorporation process of the obtained extract in yogurt were set as objectives of the present study. The effects of solvent concentration (30-100%), extraction time (15-120 min), particle size (125-710µm), and sample to solvent ratio (0.1/20-0.5/20 g/ml) on the extraction of antioxidant phenolic compounds from orange peels were assessed. Evaluated factors affect significantly ($p<0.05$) the extraction of bioactive compounds. It is established that the best extraction conditions were 70% acetone, 30 min, 250 µm, and 0.2 g/20 mL for solvent concentration, extraction time, particle size, and sample to solvent ratio, respectively. Under these optimal conditions, total phenolic content and antiradical activity were respectively 6.26 g GAE/100g DM, and 33.64 g AAE/100g DM. The incorporated extract in yogurt had demonstrated an antioxidant quality (895.39 mg AAE/100g of yogurt), and total phenolics, flavonoids, flavonols, and ortho-diphenols were determined in 100 g of yogurt with respective contents of 258.60 mg GAE, 106.86 mg QE, 17.74 mg RE, and 52.43 mg CAE. Orange peel waste has a potential source of phenolic compounds, which could play a major role in human health. As a new eventual developed functional food, the yogurt enriched with phenolic compounds of orange peels is also could be considered as a good source of natural antioxidants, free of synthetic additives, which could play good roles as protector of yogurt against oxidation.*

I. Introduction

Citrus-fruit cultivation is the greatest sector of fruit production around the world, and has expanded over the last decade with annual word production more than 90 million tons [1]. This over production is technologically transformed to other food products, such as juices, nectars and jams, in order to (1) avoid damage of fresh foods and (2) provide to the consumers these products conserved around months, and may be years. For these reasons, more than a third of this production is destined to food proceedings, particularly to juice production. However, these transformations produce annually more than 15,000 tons of wastes [2]. Generally, these by-products can be seeds, pulps residues, and citrus peels.

Citrus peels are among highly valuable constituent sources which have varied applications in food, nutraceutical and cosmetic industries, and for the

production of biofuels and biodegradable materials [3].

Among functional constituents extracted from citrus peels industrial waste, we found carotenoids, ascorbic acid, fibers, essential oils and phenolic compounds [4, 5], especially, flavonoids and phenolic acids have gained great interest by both scientific and industrial communities. Indeed, phenolic compounds are used in food industry as natural food additives such as colorants and antioxidants. In addition, scientific researchers have demonstrated the healthy effects of these bioactive compounds on colon, breast, lung, liver, cancer, and so on [6-8].

Extraction study of these phenolic compounds is needed to evaluate independently the effect of each factor on this extraction. Before proceeding to response surface methodology, by using experimental plans to determine possible quadratic and/or interactive effects, the sequential methodology is mostly used at the first time for any extraction conditions study [9-12]. Therefore, the sequential methodology is the basic study in order to estimate the extract contented by limiting the study area of each factor. Many factors such as solvent concentration, sample to solvent ratio, extraction time, and temperature can affect the extraction of phenolic compounds from plants and fruits [13-15]. In the large literature, there is scarcity in the studies that deal with the extraction conditions of the phenolic compounds from orange peel waste.

In this study, we fixed as objectives the optimization of phenolic compounds extraction from orange peels, and then preliminary assay was performed aiming to incorporate the extract in yogurt to propose a new functional food product supplemented with phenolic compounds as natural antioxidants.

II. Materials and methods

II.1. Standards and reagents

Folin–Ciocalteu was from VWR Prolabo (Fantenay-sous-Bois, France); gallic acid (≥99.99 purity), sodium carbonate (≥99 purity), acetone, sodium acetate (≥99 purity), sodium nitrite (≥99 purity) and sodium hydroxide were from Biochem, Chemopharma (Cosne-Sur-Loire, France); aluminum chloride (≥99 purity) and sodium molybdate (≥99 purity) were from VWR Prolabo (Leuven, Belgium); 1,1-diphenyl-2-picrylhydrazyl (DPPH), quercetin, rutin (≥99.99 purity) and caffeic acid (≥99.99 purity) were from Sigma Chemical (Sigma–Aldrich GmbH, Germany); ascorbic acid (≥99.53 purity) was from Sigma–Aldrich (St. Louis, USA).

II.2. Plant material

The samples of fresh Thomson oranges (*Citrus sinensis* L.) were collected in 2017 from Skikda city (Algeria). They were selected for uniformity of shape and washed carefully. The separated peels were freeze-dried (Alpha 1-2 LD plus Martin Christ®, Osterode am Harz, Germany), ground with crusher (IKA®, A 11basic, Staufen, Germany) and passed through sieves (Resch, AS 400 Control, France). The samples were stocked at 4°C until analyses.

II.3. Extraction procedure

The extraction of phenolic compounds from orange peel waste was carried out by using sequential methodology; the effect of studied factors on the extraction was evaluated independently. The four optimized factors were studied according to the following order: solvent concentration, extraction time, particle size, and sample to solvent ratio.

For extraction procedure, an aliquot of orange peels sample was weighed and homogenized in extraction solvent (30, 50, 70, and 100% (v/v) acetone). The extraction was carried out under magnetic stirring at 500 rpm (AGIMATIC-S, P-SELECTA, Spain), at different times (15, 30, 60, 90, and 120 min), using different particle sizes (125, 250, 500, and 710 µm), and finally variable sample to solvent ratios (0.1/20, 0.2/20, 0.3/20, 0.4/20, and 0.5/20 g/mL). The temperature of extraction was set at 25°C. Extract was recovered after centrifugation at 1700×g (Sigma 2–16 P centrifuge, Germany) for 20 min, and the supernatant was passed through a filter paper (F1001, Chem, Chmlab Group, Barcelona, Spain). The obtained extract was used to determine total phenolic content (TPC) and antiradical activity (AA).

II.4. Incorporation of orange peel extract in yogurt

In order to incorporate the extracted phenolic compounds in yogurt obtained under optimal extraction conditions, the extraction solvent was evaporated by using a rotary evaporator (BUCHI 11100C101, Germany). The residue was then freeze-dried, and the obtained powder was reconstituted in water. Ten milliliters of the obtained solution were incorporated in 50 g of natural yogurt. After 5 min of shaking to ensure a good homogenization and 24 hours later at 4°C, the extraction of phytochemical compounds from the enriched yogurt was carried out. For this, 2 g of yogurt were homogenized with 20 mL of aqueous acetone solvent (70%). The rest of the extraction procedure was performed as described above. Total phenolic, flavonoid, flavonol, ortho-diphenol contents, and AA were then evaluated.

II.5. Determination of phytochemical contents

TPC was determined according to Singleton and Rossi [16] as described by Benchikh et al. [17]. The extract (100 µL) was mixed with Folin-Ciocalteu reagent (1 mL), and 800 µL sodium carbonate (7.5%). Absorbance was measured at 765 nm after 30 min using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). TPC was expressed as grams gallic acid equivalent per 100 g dry matter or yogurt (g GAE/100 g DM or yogurt).

TFC was measured as reported by Dewanto et al. [18] and described by Benchikh et al. [15]. 0.3 mL of extract was mixed with sodium nitrite (5%, 0.3 mL). 0.3 mL of aluminum chloride (10%) and 2 mL of sodium hydroxide (1 M) were respectively added to the mixture. This latter was adjusted to 10 mL with distilled water. The absorbance was measured at 510 nm. TFC was expressed as milligrams quercetin equivalent per 100 grams of yogurt (mg QE/100 g of yogurt).

TFlC of yogurt was determined following Yermakov et al. [19]. 1 mL of extract was added to 1 mL of aluminum chloride (2%) and 3 mL of sodium acetate (5%). After 2 h, the absorbance was measured at 440 nm. TFlC was expressed as milligrams rutin equivalent per 100 grams of yogurt (mg RE/100 g of yogurt).

ODC of yogurt supplemented by orange peels was measured by the method of Mateos et al. [20]. Briefly, 1 µL of sodium molybdate (5%) was added to 4 mL of extract. After 15 min, the absorbance was measured at 370 nm. ODC was expressed as milligrams caffeic acid equivalent per 100 grams of yogurt (mg CAE/100 g of yogurt).

II.6. Evaluation of antiradical activity

AA was measured according to Brand-Williams et al. [21]. 100 µL of the extract was added to 1 mL of DPPH solution (60 mM). After 30 min, the absorbance was determined at 517 nm. AA was expressed as grams ascorbic acid equivalent per 100 g dry matter or yogurt (g AAE/100 g DM or yogurt).

II.7. Statistical analyses

All data reported were carried out in triplicates and expressed as means ± standard deviations. The analysis of variance with one factor (ANOVA) was performed using Statistica® 5.5 software. The relationship among all parameters in orange peels was described as Pearson correlation coefficient (r).

III. Results and discussion

The present study was carried out to determine the optimal extraction conditions of phenolic compounds and antioxidant activity from orange peels (*Citrus sinensis* L.). For this, the sequential methodology was followed to check the independent effect of each factor. The extract obtained under optimal extraction conditions was used in the incorporation in the yogurt part and the obtained product was assessed for its phytochemical contents and antioxidant activity.

III.1. Optimization of extraction conditions

The results of TPC obtained by using a graduated solvent concentration of acetone-water (30 to 70%) and pure acetone were mentioned in Table 1. It was revealed that acetone 70% was the best solvent that has extracted the maximum amount of phenolic compounds (3.76 ± 0.06 GAE/100 g DM), and its extract manifested the highest AA against DPPH (23.74 ± 0.07 g AAE/100g DM). The 50% acetone was followed in the second position. However, the lowest one was the 100% acetone with 1.36 ± 0.04 g GAE/100 g DM for TPC, and 12.04 ± 0.07 g AAE/100g DM for antioxidant activity. These differences may be due to solvent polarity variations; generally, phenolic compounds prefer an organic-aqueous solvent to be easily extracted, this can facilitate the penetration of the solvent into particles and drain with it the soluble phenolic compounds. The presence of water allows the swelling of the particles, and then the surface of contact between solvent and sample is extended [22, 13]. The best solvent concentration obtained in our study was close to that found by Dahmoune et al. [23]. By comparing our results (TPC and AA) to those cited in the literature, we can say that they were close to those obtained by Hayat et al. [24] and Dahmoune et al. [4]. Whereas, the obtained results were higher than those reported by Dahmoune et al. [23], using ultrasonic extraction at different solvent concentrations. Thus, 70% acetone was selected as the best solvent that will be fixed to continue studying the rest of extraction factors.

TPC and AA were affected significantly ($p<0.05$) by the extraction time (Table 1). The highest content of phenolic compounds and antioxidant activity were obtained at 30 min of extraction, with values of 3.79 ± 0.01 g GAE/100 g DM, and 23.64 ± 0.07 g AAE/100g DM, respectively. These values were increased from 15 to 30 min during the extraction process, and beyond 30 min of extraction, TPC and AA decreased significantly ($p<0.05$). These results can be explained by the fact that the extraction needed enough time in order to accomplish the extraction procedure; the time of contact, penetration, and draining of phenolic compounds from the inside to the outside of the particles. On the other hand, an exceed time of extraction can let to oxidation and degradation of

the phenolic compounds already extracted and existing in the extraction solvent outside the particles. The results can be also explained by Fick's second law of diffusion; after a certain time, there will be a final equilibrium between the solute concentration in the plant sample and in the extraction solvent [25, 13]. The optimal extraction time was similar to that obtained by Montero-Calderon et al. [26]. However, it was lower than that reported by Rehan et al. [27], who indicated that phenolic compounds reached their maximal extraction after 60 min using the ultrasonic method. In contrast, our extraction time was higher than that obtained by Dahmoune et al [23] for phenolic extraction by using the ultrasonic method too. TPC and AA obtained in the present study were higher than those reported by Barrales et al. [7], Dahmoune et al. [23], and Khan et al. [28]. Therefore, 30 min was selected as the best extraction time to continue studying the rest of the extraction conditions.

Table 1. *Effects of studied extraction factors on total phenolic content (TPC) and antiradical activity (AA)*

Factor	Variable	TPC	AA
Concentration solvent (aqueous acetone, %)	30	$2.89 \pm 0.01^{b***}$	$18.17 \pm 0.14^{c*}$
	50	$3.05 \pm 0.05^{b***}$	$21.86 \pm 0.16^{b*}$
	70	$\mathbf{3.76 \pm 0.06^{a***}}$	$\mathbf{23.74 \pm 0.07^{a*}}$
	100	$1.36 \pm 0.04^{c***}$	$12.04 \pm 0.07^{d*}$
Extraction time (min)	15	$2.36 \pm 0.02^{e**}$	$14.04 \pm 0.07^{e*}$
	30	$\mathbf{3.79 \pm 0.06^{a**}}$	$\mathbf{23.64 \pm 0.07^{a*}}$
	60	$3.31 \pm 0.01^{b**}$	$18.03 \pm 0.03^{b*}$
	90	$2.92 \pm 0.05^{c**}$	$15.47 \pm 0.07^{c*}$
	120	$2.64 \pm 0.01^{d**}$	$14.69 \pm 0.05^{d*}$
Particle size (µm)	125	$3.66 \pm 0.25^{b*}$	$18.41 \pm 0.11^{b**}$
	250	$\mathbf{4.11 \pm 0.29^{a*}}$	$\mathbf{24.24 \pm 0.02^{a**}}$
	500	$3.60 \pm 0.24^{b*}$	$14.76 \pm 1.03^{c**}$
	710	$3.55 \pm 0.17^{c*}$	$13.17 \pm 0.78^{c**}$
Sample to solvent ratio (g/20 mL)	0.1	$4.51 \pm 0.17^{b*}$	$24.24 \pm 0.02^{b*}$
	0.2	$\mathbf{6.26 \pm 0.11^{a*}}$	$\mathbf{33.64 \pm 0.05^{a*}}$
	0.3	$2.68 \pm 0.02^{c*}$	$11.20 \pm 0.18^{c*}$
	0.4	$2.26 \pm 0.01^{d*}$	$8.41 \pm 0.13^{d*}$
	0.5	$1.72 \pm 0.04^{e*}$	$6.73 \pm 0.11^{e*}$

Var.: variables, TPC: total phenolic content (g GAE/100 g DM), AA: antiradical activity (g AAE/100 g DM).
*Different letters represent significant, very significant or extremely difference between result at *p<0.05, **p<0.01, and ***p<0.001, respectively.*

Particle size of samples is a factor that could influence the phenolic extraction. Indeed, ground the solid samples allows obtaining small particles where they could be more exposed to the extraction solvent and easily solubilized. As shown in Table 1, the particle size effects significantly ($p<0.05$) the extraction of TPC and AA. According to these results, the highest TPC and AA were found in the extract from the 250 µm particle size of sample with the respective values of 4.11 ± 0.29 g AGE/100 g DM, 24.24 ± 0.02 g AAE/100 g DM, and the lowest one was obtained from 710 µm particle size of powder (3.55 ± 0.17g GAE/100 g DM and 13.17 ± 0.78 g AAE/100 g DM, for TPC and AA, respectively). It is also important to note that although the difference in particle size of 125 and 500 µm samples, the extraction of phenolic compounds from these samples were not significantly different ($p>0.05$), so the results obtained with the sample having particles under 125 µm size were not higher than those obtained by using 250 µm one, and the reduction rate was 12.40%. The reduction rate of the TPC from 250 µm sample to 710 µm one was 13.62%. These above results can be explained by the fact that the smallest particles allow the solvent to penetrate easily into cells and to be around the maximum surfaces of particles, and then, drain with it the highest amount of phenolic compounds. However, the reduction of diameter particles from 250 to 125 µm caused a reduction in TPC rate that was probably due to the fact that the smallest particles remained on the surface of the solvent during the extraction were not enough in contact with extraction solvent [28]. The antioxidant results of the obtained extract using different particle sizes were showed in Table 1. The extract obtained by the sample with particle size 250 µm was also demonstrated the highest and very significant effect ($p<0.01$) compared with other samples. The obtained TPC and AA were higher than those reported by Barrales et al. [7], and Khan et al. [28].

Thus, 250 µm of sample was selected as the best particle size to continue studying the last extraction factor.

The factor sample to solvent ratio affected significantly ($p<0.05$) the extraction of phenolic compounds and AA (Table 1). The highest levels of the two followed variables were obtained by the 0.2 g/20 mL sample to solvent ratio, with values of 6.26 ± 0.11g GAE/100g DM, and 33.64 ± 0.05 g AAE/100g DM, respectively. The effect of this factor was probably due to the saturation of the solvent; the amount of solvent was not enough to penetrate into the particles and to extract a maximum of phenolic compounds, and the surface contact of solvent and sample was reduced (lowest interaction between them). In addition, the impurities of the samples at higher amounts cause the reduction of the contact between particles and solvent [29, 13]. The best sample to solvent ratio obtained in the present study was important than that reported by Dahmoune et al. [4] which was 1/40 (g/mL). Our results of TPC and AA were higher than those reported by Dahmoune et al. [4] and Khan et al. [28]. The 0.2 g/20 mL was selected as the best sample to solvent ratio.

At the end of the present study section, the optimal extraction conditions of phenolic compounds from Thomson orange peel waste were respectively 70%, 30 min, 250 µm, and 0.2/20 g/mL for acetone concentration, extraction time, particle size and sample to solvent ratio. Under optimal extraction conditions, the obtained TPC and AA were 6.26 ± 0.11g GAE/100g DM and 33.64 ± 0.05g AAE/100g DM, respectively.

III.2. Correlation between phenolic compounds and antiradical activity results

As showed in Table 2, correlations between TPC and AA of each parameter were extremely significant ($p<0.001$). Coefficient correlations of studied parameters were 0.973, 0.954, 0.958, and 0.997, respectively for solvent concentration, extraction time, particle size, and sample to solvent ratio, and they have demonstrated highly goodnesses of regression correlations of 0.947 for solvent concentration, 0.909 for extraction time, 0.918 for particle size, and finally 0.994 for sample to solvent ratio. According to these correlation parameters, it seems that the extracted phenolic compounds were responsible for the antiradical activity of orange peels.

Table 2. *Correlation parameters between total phenolic content (TPC) and antiradical activity (AA)*

Factors	r	Equation	R^2	p
Solvent concentration	0.973	y = 4.968x + 5.216	0.947	0.001
Extraction time	0.954	y = 6.644x - 2.785	0.909	0.001
Particle size	0.958	y = 18.29x - 50.59	0.918	0.001
Sample to solvent ratio	0.997	y = 6.201x - 4.774	0.994	0.001

r: *correlation coefficient,* ***R^2:*** *regression coefficient,* ***p:*** *p-value.*

III.3. Phytochemical compounds of yogurt enriched with orange peels extract

Optimal extraction conditions of phenolic compounds from Thomson orange peels were determined. The antioxidant activity was also evaluated to estimate the antioxidant quality of extracts. It was demonstrated in the present study that the optimization of these conditions was essential for an accurate quantitative determination of phenolic compounds, and each factor had at least a significant effect ($p<0.05$) on TPC and AA.

Table 3. *Phytochemical contents and antiradical activity of enriched orange peels yogurt and control*

Parameters	Enriched yogurt (mg/100 g)	Control yogurt (mg/100 g)
TPC	$258.60 \pm 0.75^{a***}$	$23.60 \pm 0.06^{b***}$
TFC	$106.86 \pm 1.50^{a***}$	$6.31 \pm 0.11^{b***}$
TFlC	$17.74 \pm 0.85^{a***}$	$1.65 \pm 0.65^{b***}$
ODC	$52.43 \pm 0.58^{a***}$	$4.32 \pm 0.65^{b***}$
Antiradical activity	$895.39 \pm 0.65^{a***}$	$357.69 \pm 0.05^{b***}$

*Different letters in the same column represent the extremely significant difference between results at ***p<0.001.*

The most efficient extraction conditions were as follows: extracting solvent 70% acetone, extraction time 30 min, particle size 250 µm, and sample to solvent ratio 0.2 g/20 mL, and under optimal extraction conditions, TPC and AA of the orange peels were 6.26 ± 0.11g GAE/100g DM and 33.64 ± 0.05 g AAE/100g DM, respectively. Moreover, the incorporated extract in yogurt had demonstrated the antioxidant quality with a value of 895.39 ± 0.65 g AAE/100g of yogurt, and TPC, TFC, TFlC, and ODC were obtained in yogurt with respective contents in 100 g of yogurt 258.60 ± 0.75 mg GAE, 106.86 ± 1.5 mg QE, 17.74 ± 0.85 mg RE, and 52.43 ± 0.58 mg CAE. Orange peel waste and enriched yogurt have a potential source of phenolic compounds, which could replace synthetic antioxidants in food industries and play a major role in human health.

IV. Conclusion

Optimal extraction conditions of phenolic compounds from Thomson orange peels were determined. The antioxidant activity was also evaluated to estimate the antioxidant quality of extracts. It was demonstrated in the present study that the optimization of these conditions was essential for an accurate quantitative determination of phenolic compounds, and each factor had at least a significant effect ($p<0.05$) on TPC and AA. The most efficient extraction conditions were as follows: extracting solvent 70% acetone, extraction time 30 min, particle size 250 µm, and sample to solvent ratio 0.2 g/20 mL, and under optimal extraction conditions, TPC and AA of the orange peels were 6.26 ± 0.11g GAE/100g DM and 33.64 ± 0.05 g AAE/100g DM, respectively. Moreover, the incorporated extract in yogurt had demonstrated the antioxidant quality with a value of 895.39 ± 0.65 g AAE/100g of yogurt, and TPC, TFC, TFlC, and ODC were obtained in yogurt with respective contents in 100 g of yogurt 258.60 ± 0.75 mg GAE, 106.86 ± 1.5 mg QE, 17.74 ± 0.85 mg RE, and 52.43 ± 0.58 mg CAE. Orange peel waste and enriched yogurt have a potential source of phenolic compounds, which could replace synthetic antioxidants in food industries and play a major role in human health.

Acknowledgments

The authors are grateful to the Directorate-General of Scientific Research and Technological Development (DGRSDT, Algerian Ministry of Higher Education and Scientific Research) for the financial support, and to Dr. Anis Chikhoune for providing us some chemical standards.

Abbreviations

AA: Antiradical activity
AAE: Ascorbic acid equivalent
CAE: Caffeic acid equivalent
DM: Dry matter
GAE: Gallic acid equivalent
ODC: Total ortho-diphenol content
QE: Quercetin equivalent
RE: Rutin equivalent
TFC: Total flavonoid content
TFlC: Total flavonol content
TPC: Total phenolic content

V. References

1. USDA (United Sates Department of Agriculture), 2014. Citrus: World Markets and Trade.
2. Marín, F.R.; Soler-Rivas, C.; Benavente-García, O.; Castillo, J.; Pérez-Alvarez, J.A. By-products from different citrus processes as a source of customized functional fibres. *Food Chemistry* 100 (2007) 736–741.
3. Ledesma-Escobar, C.A.; Luque de Castro, M.D. Towards a comprehensive exploitation of citrus. *Trends in Food Science & Technology* 39 (2014) 63–75.
4. Dahmoune, F.; Boulekbache, L.; Moussi, K.; Aoun, O.; Spigno, G.; Madani, K. Valorization of *Citrus limon* residues for the recovery of antioxidants: Evaluation and optimization of microwave and ultrasound application to solvent extraction. *Industrial Crops and Products* 50 (2013) 77–87.
5. Dahmoune, F.; Moussi, K.; Remini, H.; Belbahi, A.; Aoun, O.: Spigno, G.; Madani, K. Optimization of ultrasound assisted extraction of phenolic compounds from *Citrus sinensis* L. peels using response surface methodology. *Chemical Engineering Transactions* 37 (2014) 889–888.
6. Bocco, A.; Cuvelier, M.E.; Richard, H.; Berset, C. Antioxidant activity and phenolic composition of citrus peel and seed extract. *Journal of Agricultural and Food Chemistry* 46 (1998) 2123–2129.
7. Barrales, F.M.; Silveira, P.; Barbosa, P. de P.M.; Ruviaro, A.R.; Paulino, B.N.; Pastore, G.M.; Macedo, G.A.; Martinez, J. Recovery of phenolic compounds from citrus by-products using pressurized liquids-an application to orange peel. *Food and Bioproducts Processing* 112 (2018) 9–21.
8. Huang, Y.S.; Ho, S.C. Polymethoxy flavones are responsible for the anti-inflammatory activity of citrus fruit peel. *Food Chemistry* 119 (2010) 868–873.
9. Chaalal, M.; Touati, N.; Louaileche, H. Extraction of phenolic compounds and *in vitro* antioxidant capacity of prickly pear seeds. *Acta Botanica Gallica* 159 (2012) 467–475.
10. Bachir bey, M.; Meziant, L.; Benchikh, Y.; Louaileche, H. Deployment of response surface methodology to optimize recovery of dark fresh fig (*Ficus carica* L., var. *Azenjar*) total phenolic compounds and antioxidant activity. *Food Chemistry* 162 (2014) 277–282.
11. Bachir bey, M.; Meziant, L.; Benchikh, Y.; Louaileche, H. Deployment of response surface methodology to optimize recovery of dried dark fig (*Ficus carica* L., var. *Azenjar*) total phenolic compounds and antioxidant activity. *International Food Research Journal* 21 (2014) 1477–1482.

12. Benchikh, Y.; Louailèche, H. Effects of extraction conditions on the recovery of phenolic compounds and *in vitro* antioxidant activity of carob (*Ceratonia siliqua* L.) pulp. *Acta Botanica Gallica* 161 (2014) 175–181.
13. Benchikh, Y.; Louaileche, H.; George, B.; Merlin, A. Changes in bioactive phytochemical content and *in vitro* antioxidant activity of carob (*Ceratonia siliqua* L.) as influenced by fruit ripening. *Industrial Crops and Products* 60 (2014) 298–303.
14. Saci, F.; Benchikh, Y.; Louaileche, H.; Bachir bey, M. Optimization of ultrasound-assisted extraction of phenolic compounds and antioxidant activity from carob pulp (*Ceratonia siliqua* L.) by using response surface methodology. *The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology* 42 (2018) 26–39.
15. Benchikh, Y.; Zaoui, A.; Derbal, R.; Bachir bey, M.; Louaileche, H. Optimisation of extraction conditions of phenolic compounds and antioxidant activity of *Ruta chalepensis* L. using response surface methodology. *Journal of Food Measurement and Characterization* 13 (2019) 883–891.
16. Singleton, V. L. and Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16 (1965) 144–158.
17. Benchikh, Y.; Paris, C.; Louaileche, H.; Charbonnel, C.; Ghoul, M.; Chebil, L. Comparative characterization of green and ripe carob (*Ceratonia siliqua* L.): physicochemical attributes and phenolic profile. *SDRP Journal of Food Science & Technology* 1 (2016) 1-10.
18. Dewanto, V.; Wu, X.; Adom, K.K.: Liu, R. H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry* 50 (2002) 3010–3014.
19. Yermakov, I.; Arasimov, V.V.; Yarosh, N. P. Methods of biochemical analysis of plants. *Leningrad Agropromizdat* (1987) 122–142 (in Russian).
20. Mateos, R.; Espartero, J.L.; Trujillo, M.; Rios, J.J.; León-Camacho, M.; Alcudia, F.; Cert, A. Determination of phenols, flavones, and lignans in virgin olive oils by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detect. *Journal of Agricultural and Food Chemistry* 49 (2001) 2185–2192.
21. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology* 28 (1995) 25–30.
22. Huang, W.; Xue, A.; Niu, H.; Jia, Z.; Wang, J. Optimized ultrasonic-assisted extraction of flavonoids from *Folium eucommiae* and evaluation of antioxidant activity in multi-test systems *in vitro*. *Food Chemistry* 114 (2009) 1147–1154
23. Dahmoune, F.; Moussi, K.; Remini, H.; Belbahi, A.; Aoun, O.; Spigno, G.; Madani, K. Optimization of ultrasound-assisted extraction of phenolic compounds from *Citrus sinensis* L. peels using response surface methodology. *Chemical Engineering Transactions* 37 (2014) 889-89.
24. Hayat, K.; Hussain, S.; Abbas, S.; Farooq, U.; Ding, B.; Xia, S.; Jia, C.; Zhang, X.; Xia, W. Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity *in vitro*. *Separation and Purification Technology* 70 (2009) 63–70.
25. Silva, E.; Rogez, H; Larondelle, Y. Optimization of extraction of phenolics from *Inga edulis* leaves using response surface methodology. *Separation and Purification Technology* 55 (2007) 381–387.
26. Montero-Calderon, A.; Cortes, C.; Zulueta, A.; Frigola, A.; Esteve M.J. Green solvents and Ultrasound-Assisted Extraction of bioactive orange (*Citrus sinensis*) peel compounds. *Scientific Reports* 9, (2019) 16120.
27. Rehan, M.F.; Abdel-Wahed, N.; Farouk, A.; El-Zawahry, M. Extraction of valuable compounds from orange peel waste for advanced functionalization of cellulosic surfaces. *ACS Sustainable Chemistry and Engineering* 6 (2018) 5911–5928.
28. Khan, M.K.; Abert-Vian, M.; Fabiano-Tixier, A.S.; Dangles, O.; Chemat, F. Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel. *Food Chemistry* 119 (2010) 851–858.
29. Luthria, D.L. Optimization of extraction of phenolic acids from a vegetable waste product using a pressurized liquid extractor. *Journal of Functional Foods* 4 (2012) 842–850.

Please cite this Article as:
Benchikh Y., Bachir bey M., Dahnoun H., Mohellebi N., Optimization of antioxidant phenolic compounds extraction from Thomson orange peels and their incorporation in yogurt, ***Algerian J. Env. Sc. Technology***, **8:3(2022) 2728-2734**