Optimization of pressurized liquid extraction of essential oil from *Citrus Sinesis* peels

Oyebola O. Olabinjo[1,*], Agboola S. Ogunlowo[1], Eliana S. Kamimura[2], and Alessandra L. Oliveira[2]

(1. Department of Agricultural and Environmental Engineering, Federal University of Technology, P, M, B 704, Akure, Ondo State, Nigeria; 2. Departamento de Engenharia de Alimentos, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Av. Duque de Caxias Norte, 225, 13635 900 Pirassununga, SP, Brazil)

Abstract: Optimization of extraction of essential oil from *Citrus sinesis* (sweet orange) peels by pressurized liquid extraction (PLE) was performed using response surface methodology (RSM). In order to obtain the maximum yield of essential oil, a two-factor central composite design (CCD) experiments were conducted using extraction variables: temperature 46°C - 70°C and static extraction cycles time 30 - 60 min. Subsequently, the total phenolic content and antioxidant property using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) of essential oil were evaluated. The response surface analysis gave 70°C and 60 min static extraction cycle time as the optimal condition to achieve the maximum yield of essential oil, while moderate yield was at 46°C and 45 min of static extraction cycles. The extraction yield and antioxidant property using ABTS radical scavenging ability under the above conditions were 49.3% and 22.1%; and 11.56 and 11.53 mg trolox g^{-1}. The low temperature of moderate yield was preferred which may prevent loss of some thermo sensitive compounds in the essential oil.

Keywords: sweet orange peels, PLE, essential oil, antioxidant.

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1 Introduction

Natural antioxidants are substances with different chemical compounds that can protect biologically important cellular components from oxidative processes caused by reactive oxygen species (ROS) attacks (Su et al., 2007). Antioxidants are widely distributed in plants. Thus, attention towards exploring antioxidant agents with low toxicity is shifting towards natural products and essential oils are recommended as one of the most promising natural products.

Citrus (*citrus spp*) is one of the most abundant fruit crops with worldwide production estimated at 115 million tons per year (FAO, 2012). Oranges (*Citrus sinensis*), tangerines (*Citrus tangerine*), Mandarin (*Citrus reticulate*), lemon (*Citrus limon*), limes (several species) and grape fruits (*Citrus paradis*) are the main cultivated species. Citrus fruits are notable for their fragrance, partly due to the flavanoids and limonoids contained in the rind (Manthey, 2004). Also, citrus fruits and juices are an important source of bioactive materials including antioxidants such as ascorbic acid, flavonoids and phenolic compounds that are important to human nutrition (Ghasemi et al., 2009). The endocarp is rich in soluble sugar and contains significant amounts of vitamin C, pectin, fibres, different organic acids and potassium

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***Corresponding author: Oyebola O. Olabinjo,** Department of Agricultural and Environmental Engineering, Federal University of Technology, Akure, Ondo State, Nigeria. Tel: +234 803 3561 512. Fax: Nil. Email: binjo_joko@yahoo.co.uk.

salt which give the fruits its characteristic citrus flavour (Ezejiofor et al., 2011).

During the citrus juice extraction process, thousands of tonnes of by-products are produced (Garau et al., 2007) and the food industry has shown special interest in finding the utilization for citrus industry by-products. In the industrial processing of concentrated orange juice and frozen pulp, essential oil (containing volatile oils and antioxidant activity compounds) are extracted from the peels by pressing and centrifugation. The dried peels can be pelletized to serve as a fibre source in animal feed.

2 Material and methods

2.1 Chemicals and reagents

Carbon-dioxide (CO_2), Nitrogen gases used in the experiments were 99.5% pure and procured from White Martins Gases Industrials (Campinas, BR). Ethanol, and sodium carbonate were obtained from Synth (Diadema, São Paulo, BR), methanol and chloroform from Merck (Darmstadt, GE), gallic acid from Vetec (Rio de Janeiro, BR), Folin-Ciocalteu (Haloquimica, BR) and, potassium persulfate (Synth, BR), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox, 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonate) (ABTS) and anisaldehyde were from Sigma (Aldrich, GE).

2.2 Sample preparation

Harvested *Citrus sinesis* (*Laranja lima* in Brazil) were obtained from a fruit and vegetable market center in Pirassununga SP, Brazil. The samples were separated manually into unripe and overripe fruits, thus providing fruits of the same ripeness stage. The ripe fruits were processed at the High-Pressure Technology for Natural Products Laboratory, of the University of Sao Paulo (Pirassunuga SP, Brazil). The fruits were washed to remove foreign materials from the epicarp prior to peeling of the fruits, with knife to remove peels (rinds).

The peeled rinds were dried in a forced air circulation oven (Marconi MA03515, Piracicaba, BR) at 50ºC for 54 h. The moisture content of the peels after drying were determined by gravimetric method which determines the mass loss from the sample by drying to constant weight (AOAC, 2000). Thereafter the dried peeled were then crushed in knife mill (Marconi M340 Piracicaba, BR) and kept in sealed plastic bags in frost free freezer (BVR 28 GBBNA BRASTEMP, Joinville, BR) at -22ºC until they were used in further analyses. The particle size diameter of the dried milled peels was determined from 200 g sample passed through a vertical vibrator sieve shaker (Tyler series system, Model 1868 Bertel, Caieiras, BR) with sequential openings of 10, 14, 20, 28, 35, 48, and 65 mesh.

2.3 Extraction with pressurised liquid extraction (PLE)

PLE was performed using an ASE 150 accelerated solvent extraction system (California, USA). The stainless steel extractor with a capacity of 34 mL was filled with approximately 10.00 g of the dried milled peels for each extraction procedure and with 5 g diatomaceous earth (California, USA), as adsorbent material, used to disperse the vegetal matrix in the extraction cell. The diatomaceous allows a better contact with solvent and, clarify the extract. Anhydrous ethanol was used as a solvent because it is generally recognized as safe (GRAS) (FDA, 2013). A static time of 15 min, purge time of 100 seconds, oven heat up time of 10 min, flush volume of 100% and pressure of 10 MPa were the fixed variables. The ethanol extract obtained by PLE was named crude oil extract, which was evaporated after the extraction and then prepared for analyses.

The effects of oven temperature (46ºC - 74ºC) and number of cycle (bath) (2 - 4), on the dependent variables; extract oil global yield (Y%), total phenolic content (TPC) and antioxidant property using free radical DPPH and ABTS radical scavenging abilities were investigated using statistical experimental design based on 2^2 central composite design (CCD) with four axial points and five central points.

Table 1 Levels of independent variables (T and C) for the central composite design.

	Levels				
Independent variables	- α	-1	0	1	+ α
Temperature (ºC)	46	50	60	70	74
Number of Cycles (C)	1.6(2*)	2	3	4	4.4(4*)

Note: α = ± 1.41 * real time in the equipment.

Table 1 shows the levels of the variables in coded and real units. The essential oil of dried *Citrus sinesis* peels

was extracted using PLE at moisture content of 6.5% (dry basis) following the central CCD model earlier described.

2.4 Statistical analysis

The experimental design and data analysis were carried out using response surface methodology with the Statistica software v. 7.0 (Statsoft Inc., USA). The effects of the independent variables on the responses in the extraction process were evaluated using residual error, considering 95% level of confidence for all variables. Using this methodology first and second order models were obtained for all the responses, and the percentage variation explained by the correlation coefficient (R^2) was considered.

The response surfaces and the respective mathematical models (Equation 1) were also obtained and the significance was accepted at $p \leq 0.05$.

$$Y=b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}x_1^2 + b_{22}x_2^2 \quad (1)$$

Where Y is the response of dependent variables, b_o is the constant coefficient, b_1, b_2 are the linear coefficients of independent variables, b_{11}, b_{22} are the quadratic coefficients independent variables, b_{12} is the linear-by-linear interaction coefficient independent variables, and x_1 and x_2 are coded values of the independent variables.

The main superiority of response surface analysis (RSA) is the reduced number of experiments required for the optimization process. RSA involving central composite design (CCD) and Box-Behnken design (BBD) were employed to optimize the PLE process to high yield and antioxidant property of the essential oil.

2.5 Yield calculation

The weight of essential oil yield was determined gravimetrically after collection. The extraction yield was expressed as the percent ratio of the mass of extracted oil to the mass of orange peels.

2.6 Determination of the TPC

TPC was determined using Folin-Ciocalteu method previously described by (Singleton and Rossi, 1965). Crude extracts (1 mL) were diluted in methanol (5,000 ppm) with 5 mL Folin-Ciocalteu in water (1:10, v/v). After a brief incubation at room temperature (8 minutes), anhydrous sodium carbonate (Na_2CO_3) (20%, 1.5 mL) solution was added. After 1 min of reaction, the tubes were vortexed and incubated for 120 min at room temperature. The absorbance was read at 760 nm on spectrophotometer (Biospectro SP 22, São Paulo, BR). The standard curve was prepared using 50 to 500 mg L^{-1} solutions of Gallic acid in methanol. The analyses were done in triplicates. The antioxidant activity was expressed as mg of gallic acid equivalents (GAE) per grams of extract.

2.7 Trolox equivalent antioxidant capacity (TEAC) assay

The TEAC assay was carried out according to the method established in the literature (Re et al., 1999). The ABTS radical consisted of 7 mM ABTS in 2.45 mM potassium persulfate in a volume ratio of 1:1, incubated in the dark for 16 h at room temperature. This $ABTS^+$ working solution was prepared by diluting the stock solution with methanol to an absorbance of 0.70 ± 0.05 at 734 nm. In this analysis, 5 µL of the diluted samples were mixed with 200 µL $ABTS^+$ working solution, the mixture was vortexed and its absorbance at 734 nm (Abs_s) was measured after 6 min of incubation at room temperature and the percent of inhibition was calculated. Methanol (Abs_{rb}) was used as blank and all determinations were performed in triplicates. Trolox was used as a reference standard, and the results were expressed as mg of trolox equivalent (mg TE) by grams of EO extract.

2.8 Antioxidant by DPPH

The determination of sequestering capacity of the stable free radical DPPH was based on the methodology of Brandi-Williams et al. (1995). Methanol solution of DPPH was prepared with absorbance between 0.70 ± 0.05 at 515 nm. Thereafter, 0.4 mL aliquots of each extract diluted in methanol, or methanol for the control, were added to tubes containing 3.6 mL of this DPPH solution. Analyses were performed in triplicate. The absorbance reading was taken after 2 h of incubation using a spectrophotometer (Biospectro SP 22, São Paulo, BR). The results were expressed as IC_{50} (µg/mg of extract) which is the amount of antioxidant required to cause 50% reduction of the initial concentration of DPPH (Equation 2). Majority of studies that express their results as IC_{50} value, defined it as the amount of antioxidant necessary to

decrease the initial DPPH concentration by 50%. This value is calculated by plotting inhibition percentage against extract concentration (Sokmen et al., 2004). Thus, the lower the IC_{50} value, the greater ability to scavenge DPPH free radical.

$$IC_{50} = \left(\frac{A_c - A_t}{A_c} \right) \quad (2)$$

where IC_{50} is the radical scavenging activity (%), A_c is the absorbance of control, and A_t is the absorbance of test sample.

3 Results

Dried and crushed orange peels had moisture content of 6.50% (dry basis). The registered weights were expressed in terms of free moisture contents. The percentage mass retained in the six-series Tyler sieve were 2.62%, 30.73%, 32.53%, 7.21%, 18.59%, 5.50% and 2.82% respectively. The particle size analysis resulted in a calculated average particle diameter of 0.844 mm.

Oliveira (2012) considers an average particle diameter ideal for extraction to be between 0.25 and 2 mm and it covers the results in this study.

3.1 Essential oil yield

PLE yield (consisting of 13 experiments of factorial, axial and central point levels) ranged from 21.1% - 49.3% with a mean of 27.7%. The positive axial temperature and negative axial static cycles had the highest yield of 49.3%. The lowest yield of 21.1% was recorded by the negative axial temperature and positive axial static cycles (Table 2).

3.3 TPC

The results of TPC distribution in the sweet orange peels essential oil are in Table 2. The TPC, as determined by folin Ciocalteu method, are reported as GAE by reference to standard curve (Y=0.2108x-0.0675, R^2= 0.95). The total phenolic contents varied from 350.42 – 1,319 mg GAE/g of essential oil. The central point had the highest value and positive axial cycle recorded the lowest value of TPC gallic acid equivalent/g of extract.

Table 2 CCD matrix to study temperature (T) and static cycles (C) effects on sweet orange extract yield, tannins, total phenolic content (TPC) and antioxidant capacity by ABTS and DPPH.

Test	T (ºC)	SC (min)	Yield (%) (A)	Purified extract (g/g crude extract) (B)	Tannins (g/g crude extract) (C)	TPC (mg GAE/g crude extract) (D)	ABTS (mg TE g^{-1}) (E)	DPPH IC_{50} (mg g^{-1}) (F)
1	50	2	21.6	0.916	0.084	652.13	11.47	40.64
2	50	4	25.7	0.916	0.084	1015.03	11.45	33.44
3	70	2	40.1	0.916	0.084	791.6	11.47	38.47
4	70	4	49.3	0.931	0.069	350.42	11.56	15.27
5*	60	3	28.6	0.914	0.086	1319	11.46	23.27
6*	60	3	24.8	0.924	0.076	733.25	11.46	32.15
7*	60	3	31.9	0.914	0.086	757.44	11.47	28.75
8*	60	3	24.6	0.922	0.078	780.212	11.45	18.89
9*	60	3	25.4	0.922	0.078	835.72	11.45	25.94
10	46	3	22.1	0.918	0.082	768.83	11.53	24.22
11	74	3	33	0.923	0.077	680.59	11.51	33.79
12	60	2	21.7	0.913	0.087	908.3	11.51	35.64
13	60	4	33	0.917	0.083	807.25	11.50	27.33

Note: α = ± 1.41; * central point; From 1 to 13 are PLE extracts; CCD is the central composite design.

3.4 Antioxidant using ABTS and DPPH IC_{50}.

The results of the radical scavenging ability of the EO used in this study is exhibited by their ABTS and DPPH radicals scavenging activities (Table 2). The ABTS scavenging ability is presented as trolox equivalents (TE) by reference to standard curve (Y=-0.0318x+0.684, R^2= 0.9971). The antioxidant value of ABTS ranged from 11.45 to 11.56 mg TE/g of essential oil. The highest temperature and highest static cycles (Test 4) showed the highest activity (11.56 mg TE g^{-1}) and central point the lowest (11.45 mg TE g^{-1}).

The DPPH free radical scavenging test is one of the commonly used techniques to evaluate the antioxidant potential of plant EO extracts. This is based on the reduction of methanolic DPPH solution in the presence of antioxidant resulting in the formation of non-radical

DPPH by the reaction. The stable DPPH was reduced by all the *citrus sinensis* essential oil extracts and, thus changing the colour from purple to yellow with varying degree depending on the presence of antioxidant compounds. The degree of discoloration indicates the scavenging potential of the EO extract. In the present study, among all the EO extracts tested, the highest capacity to neutralize DPPH radicals was found for the highest temperature and highest static cycles (Test 4) while moderate activity was found for other EO extracts.

For the DPPH method, the antioxidant activity of the EO extracts ranged from 40.64 mg g^{-1} to 15.27 mg g^{-1}. Positive axial cycle (4) showed the highest antioxidant activity (IC_{50} = 15.27 mg g^{-1}) and the least antioxidant activity value (IC_{50} = 40.64 mg g^{-1}) was shown by Test 1. The smaller the IC_{50} values, the higher antioxidant activity of the plant extracts (Prieto et al. 1999).

3.5 Effect of operating conditions

In this work, the influence of the operating conditions (Temperature and Static cycle of extraction) on the extraction yield, and some total phenolic content and antioxidants property were investigated (Figure 1).

3.5.1 Effect of temperature and static cycle

These chats were generated to verify the influence of the extraction variables temperature (T) number of Cycles (C) and their interaction (T×C) on the responses: extract yield (Figure 1A), purified extract (1B), tannins (1C), total phenolic content (1D), antioxidant using ABTS (1E), and DPPH (1F). Extraction temperature was in the range of 46°C to 75°C. Figure 1A and 1E showed that extraction yield and antioxidant property using ABTS increased with the temperature. The static cycle of extraction time was varied from 30 to 60 min and the essential oil components was extracted from the sample. In both cases T or T×C showed positive influence. The influence of temperature (T) on the PLE EO extracts yield was significant and positive. The effect of T×C interaction on antioxidant activity by ABTS was similar to the influence of the temperature. However, the static cycles have no influence as shown in Figure 1.

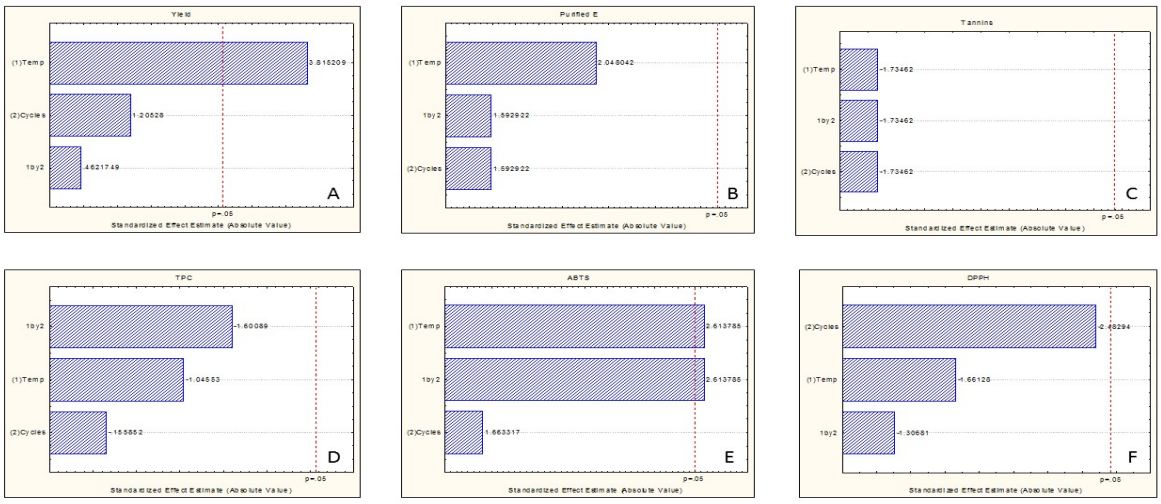

Figure 1 Pareto chart that shows the influence of extraction variables (T, C and T× C) in the extract yield (%)), ABTS (B), phenolic (C), total phenolic contents (D), and DPPH (E)

3.5.2 Optimization of PLE

As shown from the Pareto chart (Figures 1B, 1C, 1D and 1F), dependent variables that have no influence on the independent variables (T, C and T×C) were not considered in the modeling study. The dependent variables, such as extraction yield and ABTS, showed significant effects for at least one independent variables. The calculated F-test (F_{calc}) was greater than tabulated value for F-distribution (F_{tab}) at 95% confidence level ($F_{calc} > F_{tab}$, $p>0.05$). Table 3 shows the analysis of variance (ANOVA) for both first and second order experimental designs only for the two responses essential oil yield and antioxidant activity by ABTS for both dependent variables.

The coefficient of determination (R^2) (0.76 for linear and 0.72 for quadratic model) and (0.77 for linear and 0.70 for quadratic model) for the essential oil yield and

ABTS respectively, showed considerably lower values. However, the regression coefficient for both dependents variables suggests that the first and second order models could be used to predict the behavior of the experimental data showing a predicting ability for both dependent variables. The best model for predicting the extract yield and antioxidant activity by ABTS is the first order model with the higher regression of 0.76 and 0.77 respectively.

Table 3 Analysis of variance for first and second order 2^2 CCD for sweet orange extract yield (%) and antioxidant using ABTS.

Response	Source of variation	Sum square(SS)	Degree of freedom (DF)	Mean square	F_{calc}	F_{tab}
Yield (%) First order model	Model (R)	494	3	165	5.5	5.41
	Residue (r)	152	5	30		
	Total (T)	646	8			
	R^2 =0.76					
Yield (%) Second order model	Model (R)	572	5	114	3.71	3.48
	Residue (r)	219	7	31		
	Total (T)	791	12			
	R^2 =0.73					
ABTS (mg g^{-1}) First order model	Model (R)	0.0072	3	0.0024	6	5.41
	Residue (r)	0.0019	5	0.0004		
	Total (T)	0.0091	8			
	R^2 =0.77					
ABTS (mg g^{-1}) Second order model	Model (R)	0.0103	5	0.00206	3.61	3.48
	Residue (r)	0.0044	7	0.00057		
	Total (T)	0.0147	12			
	R^2 =0.70					

Note: Regression coefficient $R^2 = SS_R/SS_r$; $F_{calc} = MS_R/MS_r$. Order to be significant, $F_{calc} > F_{tab}$ to 95% of the confident level.

Table 4 Linear and quadratic models with their regression coefficients estimated for citrus peels extract yield and antioxidant using ABTS for first and second order 2^2 CCD.

Response	Regression coefficient	Equation
	Yield (%)	
Linear	Y=30.0222*+ 10.5250T*	(3)
Quadratic	Y=27.044* + 7.205T*+1.945T^2*	(4)
	ABTS	
Linear	Y=11.4711*+ 0.0.0275T* + 0.0275TC*	(5)
Quadratic	Y=11.458*+ 0.01026T*+ 0.0248T^2* + 0.0275 TC*	(6)

Note: *Coefficients with P≤0.05 were used in the model framework. Y=yield. ABTS= Antioxidant. T=temperature (ºC) and C=Static cycles (mins).

Table 4 represents the regression coefficient of the predictive model equation for essential oil yield and antioxidant activity by ABTS as a function of the independent variables (T and T×C). Regression coefficients not presenting statistical significance (p>0.05) were disregarded in these models, Equations 3-6.

In the analysis of the yield as function of process variables using RSA, two optimized regions were achieved (Figure 2). The best yield was occurred when high temperature and number of cycles (C) was used. Region with low temperature and higher number of cycles has a moderate high yield of essential oil.

4 Discussion

Pressurized liquid extraction (PLE) is presented as an excellent alternative to replace the conventional techniques of extraction. PLE have a potential use in the extraction of bioactive compounds and the assessment of food contaminants when it is coupled to an analytical technique. PLE is an attractive alternative because it allows fast extraction and reduced solvent consumption (Santos et al., 2012). Additionally, due to the small amount of organic solvent, using PLE gets broad recognition as a green extraction technique (Ibañez et al., 2012).

In general, the essential oil yields obtained in our study were higher than those reported in the literatures for the same sample of citrus *sinesis* peels. Franco-Vega et al. (2016) had essential oil yield ranging from 0.92% to 2.73%, Kamal et al. (2011) produced oil ranged between 0.24% - 1.07%, and Bourgou et al. (2012) produced 0.74% and Ademosun et al. (2015) yield 1.54%. The PLE result showed an increase in extraction yield with respect to the increase in temperatures and extraction time. This

is consistent with earlier study on sweet lime (Megha and Mumtaj, 2014). However, the higher yield of the essential oil when compared to previous studies could be due to environmental factors, such as soil type, cropping practices, stages of maturity, and types of weather can contribute to quantitative variations in the content of essential oils; in addition, they could affect the biological activity of the oils (Jing et al., 2014).

This high yield would increase the production of the essential oil and improve the availability of this oil which can be used in functional foods and nutraceuticals and cosmetics production.

4.1 TPC

The result of TPC is higher than the values reported by Ghasemi et al. (2009) with *citrus spp.* samples using Folin Ciocalteu method, which ranged from 66.5 to 396.8 mg GAE/g of EO extract. The high result from the TPC of this study implied high radical scavenging abilities of essential oil, which could be linked to the presence of the phenolic contents in the oil. This could lead to high bioactive compounds in the extracted essential oil.

The presence of strong antioxidant properties from the essential oil as indicated by the findings using ABTS and DPPH, shows that the essential oil with strong antioxidant properties could be used to prevent some diseases which can be triggered by oxidative stress.

The fact of using low temperature (T) and fewer batches or cycles (C) and thus achieve good yields (Figure 2A), is characterized as an economic condition in the extraction process, with lower energy consumption and solvent. RSA generated by the antioxidant activity when analyzed using ABTS (Figure 2B), produced similar result as EO extraction yield. Operating the equipment with low temperature leads to obtaining EO yield with good antioxidant activity and still maintains the stability of thermo-sensitive substances. Thus, the extraction with PLE can present a great advantage over conventional methods, in which sometimes must be used at high temperatures, more solvent and more extraction time. Moreover, with this use of low temperatures, less solvent and less extraction time, power consumption is less, which is economical.

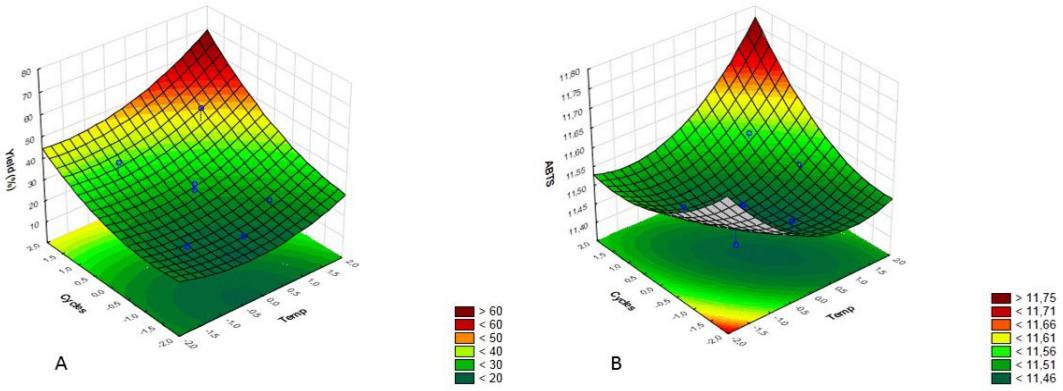

Figure 2 Response surface of essential oil extract yield (A) and antioxidants using ABTS (B) as a function of T, C and T× C generated by quadratic model

Temperature is the main parameter responsible for the extraction process acceleration. High temperatures decrease the solvent viscosity, helping with its penetration inside the matrix and consequently, improving the extraction process. The use of high temperatures increases the diffusion coefficients and the mass transference rates, furthermore helps to disrupt the compounds–matrix interactions, and then to improve extraction rate (Camel, 2001; Ibañez et al., 2012; Nieto et al., 2010, Santos et al., 2012). The temperature changes could be due to the increase of essential oil vapor pressure in the pores of the sample, which increases the mass transfer rate. The increase in the extraction yield with temperature could also be explained in terms of the increase in kinetic energy. Increase in the temperature leads to the increase in the molecules speed and their corresponding kinetic energy and consequently the rate of diffusion of CO_2 within the particles (Al-Otoom et al., 2014).

At higher temperatures (between 70℃ and 74℃), the influence of the temperature was dependent on the Static cycles extraction time. These results indicate that although the temperature is considered to be the most important parameter for ensuring good performance of the PLE process, the extraction yield is also affected by the combination of temperature and static cycle extraction time.

The duration of the static extraction time is important in the extraction efficiency since prolonging contact periods between the matrix and the solvent permits increased swelling with enhanced matrix wetting and increased penetration of solvent into the nano and micro pores resulting on greater solvation of compounds. Furthermore, an enhanced possibility of the solvent breaking specific compounds–matrix interactions is ensured (Runnqvist et al., 2010).

As seen in this study, static extraction cycles times between 45 and 60 minutes are enough to guarantee the extraction of the most compounds with a high yield, while combination of high temperatures and long extraction times could induce the degradation of the compounds and the matrix (Rizvi, 2010).

5 Conclusion

This study has shown that PLE could be an efficient, rapid, reliable and highly selective process for extracting essential oil from *citrus sinensis* peels. Generally, CCD was found to be helpful experimental design to determine the effects of extraction conditions on the efficiency of *citrus sinensis* peels essential oil and the bioactivity of the oil extract. The response surface analysis showed an adequate polynomial model for predicting essential oil yield and antioxidant using ABTS. Thus, PLE method would increase the extraction yield of essential oil from sweet orange peels and as well retain important heat-sensitive phyto-constituent which could be valuable in the functional food and nutraceuticals industries and cosmetic production.

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References

Ademosun, A. O., G. Oboh, A. J. Olupona, S. I. Oyeleye, T. M. Adewuni, and E. E. Nwanna. 2015. Comparative study of chemical composition, *In Vitro* inhibition of cholinergic and monoaminergic enzymes, and antioxidant potentials of essential oil from peels and seeds of sweet orange (C*itrus Sinensis* [L.] Osbeck) fruits. *Journal of Food Biochemistry*, 40(1): 53-60.

Al-Otoom, A., S. Al-Asheh, M. Allawzi, K. Mahshi, N. Alzenati, B. Banat, and B. Alnimr. 2014. Extraction of oil from uncrushed olives using supercritical fluid extraction method. *The Journal of Supercritical Fluids*, 95: 512–518.

AOAC. 2000. *Official Methods of Analysis of AOAC International*. 17th ed. Maryland, USA: AOAC.

Bourgou, S., F. Z. Rahali, I. Ourghemmi, and M. S. Tounsi. 2012. Changes of peel essential oil composition of four tunisian citrus during fruit maturation. *The Scientific World Journal*, 2012: Article ID 528593.

Brand-Williams, W., M. E. Cuvelier, and C. L. W. T. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. *LW Technologie-Food Science and Technology*, 28(1): 25-30.

Camel, V. 2001. Recent extraction techniques for solid matrices-supercritical fluid extraction, pressurized fluid extraction and microwave-assisted extraction: their potential and pitfalls. *Analyst*, 126(7): 1182-1193.

Ezejiofor, T. L. N., N. V. Eke, R. I. Okechukwu, R. N. Nwoguikpe, and C. M. Duru. 2011. Waste to wealth: Industrial raw materials potential of peels of Nigerian sweet orange (*Citrus sinensis*). *African Journal of Biotechnology*, 10(33): 6257-6264.

FAO. 2012. Citrus Fruit Fresh and Processed annual statistics. Rome: FAO.

FDA. 2013. Food Additives Status List. Available at: http://www.fda.gov/food/ingredientspackaginglabeling/foodadditivesingredients/ucm091048.ht. Accessed 12 November 2013

Franco-Vega, A., N. Ramirez-Corona, E. Palou, and A. López-Malo. 2016. Estimation of mass transfer coefficients of the extraction process of essential oil from orange peel using microwave assisted extraction. *Journal of Food Engineering*, 170: 135-141.

Garau, M. C., S. Simal, C. Rosselló, and A. Femenia. 2007. Effect of air-drying temperature on physicochemical properties of

dietary fibre and antioxidant capacity of orange (*Citrus aurantium* v. Canoneta) by-products. *Food Chemistry*, 104(3): 1014-1024.

Ghasemi, K., Y. Ghasemi, and M. A. Ebrahimzadeh. 2009. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pakistan Journal of Pharmaceutical Sciences*, 22(3): 277-281.

Ibañez, E., M. Herrero, J. A. Mendiola, and M. Castro-Puyana. 2012. Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates. In *Marine Bioactive Compounds: Sources, Characterization and Applications*, ed. M. Hayes, ch. 2, 55-98. New York, NY: Springer.

Jing, L., Z. Lei, L. Li, R. Xie, W. Xi, Y. Guan, L. W. Sumner, and Z. Zhou. 2014. Antifungal activity of citrus essential oils. *Journal of Agricultural and Food Chemistry*, 62(14): 3011–3033.

Kamal, G. M., F. Anwar, A. I. Hussain, N. Sarri, and M. Y. Ashraf. 2011. Yield and chemical composition of Citrus essential oils as affected by drying pre-treatment of peels. *International Food Research Journal*, 18(4): 1275-1282.

Manthey, J. A. 2004. Fractionation of orange peel phenols in ultra-filtered molasses and balance studies of their antioxidant levels. *Journal of Agricultural and Food Chemistry*, 52(25): 7586-7592.

Megha, M., and S. Mumtaj. 2014. Extraction and characterization of essential oil of sweet lime (*Citrus Limetta Risso*) peel using microwave-assisted hydro-distillation. *Research Journal of Chemical Sciences*, 4(11): 51-55.

Nieto, A., F. Borrull, E. Pocurull, and R. M. Marcé. 2010. Pressurized liquid extraction: A useful technique to extract pharmaceuticals and personal-care products from sewage sludge. *TrAC Trends in Analytical Chemistry*, 29(7): 752-764.

Oliveira, D. A. 2012. Caracterização fitoquímica e biológica de extratos obtidos de bagaço de uva (*Vitis vinifera*) das variedades merlot e syrah. Centro Tecnológico .daUniversidadeFederalde SantaCatarina, Florianópolis, SC, Brasil, 211.

Prieto, P., M. Pineda, and M. Aguilar. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E. *Analytical Biochemistry*, 269: 337–341.

Rizvi, S. S. 2010. Separation, Extraction and Concentration Processes in the Food, Beverage and Nutraceutical Industries. Cambridge, U.K.: Woodhead Pub Ltd.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, 26(9-10): 1231–1237.

Runnqvist, H., S. A. Bak, M. Hansen, B. Styrishave, B. Halling-Sørensen, and E. Björklund. 2010. Determination of pharmaceuticals in environmental and biological matrices using pressurised liquid extraction—Are we developing sound extraction methods? *Journal of Chromatography A*, 1217(16): 2447-2470.

Santos, D. T., P. C. Veggi, M. A. A. Meireles. 2012. Optimization and economic evaluation of pressurized liquid extraction of phenolic compounds from jabuticaba skins. *Journal of Food Engineering*, 108(3): 444-452.

Singleton, V. L., and J. A. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungtic acid reagents, *American Journal of Enology Viticulture*, 16(3): 144-158.

Sokmen, A., M. Gulluce, H. A. Akpulat, D. Daferera, B. Tepe, and M. Polissiou. 2004. The in vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. *Food Control*, 15(8): 627–634.

Su, L., J. Yin, D. Charles, K. Zhou, J. Moore, and L. Yu. 2007. Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. *Food Chemistry*, 100(3): 990-997.