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Comparative Evaluation of Soxhlet and Ultrasonics on the Structural Morphology and Extraction of Bioactive Compounds of Lemon (*Citrus limon* L.) Peel

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Abstract

The relative recoveries of bioactive compounds and nutritional ingredients from lemon peel by ultrasound-assisted extraction (USAE/UAE) and traditional soxhlet extraction methods under different conditions of extraction were evaluated. The effect of these treatments on the morphological structure and tissue damage were assessed by scanning electron microscopy. The overall optimal conditions for maximum extraction of total phenolic content (TPC), total flavonoids content (TFC), vitamin C and anti-oxidant activity (AOA) using USAE (200 W output) was predicted using response surface methodology to be at the combined levels of 48 ˚C extraction temperature, 60 min extraction time and 80% duty cycle. Under these optimum conditions the corresponding predicted responses for TPC, TFC, vitamin C and AOA were 67.17 mg GAE/100 g, 4.52 mg CE/100 g, 26.46 mg/100 g and 61.68% respectively. USAE proved better as compared to soxhlet in the extraction of total phenolics, flavonoids, retention of vitamin C and showing antioxidant activity. In the case of USAE, the combination of factors for maximum extraction of total phenolics (88.06 mg GAE/100 g) was found to be at a temperature of 33 ˚C, 80% duty cycle and 40 min of treatment. With reference to maximum extraction of total flavonoids (13.92 mg CE/100 g) these conditions were 40 ˚C, 60% and 50 min and 40 ˚C, 100% duty cycle, 50 min of treatment for vitamin C (58.87 mg/100 g) contents respectively. The conditions which showed the maximum antioxidant activity (69.37%) was found to be 50 ˚C, 80% duty cycle and 40 min of extraction.

Keywords

Ultrasonics extraction, Soxhlet extraction, Citrus, Lemon peel

Introduction

Lemon is an important citrus crop grown worldwide. Researchers have reported various health-promoting bioactive compounds in lemon such as phenolic compounds, flavonoids, essential oils carotenoids, vitamins, minerals and dietary fiber [1]. Many commercially available food/non-food products use lemon fruit in one form or the other for its unique properties like health-promoting, taste/flavour enhancing or visual appeal. However, the waste peel, left over from these food and non-food industries are still a source of important bioactive compounds which can be utilized in related industries like health care, cosmetics, home care products and pharmaceutical industries [1]. The role of the peel bioactives in prevention of diseases such as obesity, diabetes, blood lipid lowering, cardiovascular diseases and certain types of cancer has been reported [2]. Lemon peel is the main by-product of lemon juice processing industry as it accounts for 50 to 65% of the whole fruit weight [3]. The use of lemon by-products is challenging because of the absence of specific industries meant for the purpose and also the technological and economical costs of the available processes [4, 5]. The bioactive compounds still present in these citrus peels can act as a source of vitamin C, carotenoids, phenolic compounds and these compounds have been reported to exhibit various health promoting & antioxidant properties [2, 6]. Astell, Mathai & Su [7] reported flavonoids and phenolic acids to be a major part of bioactive compounds in citrus fruits and peels.

Polyphenols, which are secondary metabolites form the major class of these bioactive compounds and these are largely concentrated in the in-edible portions like peel and seeds where they serve varied roles some of which could be as UV protectants, insect attractants or as defence chemicals against invading pathogens [8].

The economical and efficient extraction of these bioactive compounds from lemon peels is an important area of research which can benefit the pharmaceutical and chemical industries. The extraction of bioactive compounds from peels with a solvent in a soxhlet extraction apparatus is the classical method being used in the pharmaceutical industry [8]. These classical methods of extraction like heating, boiling or refluxing are plagued with limitations like longer extraction times, lower yields, the use of large amounts of organic solvents and poor extraction efficiencies [9]. The increasing consumer interest in natural drugs has brought the need for superior extraction methods, which can extract the maximum quantity of bioactive in their native form that too economically in a short processing time. Eco-friendly cost-effective extraction procedures for the bioactives present in citrus peels are constantly being explored [10]. Novel extraction techniques like ultrasound-assisted extraction (USAE), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) have been reported [11-13] to be much superior to the conventional methods in the extraction of these bioactive compounds from by-products. Some workers have shown MAE to be better than USAE and conventional solvent extraction (CSE) with respect to recovery yield, specific antioxidant activity, shorter working time and lower solvent consumption [11], while others report USAE to be better in a comparison between USAE, MAE, CSE and superheated liquid solvent extraction [12].

The use of USAE has the potential to enhance the value of these bioactive compounds as it is an efficient, green process and such compounds will have larger appeal especially with the consumers preferring natural or nature-identical ingredients in the foods or cosmetics they use. The process is distinguished by a more effective extraction and use of moderate temperatures of extraction especially beneficial for heat-labile compounds. Several process variables, like applied ultrasonic power, frequency, extraction temperature, reactor characteristics, solvent-sample interaction are considered crucial during USAE [14] and the most amounts of bioactives are reported to be extracted in the initial few minutes of the process. Ultrasound-assisted extraction has been shown to reduce significantly the extraction times, thereby limiting power consumption, as well as enhancing extraction yields in case of many materials [15].

This study was undertaken to compare the relative recoveries of bioactive compounds and nutritional ingredients from lemon peel using USAE and the traditional soxhlet extraction methods under different conditions of extraction. Apart from comparing the efficiency of extracting polyphenols, flavonoids, ascorbic acid etc., the morphological structure and the tissue damage under the two extraction conditions were visualized by scanning electron microscopy.

Materials and Methods

Raw material

Lemon fruits (*Citrus limon* L.) of proper maturity were purchased from local market in Mysuru, Karnataka, India, washed with water twice, allowed to surface dry at room temperature and stored in refrigerated condition at 4-8 °C until used for experiments. The washed and dried fruits were peeled and the peels ground in a domestic mixture grinder for 5 min to a coarse size and filled in a storage container and kept refrigerated at 4-6 °C till further processing.

Extraction methods

The lemon peels were extracted by soxhlet apparatus and ultrasound assisted sonication for a comparative study. The process flow chart for extraction of bioactive compounds from lemon peel is given in figure 1.

Soxhlet method

In this process (5 g) of the sample was weighed and transferred into a soxhlet apparatus and extracted with 250 ml of 80% methanol (1:50 sample to solvent ratio) for about 16 hours at 65 °C [16]. The procedure was repeated to ensure complete recovery; the extracts were pooled and stored at -20 °C until analysis.

Ultrasonic assisted extraction (USAE)

Ultrasonic-assisted extraction of lemon peels was done using high intensity probe ultrasonicator, Branson Sonifier 250 (Branson Ultrasonics Corporation, Connecticut, USA) model controlling three parameters – sonication time, temperature and duty cycle. The sample: solvent ratio (80% methanol) was kept at 1:6 and the sonication was done at a constant power output of 200 W and a frequency of 30 kHz. The procedure was repeated to ensure complete recovery, the extracts pooled and was stored at -20 °C until analysis. The parameters were selected based on preliminary experiments.

Analytical methods

Ascorbic acid

Ascorbic acid content was determined by taking 1.5 g of dried lemon peel powder and subjecting to HPLC involving extraction and clean up [17, 18] before separation on a Waters Spherisorb 5 µm ODS2 analytical column, and detection using a 2489 UV/Visible Detector set at 254 nm (Waters Corporation, Milford, MA, USA). All the analyses were done for fresh, soxhlet extracted and sonicated samples of lemon peel extracts.

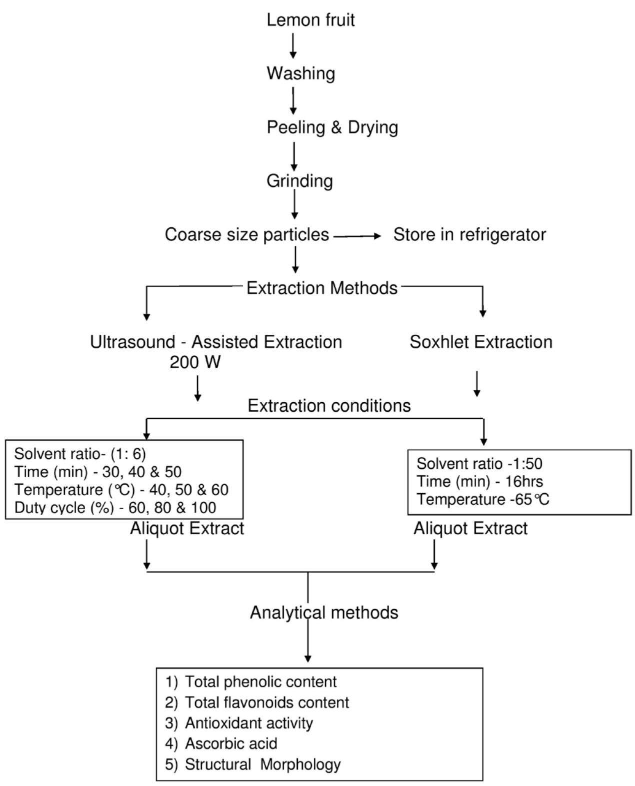

Figure 1: Process flow chart for extraction and analysis of bioactive compounds from lemon peel.

Total phenolic content (TPC)

Total phenolic content of the lemon peel was estimated using Folin-Ciocalteu reagent as per the method described by Singleton & Rossi [19].

In brief, a 1 ml of sample extract was taken in 25 ml volumetric flask. To this 4 ml of distilled water and 1 ml of Folin-Ciocalteu phenol reagent was added followed by the addition of 10 ml of 7% Na_2CO_3 solution after 5 min. This was made up to 25 ml with distilled water. The tubes were incubated for 90 minutes at room temperature and the absorbance was measured at 750 nm against a blank using UV-Visible spectrophotometer (UV-1601; Shimadzu, Japan). A calibration curve of gallic acid was plotted and the total phenolics content was calculated and expressed as mg Gallic Acid Equivalents (GAE)/100 g sample.

Total flavonoids content (TFC)

Total flavonoids concentrations were determined using the protocol outlined by Zhishen, Mengcheng & Jianming [20].

A 1 ml of sample extract was placed in 10 ml volumetric flask. To this 4 ml of distilled water and 0.30 ml of 5% $NaNO_2$ was added. $AlCl_3$ (0.3 ml) was added after 5 min followed by the addition of 2 ml of 1 N NaOH after a further period of 6 min. The solution was diluted by making up to 10 ml with distilled water. Absorbance was measured at 510 nm using a UV-Visible spectrophotometer (UV-1601; Shimadzu, Japan). A catechin standard curve was used to quantify the flavonoid concentrations and expressed as mg Catechin Equivalents (CE)/100 g sample.

Determination of antioxidant activity by DPPH radical scavenging method

The antioxidant activity of the samples was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Some modifications were made to the DPPH assay used by Brand et al. [21]. Fresh or treated lemon extract (0.1 ml) was mixed with 2.9 ml of pure methanol solution. The reaction mixture was incubated in dark at room temperature for 45 min after adding 0.5 ml of DPPH solution. The absorbance at 515 nm was read using a UV-Vis spectrophotometer (UV-1601; Shimadzu, Japan). Methanol (3 ml) and 0.5 ml DPPH solution with 3 ml methanol without sample was used as blank and control respectively. The total free radical scavenging activity was expressed as percentage of DPPH reduced which was calculated using the following equation: FRSA=100 X (initial absorbance at time zero-final absorbance after 45 minutes)/ initial absorbance. DPPH radical scavenging activity was expressed as percentage.

ORAC (oxygen radical absorbance capacity) assay

The ORAC method as described by Ou et al. [22] was employed. 15 μl of fresh or treated lemon extract (5 g/l solution in methanol) was pre incubated for 10 min at 37 °C with a fluorescent probe fluorescein (2 ml of a 26 nM solution in phosphate buffer, 75 mM pH 7.4). To this 1 ml of a 664 mM 2, 2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) solution made in phosphate buffer was added. The fluorescence intensity was measured at 2 min intervals during 40 min with excitation and emission wavelengths set at 490 and 511 nm respectively. The decrease in the intensity refers to oxidation of fluorescence by the peroxyl radicals derived from AAPH. The results were expressed as millimoles of trolox equivalents (TE) per gram of sample on a fresh weight basis (mmol TE/100 g FW). Mean values of triplicate tests are reported for both tests.

Scanning electron microscopy (SEM)

Cabinet air dried samples of control and experimentally treated lemon peel powder were gold coated in a sputter coating unit by mounting them on brass stubs. Morphological evaluation was carried out by SEM using an accelerating potential of 20 kV in a Zeis scanning electron microscope (Oxford Instrument, England).

Design of experiments

A central composite design (CCD) was used to gain maximum information about the process from least number of experiments. The three independent factors affecting extraction process included were extraction temperature (°C), Duty cycle (%) and extraction time (min). Three levels of each factor were studied. The design consisted of 20 experiments chosen in random order, including six replicates of centre point. The selected response factors were TPC (mg GAE/100 g), TFC (mg CE/100 g), vitamin C (mg/100 g) and Antioxidant activity (%). The experimental design and results were analysed using Design Expert Version 6.0 (StatEase Inc., Minneapolis, USA).

Statistical analysis of data and response surface methodology

All experiments were carried out in triplicate. Microsoft Excel software was used to calculate means and standard deviation. Regression and analysis of variance (ANOVA) with 95% confidence level was conducted for determining regression coefficients with statistical significance of model terms. The regression coefficients were used for fitting the mathematical models to the experimental data.

Response surface methodology-based optimization of the extraction of bioactive compounds from lemon peel was done. The least-squares technique [23] was used to determine the multiple regression coefficients to predict linear and quadratic polynomial models for the response variables studied. The generalized polynomial model used for predicting the response variables as function of independent variables studied is given as given below.

$$Y_i = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3$$

where Y_i is predicted response, β_0 is offset term, β_1, β_2& β_3 are the regression coefficients for linear effect terms, β_{11} β_{22}& β_{33} are quadratic effects and β_{12} β_{13}& β_{23} are interaction effects. X_1, X_2& X_3 are independent variables. F-ratio indicated the significance of the equation parameters for each response variable. The adequacy of the models was determined using model analysis and lack-of-fit test. Coefficient of determination was also calculated.

Results and Discussions

Optimization of extraction conditions for the recovery of lemon peel bioactive compounds

The pulse intensity at a particular power (200 W), the temperature of extraction and the duration of extraction were some of the variables studied for the USAE. Twenty experiments were designed using a central composite design. Table 1 shows the independent variables and responses obtained in the multivariate experiments employed in the study. Temperature (°C), ultrasonic cycles (%), time (min) on the UAE of lime peel polyphenols (mg/100 g FW), vitamin C (mg/100 g) & antioxidant activity were evaluated by response surface methodology. Twenty experiments were conducted, and the responses fitted as a function of these variables.

The quadratic regression equations in terms of actual factors were

TPC = 237.60 - 24.51 X Temperature + 10.52 X Time + 5.07 X Duty Cycle + 0.23 X Temperature^2 -0.09 X Time^2 - 0.01 X Duty Cycle^2 + 0.03 X (Temperature X Time) - 7.68E-3 X (Temperature X Duty Cycle) - 0.05 X (Time X Duty Cycle)

TFC = 53.72 - 2.76 X Temperature + 0.79 X Time + 0.18 X Duty Cycle + 0.02 X Temperature^2 + 0.00094 X Time^2 - 0.002 X Duty Cycle^2 - 0.007 X (Temperature X Time) + 0.0079 X (Temperature X Duty Cycle) - 0.005 X (Time X Duty Cycle)

Vitamin C = 5.92 + 4.40 X Temperature + 7.29 X Time - 5.48 X Duty Cycle + 0.002 X Temperature^2 - 0.10 X Time^2 + 0.03 X Duty Cycle^2 - 0.04 X Temperature X Time - 0.039 X Temperature X Duty Cycle + 0.040 X Time X Duty Cycle

Antioxidant Activity = 267.77-24.13 X Temperature + 9.12 X Time + 4.76 X Duty Cycle + 0.207 X Temperature^2 - 0.11 X Time^2 - 0.018 X Duty Cycle^2 + 0.082 X Temperature X Time + 0.00095 X Temperature X Duty Cycle - 0.047 X Time X Duty Cycle

The quadratic equations indicate a greater effect of temperature followed by time on the extraction of polyphenols, flavonoid contents, vitamin C and antioxidant activity during UAE. The main effects are significant whereas the effects of interaction between the variables are not significant in the present study. The analysis of variance resulted in an R-squared statistic for all the parameters in the range of 0.820-0.958 indicating good representation of the parameters by the model. Wang et al. [24] studied the effect of variables like power intensity of ultrasound and extraction temperature on the pectin extraction in grapefruit and reported temperature followed by power intensity of sonication as the main factors to influence the pectin extraction. Khan et al. [25] studied the effect of temperature, ultrasonic power and ethanol: water ratio on the ultrasonic extraction of orange peel polyphenols in terms of polyphenolic content, naringin and hesperidin concentrations. They reported ultrasound power, followed by temperature and ethanol: water ratio as the most important factors to influence the polyphenolic content. Similar to this study they also did not note any significant cross-product terms suggesting the absence of interactions between the studied variables. However, Dahmoune et al. [11] noted an interactive effect of time with ethanol concentration on the TPC yield during the USAE of dried lemon peels.

Multiple response optimizations were carried out to determine the optimum levels of independent variables leading to desired response goals. The response surface graphs showing the graphical interpretation of the interaction effect of independent variables on the response variables like TPC, TFC, vitamin C and Antioxidant activity of lemon peel extract are shown in figures 2-5.

A numerical optimization was carried out by the response optimizer using the Design Expert Version 6.0 software to determine the exact optimum level of independent variables leading to the overall optimized response. The responses were then analysed jointly by assigning to them the same weightage. The overall optimal condition for maximum extraction of TPC, TFC, vitamin C and AOA was predicted to be at the combined levels of 48 °C extraction temperature, 60 min extraction time and 80% duty cycle. Under these optimum conditions the corresponding predicted responses for TPC, TFC, Vitamin C and AOA were 67.17 mg GAE/100g, 4.52 mg CE/100g, 26.46 mg/100 g and 61.68% respectively. Papoutsis et al. [14] in a similar study reported an extraction time of 45 min at a temperature of 50 °C and

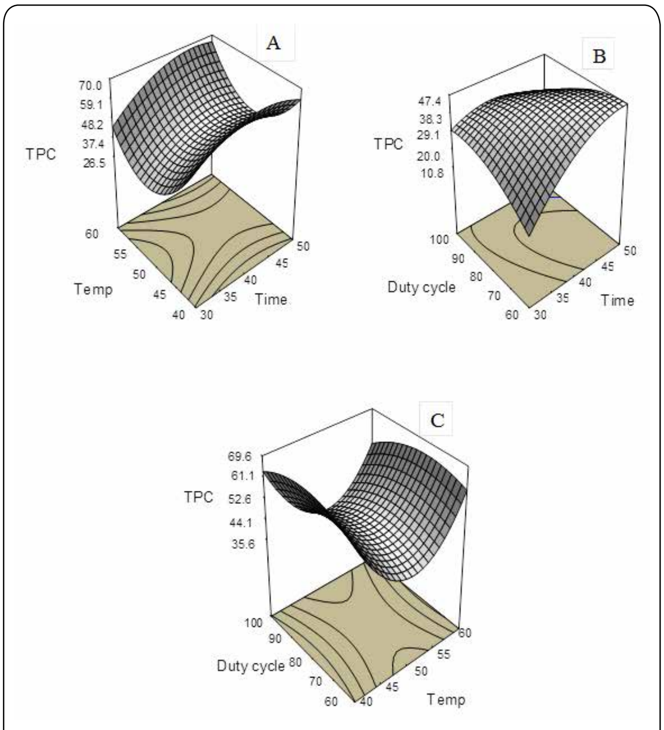

Figure 2: Response surface plots for effect of extraction temperature and time **(A)**; duty cycle and time **(B)**; duty cycle and temperature **(C)**; on total phenolic content (TPC) in lemon peel extract.

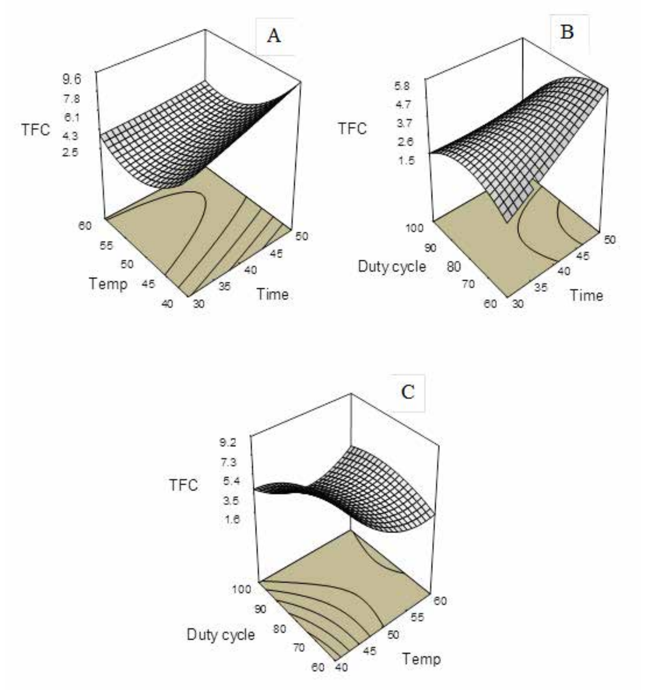

Figure 3: Response surface plots for effect of extraction temperature and time **(A)**; duty cycle and time **(B)**; duty cycle and temperature **(C)**; on total flavonoid content (TFC) in lemon peel extract.

ultrasonic power of 250 W for optimum extraction of total polyphenols. Meullemiestre et al. [26] studied the impact of ultrasound on solid-liquid extraction of phenolic compounds from maritime pine sawdust waste and reported optimum condition for polyphenols extraction by ultrasound-assisted maceration, obtained by response surface methodology, to be 0.67 W/cm^2 for the ultrasonic intensity, 40 °C for the processing temperature and 43 min for the sonication time. Dahmoune et al. [11] reported optimized values for USAE as 63.93% ethanol as extraction solvent, 40 mL/g of liquid/solid ratio, 15.05 min of holding time and 77.79% amplitude for maximum recoveries of TPC from *Citrus limon* residues.

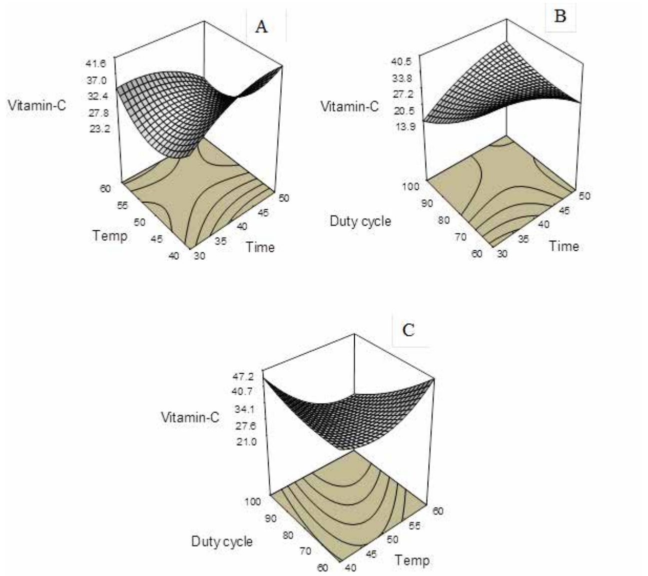

Figure 4: Response surface plots for effect of extraction temperature and time **(A)**; duty cycle and time **(B)**; duty cycle and temperature **(C)**; on Vitamin C content in lemon peel extract.

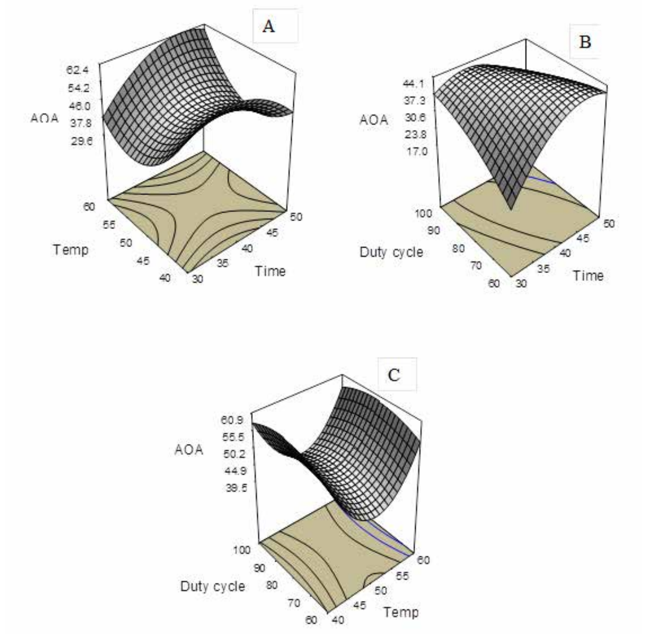

Figure 5: Response surface plots for effect of extraction temperature and time **(A)**; duty cycle and time **(B)**; duty cycle and temperature **(C)**; on antioxidant activity in lemon peel extract.

Comparison of ultrasonics assisted extraction (USAE) and conventional soxhlet extraction

USAE was compared with soxhlet extraction for recovery of total phenolics, flavonoids and ascorbic acid contents from lemon peel. The effect of these treatments on the antioxidant activity of the extract was also compared. USAE was done at a fixed power of 200 W and the pulse intensity, temperature and time were varied.

The total phenolic (TPC), flavonoid (TFC)& vitamin C contents in dried lemon peel powder by the soxhlet extraction were found to be 76.22 ± 2.22 mg GAE/100 g, 5.47 ± 0.26 mgCE/100 g and 35.04 ± 0.96 mg/100 g respectively, while the antioxidant activity was 20.27 ± 0.16%. For fresh lemon peels these values were TPC 68.36 ± 1.82 mg GAE/100 g, TFC 7.46 ± 0.19 CE/100 g, vitamin C 39.55 ± 0.71 mg/100 g and antioxidant activity 46.45 ± 0.35% (Table 1).

USAE on the other hand proved better as compared to soxhlet in the extraction of total phenolics, flavonoids, retention of vitamin C and showing antioxidant activity. In the case of USAE, the combination of factors for maximum extraction of total phenolics (88.06 mg GAE/100 g) was found to be at a temperature of 33 °C, 80% duty cycle and 40 min of treatment (Table 1). With reference to maximum extraction of total flavonoids (13.92 mg CE/100 g) these conditions were 40 °C, 60% and 50 min and 40°C, 100% duty cycle, 50 min of treatment for vitamin C (58.87 mg/100 g) contents respectively.

The conditions which showed the maximum antioxidant activity (69.37%) was found to be 50 °C, 80% duty cycle and 40 min of extraction (Table 1). USAE targeted at maximizing extraction of individual components can hence concentrate on these conditions. Similar work using an optimized aqueous ultrasound assisted extraction method for citrus peels of South American cultivars Londoño-Londoño et al. [27] reported total phenolic contents of 74.80 ± 1.90, 66.36 ± 0.75 and 58.68 ± 4.01 mg GAE/g for lime, orange and tangerine respectively. However, a comparison with conventional soxhlet extraction was not done in their study.

Various works have attempted to compare ultrasound assisted extraction with conventional extraction. Wang et al. [24] compared ultrasound assisted extraction of pectin with conventional heating extraction from various vegetable resources and noted higher efficiency and less energy consumption by ultrasound as compared to conventional heating extraction. Lopresto et al. [28] compared a high-pressure high temperature non-conventional solvent extraction with conventional soxhlet extraction and studied the influence of extraction time, temperature-pressure and matrix/solvent ratio on yield of limonene present in waste lemon. They noted

Table 1: Central Composite Design for experiments on the effect of ultrasound (1-20 Runs) and soxhlet extraction on the content of total phenolics (TPC), total flavonoids (TFC), vitamin C, antioxidant activity in lemon peel extract (LPE).

Run	Temperature (°C)	Time (min)	Duty cycle (%)	TPC (mg GAE/100 g)	TFC (mg CE/100g)	Vitamin C (mg/100 g)	Antioxidant activity (%)
1	50	40	80	33.01	3.36	9.7	56.67
2	60	50	100	55.5	4.97	15.67	55.58
3	50	40	46	44.19	2.74	31.34	38.01
4	60	30	100	43.57	2.49	17.16	42.5
5	40	30	60	29.16	4.54	43.28	35.4
6	50	23	80	31.39	3.67	19.35	34.89
7	50	40	80	33.76	4.91	18.55	40.6
8	50	40	80	32.89	3.48	18.55	28.9
9	40	50	100	38.35	2.8	58.87	30.2
10	60	30	60	37.11	3.23	50.81	26.87
11	50	40	46	39.35	2.99	47.58	43.49
12	50	40	100	37.48	2.8	20.83	35.13
13	50	40	80	40.22	2.99	29.86	34.02
14	40	50	60	69.3	13.92	28.47	53.19
15	33	40	80	88.06	9.14	31.25	64.3
16	60	50	60	73.27	2.92	25.69	62.6
17	66	40	80	48.29	3.3	47.01	54.14
18	40	30	100	61.09	4.29	32.09	65.48
19	50	40	80	72.03	3.73	50	69.37
20	50	56	80	39.97	4.48	38.06	30.28
Dried LPE (Soxhlet extraction)	65	16 hrs	--	76.22 ± 2.22	5.47 ± 0.26	35.04 ± 0.96	20.27 ± 0.16
Fresh Lemon peel *(Soxhlet extraction)*	65	65	--	68.36 ± 1.82	7.46 ± 0.19	39.55 ± 0.71	46.45 ± 0.35

that the high temperature and high pressure was better in the extraction of limonene from lemon peels. In the present study better extraction of phenolics, flavonoids and ascorbic acid contents were noted by USAE even under mild temperature conditions of 40-50 °C.

The antioxidant activity of samples extracted by ultrasound was 1.5 to 2.0 times more in comparison to the samples extracted by soxhlet extraction (Table 1). The better antioxidant activity shown by USAE samples confirms similar observations by earlier researchers [15, 29]. Dahmoune et al. [30] studied ultrasound extraction of phenolic compounds from pistachio leaves and also noted strong antioxidant activity for USAE extract compared to accelerated solvent extraction. The ultrasound assisted extraction of lemon peel hence is a promising source of natural antioxidants with applications in several foods.

USAE is particularly effective in the case of solid matrix since the ultrasound energy makes the organic and inorganic compounds to leach from plant matrix [31]. Ultrasound enhances the mass transfer and permits greater access of solvent to cell materials and matrix. Swelling of plant cells and the breakdown of plant cell walls is seen because of the process of cavitation. Mason et al. [32] suggested two main types of physical phenomena for the extraction mechanism by ultrasound viz., (a) the diffusion across the cell wall and (b) rinsing the contents of cell after breaking the walls. Among the factors which govern the action of ultrasound temperature, pressure, frequency and time of sonication are quite important and need a detailed study.

Chemat et al. [33] have reported some advantages of USAE like reduction in extraction time, energy and lesser consumption of solvent. Ultrasound energy for extraction also facilitates more effective mixing, faster energy transfer, reduced thermal gradients and extraction temperature, selective extraction, reduced equipment size, faster response to process extraction control, quick start-up, increased production and eliminates process steps [33].

Scanning electron microscopy

The microstructure of lemon peel subjected to UAE and conventional soxhlet extraction were compared by SEM. Figure 6 shows the SEM of lemon peel subjected to soxhlet extraction at 65 °C for 16 h (A) and to ultrasonics assisted extraction at the optimized condition of 48 °C extraction temperature, 60 min extraction time and 80% duty cycle (B). The physical destruction of tissues during soxhlet extraction was far more as compared to soxhlet extraction. The cell walls were damaged and the soxhlet extracted samples seemed to curve reducing the surface area in touch with the solvent, while the USAE samples seemed to spread out with each pulse allowing for the greater interaction of the extracting solvent (Figure 6-7). Apart from the increase in the abrasive effects or turbulence caused by ultrasonic waves, intensification of mass transport due to physical effects on the surface of the particles has also been suggested and the mass transfer coefficient has been shown to increase when the solid matrix is exposed to such disturbances [34]. USAE enhances mass transfer and the solvent's ease of access to the cell material. Cavitation's produce high energies around the cells leading to their breakdown and cell disruption. This leads to a high diffusion rates across the cell walls and better penetration [35].

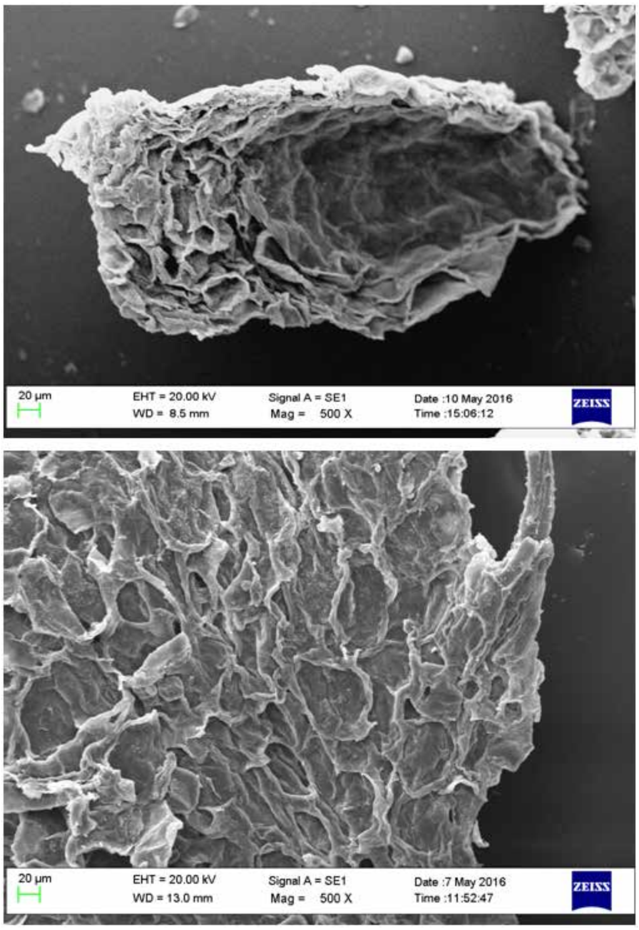

Figure 6: SEM of lemon peel subjected to soxhlet extraction at 65 °C for 16 h **(A)** and to ultrasonics assisted extraction at the optimized condition of 48 °C extraction temperature, 60 min extraction time and 80% duty cycle **(B)**.

Conclusion

The utilization of agricultural by-products is limited by uneconomical recovery techniques and dependency on chemicals thereby limiting the use of the recovered bioactives in food, pharmaceutical or health applications. Citrus peel and seed residue accounts for upto 50% of the total fruit weight [36] and the total phenolics content in them are reported to be 15% higher than those in peeled fruits [37]. With the use of newer techniques for extraction like USAE, by-products of citrus industry can prove to be major sources of phenolic compounds and can prove to be more profitable ventures compared to the traditional juice or pickling industries. The extraction efficiency of USAE is influenced by a greater effect of temperature followed by time on the extraction of polyphenols, flavonoid contents, vitamin C and on the retention of antioxidant activity. The overall optimal condition for maximum extraction of TPC, TFC, vitamin C and AOA was predicted to be at the combined levels of 48 °C extraction temperature, 60 min extraction time and 80% duty cycle of the USAE process. In this and earlier studies ultrasound assisted extraction has been shown to be promising for the extraction of plant metabolites. USAE can thus be a low cost and green chemistry approach towards utilizing agricultural by-products. However, for popularity of the technique in industries, scale up issues like availability of energy efficient ultrasound processors, suitable probes, non-reactive and anti-corrode materials of construction must be addressed. USAE can thus act as a low cost and green chemistry approach towards utilizing agricultural by-products.

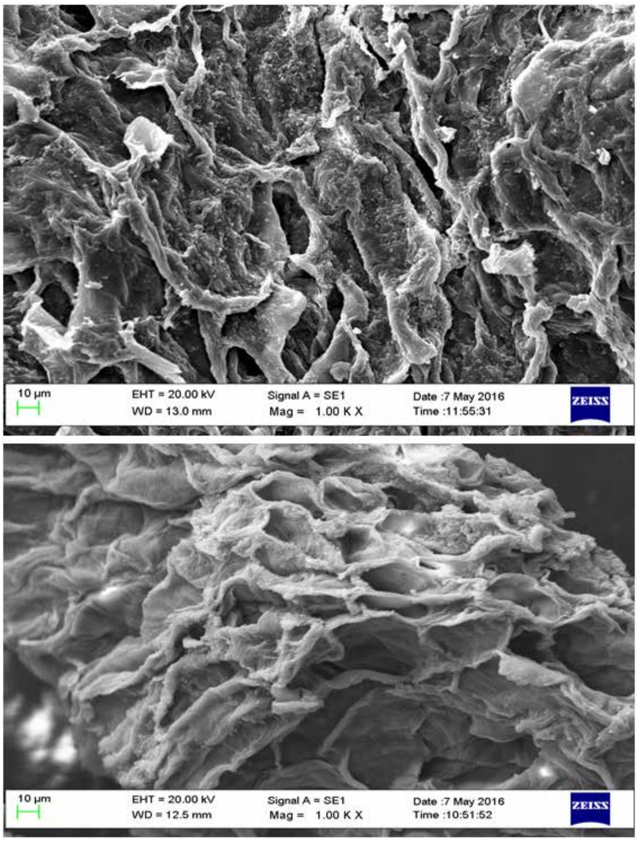

Figure 7: SEM of lemon peel subjected to ultrasonics assisted extraction at 60 °C extraction temperature, 60% duty cycle and 50 min extraction time **(A)** and at 50 °C extraction temperature, 80% duty cycle and 40 min extraction time **(B)**.

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References

1. González-Molina E, Domínguez-Perles R, Moreno DA, García-Viguera C. 2010. Natural bioactive compounds of *Citrus limon* for food and health. *J Pharm Biomed Anal* 51(2): 327-345. https://doi.org/10.1016/j.jpba.2009.07.027
2. Benavente-García O, Castillo J. 2008. Update on uses and properties of citrus flavonoids: New findings in anticancer, cardiovascular and anti-inflammatory activity. *J Agric Food Chem* 56(15): 6185-6205. https://doi.org/10.1021/jf8006568
3. Sharma K, Mahato N, Cho MH, Lee YR. 2017. Converting citrus wastes into value added products: Economic and environmentally friendly approaches. *Nutrition* 34: 29-46. https://doi.org/10.1016/j.nut.2016.09.006
4. Mandalari G, Bennett RN, Bisignano G, Saija A, Dugo G, et al. 2006. Characterization of flavonoids and pectin from bergamot (*Citrus bergamia* Risso) peel, a major byproduct of essential oil extraction. *J Agric Food Chem* 54(1): 197-203. https://doi.org/10.1021/jf051847n
5. Mandalari G, Bennett RN, Bisignano G, Trombetta D, Saija A, et al. 2007. Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. *J Appl Microbiol* 103(6): 2056-2064. https://doi.org/10.1111/j.1365-2672.2007.03456.x
6. Al-Juhaimi FY, Ghafoor K. 2013. Bioactive compounds, antioxidant and physicochemical properties of juice from lemon, mandarin and orange fruits cultivated in Saudi Arabia. *Pak J Bot* 45(4): 1193-1196.
7. Astell KJ, Mathai ML, Su XQ. 2013. A review on botanical species and chemical compounds with appetite suppressing properties for body weight control. *Plant Foods Hum Nutr* 68(3): 213-221. https://doi.org/10.1007/s11130-013-0361-1
8. Haslam E. 1986. Secondary metabolism-fact and fiction. *Nat Prod Rep* 3: 217-249. https://doi.org/10.1039/NP9860300217
9. Shirsath SR, Sonawane SH, Gogate PR. 2012. Intensification of extraction of natural products using ultrasonic irradiations - a review of current status. *Chemical Engineering and Processing: Process Intensification* 53: 10-23. https://doi.org/10.1016/j.cep.2012.01.003
10. Gómez-Mejía E, Rosales-Conrado N, León-González ME, Madrid Y. 2019. Citrus peels waste as a source of value-added compounds: extraction and quantification of bioactive polyphenols. *Food Chem* 295: 289-299. https://doi.org/10.1016/j.foodchem.2019.05.136
11. Dahmoune F, Boulekbache L, Moussi K, Aoun O, Spigno G, et al. 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. https://doi.org/10.1016/j.indcrop.2013.07.013
12. Ledesma-Escobar CA, Priego-Capote F, Luque de Castro MD. 2015. Comparative study of the effect of auxillary energies on the extraction of citrus fruit components. *Talanta* 144: 522-528. https://doi.org/10.1016/j.talanta.2015.07.011
13. Khoddami A, Wilkes MA, Roberts TH. 2013. Techniques for analysis of plant phenolic compounds. *Molecules* 18(2): 2328-2375. https://doi.org/10.3390/molecules18022328
14. Papoutsis K, Pristijono P, Golding JB, Stathopoulos CE, Bowyer MC, et al. 2018. Optimizing a sustainable ultrasound-assisted extraction method for the recovery of polyphenols from lemon by-products: comparison with hot water and organic solvent extractions. *European Food Research and Technology* 244(8): 1353-1365. https://doi.org/10.1007/s00217-018-3049-9
15. Ma YQ, Ye XQ, Fang ZX, Chen JC, Xu GH, et al. 2008. Phenolic compounds and antioxidant activity of extracts from ultrasonic treatment of Satsuma mandarin (*Citrus unshiu Marc.*) peels. *J Agric Food Chem* 56(14): 5682-5690. https://doi.org/10.1021/jf072474o
16. Ranganna S. 1986. Handbook of analysis and quality control for fruit and vegetable products, 2nd edition. Tata McGraw-Hill Publishing Company Limited, New Delhi, India.
17. Jagannath A, Raju PS, Bawa AS. 2012. Controlled lactic fermentative stabilization of ascorbic acid in amaranthus paste. *LWT - Food Science and Technology* 48(2): 297-301. https://doi.org/10.1016/j.lwt.2012.04.003
18. Moledina KH, Flink JM. 1982. Determination of ascorbic acid in plant food products by high performance liquid chromatography. *LWT - Food Science and Technology* 15: 351-358.
19. Singleton VL, Rossi J. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 16: 144-158.
20. Zhishen J, Mengcheng T, Jianming W. 1999. The determination

of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* 64(4): 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2

21. Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology* 28(1): 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5
22. Ou B, Hampsch-Woodill M, Prior RL. 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J Agric Food Chem* 49(10): 4619-4926. https://doi.org/10.1021/jf010586o
23. Myers RH, Montgomery DC, Anderson-Cook CM. 1995. Response surface methodology: process and product optimization using designed experiment. John Wiley & Sons Inc., NY, USA.
24. Wang W, Ma X, Xu Y, Cao Y, Jiang Z, et al. 2015. Ultrasound-assisted heating extraction of pectin from grapefruit peel: optimization and comparison with the conventional method. *Food Chem* 178: 106-114. https://doi.org/10.1016/j.foodchem.2015.01.080
25. Khan MK, Abert-Vian M, Fabiano-Tixier AS, Dangles O, Chemat F. 2010. Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel. *Food Chem* 119(2): 851-858. https://doi.org/10.1016/j.foodchem.2009.08.046
26. Meullemiestre A, Petitcolas E, Maache-Rezzoug Z, Chemat F, Rezzoug SA. 2016. Impact of ultrasound on solid-liquid extraction of phenolic compounds from maritime pine sawdust waste. Kinetics, optimization and large scale experiments. *Ultrason Sonochem* 28: 230-239. https://doi.org/10.1016/j.ultsonch.2015.07.022
27. Londoño-Londoño J, de Lima VR, Lara O, Gil AS, Pasa TBC, et al. 2010. Clean recovery of antioxidant flavonoids from citrus peel: optimizing an aqueous ultrasound-assisted extraction method. *Food Chem* 119(1): 81-87. https://doi.org/10.1016/j.foodchem.2009.05.075
28. Lopresto CG, Petrillo F, Casazza AA, Aliakbarian B, Perego P, et al. 2014. A non-conventional method to extract D-limonene from waste lemon peels and comparison with traditional soxhlet extraction. *Sep Purif Technol* 137: 13-20. https://doi.org/10.1016/j.seppur.2014.09.015
29. Anagnostopoulou MA, Kefalas P, Papageorgiou VP, Assimopoulou AN, Boskou D. 2006. Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*). *Food Chem* 94(1): 19-25. https://doi.org/10.1016/j.foodchem.2004.09.047
30. Dahmoune F, Remini H, Dairi S, Aoun O, Moussi K, et al. 2015. Ultrasound assisted extraction of phenolic compounds from *P. lenitscus L.* leaves: comparative study of artificial neural network (ANN) versus degree of experiment for prediction of phenolic compounds recovery. *Ind Crops Prod* 77: 251-261. https://doi.org/10.1016/j.indcrop.2015.08.062
31. Herrera MC, de Castro MD. 2005. Ultrasound-assisted extraction of phenolic compounds from strawberries prior to liquid chromatographic separation and photodiode array ultraviolet detection. *J Chromatogr A* 1100(1): 1-7. https://doi.org/10.1016/j.chroma.2005.09.021
32. Mason TJ, Paniwnyk L, Lorimer JP, 1996. The uses of ultrasound in food technology. *Ultrason Sonochem* 3(3): 253-260. https://doi.org/10.1016/S1350-4177(96)00034-X
33. Chemat F, Tomao V, Virot M. 2008. Ultrasound-assisted extraction in food analysis. In: Otles S (ed) Handbook of food analysis. instruments. ultrasound-assisted extraction in food analysis. CRC Press, Boca Raton, FL, USA, pp 85-94.
34. Castillo-Santos K, Ruiz-Lopez II, Rodriguez-Jimenes GC, Carrillo-Ahumada J, Garcia-Alvarado MA. 2017. Analysis of mass transfer equations during solid-liquid extraction and its application for vanilla extraction kinetics modelling *J Food Eng* 192: 36-44. https://doi.org/10.1016/j.jfoodeng.2016.07.020
35. Islam MS, Aryasomayajula A, Selvaganapathy PR. 2017. A Review on macroscale and microscale cell lysis methods. *Micromachines (Basel)* 8(3): 83. https://doi.org/10.3390/mi8030083
36. Bocco A, Cuvelier ME, Richard H, Berset C. 1998. Antioxidant activity and phenolic composition of citrus peel and seed extracts. *J Agri Food Chem* 46(6): 2123-2129. https://doi.org/10.1021/jf9709562
37. Gorinstein S, Martin-Belloso O, Park YS, Haruenkit R, Lojek A, et al. 2002. Comparison of some biochemical characteristics of different citrus fruits. *Food Chem* 74(3): 309-315. https://doi.org//10.1016/S0308-8146(01)00157-1