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Recovery of Bioactive Phenolic Compounds from Lemon (Citrus limon (L.) Burm. f.) and Orange (Citrus Sinensis L. Osbeck) Pomaces

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Abstract
Supercritical Fluid Extraction (SFE) from lemon (*Citrus limon (L.) Burm. f.*) and orange (*Citrus Sinensis L. Osbeck*) pomaces was applied to obtain extracts with high phenolic content and potent antioxidant activity. The effects of extraction conditions on SFE were analyzed, temperature (40 – 60°C), pressure (100 – 200 bar), co-solvent (25% (v/v), ethanol) for lemon pomace and pressure effect (100 – 200 bar) on SFE was investigated at 40°C in the presence of 25% (v/v) ethanol for orange pomace using the samples were 100 – 200 µm of average particle size. Moreover, in order to compare experimental data obtained by SFE, Soxhlet extractions were performed using methanol. Total phenolic contents, total flavonoid contents and antioxidant potential of extracts were assessed with Folin-Ciocalteu method, $AlCl_3/NaNO_2$ colorometric procedure and DPPH method, respectively. Experimental results showed that SC-CO_2 extraction allows obtaining phenolic extracts have antioxidant activity but it presents lower yield than soxhlet extraction. In SFE process, the highest extraction yield determined at the conditions of 100 bar, 50°C and 200 bar, 40°C for lemon and orange pomaces, respectively. Despite the lower extraction yields, SFE is considered as an alternative green technology which provides extensive advantages on the extraction of natural compounds.
Keywords: Lemon, Orange, Supercritical fluid extraction, Antioxidant activity, Phenolic content, Reuse of waste

1. Introduction

Nowadays, agri-food wastage has been considered a serious social, economic and environmental problem hence evaluation of wastes are becoming more and more important issue. According to the Food and Agriculture Organization (FAO) data, approximately one-third of all food for consumption in the world wasted, which equates to approximately 1.6 billion tons per year and approximately 40–50% of root crops, fruits and vegetables are lost in the weight basis (Viganó et al., 2015).

According to Turkish Statistical Institute 2013 data, Turkey is the 9th citrus manufacturer in the world with approximately 3.6 million tons of citrus production; oranges (1.78 million tons) and lemons (726 thousand tons) are among the most important ones, besides the annual production of citrus is approximately 131 million tons in the world (TÜİK., 2014). The large amount of production and consumption of citrus at industrial and domestic areas, especially in juice production, leads to considerable amount of by-products such as pomace is referred to as citrus waste, which represent half of citrus mass; is estimated to be worldwide 15 million tons (Ledesma- Escobar and Luque de Castro., 2014).

Orange and lemon pomaces have high commercial importance due to the presence of various valuable substances, not completely extracted during juice production, like the aromatic and the antioxidant components (Benelli et al., 2010). Among these compounds, polyphenols and more specifically to flavonoids has been gaining special attention due to their health benefits and significant world market is estimated approximately $200 million by Leatherhead Food Research, 2009, (Wijngaard et al., 2012). Therefore the valorization of these by-products are not only environmental but also promising for adding value to these materials that may be useful for many industries.

The demand for the replacement of synthetic antioxidants by natural ones has been increased due to the toxic effects of them and therefore limitations on use, high production costs and increased consumer concerns about the safety (Moure et al., 2001; Martinez., 2007; Balasundram et al., 2006). Antioxidants, added as an additive to oily products in order to elimination of bitterness caused by lipid oxidation, preventing or reducing the formation of toxic compounds, maintaining nutritional quality and extending the shelf life of the foods, especially in the food industry. Furthermore, antioxidants play a crucial role for protection of tissues or compounds from the destructive effects of free radicals. Therefore, they can reduce the risk of chronic diseases; such as cancer, cardiovascular diseases, diabetes, asthma and Alzheimer's disease (Abeysinghe et al., 2007). So it is now well accepted that, consumption of fruit and vegetables is important cause of their health promoting effects. Among the antioxidant compounds, polyphenols in fruits and vegetables include mainly phenolic acids (hydroxybenzoic and hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanones, isoflavones, flavanols, and anthocyanins) (Adil et al., 2007). They are synthesized as a defense mechanism against microorganisms and

strong ultraviolet (UV) radiation in plant-derived matrix and they have shown very strong antioxidant activity against radical reactions in vitro tests owing to their structures, hydrogen-donor potentials and metal chelation abilities. Therefore, they are considered as bioactive compounds exhibits antioxidant, anticancer, anti-inflammatory, antiviral and neurosedative activities (Martinez., 2007; Abeysinghe et al., 2007). Fruits and vegetables, seeds, grains and leaves are the most studied sources among the phenolic antioxidants. Additionally, recovery of phenolic antioxidants from by-products of food processing plants, agricultural and industrial wastes has been receiving special attention in recent years, because they are renewable, inexpensive and relatively easily obtained sources.

The traditional extraction methods which have been used for a long time, offer high recovery of natural antioxidant, bioactive compounds from these cheap and alternative sources, however they have several drawbacks, such as the use of large amounts of toxic solvents, long time consuming and low extraction selectivity. Moreover final product is obtained from the conventional methods contains solvent residue and also they aren't suitable for thermally labile and also, easily oxidized compounds due to the possibility of chemical degradation of target compounds (Herrero et al., 2006). Therefore; in order to overcome these disadvantages of solvent-based extraction techniques and better use of by-products and also improving the quality and safety of final products. Nowadays, there is a growing interest in development of novel and alternative technological extraction processes with minimized environmental impact (Knez et al., 2014). In this respect, supercritical fluid extraction (SFE) technique, is regarded as one of the sustainable greeen technologies with GRAS (Generally Recognized As Safe) solvents like supercritical CO_2 (SC-CO_2) has a privilege position, since it offers new attractive opportunities in food, nutraceutical, pharmaceutical, cosmetic, textile and other industry applications due to its unique properties (Salgın and Salgın, 2013; Knez et al., 2014; R.Vardanega et al., 2015; Bimakr et al., 2011). The unique characteristic of this system is application of liquid-like (solvent power, negligible surface tension) as well as gas-like transport properties (high diffusivity, low viscosity) which are easily manipulated by pressure and temperature of supercritical fluids to extract selective soluble components from a raw material (Wang and Weller, 2006). SC-CO_2 which is generally recognized as safe by FDA and EFSA, has been frequently used supercritical fluid to extract bioactive compounds because of its being cheap, nontoxic and inflammable characteristics and mild critical properties. Besides it is readily available, easy to remove from the final product, does not create major disruptions in biocompounds and its biological properties can be preserved (F.A.Espinosa-Pardo et al., 2014; Mendiola, José A., et al., 2007).

In this context, the present work aimed to evaluating the reuse of orange and lemon pomaces remained as waste after especially juice production and domestic consumption. For this purpose, supercritical fluid extraction of peculiar phenolic compounds from pomaces was carried out using green solvents, SC-CO_2 and as a co-solvent ethanol and the effects of main process parameters such as operating pressure and temperature, extraction time and co-solvent effect on extraction yield were investigated.

The pomace extracts were examined for their total phenolic content (TPC), total flavonoids (TF) and antioxidant activity by free radical scavenging activity. Finally, the results were compared with conventional soxhlet extraction.

2. Materials and methods

2.1 Materials

Carbon dioxide with a purity of 99.9% was supplied by Linde Company (Adana, Turkey). Ethanol and methanol (HPLC grade, ≥ 99.9%) was provided from Merck (Darmstadt, Germany). Folin-Ciocalteu's phenol reagent (2N), Sodium carbonate, Sodium nitrite, Sodium hydroxide and Aluminium chloride were analytical grade and obtained from Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97% (Trolox), gallic acid and quercetin were purchased from Sigma Aldrich Chemie.

2.2 Method

2.2.1 Raw material and pretreatment

Lemon and orange pomaces composed of peel, albedo and segment pulp residues were provided by Anadolu Etap Ltd. Mersin, Turkey. The raw materials were air-dried at ambient temperature away from direct sun-light in the shade for 48 – 72 h and than in oven at 40°C for 6 –7 h until they reached constant weight. After drying, the samples were ground using a laboratory type blender and sieved to obtain desired particle size range by means of a vibratory sieve shaker (Fritsch Analysette 3Spartan Model, Germany). Samples vacuum packed and stored at ambient temperature in the dark and dry place until the extractions were performed.

2.2.2 Soxhlet Extraction

Soxhlet extraction (SOX) was carried out using methanol as solvent to compare SC-CO_2 extraction with a traditional method. Briefly, 5 g sample powder (dp, >100 μm particle size of lemon and orange pomaces) was placed into a soxhlet apparatus and extracted 150 ml of methanol for 8 and 16 h at the boiling point of methanol

for lemon and orange samples respectively. Polyphenolic extracts were separated reduced pressure by evaporating methanol on a rotary evaporator (Büchi, Rotavapor R-II Model, Switzerland). All experiments were repeated three times for each particle size of sample and arithmetic average data of yields were used.

2.2.3 Supercritical Fluid Extraction

Supercritical fluid extraction process of pomaces using SC-CO_2 as solvent were carried out exactly the same extraction equipment was depicted in Yabalak and Gizir (2013). The extraction system consists of a programmable oven (Teknosem, TF R400), a control unit and two high pressure syringe pumps (Teledyne ISCO 260 D series) have 266 mL of internal volume also, can supply 0 – 517 bar pressure and provide 0.001 – 107 mL/min of adjustable flow rate when used separately.

In SFE experiments of lemon and orange pomaces, approximately 2 g of sample loaded into the stainless steel cylindrical extraction cell (250 x 4.6 mm). Cotton wool was packed at exit end of the cell to avoid the samples being carried over the cell and clogging the system. After the extraction cell was charged into the oven, selected operating pressure and temperature of the supercritical fluid were obtained by pump and oven. Afterwards, a static extraction time (210 min) was started in order to increase the dissolving the phenolic components in SC-CO_2.

Despite soxhlet extraction experiments carried out with >100 µm particle size of samples, 100 – 224 µm particle size of samples were used in SFE experiments because, the dp,>100 µm particle size of samples lead to channelling problem with increasing pressure in the extraction cell, hence solvent could not interact with material to be extracted and the process efficiency was effected, negatively.

The supercritical fluid extractions were conducted with samples were 100 – 224 µm particle size at different temperature and pressure values; 40, 50 and 60°C and 100, 150 and 200 bar respectively in the constant pressure mode. Moreover, effect of co-solvent, using 25% (v/v) ethanol was investigated on SC-CO_2 extraction.

Obtained extracts were collected in collection vials contained glass wool and the total extracted mass of polyphenolic compound was calculated gravimetrically after removing co-solvent contents in the oven. The yield was defined as the amount of extracted polyphenolic compound divided by the amount of dry raw material (g extract /g dry matter). The experiments were performed at least twice to ensure the accuracy of the experimental data and arithmetic averages of data were shown in all figures.

2.2.4 Determination of Total Phenolic Content

The Folin-Ciocalteu assay, adapted from Slinkard and Singleton (1977) was used for the determination of total phenolics present in pomace extracts. To 100 µL of extract, 7900 mL of distilled water and 500 µL Folin-Ciocalteu reagent were added and solution was mixed well. After 2 min 1.5 mL of 20% sodium carbonate was added. Tube contents were vortexed before being incubated for 2 h at room temperature in the dark. The absorbance of blue coloration formed was measured at 765 nm against the blank. Total phenolic contents were expressed as gallic acid equivalent (µg of gallic acid/g dry matter), using the standart curve prepared at different concentrations of gallic acid. All of the absorbance measurements were done at least in a dublicate.

2.2.5 Determination of Total Flavonoid Content

Total flavonoid contents of pomace extracts were measured according to a colorimetric assay described by Kim et al. (2003). To tube contained 4 mL of distilled water, properly diluted of 500 µL extract was added followed by 300 µL of 5% aqueous $NaNO_2$ and mixture was vortexed well. After 5 min, 300 µL of 10% aqueous $AlCl_3$ was added. 2 mL of 1 M NaOH was added 1 min after the addition of aluminium chloride and final tube volume was adjusted to 10 mL with distilled water. Solution was mixed and the absorbance was read at 510 nm against the blank. Total flavonoid contents were calculated with respect to quercetin standart curve and results are expressed as µg of quercetin/g dry matter. Each analysis were done at least two times.

2.2.6 Determination of Antioxidant Activity

The free radical scavenging activities of extracts were evaluated using 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenger methodology reported by Tuberoso et al. (2007) with modifications. The principle of this method is based on color change determined at 517 nm caused by reduction of DPPH radical in the precense of antioxidant compounds. Briefly, 50µL extract was added to freshly prepared 2 mL of DPPH solution in methanol (8 x 10^{-5} M) and wortexed. After incubation at room temperature in the dark for 30 min, the decrease of absorbance of DPPH was measured at 517 nm against the blank. The DPPH assay of extracts compared with synthetic antioxidant Trolox, the water-soluble vitamin E analouge by means of prepared Trolox calibration curve and data were expressed as Trolox equivalent antioxidant activity (µmol Trolox /g dry matter). Spectrophotometric readings were carried out at least dublicates.

3. Results and discussions

3.1. Preliminary study of phenolic compound extraction from lemon pomace

3.1.1 Determination of static extraction time

In this study, in order to determine optimum static extraction time for obtaining phenolic extracts, experiments were performed under the following operating conditions: 40°C, 100 bar for 210 min and 360 min. In the

literature, Lee et al. (2010) studied extraction of two polymethoxyflavones (PMFs), nobiletin and tangeretin from Citrus depressa Hayata peels by supercritical fluid extraction at 80 min and Benelli et al. (2010) carried out oil extraction from orange pomace via SFE at 300 min. Figure 1 illustrates that the amount of phenolic compounds extracted at the end of 210 and 360 min static extraction time, almost unchanged. Extraction time is closely related to type of material and the amount of desired compound in the structure. Thus, it was considered that 210 min static extraction time is enough for phenolic compounds to reach their saturation concentrations in SC-CO_2. So, influence of other operation parameters investigated in 210 min static extraction time conditions.

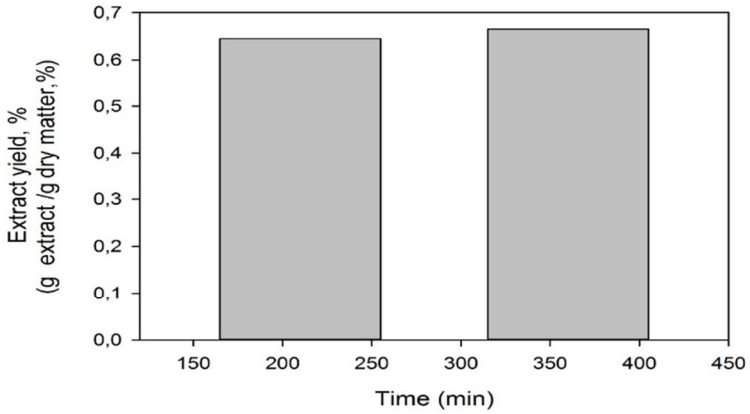

Figure 1. Effect of static extraction time on the extraction yield of lemon phenolic compounds (T = 40°C, P = 100 bar, 210 min extraction time, dp; 100 – 224 µm)

3.1.2 Supercritical fluid extraction with pure CO_2

The solvation power of SC-CO_2 based on solvent density which increases with pressure at constant temperature and decreases with temperature at constant pressure. As presented in Figure 2 and Table 1, extract yield (%) was confirmed with the above-mentioned behavior at 40, 50 and 60°C temperature and 100 bar constant pressure conditions. It can be observed again Figure 2, the highest extraction yield for SFE of lemon pomace was 0.645±0.00%, obtained at 40°C and 100 bar. The temperature increase at constant pressure, decreases extraction yield due to severe diminish in SC-CO_2 density and consequently in solvent power.

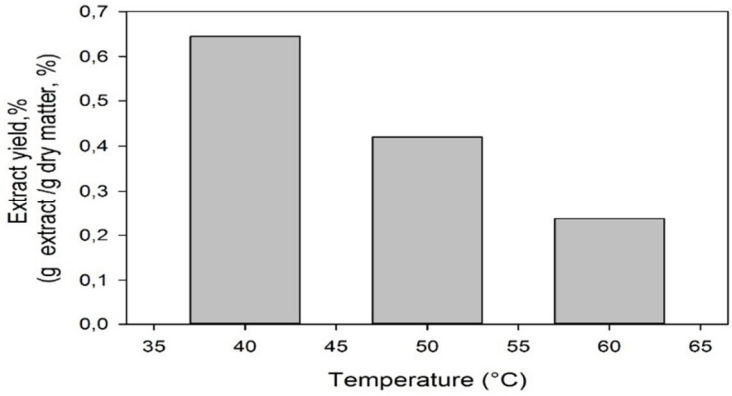

Figure 2. Effect of temperature on the extraction yield of lemon phenolic compounds using pure SC-CO_2 (P= 100 bar, 210 min extraction time, dp; 100 – 224 µm)

According to Table 1, SC-CO_2 density along with the massive amount of solvent was sent to the extraction cell reduced significantly by increasing temperature, resulted in an alteration in solubility of polar phenolic compounds in SC-CO_2. Despite the solute solubility data were near to each other, why the observed a sharp decrease in the extraction yield was considered that related to inconstancy of the massive amount of solvent (SC-CO_2). Similar temperature effect was observed in supercritical fluid extraction from dried banana peel by Rosso Comim et al. (2010).

Table 1. Effect of temperature on ρSC-CO_2 extract yield, the amount of solvent and solubility of phenolic compounds in SC-CO_2 for lemon pomace at 100 bar

Pressure (Bar)	Temperature (°C)	ρ_{SC-CO2} (kg/m^3)	Extract yield (%) (t = 210 min)	The amount of solvent (g sc-co_2)	Solubility (g extract /kg CO_2)
100	40	627	0.645 ± 0.000	2.414	5.344
	50	402	0.420 ± 0.021	1.548	5.426
	60	301	0.237 ± 0.018	1.159	4.098

3.1.3 Influence of extraction with SC-CO_2 plus co-solvent

The main limiting factor in separation of polar phenolic compounds is SC-CO_2 behaves a non-polar solvent and to cope with this disadvantage, some modifiers can be added in SC-CO_2. The effect of co-solvent (25% (v/v) EtOH) on the extraction yield was investigated at 40°C, 100 bar, 210 min operating conditions. In this study, EtOH which was preferred in 53% of the SFE works involving modifier, was selected as co-solvent because it is both more advantageous than n-hexane and methanol in terms of human health and environmental and considerably polar to provide increasing the polarity of supercritical CO_2 by addition of small amounts (De Melo et al., 2014). Furthermore, it is widely used to improve the extraction efficiency of relatively polar compounds such as phenolic acids and flavonoids.

Adil et al. (2007) indicated that certain polyphenolic compounds such as; hydroxycinnamic acids (p-coumaric acid, caffeic acid and ferrulic acid, and coumeric acid isomers (o-,m-,p-coumaric acids), are little soluble in supercritical CO_2 without addition of a co-solvent but in order to increase the solubility of quercetin, catechin, epicatechin and resveratrol in supercritical CO_2 5–30% ethanol must be added, in the study aimed the extraction of subcritical (carbon dioxide+ethanol) extraction of polyphenols from apple and peach pomaces.

Figure 3 shows that, the yield of phenolic compounds was significantly increased with presense of co-solvent ethanol. While, the maximum extraction yield was determined as 0.645±0.00% (w/w) in SFE experiments performed with only SC-CO_2 the highest extraction yield determined with ethanol as a co-solvent was reached 1.765± 0.290% at the same operation conditions. At 40°C and 100 bar the density of pure SC-CO_2 was 627 kg/m^3 but density of new mixtrure obtained adding ethanol 25% (v/v) was enhanced as 893kg/m^3. Additionally, the addition of co-solvent changes not only solubility but also transport properties, and intraparticle resistance in the matrix (Rawson et al., 2012). Therefore, enlargement of density of extraction solvent, increasing solvent power and decreasing intraparticle resistances caused improving yields of polar compounds. In fact, increased co-solvent concentrations may improve the extraction yields, but the temperature necessary to reach the supercritical state is higher and could be not suitable for natural products sensitive to heat (M'hiri et al., 2014). In this study, critical temperature and pressure of mixture (CO_2+ethanol) are T_c = 97.94°C and P_c = 69.29 bar, respectively. When these critical points were compared with the critical parameters of pure supercritical carbon dioxide (T_c = 31.2°C and P_c = 72.8 bar), it was seen that this study were accomplished in near subcritical region.

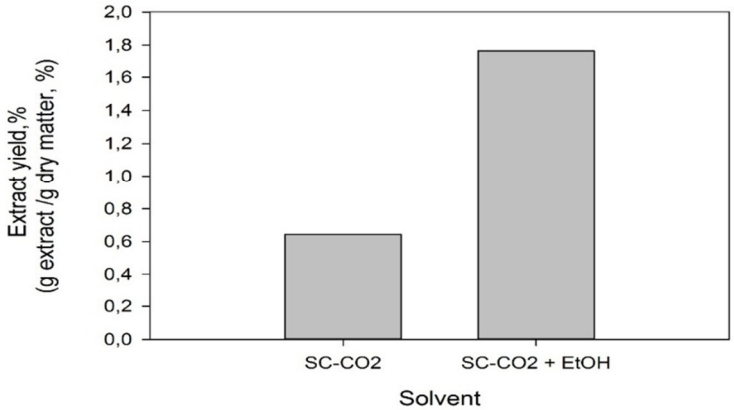

Figure 3. Effect of co-solvent on extraction yield of lemon phenolic compounds (T = 40°C, P = 100 bar, 210 min dp; 100 – 224 μm)

3.1.4 Effect of temperature

In the literature, optimal temperature ranges between 58.6°C and 80°C for a pressure varying from 9.5 to 40 MPa for supercritical fluid extraction from citrus peel phenolics (M'hiri et al., 2014) Giannuzzo et al. (2003) determined the optimal temperature as 58.6°C for 45 min at 9.5 MPa for supercritical fluid extraction of naringin from grapefruit peel and Toledo-Guillen et al. (2010) reported that the optimum process conditions for flavonoid extraction from orange peel via SFE, were a temperature of 60°C at 40 MPa.

In this study, the temperature effect on supercritical fluid extraction from lemon phenolic compounds was studied at three temperature levels of 40, 50 and 60°C at pressure from 100 to 200 bar, using SC-CO_2 + 25 % (v/v) ethanol at 210 min static extraction time; as shown in Figure 4 (a – c) and all results were presented in Table 2.

The density of SC-CO_2 at constant pressure is reduced with increasing temperature and leads to reduce the solvent power of supercritical CO_2 and this phenomenon has a negative effect on extraction yield. However, the increase in operation temperature will also accelerate mass transfer and improve the extraction yield as a result of decreasing viscosity and increasing mass transfer coefficient which are the resistance parameters of the extraction. Thus, supercritical CO_2 shows better penetration behavior in solid matrix and its interaction with target substances enhances by decreasing internal and external mass-transfer resistances. For this reason, the extraction yield closely depends on negative and positive effects which one of the dominant on extraction process (Döker et al., 2004; Wang et al., 2008).

Figure 4(a) shows that, at low operation pressure (100 bar), firstly; extraction yield was increased, then showed a slightly decreasing tendency by increasing temperature. The highest yield obtained from SFE was performed at 100 bar and 50°C (3.460 ± 1.014%, w/w). The lowest SFE yield was 1.765 ± 0.290%, (w/w) at 100 bar and 40°C. Acccording to Table 2, despite the increasing temperature produced a decrease density and the massive amount of solvent, the solute solubility showed the same trend with extraction yield. Therefore, from 40 to 50°C, positive effect occured by decreasing mass-transfer resistances is dominant. Otherwise, from 50 to 60°C, decline in density of supercritical fluid was higher and diminished the positive effect of decreasing viscosity and increasing mass transfer coefficient.

At medium operation pressure (150 bar), the temperature effect was shown in Figure 4(b) and it was observed that, the increase in extracting temperature did not provided a significant effect on product yield. The highest yield determined at 150 bar, 50°C as 2.820 ± 0.127%, (w/w). This behavior could be explained by due to the changes in solvent density and mass-transfer resistances were balanced each other.

Figure 4(c) showed that, at high pressure condition (200 bar) from 40 to 60°C, extraction yield was decreased due to the solvent density which was more dominant than mass-transfer resistances. The highest extraction yield reached at 200 bar and 40°C operation conditions (1.965±0.219%, w/w). Moreover, according to Table 2, despite the solubility of phenolic compounds did not changed by increasing temperature, extraction yield reduced because of the decreasing of massive amount of solvent.

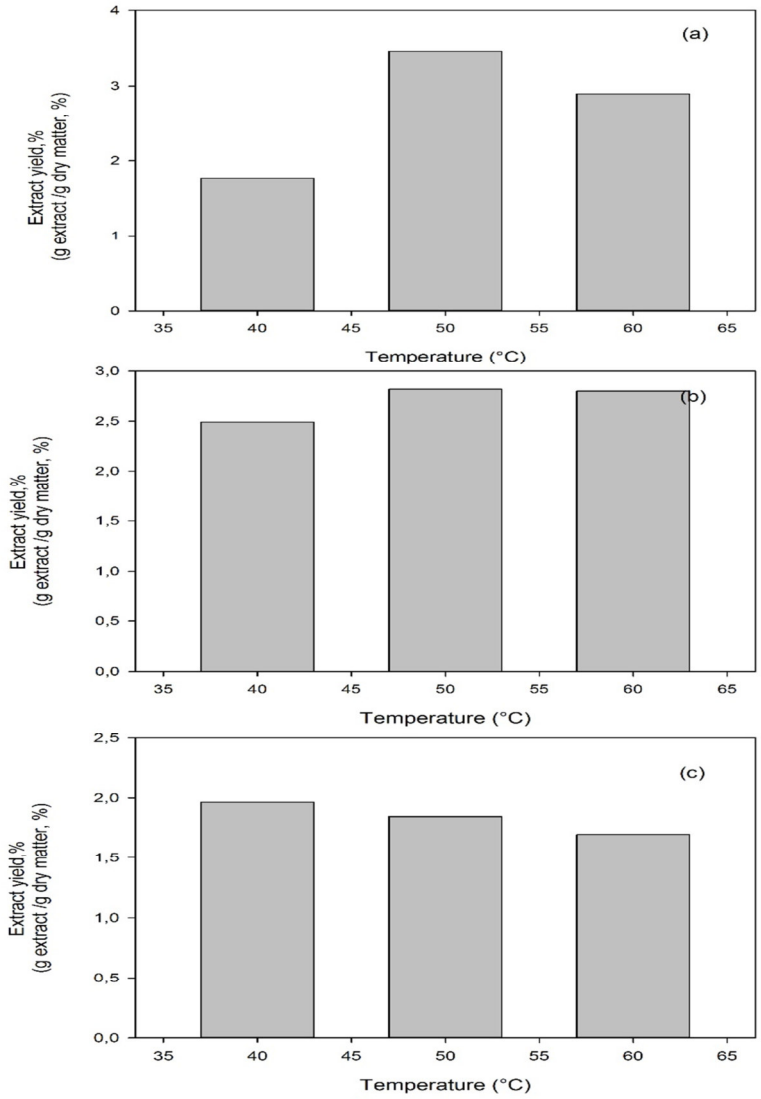

Figure 4. Effect of extraction temperature on extraction yield of lemon phenolic compounds at 210 min static extraction time and 100 – 224 µm mean particle size, 25% (v/v) ethanol co-solvent
(a)100 bar; (b) 150 bar; (c) 200 bar

Table 2. Effect of temperature on ρSC-CO_2 extract yield, the amount of solvent and solubility of phenolic compounds in SCF for lemon pomace at different operation pressures

Pressure (Bar)	Temperature (°C)	ρ_{SCF} (kg/m^3)	Extract yield (%) t= 210 min	The amount of solvent (g SCF)	Solubility (g extract /kg SCF)
100	40	893	1.765 ± 0.290	3.438	10.267
	50	864	3.460 ± 1.014	3.326	20.803
	60	836	2.898 ± 1.043	3.219	18.005
150	40	792	2.491 ± 1.278	3.049	17.628
	50	711	2.820 ± 0.127	2.737	20.604
	60	613	2.800 ± 0.650	2.360	23.728
200	40	849	1.965 ± 0.219	3.269	12.023
	50	793	1.845 ± 0.085	3.053	12.086
	60	733	1.694 ± 0.000	2.822	12.013

3.1.5 Effect of presssure

Generally there is a consensus idea that increasing operating pressure has a positive effect on the extraction yield as an increase in pressure at constant temperature, provides the density of SC-CO_2 increase, which enhances its solvent power and, ultimately, the extraction rate or yield. Nevertheless, the economic feasibility of working at

high pressure has to be assessed on a case-by-case, since any increase in pressure is associated with an increase in energy consumption (K.S. Duba and L. Fior, 2015).

In this work, the influence of the pressure on extraction process was investigated at 100, 150 and 200 bar values at temperature between 40 and 60°C, using SC-CO_2 + 25% (v/v) ethanol at 210 min static extraction time; as shown in Figure 5 (a – c) and all results were presented in Table 3.

An increasing of pressure can result in an increase in solvent density which modifies solute solubility. Concurrently, the viscosity of supercritical fluid increases and the mass transfer coefficient decreases by the way of increasing pressure. For this reason, internal interactions between matrix and supercritical fluid decrease and penetration of supercritical fluid to the pores of matrix is getting difficult and internal and external mass-transfer resistances enhance. As a result, increasing of solute solubility shows a positive effect on extraction process whether, increasing of mass-transfer resistances show a negative effect (Döker et al., 2004).

The results from our study show that the best operation pressure 100 bar at 50°C reached the maximum extraction yield as 3.460 ±1.014% (w/w). As shown in Figure 5(a) at 40°C operating temperature, an increase pressure from 100 to 150 bar resulted the increase on extraction yield approximately by 2-fold, owing to enhancing of density and solvent power of CO_2 + ethanol mixture, but when the operating pressure was increased from 150 to 200 bar, a reduction on extraction yield was observed due to the negative effect caused by increasing of viscosity and decreasing of diffusion on mass transfer.

At 50°C operating temperature as presented Figure 5(b), an increase in the pressure from 100 to 200 bar, had a negative effect on extract yield, despite increment the solubility of phenolic compounds in supercritical fluid, the above mentioned negative effects overcome the positive effect that related to solvent density. Similar behavior on extraction yield was determined at high extracting temperature 60°C, illustrated in Figure 5(c) by rising the pressure, firstly there was not detected a significant change in yield but continue to increase pressure caused an important reduction in extraction yield later.

Similar trend was observed by Lee et al. (2010). The researchers studied the effect of pressure on extraction yield of phenolic compounds from Citrus depressa Hayata and reported that at 80°C, the amount of nobiletin and tangeretin extracted increased with increasing pressure from 20 to 30 MPa. However, the extraction yield decreased significantly as the pressure increased from 30 to 40 MPa, respectively.

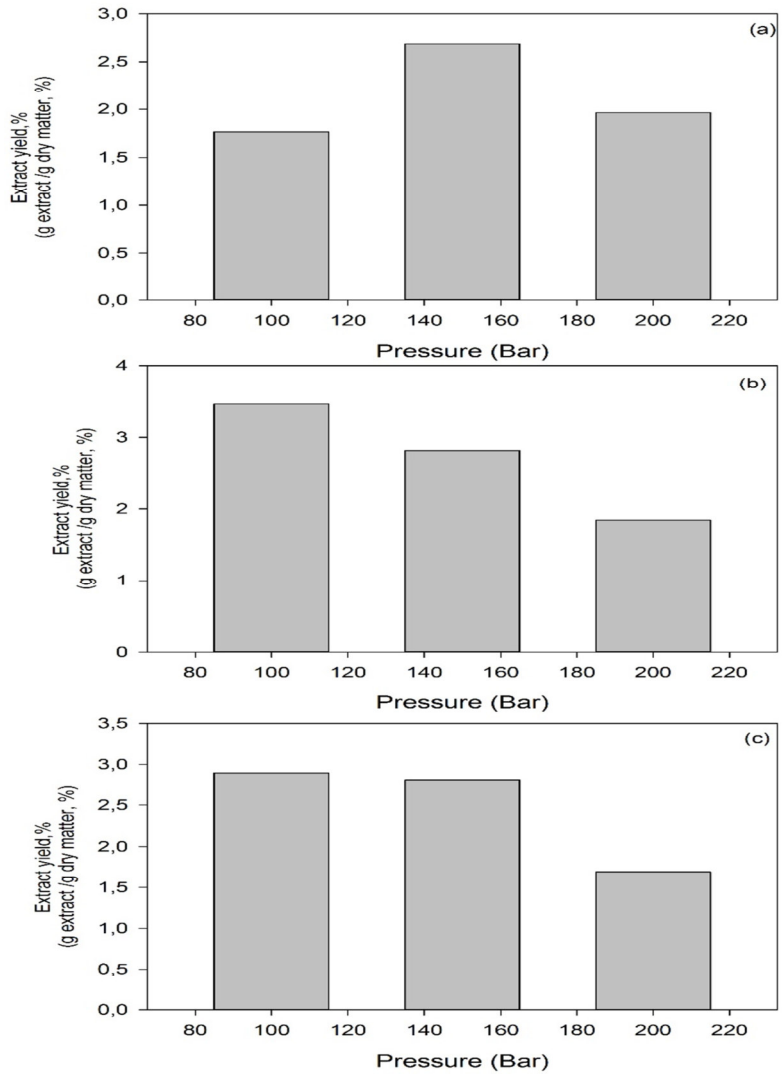

Figure 5. Effect of extraction pressure on extraction yield of lemon phenolic compounds at 210 min static extraction time and 100 – 224 µm mean particle size, 25% (v/v) ethanol co-solvent (a) 40°C; (b) 50°C; (c) 60°C

Table 3. Effect of pressure on ρSC-CO_2 extract yield, the amount of solvent and solubility of phenolic compounds in SCF for lemon pomace at different operation temperatures

Temperature (°C)	Pressure (Bar)	ρ_{SCF} (kg/m^3)	Extract yield (%) t= 210 min	The amount of solvent (g SCF)	Solubility (g extract / kg solvent)
40	100	893	1.765 ± 0.290	3.438	10.267
	150	901	2.687 ± 1.743	3.469	15.495
	200	909	1.965 ± 0.219	3.500	11.230
50	100	864	3.460 ± 1.014	3.326	20.803
	150	876	2.819 ± 0.127	3.373	16.723
	200	884	1.845 ± 0.085	3.403	10.842
60	100	836	2.898 ± 1.043	3.219	18.005
	150	853	2.800 ± 0.650	3.284	17.052
	200	859	1.694 ± 0.00	3.307	10.251

3.2 Supercritical fluid extraction from orange pomace

Supercritical fluid extraction from orange phenolic compounds was carried out at 40°C at three pressure levels of 100, 150 and 200 bar, precense of co-solvent ethanol 25% (v/v). Acccording to the obtained results were presented in Figure 6 and Table 4; the extraction yield increased with pressure from 100 to 200 bar, which was due to increase of supercritical fluid density which was more dominant than the increasing mass transfer resistances at higher pressure. The highest yield was obtained at 40°C and 200 bar as 2.784 ± 1.173% (w/w). Acccording to the obtained results were presented in Figure 6 and Table 4; the extraction yield increased with

pressure from 100 to 200 bar, which was due to increase of supercritical fluid density which was more dominant than the increasing mass transfer resistances at higher pressure. The highest yield was obtained at 40°C and 200 bar as 2.784 ± 1.173% (w/w).

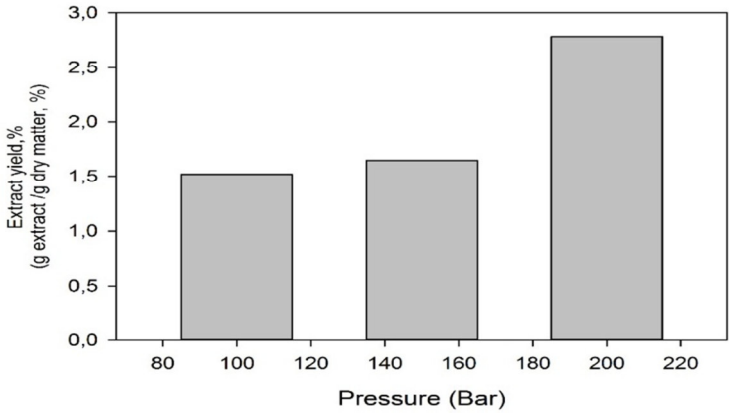

Figure 6. Effect of extraction pressure on extraction yield of orange phenolic compounds at 210 min static extraction time and 100 – 224 µm mean particle size, 25% (v/v) ethanol co-solvent

Table 4. Effect of pressure on ρSC-CO_2, extract yield, the amount of solvent and solubility of phenolic compounds in SC-CO_2 for orange pomace at 40°C

Temperature (°C)	Pressure (Bar)	ρ_{SCF} (kg/m^3)	Extract yield (%) t= 210 min	The amount of solvent (g SCF)	Solubility (g extract / kg solvent)
40	100	893	1.520 ± 0.085	3.438	8.842
40	150	901	1.647 ± 0.081	3.469	9.499
40	200	909	2.784 ± 1.173	3.500	15.916

3.3 Total phenolic and flavonoid contents of extracts

The total phenolic contents (TPC) of the lemon and orange pomace extracts in presented in Table 5 and 6. As seen from Table 5 and 6, the amount of total phenolics ranged from 778.75 – 1164.71 µg GAE/g dry matter and 1268.69 – 1839.54 µg GAE/g dry matter for lemon and orange samples respectively, in SFE experiments.

The best TPC results for SFE were obtained at 40°C-150 bar added with 25% (v/v) of co-solvent ethanol for lemon pomace samples. The effect of temperature and pressure on total phenolic content of lemon pomace extracts obtained from SFE was presented in Figure 7. According to Figure 7, it was seen that, operation pressure and temperature were significant factors effect the amount of phenolic content and selective separation of phenolic compounds can be possible by changing the operation conditions since the solvent strength of SC-CO_2 is a function of density.

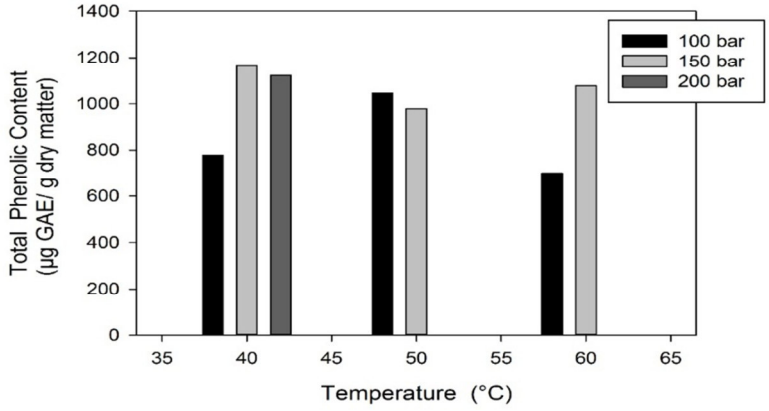

Figure 7. Effect of temperature and pressure on total phenolic content of lemon pomace extracts obtained from SFE

The highest phenolic content determined at 40°C-200 bar presense of co-solvent for orange pomace in SFE experiments. The effect of pressure on total phenolic content of orange pomace extracts obtained from SFE at 40°C temperature was presented in Figure 8. According to Figure 8, an increase was observed in the amount

of phenolic content varying the pressure condition from 100 bar to 200 bar, as a result of enhancing the solute solubility.

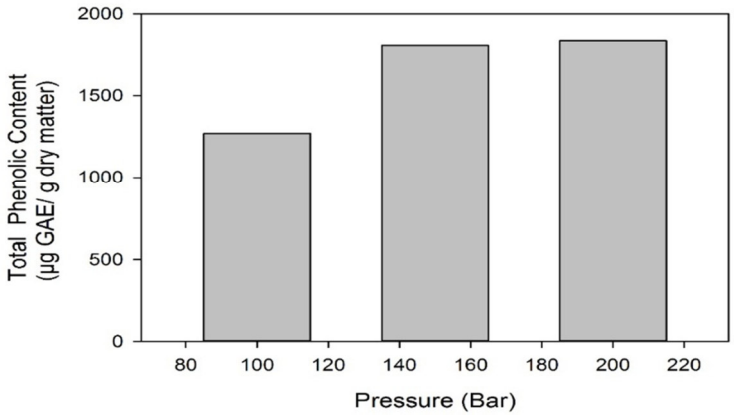

Figure 8. Effect of pressure on total phenolic content of orange pomace extracts obtained from SFE at 40°C temperature

The total flavonoid contents of the lemon and orange pomace extracts in presented in Table 5 and 6, respectively. In SFE experiments the amount of total flavonoid compounds ranged from 3.31 – 3.93 mg quercetin/g dry matter, and 3.49 – 4.53 mg quercetin/g dry matter, for lemon and orange samples respectively.

The effect of temperature and pressure on total flavonoid content of lemon pomace extracts obtained from SFE was given in Figure 9. According to Figure 9, there was no a significant change in the amount of flavonoid content of lemon samples by the way of increasing temperature and pressure. The highest flavonoid content obtained at 60°C-150 bar for lemon samples.

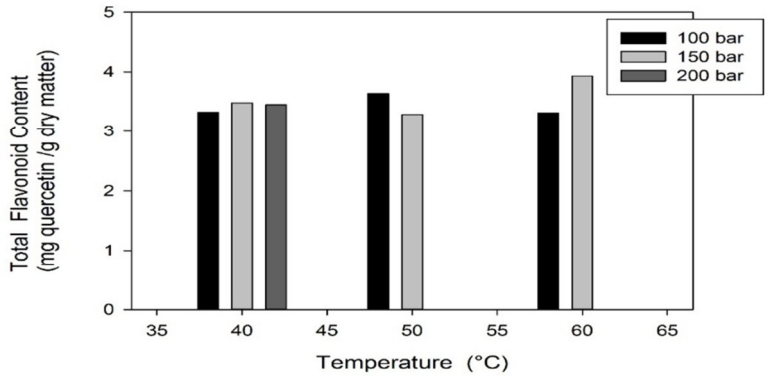

Figure 9. Effect of temperature and pressure on total flavonoid content of lemon pomace extracts obtained from SFE

The effect of pressure on total flavonoid content of orange pomace extracts obtained from SFE at 40°C temperature. The highest amount of total flavonoid content determined as 4.53 mg quercetin/g dry matter at 40°C-100 bar operating conditions for orange pomace samples. Figure 10 shows that, an increase in pressure caused a decrease in flavonoid content of orange samples.

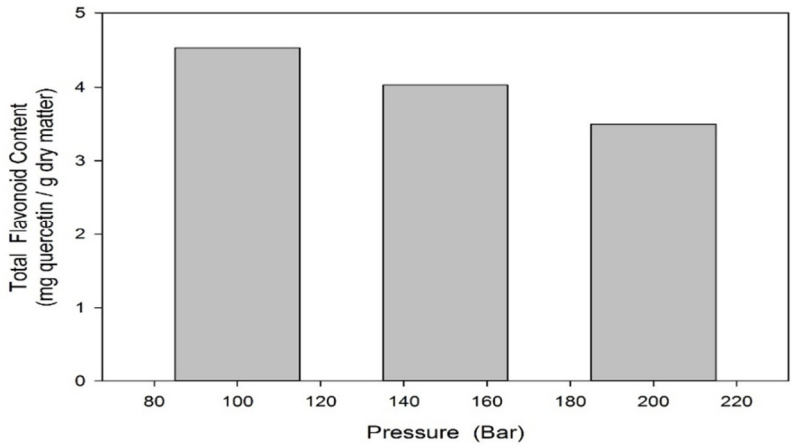

Figure 10. Effect of pressure on total flavonoid content of orange pomace extracts obtained from SFE at 40°C temperature

Table 5. Extraction yields, total phenolic, flavonoid contents and antioxidant activities of lemon pomace extracts obtained from SFE operating conditions and Soxhlet method

Operating conditions	Extraction yield%, (g extract/g dm)	Total Phenolic Content (µg GAE/g dm)	Total Flavonoid Content (mg quercetin /g dm)	Antioxidant Activity (µmol trolox/g dm)
40°C, 100 bar	1.765 ± 0.290	778.75	3.32	2.407
50°C, 100 bar	3.460 ± 1.014	1046.25	3.64	2.478
60°C, 100 bar	2.898 ± 1.043	699.79	3.31	2.003
40°C, 150 bar	2.491 ± 1.278	1164.71	3.48	2.415
50°C, 150 bar	2.820 ± 0.127	978.65	3.28	2.189
60°C, 150 bar	2.800 ± 0.650	1078.43	3.93	2.216
40°C, 200 bar	1.965 ± 0.219	1122.33	3.45	2.398
50°C, 200 bar	1.845 ± 0.085	-	-	-
60°C, 200 bar	1.694 ± 0.000	-	-	-
Soxhlet	36.715 ± 0.284	8790.00	28.46	23.80

Table 6. Extraction yields, total phenolic, flavonoid contents and antioxidant activities of orange pomace extracts obtained from SFE operating conditions and Soxhlet method

Operating conditions	Extraction yield%, (g extract/g dm)	Total Phenolic Content (µg GAE/g dm)	Total Flavonoid Content (mg quercetin /g dm)	Antioxidant Activity (µmol trolox/g dm)
40°C, 100 bar	1.520 ± 0.085	1268.69	4.53	3.067
40°C, 150 bar	1.647 ± 0.081	1811.25	4.03	3.369
40°C, 200 bar	2.784 ± 1.173	1839.54	3.49	3.239
Soxhlet	37.63 ± 1.07	9599.42	19.67	25.74

3.4 Antioxidant Activity

Table 5 and 6, shows the antioxidant activity results according to analyses of DPPH, achieved by samples of lemon and orange extracts obtained from SFE. According to results, the trolox equivalent antioxidant activity ranged from 2.003 – 2.415 µmol trolox /g dry matter and 3.067 – 3.369 µmol trolox /g dry matter, for lemon and orange samples respectively, in SFE experiments.

The effect of temperature and pressure on antioxidant activity of lemon pomace extracts obtained from SFE was shown in Figure 11. According to Figure 11, the best conditions were determined as 50°C-100 bar operating conditions for obtaining the extracts that exhibit highest antioxidant activity in terms of free radical scavenging property for lemon pomace samples.

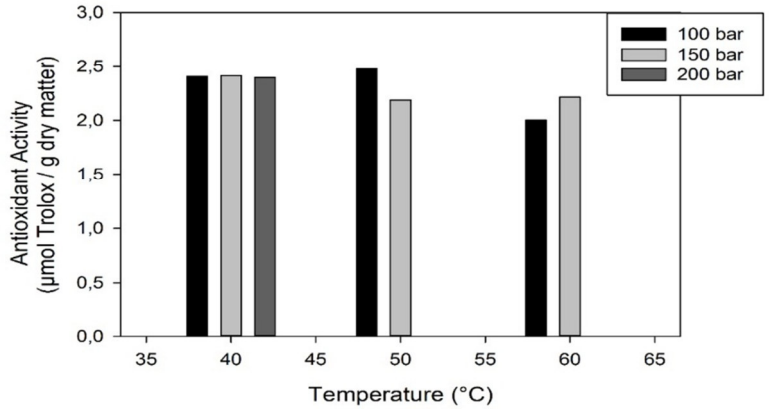

Figure 11. Effect of temperature and pressure on antioxidant activity of lemon pomace extracts obtained from SFE

The pressure effect on antioxidant activity of orange pomace extracts obtained from SFE at 40°C temperature was presented in Figure 12. As seen from Figure 12, the best antioxidant activity values for orange pomace was detected as 40°C-150 bar conditions as well. However, the obtained results showed that, the extracts obtained at all operation pressure and temperature parameters showed quite close activity values. It is considered that, these conclusions are related to selectivity of the SFE process.

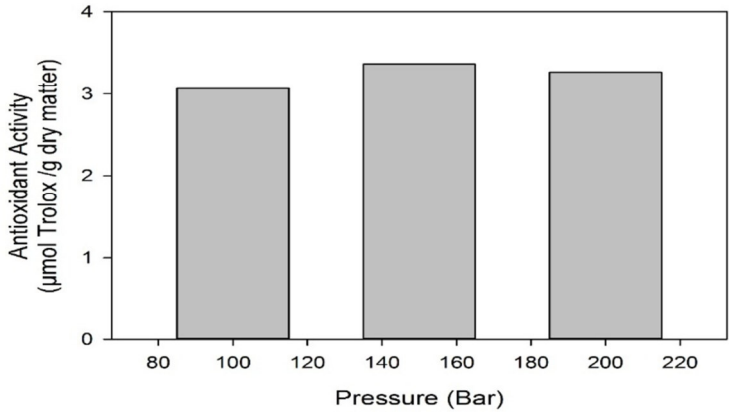

Figure 12. Effect of pressure on antioxidant activity of orange pomace extracts obtained from SFE at 40°C temperature

3.5 Conventional soxhlet extraction method

The conventional soxhlet extraction was conducted in parallel for comparison in order to evaluate the supercritical fluid extraction method in terms of extraction efficiency and chemical characterization of extracts. For this purpose, the following variables were compared: extraction yield, total phenolic content (TPC), total flavonoid content (TF) and antioxidant activity (AA).

The literature showed that, polar solvents as methanol and appropriate mixtures of these solvents with water are suitable to extract citrus polyphenols and reducing polyphenol oxidase (PPO) activity. Moreover, it was reported that methanol and DMSO are generally preferred solvents to extract phenolic acids (González-Molina vd., 2010). For this reason methanol is selected as a solvent in soxhlet experiments to extract phenolic compouds from lemon and orange pomaces due its high polarity.

Regarding soxhlet extractions fulfilled with methanol, the average extraction yield was determined as 36.715±0.284% (w/w) for lemon pomace samples after 8 h extraction time and polyphenolic extract yield found as 37.63±1.07% (w/w) for orange pomace samples at the end of 16 h extraction time.

Comparable extraction yields were detected from orange pomace by Benelli et al. (2010) during the soxhlet experiments carried out for 360 min using water and ethanol as the solvents and 39.7± 0.6% (w/w) and 37±1% (w/w) extract yields were reached for water and ethanol respectively. The researchers indicated that, this behaviour may be related to the high temperature, solvent recirculation and solute–solvent interactions found in Soxhlet extraction method.

According to results; total phenolic contents, flavonoid contents and antioxidant activities of extracts obtained from lemon and orange pomaces via soxhlet extraction method was quite better yield than SFE because

of methanol was more effective than ethanol modified SC-CO_2 for separation polar compounds. However, methanol is a toxic solvent and less consumed in food industry.

4. Conclusions

The known fact that, annually billions of tons of non-edible residues and by-products which can cause pollution, management, and economic problems worldwide are produced by food industries. Global concerns about the environmental damage is the main reason for development new strategies for utilization, agricultural, and industrial residues as source of new products. Sub- and supercritical fluid extraction can meet requirements of "Green Chemistry" concept since, it is environmentally friendly technique and generally green solvents are used in the process. Additionally, these residues are renewable and aboundant sources of bioactive compounds. In the light of these, the extraction of polyphenols from lemon and orange pomaces are discarded as waste in juice industry is managed a green approach using SC-CO_2 and ethanol in this study. Literature data about obtaining phenolic extracts from orange by-products using SFE are quite limited and, also the possibility of separation lemon phenolic compounds via SFE and main process parameters effect the extraction efficiency were investigated for the first time for lemon pomace. Therefore, it is thought that, the outputs of this study make some important contributions to this field.

According the experimental results, the experiments were carried out only SC-CO_2 was used as a solvent, quite low yields were obtained in the preliminary studies of lemon pomace. Because, CO_2 is a non-polar fluid and it is not an effective solvent to separation polar phenolic compounds. That's why ethanol is a food grade solvent was used as a modifier to SC-CO_2 following SFE experiments in order to increase the polarity of extraction solvent. Thereby, better extraction yields were obtained and the best operation conditions were determined as 100 bar - 50°C, co-solvent ethanol 25% (v/v) for lemon pomace and 200 bar - 40°C, co-solvent ethanol 25% (v/v) for orange pomace. In SFE experiments, extraction yields (g extract/g dry matter, %) were determined as 1.694±0.000% – 3.460±1.014% and 1.520±0.085% – 2.784±1.17% after 210 min extraction time for lemon and orange pomaces, respectively. Moreover, the higher extraction yields were obtained than SFE by conventional soxhlet extraction method and extraction yields were obtained as 36.715±0.284% g extract/g dry matter and 37.63±1.07% g extract/g dry matter for lemon and orange pomaces after 8 and 16 h extraction time, respectively.

Total phenolic contents, flavonoid contents and antioxidant activities of extracts obtained from lemon pomaces by SFE and Soxhlet extraction processes were determined as, 778.75 – 1164.71 and 879 µg GAE/g dry matter, 3.31 – 3.93 and 28.46 mg quercetin/g dry matter, 2.003 – 2.415 and 23.80 µmol trolox /g dry matter, respectively.

Total phenolic contents, flavonoid contents and antioxidant activities of extracts obtained from orange pomaces by SFE and Soxhlet extraction processes were assessed as, 1268.69 – 1839.54 and 9599.42 µg GAE/g dry matter, 3.49 – 4.53 and 19.67 mg quercetin/g dry matter, 3.067 – 3.369 and 25.74 µmol trolox /g dry matter, respectively.

Consequently, despite good results obtained with the conventional soxhlet extraction, supercritical fluid extraction was tested to search extraction phenolic compounds from lemon and orange pomaces. It was concluded that, SFE can be feasible method for recovering valuable non-polar compounds from food processing residues, but; extraction polar compounds like polyphenols are depend on solubility in SC-CO_2. Additionally, the use of lemon and orange pomaces as a raw material, promising for evaluation of industrial residues have commercial high quality of substances that remain these industrial wastes.

Acknowledgements

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Nomenclature

AA : Antioxidant activity,
DMSO : Dimethyl sulfoxide,
DPPH : 2,2-Diphenyl-1-picrylhydrazyl,
EFSA : European Food Safety Authority,
EtOH : Ethanol,
FAO : Food and Agriculture Organization,
FDA : U.S. Food and Drug Administration,
GAE : Gallic acid equivalent,
GRAS : Generally Recognized As Safe,
P_c : Critical pressure,
PMs : Polymethoxy flavones,
PPO : Polyphenol oxidase,
SC-CO_2 : Supercritical carbondioxide,
SCF : Supercritical fluid,
SFE : Supercritical fluid extraction,
SOX : Soxhlet extraction,
T_c : Critical temperature,
TF : Total flavonoids,
TPC : Total phenolic content