**Original Article****Supercritical Carbon Dioxide Extraction of Bioactive Compounds from Feijoa (*Feijoa sellowiana*) Leaves**Mitra Mousavi¹, Mandana Bimakr^{2*}, Seyyed Mohammad Ghoreishi³, Ali Ganjloo²

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A B S T R A C T

Background and Objectives: Supercritical fluid extraction (SFE) technique has been studied for extraction of bioactive compounds from *Feijoa sellowiana* leaves. Results were compared with those obtained from conventional ethanolic extraction (CE).

Materials and Methods:

Results: The best supercritical carbon dioxide (SC-CO₂) extraction conditions was determined as 250 bar of pressure, 50 °C of temperature, and 90 min dynamic extraction time. Under these conditions, the crude extraction yield (CEY) of bioactive compounds was 38.14 ± 0.17 mg g⁻¹. Spectrophotometric analysis revealed that the extract possesses strong radical scavenging activity (78.18 ± 0.12%, 74.19 ± 0.14%, 49.38 ± 0.18% inhibition of DPPH[·], ABTS^{·+}, and OH[·], respectively). The HPLC analysis revealed that gallic acid, catechin, rutin, ferulic acid, apigenin, and quercetin are the major phenolic compounds present in the extract. The CEY obtained using the best conditions of SCE process was around 70% of those obtained with CE. However, the quality of extracts regarding radical scavenging activity and bioactive phenolic content was higher than those obtained by CE.

Conclusions: *F. sellowiana* leave is a potential source of bioactive compounds with strong radical scavenging activity and the SC-CO₂ extraction can be considered as a green technique to extract bioactive compounds.

Keywords: Supercritical carbon dioxide extraction, *Feijoa sellowiana* leaves, Conventional extraction, Radical scavenging activity, High-performance liquid chromatography

Introduction

Supercritical carbon dioxide extraction (SCE) is becoming popular as a practical and environmental friendly technique for bioactive compounds extraction from herbal sources. In recent years, possible applications of the SCE process which offers advantages over conventional extraction methods have been investigated by several researchers (1,2). Supercritical fluids contain both liquid-like and gas-like properties, and the dual effect of such fluids provide good conditions for targeted compounds extraction (3). Supercritical carbon dioxide (SC-CO₂) is an alternative technique for the extraction of different valuable compounds from natural sources (4, 5).

Carbon dioxide (CO₂) is a non-toxic, environmentally friendly solvent, which allows SFE at moderate temperature and pressure. The products obtained by SCE have good quality, and the yields are comparable with those by classic methods (6, 7). Therefore, SCE may be considered as a promising technique in food technology. Over the last few years, numerous applications of SCE for the extraction of valuable compounds from different raw materials such as coriander seed (5), *Morus* leaves (8), guava leave (9), green tea (10), tomato (11), spearmint leaves (12), licorice plant root (13), oilseeds (14) as well as grape seed oil (15) have been reported in the literature.

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Free radicals are unstable molecules with one or more unpaired electron in its outer shell. Cells may damage if a free radical reacts with cellular components (16). Therefore, it is necessary to scavenge these unstable molecules to prevent their harmful effects. Plant extracts which are rich in bioactive compounds with strong radical scavenging activity can be a well-considered choice for the prevention of the harmful effects of free radicals on human health.

Feijoa (*Feijoa sellowiana*) is an evergreen tree belonging to *Myrtaceae* family and is indigenous to western highlands of Paraguay, southern Brazil, Uruguay and northern Argentina (17). This plant entered the Mediterranean region in the late nineteenth century and also entered Islamic Republic of Iran in 1973 through the Republic of Azerbaijan. This shrub is just grown on the northern strip of Iran. The fruit of Feijoa contains a high value of vitamin C, fiber, minerals and calories and it could be considered as a good source of iodine. The leaves of *F. sellowiana* plant are waste materials without any utilization (18-20). The recent growth in the knowledge of free radicals increase the consumer demand to replace controversial synthetic antioxidants which have shown adverse effects on human health (Bimakr *et al.*, 2015). In this regard, finding cheap and alternative sources of natural bioactive compounds to scavenge free radicals would be favorable. These valuable natural bioactive compounds can be consumed in food products to inhibit the lipid oxidation.

To the best of our knowledge no information has been reported on the SCE of valuable bioactive compounds from *F. sellowiana* leaves. Therefore, this study was performed to investigate the influences of some SCE process variables namely temperature, pressure and dynamic extraction time on crude extraction yield (CEY). The radical scavenging activity of extracts obtained under the best extraction conditions was determined regarding DPPH[•], OH[•], and ABTS^{•+} free radicals. The major phenolic compounds were qualified and quantified using HPLC. Furthermore, the results were compared with those obtained from ethanolic extraction method.

Materials and Methods

Raw material and reagents: Fresh leaves of *F. sellowiana* were harvested from a garden in

Mazandaran, Iran. The leaves were washed and then dried at 40 °C to a final moisture content of 9 ± 0.88%. The samples were ground in grinder mill (Ginsong, Zhejiang, China) to produce a sample with a particle size of 0.80-1.00 mm. The following chemicals were used: Methanol and ethanol (analytical grade) were purchased from Merck, Germany. ABTS^{•+}, DPPH[•], and potassium persulphate were purchased from Sigma-Aldrich Chemie GmbH, Germany. All phenolic compound standards were purchased from Sigma-Aldrich Chemie GmbH, Germany. Carbone dioxide (SFE grade) was purchased from Ardestan company, Iran.

Supercritical carbon dioxide extraction: The supercritical carbon dioxide flow rate, the pressure and temperature were measured by the equipment software, and the extraction time was adjusted by the stopwatch. 2 g of *F. sellowiana* leaves samples were weighed accurately, and then put into the sample cell. The liquefied CO₂ was transferred to the extraction vessel by a high-pressure pump (TESCOM, 26-1762-24). The CO₂ liquid was then heated until it reached supercritical state. The supercritical CO₂ flow rate was adjusted at 1.00 mL min⁻¹. The modifier flow rate was kept at 2 g min⁻¹ based on the preliminary experiments. Extractions were carried out at different levels of pressure (150-350 bar), temperature (40-60 °C) and dynamic extraction time (60-120 min). After extraction, the modifier was removed using vacuum rotary evaporator (R-250, Flawil, Switzerland) and flushed by nitrogen (99.9%).

Conventional extraction method: Three gram (3 g) of ground *F. sellowiana* leaves were used in each extraction. Approximately, 150 mL of ethanol (99.5%) was added to the extraction flask. Each extraction was performed in triplicate for 6 hr. After conventional extraction (CE), the solvent was removed at 40 °C using vacuum rotary evaporator (R-250, Flawil, Switzerland).

Determination of crude extraction yield of bioactive compounds: The crude extraction yield (CEY) was calculated as mg g⁻¹ using Eq.1:

$$CEY = \frac{m_e}{m_s} \times 1000 \quad (\text{Eq.1})$$

Where m_e is the crude extract mass (g) and m_s is the sample mass (g) (21, 22).

DPPH[•] radical scavenging assay: This assay was carried out as described by Zengin *et al.* (24). Absorbance measurements performed in a 1 cm quartz cell using a UV-Vis spectrophotometer (Specord 250, AnalytikJena, Germany). The inhibition percent of scavenged DPPH[•] (%DPPH_{sc}) was calculated as $100 \times (A_b - A_s) / A_b$, where A_b was the absorbance of the blank and A_s was the absorbance of the sample.

ABTS^{•+} radical scavenging activity assay: The procedure was the same as reported by Bimakr *et al.* (2013). Absorbance measurements were performed using a UV-Vis spectrophotometer (Specord 250, AnalytikJena, Germany) at 734 nm. The inhibition percent of scavenged ABTS^{•+} (%ABTS_{sc}^{•+}) was calculated as $100 \times (A_b - A_s) / A_b$, where A_b was the blank absorbance and A_s was the sample absorbance (25).

Hydroxyl radical scavenging activity assay: The procedure was the same as reported by Boulekbache-Makhlouf *et al.* (2013) with slight modification (26). 1.5 mL of test sample (1 mg mL^{-1}) was mixed with 0.02 mL of 30% of H₂O₂ solution. Absorbance was read at 530 nm at different times (5-60 min) UV-Vis spectrophotometer (Specord 250, AnalytikJena, Germany). The inhibition percent of scavenged hydroxyl radicals (%OH_{sc}[•]) was calculated as $100 \times (A_b - A_s) / A_b$, where A_b was the blank absorbance and A_s was the sample absorbance.

High-performance liquid chromatographic condition: A High-performance liquid chromatography (HPLC) system equipped with a Varian 9012 HPLC pump (CA, USA) and UV-Vis detector were used to identify the phenolic compounds. The volume of sample loop was 20 μL . Data were analyzed using Chromana software (version 3.6.4). The separation was obtained by an Eclipse RP- C18 column (25 cm \times 4.6 mm \times 5 μm , Supelco, USA) with trifluoroacetic acid (2.5 pH) as solvent A and methanol as solvent B. The bioactive phenolic compounds were detected at 280 nm. Elution flow rate was 1 mL min⁻¹. Table 1 lists the solvent gradient conditions. The calibration curves were established by plotting the peak areas obtained from the injections against the concentrations. The standard of phenolic compounds were gallic acid (0.05 – 380 $\mu\text{g/mL}$), catechin (0.05 – 250 $\mu\text{g/mL}$), quercetin (0.07 – 100 $\mu\text{g/mL}$), rutin (0.07 – 200

$\mu\text{g/mL}$), apigenin (0.07 – 200 $\mu\text{g/mL}$), and ferulic acid (0.10 – 300 $\mu\text{g/mL}$).

Table 1. Gradient elution program of HPLC mobile phase for analysis of major phenolic compounds from *F. sellowiana* leaves

| Time (min) | A% | B% |
|------------|-----|----|
| 0 | 100 | 0 |
| 5 | 70 | 30 |
| 20 | 50 | 50 |
| 30 | 40 | 60 |
| 40 | 100 | 0 |

Experimental designs and statistical analysis: A 3 \times 3 \times 3 full factorial design (3 levels of extraction pressure \times 3 levels of temperature \times 3 levels of dynamic extraction time) in a frame of Completely Randomized Design (CRD) was adopted (Table 2). An analysis of variance (ANOVA) following the Turkey test was carried out to identify significant effects ($p < 0.05$) among treatments. All experiments were performed in triplicate ($n=3$). The statistical analysis was performed using MINITAB software V.14 (Minitab Inc. State College, PA, USA).

Table 2. Experimental levels of the independent variables used in Completely Randomized Design (CRD) full factorial

| Independent variables | Levels | | |
|------------------------------------|--------|-----|-----|
| | 1 | 2 | 3 |
| Pressure (bar) | 150 | 250 | 350 |
| Temperature ($^{\circ}\text{C}$) | 40 | 50 | 60 |
| Dynamic extraction time (min) | 60 | 90 | 120 |

Results

Effects of SC-CO₂ extraction process variables on the CEY of bioactive compounds: In the current study, the CEY obtained under studied conditions varied from 14.00 ± 0.26 to $38.81 \pm 0.23 \text{ mg g}^{-1}$ suggested the potentiality of the SC-CO₂ extraction process for extraction of valuable bioactive compounds from *F. sellowiana* leaves. The analysis of variance (ANOVA) was done to evaluate the main effects of independent variables on the CEY of bioactive compounds. Considering the probability value (p -value), all process variables had significant ($p < 0.05$) effects on the CEY. In Table 3, the average response of each level about CEY of bioactive compounds is shown. Based on the R -value, it can be stated that the effect of variables on the CEY of bioactive compounds followed the decreased order of pressure, dynamic extraction time and temperature.

Table 3. Results obtained at the experimental conditions using Complete Randomized Design (CRD) full factorial

| Parameter | CEY ^c (mg g ⁻¹) L1 ^a | CEY ^c (mg g ⁻¹) L2 ^a | CEY ^c (mg g ⁻¹) L3 ^a | R ^b |
|-------------------------------|---|---|---|----------------|
| Pressure (bar) | 22.51 ± 4.43 | 33.59 ± 3.21 | 30.64 ± 4.72 | 11.08 |
| Temperature (°C) | 24.32 ± 5.32 | 31.18 ± 5.61 | 31.25 ± 5.43 | 6.93 |
| Dynamic extraction time (min) | 24.70 ± 3.45 | 29.76 ± 4.43 | 32.28 ± 6.3 | 7.58 |

^a Average responses of each level about CEY of bioactive compounds

^b R-value means range between three average responses of each level about CEY of bioactive compounds

^c Values are mean ± SD of triplicate runs

Moreover, Figure 1 (A-C), presented the effect of extraction temperature at different pressure levels (150-350 bar) on the CEY of bioactive compounds from *F. sellowiana* during 120 min dynamic extraction time. Considering Figure 1 (A), it was clear that at the lower pressure (150 bar) the CEY increased with temperature from 40 to 60°C until 120 min of dynamic extraction time. From Figure 1 (B and C), it was found that application of higher pressure levels (250 and 350 bar) at 50 and 60 °C leads to an increase of CEY with increasing dynamic extraction time up to 90 min, after that significant changes ($p>0.05$) were not detected. These results revealed the effective role of higher pressure to reduce the required dynamic extraction time. Higher values of CEY were obtained applying higher pressures (250 and 350 bar) during 90 min of dynamic extraction time. The results of CEY of bioactive compounds obtained using 350 bar pressure at the constant temperature was lower than those obtained at 250 bar. In the current study, regarding the effect of extraction temperature, it was found that at the constant level of pressure (for example 250 bar) during 90 min of dynamic extraction time (Figure 1 (b)), the CEY of bioactive compounds were increased with increasing temperature from 40 °C ($28.90 \pm 0.11 \text{ mg g}^{-1}$) to 50°C ($38.14 \pm 0.15 \text{ mg g}^{-1}$) and there were no significant ($p>0.05$) difference beyond that. Referring to the results obtained, it was clear that using 350 bar pressure was not satisfactory. It is interesting to know that using a pressure of 350 bar and high temperature (60 °C), the CEY of bioactive compounds reduced, increasing dynamic time from 90 ($34.18 \pm 0.16 \text{ mg g}^{-1}$) to 120 min ($34.03 \pm 0.14 \text{ mg g}^{-1}$), however, the changes were not significant ($p>0.05$).

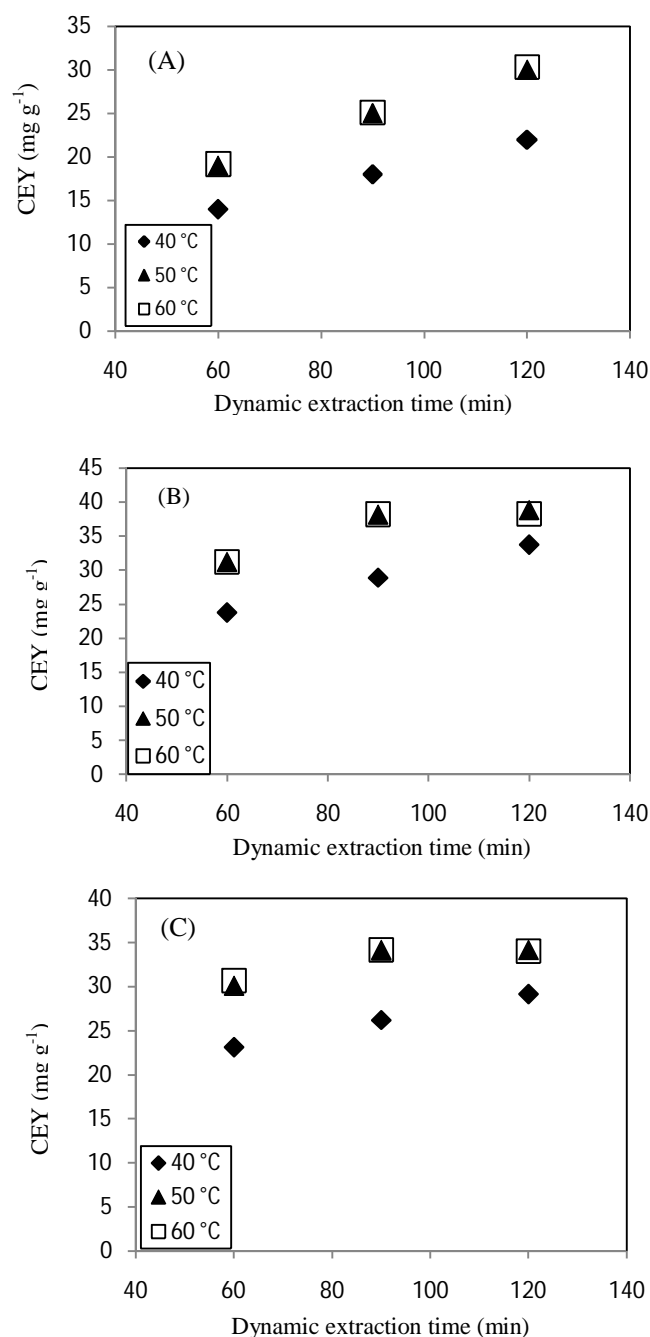


Figure 1: Effect of temperature at (A) 150 bar pressure; (B) 250 bar pressure and (C) 350 bar pressure on the CEY (mg g⁻¹) at different dynamic extraction time (standard deviation bars are smaller than the symbol size).

The dynamic extraction time has been shown to be another effective independent variable that significantly ($p < 0.05$) affected the SC-CO₂ extraction process. From Figure 1, it was observed that at 150 bar of pressure the CEY of bioactive compounds was increased until 120 min of dynamic extraction time ($22.00 \pm 0.11 \text{ mg g}^{-1}$) which could be due to the lower solvent power. But at higher pressures (250 and 350 bar) due to the higher extraction rate, the CEY of bioactive compounds was increased until 90 min of dynamic extraction time, and no significant ($p > 0.05$) increase was observed beyond that. Referring to Figure 1 (B and C), it was clear that 90 min of dynamic extraction time was adequate to complete the SC-CO₂ extraction process of bioactive compounds from *F. sellowiana* leaves. Finally, it can be stated that the highest value of CEY of bioactive compounds from *F. sellowiana* leaves was obtained at 250 bar of pressure, 50 °C of extraction temperature and 90 min dynamic extraction time ($38.14 \pm 0.17 \text{ mg g}^{-1}$).

Characterization of the RSA of extracts: Table 4 showed the RSA of *F. sellowiana* leaves extracts obtained under the best conditions of the SC-CO₂ extraction process (250 bar of pressure, 50°C of extraction temperature and 90 min dynamic extraction time). Furthermore, the RSA of bioactive compounds of *F. sellowiana* leaves extracts obtained under minimum level (pressure of 150.00 bar, extraction temperature of 40.00°C and 60.00 min dynamic extraction time) and maximum level

(pressure of 350.00 bar, extraction temperature of 60.00°C and 120.00 min dynamic extraction time) of each independent variable studied was compared with those obtained under the best level (pressure of 250.00 bar, extraction temperature of 50.00°C and 90.00 min dynamic extraction time). There was significant ($p < 0.05$) difference between the RSA of extracts (regarding DPPH[·], ABTS^{·+}, and OH[·] radical scavenging activity) obtained under different conditions of the SC-CO₂ extraction process. From the results, the highest value of RSA (78.18 ± 0.12 , 74.19 ± 0.14 and $49.38 \pm 0.18\%$ scavenging of DPPH[·], ABTS^{·+} and OH[·] radicals, respectively) can be obtained using the best conditions of the SC-CO₂ extraction technique. It should be noted that using the maximum levels of each independent variable, leads to reduction in the RSA of extracts which could be due to the thermo-degradation and vitality of bioactive phenolic compounds which contributed in the RSA of *F. sellowiana* leaves extracts. Furthermore, applying the highest level of pressure (350 bar) resulted in reduction of the RSA, which were correlated with the lower CEY of bioactive compounds at the highest pressure. Applying the lower pressure and extraction temperature during the shorter dynamic extraction times, resulted in the lower RSA of extracts (33.11 ± 0.11 , 29.46 ± 0.16 and $18.88 \pm 0.17\%$ scavenging of DPPH[·], ABTS^{·+} and OH[·] radicals, respectively) which could be due to the lower extraction rate and recovery of valuable bioactive compounds from *F. sellowiana* leaves.

Table 4. Radical scavenging activity of *F. Sellowiana* leaves extracts obtained using CE and SC-CO₂ extraction techniques

| Extraction mode | | Radical scavenging activity | | |
|-------------------------------|-----------------------|-----------------------------|---------------------|-------------------|
| | | %DPPH _{sc} | %ABTS _{sc} | %OH _{sc} |
| SC-CO ₂ extraction | Type I ^a | 33.11 ± 0.11 | 29.46 ± 0.16 | 18.88 ± 0.17 |
| | Type II ^b | 78.18 ± 0.12 | 74.19 ± 0.14 | 49.38 ± 0.18 |
| | Type III ^c | 76.98 ± 0.10 | 71.58 ± 0.17 | 47.08 ± 0.11 |
| CE | | 16.14 ± 0.14 | 14.21 ± 0.15 | 10.62 ± 0.10 |

^a Minimum level of each studied parameter (40 °C, 150 bar and 60 min)

^b Optimum level of each studied parameter (50 °C, 250 bar and 90 min)

^c Maximum level of each studied parameter (60 °C, 350 bar and 120 min)

The contents of major bioactive phenolic compounds of *F. sellowiana* leaves extracts obtained under the best conditions of the SC-CO₂ extraction process (pressure of 250.00 bar, extraction temperature of 50.00 °C and 90.00 min dynamic extraction time) are given in Table 5. As presented in Figure 2, the main phenolic compound was gallic acid, which reached the maximum value of 133.25 ± 0.15 mg g⁻¹ in the extract obtained under the best conditions followed with ferulic acid, catechin, quercetin, apigenin, and rutin. As given in Table 5, the contents of bioactive phenolic compounds obtained under the minimum level (pressure of 150.00 bar, extraction temperature of 40.00°C and 60.00 min dynamic extraction time) and maximum level (pressure of 350.00 bar, extraction temperature of 60.00 °C and 120.00 min dynamic extraction

time) of each independent variable studied was compared with those obtained under the best conditions. The results showed the highest value of bioactive phenolic compounds that could be obtained under the best conditions of the SC-CO₂ extraction process. It was clear that with applying the highest levels of each variable studied; the bioactive phenolic compounds were decreased compared with those obtained under the best conditions of the SC-CO₂ extraction process. This phenomenon could be due to the thermo-sensitivity of bioactive phenolic compounds and the negative effect of the higher pressure for extraction purpose of bioactive compounds. Furthermore, these findings are consistent with the results of RSA obtained in the current study.

Table 5. Identification and quantification of the major bioactive phenolic compounds extracted by the SC-CO₂ and CE techniques

| Extraction Mode | Phenolic compounds (mg g ⁻¹) | | | | | |
|-----------------------|--|--------------|--------------|--------------|--------------|--------------|
| | Gallic acid | Catechin | Rutin | Ferulic acid | Apigenin | Quercetin |
| Type I ^a | 98.18 ± 0.12 | 37.37 ± 0.13 | 24.10 ± 0.15 | 34.73 ± 0.13 | 19.67 ± 0.10 | 22.34 ± 0.11 |
| Type II ^b | 133.25 ± 0.15 | 67.12 ± 0.10 | 40.13 ± 0.12 | 75.33 ± 0.13 | 44.60 ± 0.13 | 49.35 ± 0.14 |
| Type III ^c | 130.15 ± 0.13 | 64.11 ± 0.06 | 39.13 ± 0.19 | 72.33 ± 0.14 | 41.65 ± 0.13 | 46.30 ± 0.12 |
| CE | 10.18 ± 0.12 | tr | - | 13.48 ± 0.11 | - | - |

^a Minimum level of each studied parameter (40 °C, 150 bar and 60 min)

^b Optimum level of each studied parameter (50 °C, 250 bar and 90 min)

^c Maximum level of each studied parameter (60 °C, 350 bar and 120 min)

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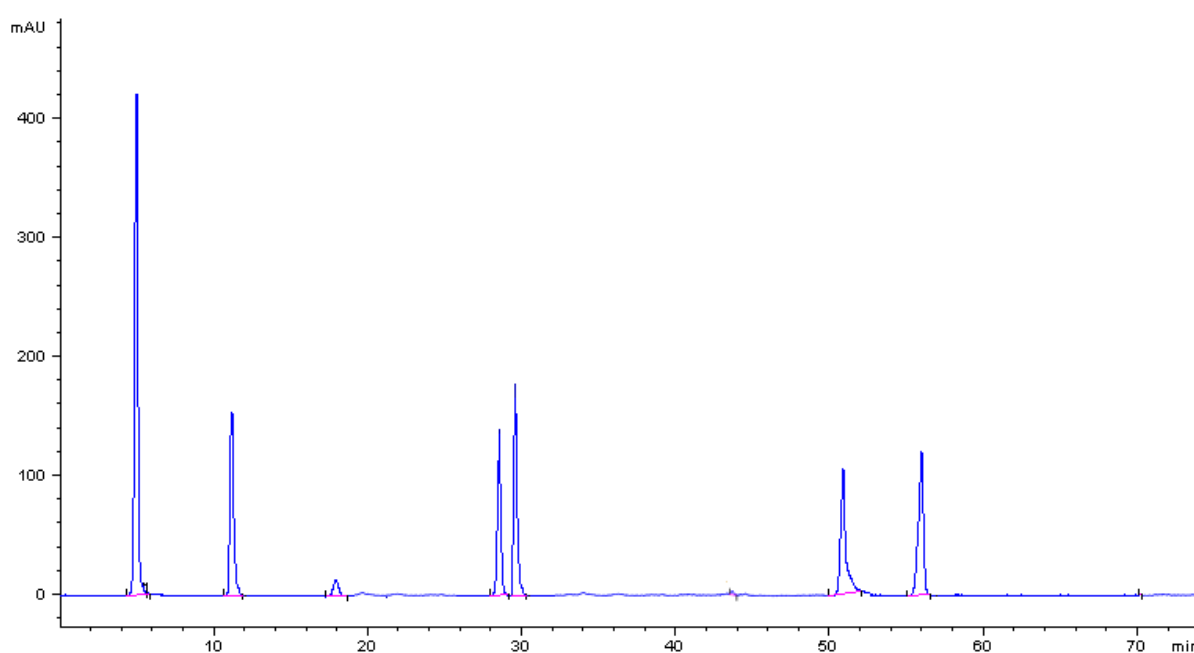


Figure 2. HPLC chromatogram of extract obtained using the best conditions of SC-CO₂ technique (250 bar of pressure, 50°C of temperature, and 90 min dynamic extraction time)

Conventional extraction versus the SCE technique for extraction of bioactive compounds from *F. sellowiana* leaves

Different valuable compounds are widely distributed in plants and according to the current study, *F. sellowiana* leave is a natural source of valuable bioactive compounds. In the current study, the CEY of bioactive compounds obtained using CE method was $54.48 \pm 0.38 \text{ mg g}^{-1}$. From the results, it was clear that the best conditions of the SC-CO₂ extraction process produced fewer amounts of crude extracts with higher quality regarding radical scavenging activity compared with ethanolic CE (Table 3). As given in Table 5, the extract obtained under the best conditions of the SC-CO₂ extraction is rich in bioactive phenolic compounds compared with that obtained using CE. Gallic acid and ferulic acid in low concentrations were detected in extracts obtained using CE. This finding could be due to the thermo-degradation of bioactive phenolic compounds resulted in using high temperature for the long extraction time.

Discussion

The effective role of higher pressure to reduce the required dynamic extraction time has been revealed in this study. It was documented that at higher pressures, the density of supercritical CO₂ increases, leading to a higher solvent power and mass transfer rate of solute (2, 3). It can be concluded that at 150 bar pressure, the solvent power of supercritical CO₂ density is reduced due to the lower CO₂ density and maximum CEY was obtained during longer dynamic extraction time (120 min). Considering the scientific evidence, at higher pressures, solvent power of CO₂ increases due to its higher density leading to higher extraction rate of bioactive compounds (27). Therefore, in the current study, higher values of CEY were obtained applying higher pressures (250 and 350 bar) during 90 min of dynamic extraction time. Similar results were found by Topal *et al.* (2006) (28). Moreover, the same behavior was reported by Liu *et al.* (2009) in SCE of pomegranate (*Punica granatum* L.) seeds (29). It was revealed that an elevation of pressure (up to around 320 bar) caused significant increase of crude yield extract. They mentioned that it could be due to the improvement of solute solubility resulted from the increased solvent density. There was an unexpected result regarding the effect of pressure on bioactive compounds

extraction since reduced value of CEY was obtained applying the highest level of pressure studied. One possible explanation for the low efficiency of SCE process at high pressure is the compressing of cell structures at higher pressure (30). Luengthanaphol *et al.* (2004) obtained the same behavior regarding the effect of high pressure on bioactive compounds extraction from tamarind seeds. They stated that increasing pressure might compress the tamarind seeds coat and cause reduction in the ability for the supercritical fluid to diffuse inside the seed particles (31, 32). In the current study, a dual effect of temperature on bioactive compounds extraction from Feijoa leaves was observed. In another study which conducted by Biamkr *et al.* (2012) the same results were obtained about the effect of extraction temperature on supercritical carbon dioxide extraction of bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves (22). Reduction of CEY of bioactive compounds applying the highest levels of pressure and temperature could be due to the volatility and sensitivity of bioactive extracted compound to high pressure or more likely due to thermo-sensitivity of valuable bioactive compounds presented in the extracts which are degraded using high temperature in a long time (1). These findings revealed that the SC-CO₂ extraction is a potential and effective novel extraction method to obtain extracts concentrated in valuable bioactive compounds with strong radical scavenging activity. These findings are in accordance with Bimacr *et al.* (2016).

Conclusion

This study showed the potential application of the SC-CO₂ extraction process as an effective extraction method for the isolation of valuable bioactive compounds from *F. sellowiana* leaves. It was illustrated that it might be possible to concentrate the valuable bioactive compounds by manipulating SCE extraction condition. Based on the results obtained, pressure, temperature and dynamic extraction time had significant ($p < 0.05$) effects on the CEY of bioactive compounds. The highest value of CEY of bioactive compounds and radical scavenging activity of extracts were determined in those obtained under the best SC-CO₂ extraction conditions (250 bar of pressure, 50 °C of temperature and 90 min dynamic extraction time). According to the results of HPLC analysis, *F. sellowiana* leaves are the potential

source of valuable phenolics such as gallic acid, ferulic acid, catechin, apigenin, quercetin, and rutin. Considering the comparison results between the SC-CO₂ extraction and CE method, it was observed that the quality of the SC-CO₂ extracts was significantly ($p<0.05$) improved compared to those obtained using the conventional extraction. The extraction time using the best conditions of the SC-CO₂ extraction, significantly ($p<0.05$) reduced to 90 min as compared with the 6 h in CE. In the current study, it was demonstrated that *F. sellowiana* leaves which are considered as agricultural waste, are rich source of bioactive phenolic compounds with high radical scavenging activity. These compounds could be consumed in food and pharmacy industries to replace harmful synthetic antioxidants. Finally, SCE process can be suggested as an effective and promising novel extraction technique to obtain bioactive compounds in good yield with strong RSA from *F. sellowiana* leaves.

Financial disclosure

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