



## Process optimization of supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction parameters for extraction of deoxynojirimycin (1-DNJ) from mulberry (*Morus alba* L.) leaves

V. Ramya<sup>1</sup>, Udaykumar Nidoni<sup>1\*</sup>, Sharangouda Hiregoudar<sup>1</sup>, C. T. Ramachandra<sup>1</sup>, J. Ashoka<sup>2</sup> and V. Lavanya<sup>1</sup>

<sup>1</sup>Department. of Processing and Food Engineering, College of Agricultural Engineering, University of Agricultural Sciences, Raichur (Karnataka), INDIA.

<sup>2</sup>Department of Entomology, College of Agriculture, University of Agricultural Sciences, Raichur (Karnataka), INDIA.

\*Corresponding author. E-mail: [udaykumarnidoni@yahoo.co.in](mailto:udaykumarnidoni@yahoo.co.in)

Received: July 18, 2015; Revised received: December 16, 2015; Accepted: March 12, 2016

**Abstract:** In the present study, supercritical fluid extraction (SFE) technology was applied to extract deoxynojirimycin (1-DNJ) from mulberry leaf powder using carbon dioxide (CO<sub>2</sub>) as major extraction solvent with ethanol as co-solvent, and extraction parameters such as pressure (100, 150 and 200 bar), temperature (40, 50 and 60 °C) and dynamic extraction time (40, 60 and 80 min) were systematically investigated by full factorial design to obtain the optimum extraction efficiency and extraction yield. Under optimized conditions (pressure of 200 bar, temperature of 50 °C and dynamic extraction time of 80 min), DNJ enriched extract was obtained with high extraction efficiency (96.46 %) and extraction yield (13.41 %), enabling this product to use for nutraceutical purpose. The results indicated that SC-CO<sub>2</sub> extraction is a promising and alternative process for recovering the bioactive compounds from mulberry leaves.

**Keywords:** Carbon dioxide, Deoxynojirimycin, Extraction efficiency, Extraction yield, Mulberry, Supercritical fluid extraction

### INTRODUCTION

Mulberry leaves are rich in flavonoid, alkaloid and polysaccharide components which are known to be the major bioactive compounds from chemical constituent investigations. Among those, deoxynojirimycin (1-DNJ), which is an alkaloid belonging to polyhydroxylated piperidine family, exists in mulberry leaves and its roots (Tsuduki *et al.*, 2013). Due to its potent  $\alpha$ -glycosidase inhibitory activity and unique distribution in mulberry, DNJ extracted from mulberry leaves has been known to be one of the major blood glucose lowering substances among a diverse mulberry extract and a powerful competitive inhibitor for  $\alpha$ -glycosidase, thereby potentially preventing diabetes (Kim *et al.*, 2001). The future use of mulberry DNJ can therefore be anticipated, but the amount of the key compound in mulberry leaves is as low as 0.1 % (Vichasilp *et al.*, 2009). Hence, more effective extraction of mulberry DNJ has become one of the important food processing challenges.

Conventional extraction methods, such as steam distillation and organic solvent extraction, have been used to extract bioactive compounds from plant materials for a long time. These methods usually require a long

time, a large amount of solvent and high temperatures (Liza *et al.*, 2010). Therefore, developing alternative extraction techniques with high efficiency and moderate peculiarity is highly desirable. Supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction has received a great deal of attention because it is usually performed at low temperatures, costing short extraction time and a small amount of solvent (Li *et al.*, 2010). Generally, addition of a small amount of a liquid polar modifier (methanol or ethanol) can significantly enhance extraction efficiency (Lang and Wai, 2001).

Considering the fact that the content of 1-DNJ in mulberry leaves might be too low to achieve its bioavailability (Wang *et al.*, 2014), the extraction and concentration of mulberry DNJ by SFE method represents something clinically meaningful. To date, there are no publications found on supercritical fluid extraction of deoxynojirimycin (1-DNJ) from mulberry (*Morus alba* L.) leaves. On the basis of these factors, the present investigation was aimed to determine the optimum supercritical carbon dioxide parameters for maximal extraction of 1-DNJ from mulberry leaf powder.

### MATERIALS AND METHODS

**Study materials:** The leaves of *Morus alba* were pro-

cured from the mulberry garden of Sericulture section, College of Agriculture, UAS, Raichur, Karnataka, India. The leaves were separated from the stalk, thoroughly washed with tap water. The leaves were dried at room temperature ( $28 \pm 2$  °C) until a constant weight was reached and then ground into powder manually. The powder was sieved using IS 500  $\mu\text{m}$  sieve to get a fine dust.

Standard DNJ was purchased from m/s. USA Sigma-Aldrich, USA. Water (HPLC grade), acetic acid (HPLC grade), acetonitril (HPLC grade) and ethanol (analytical grade) were purchased from Sd Fine-Chem Ltd., Bangalore. 9-fluorenylmethyl chloroformate was purchased from Himedia.

**SC-CO<sub>2</sub> Extraction:** The supercritical carbon dioxide extraction system (Make: Waters Thar; Model: SFE 500) used for extraction of 1-DNJ. Extractions were performed at three levels of pressure (100, 150 and 200 bar), temperature (40, 50 and 60 °C) and dynamic extraction time (40, 60 and 80 min). The extracts were collected in a glass vial at room temperature and atmospheric pressure. The modifier was removed completely by a vacuum rotary evaporator (Make: Superfit, Rotavap; Model: PBU-6D) at 40 °C (water-bath temperature). The dry extracts were adjusted to 50 ml with absolute ethanol as samples for further analysis.

**Determination of 1-DNJ by high performance liquid chromatography (HPLC):** The standard solutions of 1-DNJ or the extracted solutions were dissolved with appropriate amount of potassium borate buffer (0.4 M, pH 8.5), and then 40  $\mu\text{l}$  FMOC-Cl (5 mmol/l) in CH<sub>3</sub>CN was added. The reactant was mixed immediately and allowed to react at 20 °C for 20 min in a water bath. 10  $\mu\text{l}$  glycyl (0.1 M/l) was added to terminate the reaction by quenching the remaining FMOC-Cl. The mixture was diluted with 1 ml of 0.1 % (v/v) aqueous acetic acid to stabilize the DNJ-FMOC, and filtered by a 0.22  $\mu\text{m}$  PTFE filter (Kim *et al.*, 2001). A 20  $\mu\text{L}$  aliquot of the filtrate was injected into the HPLC system. The mobile phase consisted of acetonitril and 0.1 per cent of aqueous acetic acid (40:60, v/v). The flow rate was adjusted to 0.5 ml/min and the column (Shimpack-XR-ODS-III 150  $\times$  3.0mm, 2.1 $\mu\text{m}$ ) temperature was maintained at 25 °C. DNJ was detected as an FMOC-Cl derivative by fluorescence detector (excitation at 254 nm and emission at 322 nm).

**Extraction efficiency:** The extraction efficiency was calculated as per the method described by the Olawale (2012). Extraction efficiency (DNJ yield) is the quantity (mg) of 1-DNJ present in extract residue per 100 g of dry leaf powder.

$$\text{Extraction efficiency (\%)} = \frac{\text{Quantity of 1-DNJ recovered after extraction}}{\text{Known quantity of 1-DNJ in mulberry leaf powder}} \times 100$$

**Extraction yield:** The extraction yield was calculated as per the method described by the Liza *et al.* (2010). The extraction yield of SFE unit was calculated by using the following expression

$$\text{Extraction yield (\%)} = \frac{M_{\text{extract}}}{m_{\text{feed}}} \times 100$$

where,

$M_{\text{extract}}$  = Weight of crude extract, g

$m_{\text{feed}}$  = Weight of feed, g

## RESULTS AND DISCUSSION

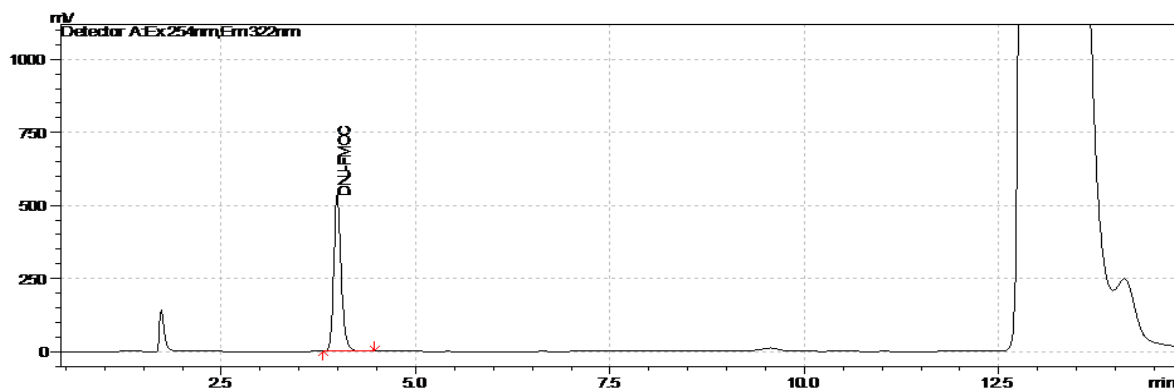
**Quantification of 1-DNJ:** The 1-DNJ content in supercritical fluid extract of mulberry leaf powder obtained for different temperature, pressure and time combinations are presented in Table 1. It is observed that the 1-DNJ content in supercritical fluid extract was in the range of 0.37 to 1.64 mg/g of dry material. Among the different treatment combinations, the highest DNJ content of 1.64 mg/g was recorded at SC-CO<sub>2</sub> pressure of 200 bar, temperature of 50 °C and dynamic extraction time of 80 min, which is considered as the optimum and best SC-CO<sub>2</sub> extraction condition for obtaining the highest DNJ yield from mulberry leaf powder. The lowest DNJ content of 0.37 mg/g of dry material was observed at SC-CO<sub>2</sub> pressure of 100 bar, temperature of 40 °C and dynamic extraction time of 40 min which is considered as a minimum condition. (The HPLC chromatogram of 1-DNJ standard, optimised condition and minimum condition is given in Fig. 1-3).

When compared with the result obtained by SFE and solvent extraction, SC-CO<sub>2</sub> extraction had better recovery (1.64 mg/g) than solvent extraction (0.58 mg/g). The DNJ content in acetonitrile with water extraction was found to be 140 mg/100 g (Kimura *et al.*, 2004). Yatsunami *et al.* (2011) extracted 1-DNJ with 75 per cent ethanol and found the DNJ content of 80-120 mg/100 g of dry material. It was reported that there are several methods for extracting 1-DNJ from plant sources. Lou *et al.* (2011) compared four different extraction methods namely, aqueous HCl (0.05 mol/l), hot water, microwave-assisted hot water and aqueous ethanol (65%) extraction. The aqueous HCl immersion extracted relatively high DNJ content of 132 mg/100 g compared to the other three methods. SFE provide a superior yield of 1-DNJ content with higher extraction efficiency and extraction yield. The developed technology could be used for commercial production of 1-DNJ from mulberry leaf powder.

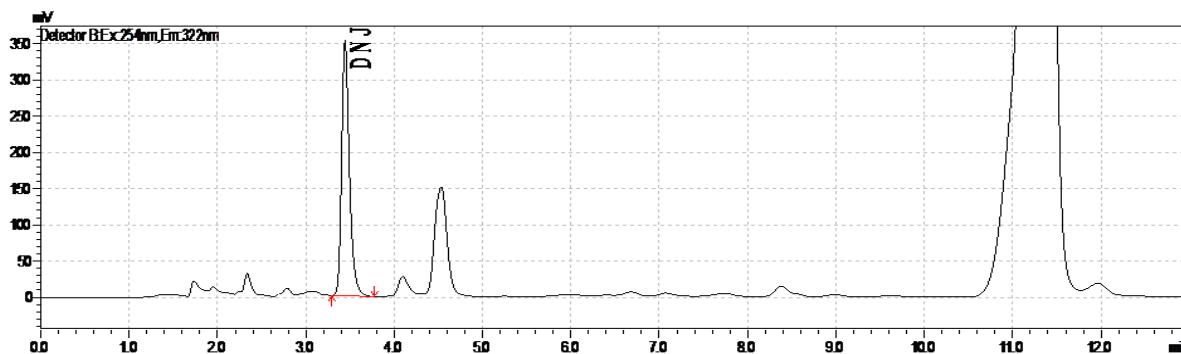
**Effect of pressure and temperature on SC-CO<sub>2</sub> extraction efficiency and yield:** The effect of various pressures and temperatures on extraction efficiency and extraction yield from mulberry (*M. alba*) leaf powder are presented in Fig. 4. It is evident from the figure that as pressure increased from 100 to 200 bar, the extraction efficiency and extraction yield also increased. This might be due to increase in pressure which increased the density of SC-CO<sub>2</sub> thereby increasing the solvent strength and solubility of the analytes in CO<sub>2</sub>. It is also observed from the figure that raising the extraction temperature from 40 to 50 °C

**Table 1.** Effect of process variables on SC-CO<sub>2</sub> extraction of 1-DNJ from mulberry leaf powder.

Treatment	Pressure (bar)	Temperature (°C)	Time (min)	1-DNJ (mg/g)	Extraction efficiency (%)	Extraction yield (%)
T <sub>0</sub>	Control			0.58	34.11	10.87
T <sub>1</sub>			40	0.37	21.57	1.12
T <sub>2</sub>	100	40	60	0.39	22.83	1.61
T <sub>3</sub>			80	0.46	26.76	1.50
T <sub>4</sub>			40	0.54	31.88	2.64
T <sub>5</sub>	100	50	60	0.62	36.75	3.11
T <sub>6</sub>			80	0.65	38.32	3.91
T <sub>7</sub>			40	0.38	22.58	1.82
T <sub>8</sub>	100	60	60	0.42	24.53	2.15
T <sub>9</sub>			80	0.47	27.73	2.52
T <sub>10</sub>			40	0.54	31.60	3.83
T <sub>11</sub>	150	40	60	0.62	36.28	4.13
T <sub>12</sub>			80	0.69	40.78	4.65
T <sub>13</sub>			40	0.73	43.04	5.10
T <sub>14</sub>	150	50	60	0.79	46.66	5.63
T <sub>15</sub>			80	0.84	49.35	6.06
T <sub>16</sub>			40	0.65	38.24	3.50
T <sub>17</sub>	150	60	60	0.70	41.11	3.77
T <sub>18</sub>			80	0.84	49.30	4.18
T <sub>19</sub>			40	0.91	53.81	8.13
T <sub>20</sub>	200	40	60	1.07	62.71	8.42
T <sub>21</sub>			80	1.25	73.56	9.94
T <sub>22</sub>			40	1.51	88.70	12.05
T <sub>23</sub>	200	50	60	1.60	93.90	12.97
T <sub>24</sub>			80	1.64	96.46	13.41
T <sub>25</sub>			40	0.60	35.29	8.43
T <sub>26</sub>	200	60	60	0.72	42.36	9.71
T <sub>27</sub>			80	0.98	57.40	10.53



**Fig. 1.** HPLC Chromatogram for 1-DNJ standard (39.7 ppm).



**Fig. 2.** HPLC Chromatogram for SC-CO<sub>2</sub> extraction of 1-DNJ at 200 bar, 50 °C and 80 min (optimised condition).

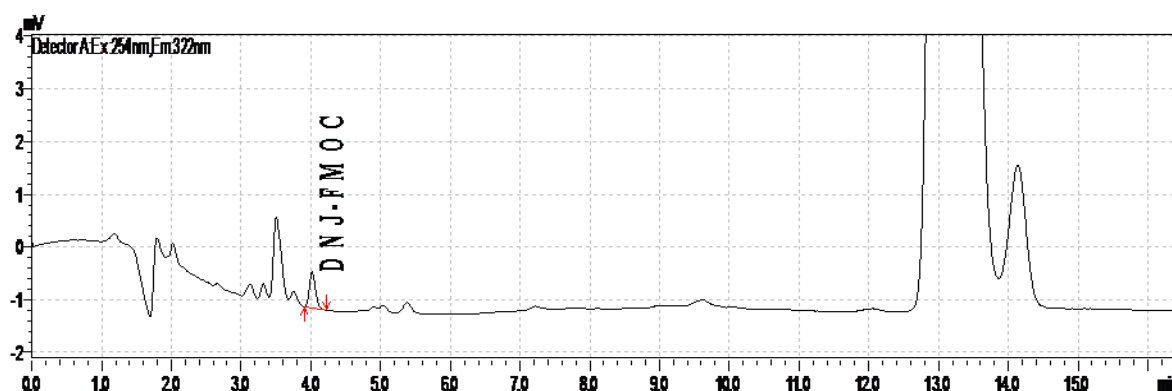


Fig. 3. HPLC Chromatogram for SC-CO<sub>2</sub> extraction of 1-DNJ at 100 bar, 40 °C and 40 min (minimum condition).

Table 2(a). ANOVA for interaction effect of temperature, pressure and time on extraction of 1-DNJ from mulberry leaf powder.

Source	Sum of Squares	DF	Mean Sum of Square	F Value	p-value Prob > F	SE±	CD @ 1%
Model	6.74	26	0.259	153.00	< 0.0001	significant	
A-Temp	1.28	2	0.641	378.30	< 0.0001	0.008	0.022
B-Pressure	4.08	2	2.039	1204.21	< 0.0001	0.008	0.022
C-Time	0.28	2	0.140	82.38	< 0.0001	0.008	0.022
AB	1.00	4	0.249	146.89	< 0.0001	0.011	0.031
AC	0.02	4	0.006	3.55	0.0188	0.011	0.031
BC	0.06	4	0.014	8.19	0.0002	0.011	0.031
ABC	0.02	8	0.003	1.72	0.1388	0.016	0.044
Pure Error	0.05	27	0.002				
Cor Total	6.78	53					

SD = 0.04; Mean = 0.78; C V % = 5.30; R<sup>2</sup> = 0.99

Table 2(b). ANOVA for interaction effect of temperature, pressure and time on extraction efficiency.

Source	Sum of Squares	DF	Mean Sum of Square	F Value	p-value Prob > F	SE±	CD @ 1%
Model	23311.01	26	896.58	153.00	< 0.0001	significant	
A-Temp	4433.56	2	2216.78	378.30	< 0.0001	0.47	1.29
B-Pressure	14112.98	2	7056.49	1204.21	< 0.0001	0.47	1.29
C-Time	965.49	2	482.74	82.38	< 0.0001	0.47	1.29
AB	3443.09	4	860.77	146.89	< 0.0001	0.66	1.82
AC	83.21	4	20.80	3.55	0.0188	0.66	1.82
BC	191.98	4	48.00	8.19	0.0002	0.66	1.82
ABC	80.70	8	10.09	1.72	0.1388	0.93	2.57
Pure Error	158.22	27	5.86				
Cor Total	23469.22	53					

SD = 2.42; Mean = 45.69; C V % = 5.30; R<sup>2</sup> = 0.99

Table 2(c). ANOVA for interaction effect of temperature, pressure and time on extraction yield. [

Source	Sum of Squares	DF	Mean Sum of Square	F Value	p-value Prob > F	SE±	CD @ 1%
Model	722.68	26	27.795	144711	< 0.0001	significant	
A-Temp	60.14	2	30.068	156544	< 0.0001	0.003	0.01
B-Pressure	633.57	2	316.78	1649266	< 0.0001	0.003	0.01
C-Time	11.17	2	5.587	29086	< 0.0001	0.003	0.01
AB	14.46	4	3.615	18822	< 0.0001	0.004	0.01
AC	0.15	4	0.038	198	< 0.0001	0.004	0.01
BC	1.84	4	0.461	2400	< 0.0001	0.004	0.01
ABC	1.35	8	0.168	876	< 0.0001	0.005	0.01
Pure Error	0.01	27	0.000				
Cor Total	722.68	53					

SD = 0.01; Mean = 5.73; C V % = 0.24; R<sup>2</sup> = 0.99

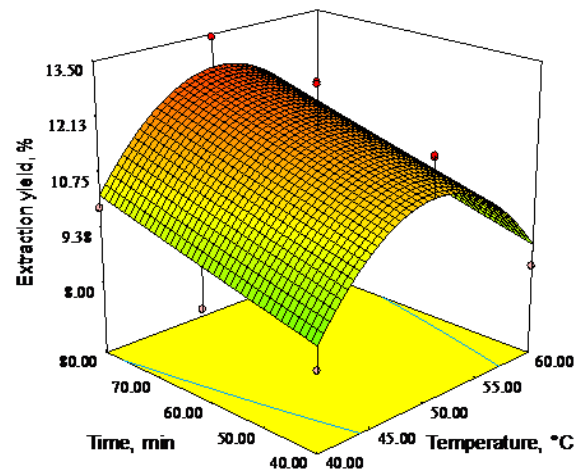
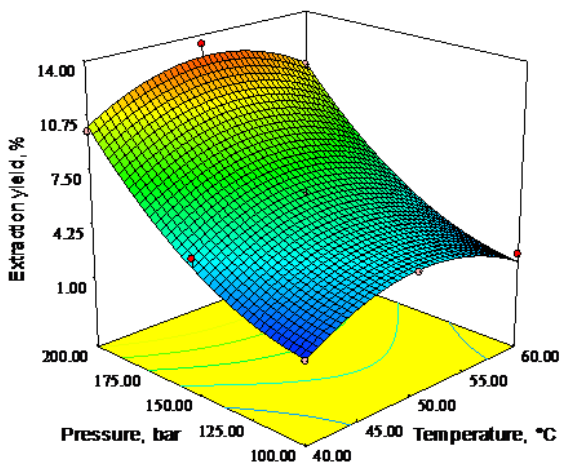
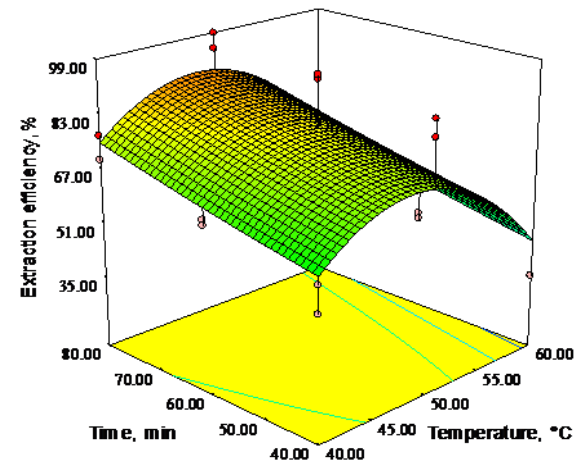
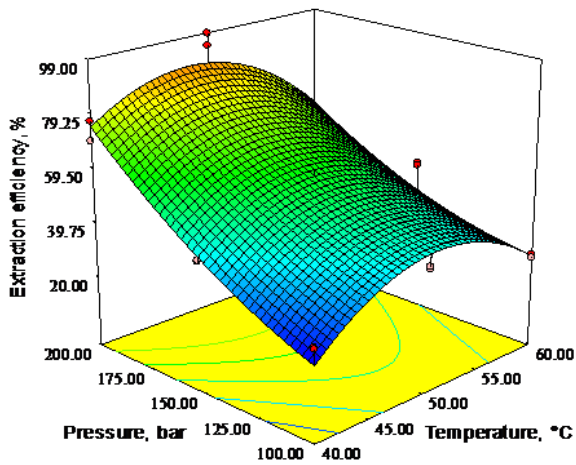
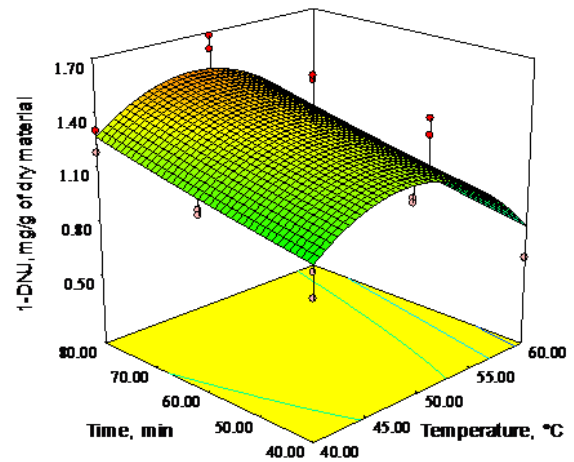
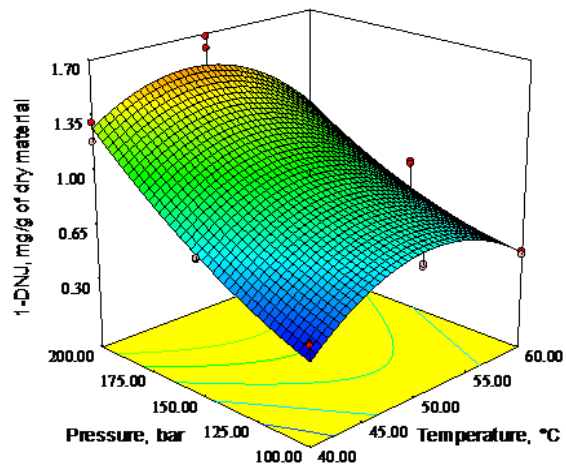


Fig. 4. Effect of pressure and temperature on SC-CO<sub>2</sub> Extraction of 1-DNJ, Extraction efficiency and Extraction yield.

Fig. 5. Effect of temperature and time SC-CO<sub>2</sub> Extraction of 1-DNJ, Extraction efficiency and Extraction yield.

increased the extraction efficiency and extraction yield. However, a temperature increase from 50 to 60 °C caused a decrease in the extraction efficiency and extraction yield which probably was due to reduction in the density of CO<sub>2</sub>. These results demonstrated that

1-DNJ solubility was the prevalent extraction parameter resulting in higher extraction yields by maintaining pressure of 200 bar and temperature of 50 °C. Similar effect of pressure and temperature on extraction of bioactive compounds from *Ampelopsis grossedentata*

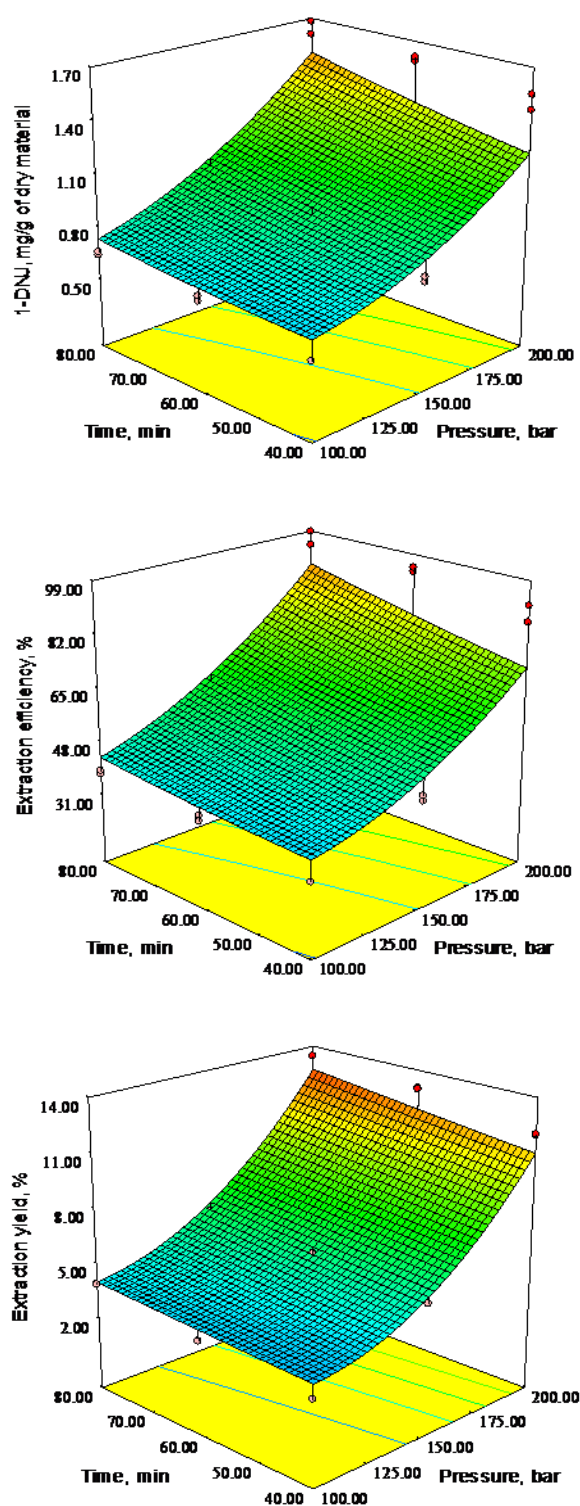


Fig. 6. Effect of extraction pressure and time SC-CO<sub>2</sub> Extraction of 1-DNJ, Extraction efficiency and Extraction yield.

stems was reported by Wang *et al.* (2011). This can be justified that limited amounts of the bioactive compounds present in the plants are easily accessible to the supercritical fluid environment by varying pressure and temperature, thus increases the higher recovery of

targeted compound (Ebrahimzadeh *et al.*, 2003).

**Effect of temperature and time on SC-CO<sub>2</sub> extraction efficiency and yield:** The effect of various temperature and time on SC-CO<sub>2</sub> extraction efficiency and yield are presented in Fig. 5. It is clear from the figure that the extraction efficiency and yield was increased as temperature rises from 40 to 50 °C. Generally, temperature has a double effect on the SC-CO<sub>2</sub> extractions. Higher temperature increased the vapor pressure of the solute and improved the extraction yield, while higher temperature could also reduce the density of carbon dioxide, decreased the extraction yield (Wang *et al.*, 2011). It is also observed from the figure that by increasing the dynamic extraction time, the 1-DNJ yield was enhanced. The effect of temperature and time in present investigation was in line with the results obtained by Ebrahimzadeh *et al.* (2003) for chemical composition of the essential oil and supercritical CO<sub>2</sub> extracts of *Zataria multiflora* Boiss., Bimakr *et al.* (2009) for extraction of bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves and Liza *et al.* (2010) for extraction of bioactive flavonoids from *Strobilanthes crispus*.

**Effect of pressure and time on SC-CO<sub>2</sub> extraction efficiency and yield:** The effect of various pressure and time on SC-CO<sub>2</sub> extraction efficiency and yield are presented in Fig. 6. It is observed from the figure that, as pressure increased from 100 to 200 bar, the extraction efficiency and yield also increased. The same phenomenon was observed in SC-CO<sub>2</sub> extraction of the bioactive flavonoid compounds from *Strobilanthes crispus* (Liza *et al.*, 2010) and for effects of co-solvents on the decaffeination of green tea by supercritical carbon dioxide (Park *et al.*, 2007). This could be explained by that a higher CO<sub>2</sub> density at higher pressures increases CO<sub>2</sub> power to dissolve the solute and thus more bioactive compounds were extracted. The highest extraction time of 80 min was found to be the optimum as the extract obtained was maximum.

**Optimization of SC-CO<sub>2</sub> Extraction Process:** The experiment was conducted to optimize SFE process parameters at a CO<sub>2</sub> flow rate of 25 g/min and a modifier (ethanol) flow rate of 3 g/min and the results are presented in Table 1. The results showed that the maximum 1-DNJ content, extraction efficiency and extraction yield of the extracts were 1.64 mg/g dry material, 96.46 % and 13.41 %, respectively. Since ANOVA (Tables 2 a, b and c) demonstrated good statistical parameter with a significant ( $p < 0.01$ ) effect on 1-DNJ content, extraction efficiency and extraction yield. The best SC-CO<sub>2</sub> extraction conditions for extraction from mulberry (*M. alba*) leaves was 200 bar, 50 °C and 80 min. Optimized parameters had desirability of 0.99 depicting efficient extraction of desired product.

## Conclusion

In conclusion, SFE has a potential for mulberry DNJ extraction to improve efficiency and extraction yield.

Based on the results obtained, the optimal extraction condition to obtain highest extraction efficiency and yield from mulberry leaf powder was found to be SC-CO<sub>2</sub> pressure of 200 bar, temperature of 50 °C and dynamic extraction time of 80 min. Under the optimum condition the highest 1-DNJ content of 1.64 mg/g of dry material was extracted with the extraction efficiency of 96.46 % and extraction yield of 13.41 %. The identification of 1-DNJ by HPLC in this study clearly revealed that temperature of 50 °C is more convenient for SC-CO<sub>2</sub> extraction and to avoid thermal degradation of the sample. SC-CO<sub>2</sub> extraction has been found as an efficient (consumes comparatively less solvent) and more convenient method for extraction of bioactive compounds.

## REFERENCES

- Bimakr, M., Rahmana, R.A., Taipa, F.S., Chaun, L.T., Ganjloo, A., Selamat, J. and Hamid, A. (2009). Supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction of bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves. *European Journal of Scientific Research*, 33: 679-690.
- Ebrahimzadeh, H., Yaminib, Y., Sefidkonec, F., Chalooosid, M. and Pourmortazavib, S. M. (2003). Chemical composition of the essential oil and supercritical carbon dioxide extracts of *Zataria multiflora* Boiss. *Journal of Food Chemistry*, 83: 357-361.
- Kim, I., Kim, J.W., Lee, H.S., Ha, N.K. and Ryu, K.S. (2001). Regional and varietal variation of 1-deoxyojirimycin (DNJ) content in the mulberry leaves. *International Journal of Industrial Entomology*, 2: 141-147.
- Kimura, T., Nakagawa, K., Saito, Y., Yamagishi, K., Suzuki, M., Yamaki, K., Shinmoto, H. and Miyazawa, T. (2004). Determination of 1-deoxyojirimycin in mulberry leaves using hydrophilic interaction chromatography with evaporative light scattering detection. *Journal of Agricultural and Food Chemistry*, 52: 1415-1418.
- Lang, Q.Y. and Wai, C.M. (2001). Supercritical fluid extraction in herbal and natural product studies – A practical review. *Talanta*, 53: 771-782.
- Li, B., Xu, Y., Yu-Xia, J, Wu, Yuan-Yuan, W and Tu, Y.Y. (2010). Response surface optimization of supercritical fluid extraction of kaempferol glycosides from tea seed cake. *Industrial Crops and Products*, 32: 123-128.
- Liza, M.S., Abdul, R.R., Mandana, B., Jinap, S., Rahmat A., Zaidul, I.S.M. and Hamid, A. (2010). Supercritical carbon dioxide extraction of bioactive flavonoid from *Strobilanthes crispus* (Pecah Kaca). *Journal of Food and Bioproducts Processing*, 88: 319-326.
- Lou, D.S., Zou, F.M., Yan, H. and Gui, Z. (2011). Factors influencing the biosynthesis of 1-deoxyojirimycin in *Morus alba* L. *African Journal of Agricultural Research*, 6(13): 2998-3006.
- Olawale, A.S. (2012). Solid-liquid extraction of oils of African elemi's (*Canarium schweinfurthii*'s) fruit. *International Journal of Agricultural Engineering: CIGR*, 14 (2): 155-160.
- Park, H.S., Lee, H.J., Shin, H.M., Lee, K. W., Lee, H., Kim, Y.S., Kim, K.O. and Kim, K.H. (2007). Effects of co-solvents on the decaffeination of green tea by supercritical carbon dioxide. *Journal of Food Chemistry*, 105: 1011-1017.
- Tsudoku, T., Kikuchi, I., Kimura, T., Nakagawa, K. and Miyazawa, T. (2013). Intake of mulberry 1deoxyojirimycin prevents diet-induced obesity through increases in adiponectin in mice. *Journal of Food Chemistry*, 139: 16-23.
- Vichasilp, C., Nakagawa, K., Sookwong, P., Suzuki, Y., Kimura, F., Higuchi, O. and Miyazawa, T. (2009). Optimization of 1-deoxyojirimycin extraction from mulberry leaves by using response surface methodology. *Journal of Bioscience Biotechnology Biochemistry*, 73 (12): 2684-2689.
- Wang T, Li C, Zhang H and Li J. (2014). Response surface optimized extraction of 1-deoxyojirimycin from mulberry leaves (*Morus alba* L.) and preparative separation with resins. *Journal of Molecules*, 19: 7040-7056.
- Wang, Y., Ying, L., Sun, D., Zhang, S., Zhu, Y. and Xu, P. (2011). Supercritical carbon dioxide extraction of bioactive compounds from *ampelopsis grossedentata* stems: process optimization and antioxidant activity. *International Journal of Molecular Sciences*, 12: 6856-6870.
- Yatsunami, K., Murata, K. and Kamei, T. (2011). 1-Deoxyojirimycin content and alfa-glucosidase inhibitory activity and heat stability of 1-deoxyojirimycin in silkworm powder. *Journal of Food and Nutritional Sciences*, 2: 87-89.