研 究 报 告

Simultaneous Separation of Solanesol and Coenzyme Q_{10} from Tobacco(*Nicotiana tabacum* L.) Extract Using Supercritical Carbon Dioxide

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Abstract: A method for simultaneous separation of solanesol and coenzyme Q_{10} from tobacco (*Nicotiana tabacum* L.) extract using supercritical carbon dioxide (SC-CO_2) was developed. The effects of extraction time, pressure, temperature and CO_2 flow rate on yield of solanesol and coenzyme Q_{10} were studied. The optimum conditions are met for extraction time 60 min, pressure 36 MPa, temperature 59 ℃ and CO_2 rate of 10 kg/h. Under the optimized SC-CO_2 separation, the extraction yields of solanesol and coenzyme Q_{10} are 1.84 % and 2.07 mg/g, respectively. The contents of solanesol and coenzyme Q_{10} in the extract obtained by optimized SC-CO_2 are 52.3 % and 3.6 %, respectively.

Key words: solanesol; coenzyme Q_{10}; tobacco; SC-CO_2

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超临界二氧化碳同时分离烟草提取物中的茄尼醇和辅酶 Q_{10}

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摘 要:开发了一种应用超临界二氧化碳技术从烟草提取物中同时分离茄尼醇和辅酶 Q_{10} 的方法。研究了萃取时间、压力、温度和二氧化碳流量对茄尼醇和辅酶 Q_{10} 收率的影响。结果表明,最优的提取条件为:萃取时间 60 min,萃取压力 36 MPa,萃取温度 59 ℃,二氧化碳流量 10 kg/h。在优化的超临界二氧化碳提取条件下,茄尼醇和辅酶 Q_{10} 的提取率分别为 1.84 % 和 2.07 mg/g,茄尼醇和辅酶 Q_{10} 在超临界二氧化碳萃取物中的含量分别为 52.3 % 和 3.6 %。

关键词:茄尼醇;辅酶 Q_{10};烟草;超临界二氧化碳

Solanesol itself can be used as antiulcer and hypertension treating agent[1-2]. In addition, solanesol is a necessary medical intermediate in the industrial synthesis of coenzyme Q_{10}[3], which is an excellent medicine in cardiovascular disease, atherosclerosis and so on[4-6]. Solanesol and coenzyme Q_{10} are in fact found in many plants from the *Solanaceae* family, one member of which is the tobacco plants. Other members of the family known to contain solanesol and coenzyme Q_{10} include tomato plants, potato plants, egg plants and pepper plants[7-8]. However, it was reported that the contents of solanesol and coenzyme Q_{10} in tobacco were considerably

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higher than those in other plants and thus this plants represented the most convenient source for large-scale isolation of solanesol and coenzyme Q_{10}. Many extraction methods were once reported to extract solanesol and coenzyme Q_{10}[9-12]. In the above methods, solanesol and coenzyme Q_{10} have been mainly extracted with organic solvents. The use of organic solvents may cause adverse health effects and environmental problems in both solvents handling and disposal. In addition, there are also some drawbacks such as low purity, long operation procedure and time-consuming. Nowadays, supercritical fluid extraction (SFE) has become an acceptable extraction technique used in many areas[13-16]. As a process, SFE offers numerous potential advantages over conventional solvent extraction, including shortened extraction time, excluding of organic solvent and more selective extraction[17]. Supercritical carbon dioxide (SC-CO_2) has been developed to extract solanesol in tobacco leaves or coenzyme Q_{10} in palm oil[18-20]. However, there is no report on the simultaneous separation of solanesol and coenzyme Q_{10} from tobacco (*Nicotiana tabacum* L.) extract using supercritical carbon dioxide. In this study, a method for simultaneous separation of solanesol and coenzyme Q_{10} from tobacco extract using SC-CO_2 was proposed. The effect of various extraction parameters such as extraction time, pressure, temperature and CO_2 flow rate on the yield of solanesol and coenzyme Q_{10} was investigated.

1 Materials and methods

1.1 Reagents and materials

Reagents: acetonitrile, isopropanol, HPLC grade, Krackeler Scientific, Inc., Albany, USA; Ethanol, hexane, analytical grade, Beijing Chemical Reagents Company, China; Carbon dioxide (99.98 %), Liming Gas Corporation, China; Solanesol (+90 %), coenzyme Q_{10} (98 %) standards, SIGMA Company, USA.

Materials: 80 % ethanol tobacco extract (made in our lab). The contents of solanesol and coenzyme Q_{10} in the tobacco extract are 2.1 % and 2.4 mg/g, respectively.

1.2 Apparatus

HA121-50-01 SFE device, Hua'an Supercritical Fluid Extraction Corporation, Nantong, China; HPLC system consists of Empower Software, Model Waters Delta 600 pump and a Model Waters 2996 Diode Array Detector, Waters Company, USA.

1.3 Experimental procedure

HA121-50-01 SFE device was used to separate solanesol and coenzyme Q_{10} from tobacco extract. The apparatus is shown schematically in Fig. 1. In this extraction unit, liquid carbon dioxide was cooled in cooling unit before it was pressurized and passed into the system. Each experiment was started by pre-pressurizing the entire system, during which the pump speed was adjusted to obtain the desired carbon dioxide flow rate. The flow rate was continuously measured using a mass flowmeter.

200 g of tobacco extract was charged into a cylindrical container (1 L volume) which was equipped with steel mesh filters on both ends, thus enabling carbon dioxide to pass the cylinder without loosing solids to the exterior. The filled cylinder was subsequently placed inside extractor, then carbon dioxide was introduced into the extractor. The temperature and pressure of the extractor were controlled. When the scheduled extraction time was reached, the extractor was depressurized. The extract was collected from separator and solid residue was removed from the extractor. Subsequently, both separators were rinsed with hexane. Extract was evaporated to dryness under reduced pressure and was dissolved into mobile phase prior to HPLC.

1.4 Experimental design

Orthogonal array design (OAD) is a type of fractional factorial design in which orthogonal array is used to assign factors to a series of experimental combinations, whose results can then be analyzed using a common mathematical procedure[21].

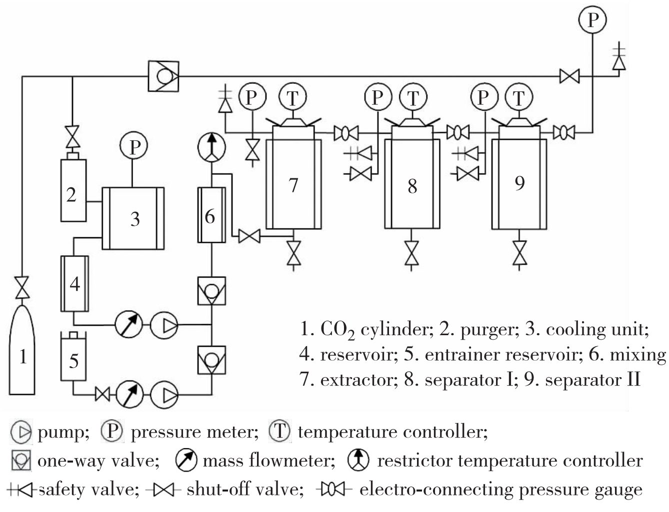


Fig. 1　Schematic representation of the SFE-apparatus

The effects of three factors, namely: extraction pressure (P), extraction temperature (T) and CO_2 flow rate (C) on the yields of solanesol and coenzyme Q_{10} were studied using a four-level experimental design. Main effects and all interactions are clear of each other (not confounded). The orthogonal array design of L_{16} (4^5) was used (the unassigned column 4 and 5 was used for estimating error variance). By using this design, the three variables were tested at four different experimental levels: extraction pressure at 20, 25, 30, 35 MPa, extraction temperature at 40, 50, 60, 70 ℃, CO_2 flow rate at 10, 15, 20 and 25 kg/h. The response variables selected were the yields of solanesol (%) and coenzyme Q_{10} (mg/g).

1.5　Quantification of solanesol and coenzyme Q_{10} by HPLC

Quantification of solanesol and coenzyme Q_{10} by HPLC has been described elsewhere[22]. All chromatographic operations were carried out at ambient temperature.

2　Results and discussion

The aim of this work was to get the experimental conditions providing the optimum SC-CO_2 separation of solanesol and coenzyme Q_{10}. Since various factors potentially influence the SC-CO_2 extraction process, the optimization of experimental conditions represents a critical step in the development of a SC-CO_2 extraction method. So we first had to test the factors that may influence the yields of solanesol and coenzyme Q_{10}.

2.1　The effect of extraction time

The effect of extraction time on the yields of solanesol and coenzyme Q_{10} is shown in Fig. 2.

As indicated in Fig. 2: the six curves obtained by SC-CO_2 showed similar trend, that was in the range of 0–60 min, with the increase of extraction time, the yields of solanesol and coenzyme Q_{10} increased steadily. When extraction time was longer than 60 min, the yields of solanesol and coenzyme Q_{10} increased slowly. In general, extraction time of 60 min gave the highest yields of solanesol and coenzyme Q_{10}. Thereby, 60 min was selected as the most suitable extraction time.

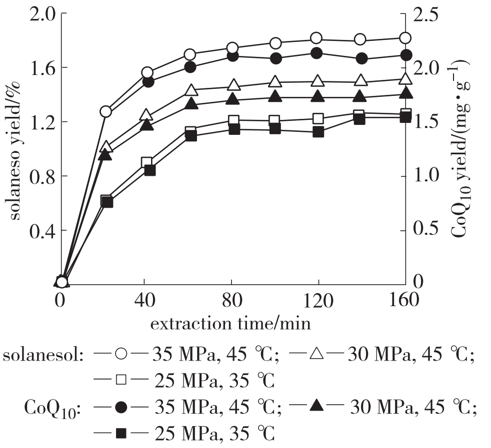


Fig. 2　Effect of extraction time on yields of solanesol and coenzyme Q_{10}

2.2 Effect of pressure, temperature and CO_2 flow rate

On the basis of preliminary experiments, extraction time (60 min) was not varied. While the effects of other three factors including extraction pressure, temperature and CO_2 flow rate on the yields of solanesol and coenzyme Q_{10} were investigated using an orthogonal array design with an $L_{16}(4^5)$ matrix (the unassigned column 4 and 5 was used for estimating error variance). Pressure, temperature and CO_2 flow rate were used as factors, the extraction yields of solanesol and coenzyme Q_{10} were used as response variables. The results are reported in Table 1. The analysis of variance (ANOVA) of results is shown in Tables 2 and 3. The results indicate that pressure has the most significant effect on the yields of solanesol and coenzyme Q_{10}, extraction temperature has significant effect and the CO_2 flow rate has little effect.

Table 1 Results of orthogonal experimental design for the separation of solanesol and coenzyme Q_{10}

No.	A pressure/MPa	B temp./℃	C CO_2 flow rate/(kg·h^{-1})	D	E	solanesol yield /%	CoQ_{10}/(mg·g^{-1}) yield
1	20	40	10	1	1	0.77	0.85
2	20	50	15	2	2	0.90	1.01
3	20	60	20	3	3	0.92	0.98
4	20	70	25	4	4	0.91	0.98
5	25	40	15	3	4	1.21	1.30
6	25	50	10	4	3	1.37	1.52
7	25	60	25	1	2	1.37	1.56
8	25	70	20	2	1	1.27	1.39
9	30	40	20	4	2	1.46	1.58
10	30	50	25	3	1	1.68	1.81
11	30	60	10	2	4	1.70	1.89
12	30	70	15	1	3	1.63	1.82
13	35	40	25	2	3	1.51	1.77
14	35	50	20	1	4	1.82	2.04
15	35	60	15	4	1	1.74	1.98
16	35	70	10	3	2	1.69	1.93

Table 2 ANOVA table for the SFE of solanesol

variance sources	sum of squares	degree of freedom	mean square	F value	P
A	1.653	3	0.5510	236.1429	0.0000
B	0.107	3	0.0357	15.2857	0.0032
C	0.001	3	0.0003	0.1429	0.9306
D	0.006	3	0.0023		
E	0.008	3			
total	1.775	15			

$F_{0.01}(3,6)=9.7795$, $F_{0.05}(3,6)=4.7571$, $F_{0.10}(3,6)=3.2888$

Table 3 ANOVA table for the SFE of coenzyme Q_{10}

variance sources	sum of squares	degree of freedom	mean square	F value	P
A	2.232	3	0.7440	318.8571	0.0000
B	0.134	3	0.0447	19.1429	0.0018
C	0.005	3	0.0017	0.7143	0.5784
D	0.010	3	0.0023		
E	0.004	3			
total	2.385	15			

$F_{0.01}(3,6)=9.7795$, $F_{0.05}(3,6)=4.7571$, $F_{0.10}(3,6)=3.2888$

Table 1 shows the experimental matrix design, with the experimental levels of the independent variables (factors), along with the yields of solanesol and coenzyme Q_{10} (response variable). Since CO_2 flow rate had little effects on the yields of solanesol and coenzyme Q_{10}, only extraction temperature and pressure were considered. The models for response variable (Y_1 , Y_2) were proposed as follows:

$$Y_1 = \alpha_1 P + \alpha_2 T + \alpha_3 P^2 + \alpha_4 T^2 + \alpha_5 PT + \alpha_6 \quad (1)$$

$$Y_2 = \beta_1 P + \beta_2 T + \beta_3 P^2 + \beta_4 T^2 + \beta_5 PT + \beta_6 \quad (2)$$

Where: Y_1—yield of solanesol, % ; Y_2—yield of coenzyme Q_{10}, mg/g; α_1, α_2, α_3, α_4, α_5, α_6, β_1, β_2, β_3, β_4, β_5, β_6—coefficients; P—extraction pressure, MPa; T—temperature, ℃.

The parameters of the model were estimated by multiple regression using the statistica 5.5 program. α_1 = 0.249 304, α_2 = 0.075 148, α_3 = −0.003 587, α_4 = −0.000 661, α_5 = 0.000 055, α_6 = −4.795 600; β_1 = 0.240 894, β_2 = 0.082 016, β_3 = −0.003 325, β_4 = −0.000 735, β_5 = 0.000 132, β_6 = −4.876 140.

The goodness of fit of the models was evaluated using the regression coefficients and the residual standard deviations. The plots of observed values versus predicted values for the estimated multiple models are shown in Fig. 3. From Fig. 3, it can be seen that the correlation was good (R = 0.993 8 for solanesol, R = 0.995 5 for coenzyme Q_{10}). The results show that numerical model is successful due to the goodness of fit between the obseved and predicted values. Furthermore, it is the possibility of using the mathematical model to predict the response values in the experimental domain.

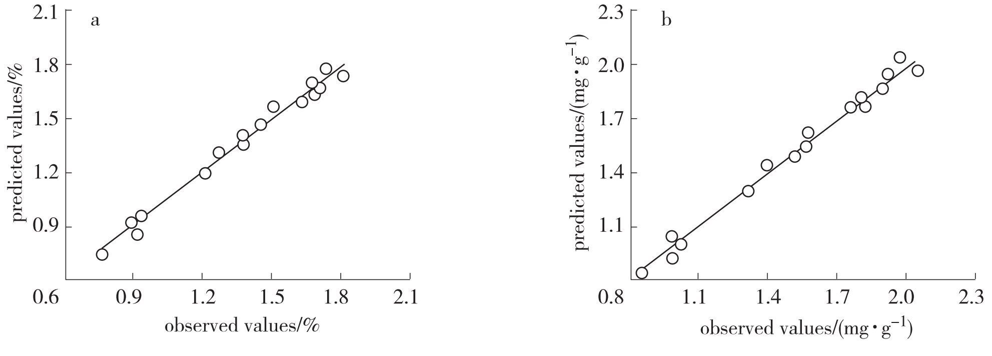
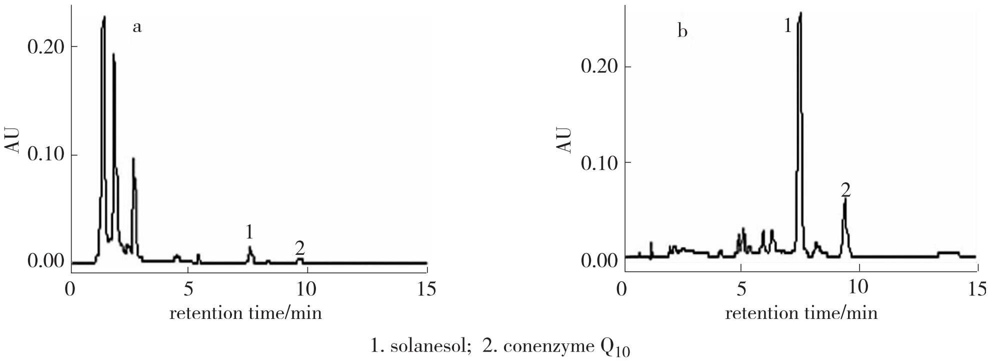


Fig. 3　Observed values obtained from estimated model solanesol (a) and coenzyme Q_{10} (b)

The predicted value by Eq(1) was: P = 35.2 MPa, T = 58.3 ℃, Y_1 = 1.78 mg/g. The predicted value by Eq(2) was: P = 37.3 MPa, T = 59.2 ℃, Y_2 = 2.05 mg/g. Considering the yields and character parameters of SC-CO_2 device, under the conditions of P = 36 MPa, T = 59 ℃, solanesol and coenzyme Q_{10} were extracted by SC-CO_2. After triplicate experiments, the extraction yields of solanesol and coenzyme Q_{10} are 1.84 % and 2.07 mg/g, respectively. The contents of solanesol and coenzyme Q_{10} in the extract obtained by optimized SC-CO_2 are 52.3 % and 3.6 %, respectively. That means experimental value is close to the predicted value. Thereby, the optimized results are believable. The HPLC chromatograms of sample obtained by the optimized SC-CO_2 methods are shown in Fig. 4.



1. solanesol; 2. conenzyme Q_{10}

Fig. 4　HPLC chromatograms of raw material (a) and tobacco extracts by SC-CO_2 (b)

3 Conclusion

In the present work, a method for the simultaneous separation of solanesol and coenzyme Q_{10} using SC-CO_2 has been presented. Solanesol and coenzyme Q_{10} in tobacco extract were extracted using optimized SC-CO_2 conditions and the contents of solanesol and coenzyme Q_{10} were analyzed by HPLC. Anyhow, SC-CO_2 extraction is an efficient method for extraction of solanesol and coenzyme Q_{10} and provides a reference for the separation of solanesol and coenzyme Q_{10} in other plant samples.

References:

[1]TAHARA Y,NAGAI M,KOGURE K,et al. Antiulcer compounds:US,4221810[P]. 1980-09-09.

[2]KIJIMA S,YAMATSU I,INAI Y,et al. Hypertension treating agent containing polyprenyl alcohol:US,4175139[P]. 1979-11-20.

[3]LIPSHUTZ B H,LOWER A,BERL V,et al. An improved synthesis of the 'miracle nutrient' coenzyme Q_{10}[J]. Organic Letters,2005,7(19):4095-4097.

[4]RUNDEK T,NAINI A,SACCO R,et al. Atorvastatin decreases the coenzyme Q_{10} level in the blood of patients at risk for cardiovascular disease and stroke[J]. Archives of Neurology,2004,61(6):889-892.

[5]WEANT K A,SMITH K M. The role of coenzyme Q_{10} in heart failure[J]. The Annals of Pharmacotherapy,2005,39(9):1522-1526.

[6]YALCIN A,KILINC E,SAGCAN A,et al. Coenzyme Q_{10} concentrations in coronary artery disease[J]. Clinical Biochemistry,2004,37(8):706-709.

[7]DOUCE D S,CLENCH M R,FROST B. Variations in the estimation of the contribution of environmental tobacco smoke(ETS) to respirable (≤5 mm) indoor air particulates obtained by the use of different analytical methods[J]. Journal of Environmental Monitoring,2001,3(3):295-301.

[8]LENAZ G. Coenzyme Q: Biochemistry, Bioenergetics and Clinical Applications of Ubiquinone[M]. New York: John Wiley & Sons,1985:68-78.

[9]ZHOU H Y,LIU C Z. Microwave-assisted extraction of solanesol from tobacco leaves[J]. Journal of Chromatography A,2006,1129(1):135-139.

[10]李玉山,王经安. 茄尼醇的纯化工艺研究[J]. 林产化学与工业,2010,30(2):103-106.

[11]SEVERSON R F,ELLLNGTON J J,SCHLOTZHAUER P F,et al. Gas chetomatographic method for the determination of free and total solanesol in tobacco[J]. Journal of Chromatography,1977,139(2):269-282.

[12]吕春茂,李英华,安艳秋,等. 辅酶 Q_{10}几种提取工艺的优化研究[J]. 食品科学,2007,28(12):132-135.

[13]SUN M,TEMELLI F. Supercritical carbon dioxide extraction of carotenoids from carrot using canola oil as a continuous co-solvent[J]. The Journal of Supercritical Fluids,2006,37(3):397-408.

[14]CHAFER A,PASCUAL-MARTI M C,SALVADOR A,et al. Supercritical fluid extraction and HPLC determination of relevant polyphenolic compounds in grape skin[J]. Journal of Separation Science,2005,28(16):2050-2056.

[15]张玉红,温慧. 黄檗中小檗碱的超临界二氧化碳萃取工艺研究[J]. 林产化学与工业,2010,30(4):103-106.

[16]潘炘,谷西荣,庄晓伟,等. 银杏叶超临界 CO_2 萃取银杏内酯动态变化的研究[J]. 生物质化学工程,2010,44(3):28-31.

[17]MENDES R L,REIS A D,PALAVRA A F. Supercritical CO_2 extraction of γ-linolenic acid and other lipids from *Arthrospira*(*Spirulina*) *maxima*: Comparison with organic solvent extraction[J]. Food Chemistry,2006,99(1):57-63.

[18]张歆,倪晋仁,黄文. 超临界 CO_2 萃取烟草中茄尼醇[J]. 精细化工,2006,23(5):480-482.

[19]李雪莹,武永刚,付玉杰,等. 超临界萃取-高效液相色谱法测定烟叶中茄尼醇的含量[J]. 内蒙古林业科技,2007,33(1):14-15.

[20]NG M H,CHOO Y M,MA A N,et al. Separation of coenzyme Q_{10} in palm oil by supercritical fluid chromatography[J]. American Journal of Applied Sciences,2006,3(7):1929-1932.

[21]MOORE L M,MCKAY M D,CAMPBELL K S. Combined array experiment design[J]. Reliability Engineering & System Safety,2006,91(10/11):1281-1289.

[22]李春英,赵春建,祖元刚. 高效液相色谱法同时测定烟叶中茄尼醇和辅酶 Q_{10}的含量[J]. 应用化学,2006,23(5):524-527.