β环糊精包合花椒挥发油的新工艺研究

张华, 齐香君, 史卓强 (陕西科技大学生命科学与工程学院, 陕西西安 710021)

摘要　[目的] 提高花椒挥发油在制剂和食品加工过程中的稳定性。[方法] 以花椒与β环糊精的投料比、包合温度、包合时间为考察因素进行正交试验,研究β环糊精包合花椒挥发油的新工艺。[结果] 该研究一步完成了花椒挥发油的提取和包合,与原工艺相比,液-液包合法的包合率明显升高,而且省去了单独提取和分离挥发油的过程,减少了挥发油的损失,缩短了挥发油的制备时间。花椒与β环糊精的投料比对花椒挥发油包合率的影响最大,其次是包合时间,包合温度的影响最小。[结论] β环糊精包合花椒挥发油的最佳工艺为:花椒与β环糊精的投料比15∶10,包合温度95 ℃,包合时间60 min,而且该工艺重现性好,产品质量稳定。

关键词　花椒挥发油;β环糊精;包合工艺
中图分类号　S573+.9　　文献标识码　A　　文章编号　0517-6611(2008)27-11630-01

Study on New Technology of Inclusion Compound of β-cyclodextrin and *Zanthoxylum bungeanum* (Maxim) Volatile Oil

ZHANG Hua et al (College of Life Science and Engineering, Shaanxi University of Science and Technology, Xi'an, Shaanxi 710021)

Abstract　[Objective] The aim was to improve the stability of *Zanthoxylum bungeanum* (Maxim) volatile oil in the process of preparation and food processing. [Method] With the feed ratio of *Z. bungeanum* to β-cyclodextrin, inclusion temperature and inclusion time as investigation factors, the orthogonal test was made to study the new technology of inclusion compound of β-cyclodextrin and *Z. bungeanum* volatile oil. [Result] The study completed the extraction and inclusion of *Z. bungeanum* volatile oil by one step. Compared with the original technology, the inclusion rate by liquid-liquid inclusion method was obvious increased and the technology saved the processes of solely extracting and separating volatile oil, decreased the loss of volatile oil and shortened the preparation time of volatile oil. The feed ratio of *Z. bungeanum* to β-cyclodextrin had greatest influence on the inclusion rate of *Z. bungeanum* volatile oil, followed by the inclusion time, and the inclusion temperature had least influence. [Conclusion] The optimum technology of inclusion compound of β-cyclodextrin and *Z. bungeanum* volatile oil was that the feed ratio of *Z. bungeanum* to β-cyclodextrin was 15∶10, the inclusion time was 60 min and the inclusion temperature was 95 ℃. The technology had good reproducibility and the quality was stable.

Key words　*Zanthoxylum bungeanum* (Maxim) volatile oil; β-cyclodextrin; Inclusion technology

花椒(*Zanthoxylum bungeanum*)是芸香科植物,具有温中助阳、散寒燥湿、行气止痛、杀虫止痒、抗氧调味之功效,不仅可以作为中药材,而且是一种具有麻香味的调味食用香料,在食品添加剂领域有广泛的应用,被誉为"八大味"之一。花椒油是从花椒籽核和籽皮中提取的一种天然挥发油,其主要成分烯烃随产地不同而有明显的差异。为了提高花椒油在制剂和食品加工过程中的稳定性,笔者对β环糊精包合花椒挥发油的工艺进行了研究。

1　材料与方法

1.1　材料及试剂　花椒(陕西韩城大红袍)。β环糊精、氯仿、无水乙醇均由天津化学试剂公司生产;活性炭。

1.2　仪器　UV-9100型紫外可见光光度计(北京瑞利分析仪器公司);JJ-3型控温电动搅拌器(常州国化电器有限公司);KQ-500DE型数控超声波清洗器(昆山市超声仪器公司);DHG-9123A型电热恒温鼓风干燥箱(上海精密实验设备有限公司)。

1.3　方法

1.3.1　花椒挥发油最大吸收波长的测定。利用水蒸气蒸馏法提取少量花椒挥发油,将其用氯仿溶解、稀释,并用氯仿为空白对照,在波长200～400 nm处扫描,测得花椒挥发油的最大吸收峰在268 nm。

1.3.2　花椒挥发油的包合工艺[1]。称取花椒与β环糊精适量(注意两者的投料比),置于烧瓶中,加入适量水;在一定温度下,加热、搅拌一定时间后,热过滤,冷却,静置24 h后过滤,包合物用乙醇进行洗涤直至表面无油迹。将过滤后的产物低温(30 ℃)烘干,即得β环糊精包合物。

作者简介　张华(1979-),男,陕西西安人,在读硕士,助理工程师,从事生物制药研究。

收稿日期　2008-08-01

1.3.3　包合物中挥发油含量的测定方法[2]。称取干燥后的包合物0.2 g于50 ml三角瓶中,加入20 ml蒸馏水和20 ml氯仿,用塑料纸包好,于超声波清洗器中,在60 ℃、70%功率下超声30 min。静置分层后,用移液管移取0.25 ml氯仿液于20 ml具塞试管中,用氯仿定容(即稀释80倍),在268 nm下测定吸光度。根据吸光度值(*A*)计算包合物中挥发油的含量。包合物中挥发油的含量(ml)=(挥发油浓度×20×包合物的重量)/0.2。

1.3.4　包合率的计算方法。包合物质量鉴定的重要考察指标就是包合物的包合率,挥发油的包合率=包合物中挥发油量(ml)/花椒中挥发油的量(ml)×100%。

2　结果与分析

通过预试验的单因素考察,并参考相关文献资料[3],选择了花椒与β环糊精的投料比、包合温度、包合时间为考察因素,按$L_9(3^3)$正交表进行正交试验,因素水平见表1。正交试验结果见表2。

表1　正交试验的因素及水平

Table 1　The factors and levels of the orthogonal test

水平 Level	花椒∶β-CD(A) Ratio of Chinese prickly ash to β-CD	包合温度(B)/℃ Inclusion temperature	包合时间(C)/min Inclusion time
1	10∶10	85	45
2	15∶10	90	60
3	20∶10	95	75

由表2可见,$R_A > R_C > R_B$,所以因素对试验指标影响的主次顺序为A>C>B,即投料比的影响最大,其次是包合时间,而包合温度的影响最小。对正交试验及其结果进行分析可以看出,在所选的各因素水平中最优的组合是$A_2B_3C_2$,即花椒与β环糊精的投料比为15∶10,在95 ℃的条件下回流60 min。

(下转第11655页)

但却在常染色体上发现了其同源基因 SlAP3A,其序列间的分歧表明这种基因重复是在性染色体进化后发生的。SlAP3A 在花瓣中表达,而 SlAP3Y 在发育的雄蕊中强烈表达,这种不同的表达模式很可能是由于调控其表达的上游控制元件在进化过程中发生的改变所引起的。这一发现也将 MADS-box 基因与 Y 染色体的进化联系了起来,随着 Y 染色体上基因的退化[20],SlAP3Y 则获得了雄性特异性表达。最近,Roman Hobza 等报道了研究染色体的方法即激光显微解剖分析法,可以用来构建性染色体文库[21],这将有利于对其性别决定与分化有更深入的认识。

参考文献

[1] SHORE P, SHARROCKS A D. The MADS-box family of transcription factors [J]. Eur J Biochem, 1995, 229: 1 - 13.

[2] LILJEGREN S J, DITTA G S, ESHED Y, et al. SHATTERPROOF MADS-box genes control seed dispersal in Arabidopsis [J]. Nature, 2000, 404: 766 - 770.

[3] ALVAREZ-BUYLLA E R, PELAZ S, LILJEREN S J, et al. An ancestral MADS-box gene duplication occurred before the divergence of plants and animals [J]. Proc Natl Acad Sci USA, 2000, 97(10): 5328 - 5333.

[4] ANGENENT G C, COLOMBO L. Molecular control of ovule development [J]. Trends Plant Sci, 1996(1): 228 - 232.

[5] HARDENACK S, YE D, SAEDLER H, et al. Comparison of MADS box gene expression in developing male and female flowers of the dioecious plant white campion [J]. Plant Cell, 1994, 6(12): 1775 - 1787.

[6] PARK J H, ISHIKAWA Y, YOSHIDA R, et al. Expression of AODEF, a B-functional MADS-box gene, in officinalis [J]. Plant Mol Biol, 2003, 51(6): 867 - 875.

[7] LOSA A, CAPORALI E, SPADA A. AOM3 and AOM4: MADS box genes expressed in reproductive structures of Asparagus officinalis [J]. Sex plant Reprod, 2004, 16: 215 - 221.

[8] AINSWORTH C, CROSSLEY S, BUCHANAN-WOLLASTON V, et al. Male and female flowers of the dioecious plant sorrel show different patterns of MADS box gene expression [J]. Plant Cell, 1995, 7(10): 1583 - 1598.

[9] PFENT C, POBURSKY K J, SATHER D N, et al. Characterization of SpAPETALA3 and SpPISTILLATA, B class floral identity genes in Spinacia oleracea, and their relationsip to dexual dimorphism [J]. Dev Genes Evol, 2005, 215(3): 132 - 142.

[10] SATHER D N, YORK A, POBURSKY K J. Sequence evolution and sex-specific expression patterns of the C class floral identity gene, SpAGAMOUS, in dioecious Spinacia oleracea L. [J]. Planta, 2005, 222: 284 - 292.

[11] COEN E S, MEYEROWITZ E M. The war of the whorls: Genetic interactions controlling flower development [J]. Nature, 1991, 353: 31 - 37.

[12] SCHULTZ E A, PICKETT F B, HAUGHN G W. The Flo10 gene product regulates the expression domain of homeotic genes AP3 and P1 in Arabidopsis flowers [J]. Plant Cell, 1991, 3: 1221 - 1237.

[13] BOWMAN J L, SAKAI H, JACK T, et al. SUPERMAN, a regulator of floral homeotic genes in Arabidopsis [J]. Development, 1992, 114: 599 - 615.

[14] JACK T, FOX G L, MEYEROWITZ E M. Arabidopsis homeotic gene APETALA3 ectopic expression: Transcriptional and posttranscriptional regulation determine floral organ identity [J]. Cell, 1994, 76: 703 - 716.

[15] MATSUNAGA S, UCHIDE W. Characterization of two SEPALLATA MADS-box genes from the dioecious plant Silene latifolia [J]. Sex Plant Reprod, 2004, 17: 189 - 193.

[16] DI STILIO V S, KRAMER E M, BAUM D A. Floral MADS box genes and homeotic gender dimorphism in Thalictrum dioicum (Ranunculaceae) - a new model for the study of dioecy [J]. The Plant Journal, 2005, 41: 755 - 766.

[17] DELONG A, CALDERON A, DELLAPORTA S L. Sex determination gene TASSELSEED2 encodes a short-chain alcohol dehydrogenase required for stage-specific floral organ abortion [J]. Cell, 1993, 74(4): 757 - 768.

[18] CHEN X, MEYEROWITZ E M. HUA1 and HUA2 are two members of the floral homeotic AGAMOUS pathway [J]. Mol Cell, 1999, 3(3): 349 - 360.

[19] HILL K, WANG H, PERRY S E. A transcriptional repression motif in the MADS factor AGL15 is involved in recruitment of histone deacetylase complex components [J]. Plant J, 2008, 53: 172 - 185.

[20] GABRIEL A B, MICHAEL N C LAS. Evidence for degeneration of the Y chromosome in the dioecious plant Silene latifolia [J]. Current Biology, 2008, 18(7): 545 - 549.

[21] ROMAN H, BORIS V. Laser microdissection-Based analysis of plant sex chromosomes [J]. Methods in Cell Biology, 2007, 82: 433 - 453.

(上接第11630 页)

方差分析结果表明:所选的3 个因素对该试验结果均有显著影响,其中 A 因素影响最大,C、B 因素次之。

表2　正交试验结果与分析

Table 2　The results and analysis of the orthogonal test

序列号 Serial number	因素 Factors A	B	C	产量/g Yield	产率/% Yield rate	吸光度(A) Absorbency	相对含量/% Relative content	总指标/% Total index
1	1	1	1	3.23	32.3	0.683	68.8	22
2	1	2	2	3.88	38.8	0.821	82.7	32
3	1	3	3	4.50	45.0	0.687	69.2	31
4	2	1	2	4.55	45.5	0.993	100.0	45
5	2	2	3	3.85	38.5	0.905	91.1	35
6	2	3	1	4.60	46.0	0.933	94.0	43
7	3	1	3	4.30	43.0	0.726	73.1	31
8	3	2	1	4.16	41.6	0.845	85.1	35
9	3	3	2	6.24	62.4	0.855	86.1	54
R	12.7	10.0	11.4					

3　小结与讨论

(1) 以往都是采用先水蒸气回流提取出挥发油,再进行包合,而该试验笔者将提取和包合的过程一步完成,即在回流药品的同时,加入β环糊精进行包合。与原工艺相比,液-液包合法的包合率显著升高,而且省去了单独提取和分离挥发油的过程,减少了挥发油的损失,简化了工艺,缩短了制备时间,更适合实际生产的需要。

(2) 通过正交试验优选最佳包合工艺为:花椒与β环糊精的投料比为15∶10,在95 ℃的条件下回流60 min。该工艺重现性好,产品质量稳定。

参考文献

[1] 崔福德. 药剂学——包合技术[M]. 北京:人民卫生出版社,2003:350 - 355.

[2] 王亚娜,孙俊梅,余丽丽,等. 环糊精及衍生物 药物包合常数的测定方法及其应用[J]. 药学进展,2004(1):23 - 28.

[3] 林维宣. 实验设计方法——正交实验的设计[M]. 大连:大连海事大学出版社,1995.