

不同叶色紫苏花青素含量与成分研究

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摘要:选取不同叶色紫苏资源,统一田间栽培管理,待紫苏成熟期收获紫苏叶,并对紫苏叶中花青素含量进行分析。紫苏花青素传统的提取方法中含有大量的副产物如糖、有机酸和蛋白质等副产物,这些杂质可能加速花青素的降解。本研究比较不同提取介质的提取能力及选择性,其中乙醇-酸化水(50%, v/v)提取花青素的含量最高(4.7%)。采用不同的吸附剂进行吸附纯化,实验表明 XAD-7HP 吸附树脂表现出较好的吸附能力和解吸能力,利用 LCMS 对花青素苷成分进行分析,花青素苷中丙二酸单酰基紫苏宁含量最高。

关键词:紫苏;花青素;提取;纯化;分析

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The Contents and Constituents of Anthocyanidins from Different Perilla Leaves

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Abstract: With perilla as raw material, the anthocyanins in the leaves of different perilla varieties were studied. Perilla leaves were harvested at mature stage and analysis of anthocyanin content in the leaves of perilla varieties. Conventional extraction methods of anthocyanin from plant material are nonselective and yield pigment solutions with large amounts of byproducts such as sugars, organic acids and proteins. Some of these impurities may accelerate anthocyanin degradation. Different extracting media were used and the mixture of ethanol and acidified (50%, v/v) water resulted in maximum anthocyanin content (4.7%). In order to obtain anthocyanins in a purified form, adsorption was carried out with different adsorbents. Among these, nonionic acrylic ester adsorbent, namely Amberlite XAD-7HP showed the highest adsorption capacity and desorption ratio. The main components of anthocyanin were characterized by LCMS. The main components were malonylshisonin.

Key words: Perilla; anthocyanins; extraction; purification; analysis

紫苏,唇形科一年生草本植物,在亚洲国家广泛种植,应用于食用和药用领域已经有几百年的悠久历史^[1]。紫苏叶片有红色、绿色和面青背紫三种颜色,红色叶片中含有丰富的花青素,如花色苷、丙二酰基紫苏宁(MS)、紫苏苷(S)和类花色苷结构的化合物^[2]。天然资源的花青素作为食品工业的着色剂和添加剂有着广泛地应用。此外,花青素还具有多种生物活性,如具有抑制脂质过氧化和氧自由基清除作用^[3],紫苏叶片中的酚类化合物有抗过敏、抗炎^[4,5]和抗氧化性能^[6],神经保护作用^[7],对糖尿病和肥胖症的改善有潜在应用价值^[8]。绿紫苏叶

片中含有花青素以外的其他色素,可应用于食品、护肤霜和过敏性皮炎药物领域^[9]。

近年来人们对天然资源越来越重视,相比于化学合成色素天然色素更加安全,人们对天然色素需求越来越多,高含量色素紫苏资源研究已经成为研究热点。随着现代分析技术的不断发展,液相色谱-质谱联用(LCMS)已成为解析复杂组分的常用手段之一。LCMS已经被广泛用于天然产物资源的成分分析、质量控制和体内过程研究等^[10-12],并发展出了许多适用于复杂组分的解析策略^[13,14]。本文通过选取不同叶色紫苏资源进行栽培选育,并对花青素含量进行分析,同时对紫苏花青素的纯化工艺进行研究,比较不同大孔吸附树脂的选择性和吸附解析能力,利用LCMS对花青素的主要成分进行结构解析。

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1 材料与方法

1.1 材料与试剂

紫苏栽培选择排水良好的平川地或二洼地,质地为壤土,偏中性,秋翻和春季顶浆整地,耙、耩、压连续作业,达到良好播种状态。选取不同叶色紫苏资源在相同的种植模式下统一田间管理,在开花末期到种子灌浆初期进行收割,取样部位为植株三分之二以上高度叶片,叶片经自然晾干、去杂后,保存备用。ZS-8、ZII-2、ZS-5、ZS-4、ZS-7、ZX-1、ZS-10、ZS-2、ZS-34 为正背面均为紫色的紫苏资源,Z31、Z11、Z1H 为正面绿色背面紫色的紫苏资源,ZS-5、ZS-13、ZS-12、ZS-9、ZS-6、Z-2 为正面绿色背面浅紫色的紫苏资源。

甲醇、乙醇、醋酸等为国产分析纯级试剂;AB-8、D101、X-5、DN130、XAD16、XAD7HP、NKA-II、H103 吸附树脂;乙腈(色谱纯)、甲酸(色谱纯)去离子水,北京百灵威科技有限公司。

1.2 仪器与设备

液质联用仪(LCMS),LCMS-2020;旋转蒸发器(RE-2000),上海亚荣生化仪器厂;可见分光光度计(721S),上海精科仪电公司;天平(ME2014E),梅特勒-托利多公司;鼓风干燥箱(DHG-9070A),上海仪器仪表有限公司。

1.3 方法

1.3.1 紫苏花青素提取

紫苏叶处理,紫苏叶在自然的条件下晾干去杂(含水量<5%),花叶粉碎包装待用。称取1g处理好的紫苏叶样品,装入50mL试管中,加入提取试剂醋酸水-乙醇(醋酸水:乙醇=1:1,体积比,醋酸水中醋酸含量7%)30mL,提取温度为35℃、超声震荡30min,震荡频率100HZ、提取3次,抽滤,收集滤液浓缩除去乙醇,低温保存待测。

1.3.2 紫苏花青素的含量分析

pH差示法:取紫苏花色素苷母液,用0.025mol/L的氯化钾缓冲液(pH1.0)稀释,用510nm波长测吸光值,使其吸光值在0.4~1.2的范围内,确定稀释倍数。同样的稀释倍数用0.4mol/L醋酸钠缓冲液(pH4.5)稀释母液,以蒸馏水为空白对照,使用1cm比色皿分别测两管稀释液在510nm和700nm处的吸光值。

$$\text{总花青素的含量}(\text{mg/L}) = \frac{AMWDF 10^3}{\varepsilon L}$$

计算: $A = (A_{510} - A_{700}) \text{pH}_{1.0} - (A_{510} - A_{700}) \text{pH}_{4.5}$, MW是花青素的分子量(449.2g/mol),DF是稀释倍数, ε 是摩尔吸光系数(26900L/mol·cm),L是光长度(1cm)。

缓冲液的配制:pH1.0的缓冲液:称取1.49gKCl,用蒸馏水溶解,并定容至100mL,得浓度为0.02mol/L的KCl溶液。准确称取1.7mL浓盐酸于100mL容量瓶中,并用蒸馏水定容至刻度线,得浓度为0.2mol/L的盐酸溶液,将0.02mol/L的KCl溶液与0.2mol/L盐酸溶液以体积比为25:67的比例混合,用KCl溶液调pH(1.0±0.1)即得。pH4.5的缓冲液配制:称取1.64g无水醋酸钠,蒸馏水溶解,定容至100mL容量瓶中,用盐酸调pH(4.5±0.1)。

1.4 紫苏花青素成分分析

1.4.1 色谱和质谱条件

色谱条件,色谱柱:Agilent ZorbaxSB-C₁₈(4.6mm×150mm,5.0μm);流动相:0.05%甲酸水溶液(A)-乙腈(0.05%甲酸,B),梯度洗脱(0~25min,98%A→60%A;25~40min,60%A→40%A;40~50min,40%A→5%A;50~60min,5%A);流速:0.5mL·min⁻¹;柱温:30℃;进样体积:20μL,波长520nm。

质谱条件,质谱仪,LCMS-2020IT/MS;离子源ESI;离子扫描方式,正离子扫描(ESI+),扫描范围m/z90~900;检测器电压,1.10kV(ESI+);加热模块温度,250℃;脱溶剂管温度,250℃;雾化器流速,1.5L/min;干燥气流速,15L/min。

2 结果与分析

2.1 提取介质的选择

2.1.1 醋酸浓度对花青素提取量的影响

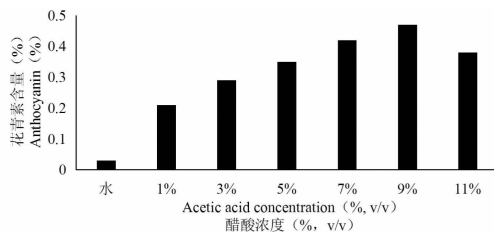


图1 醋酸浓度对提取量的影响

Fig. 1 Effect of acetic acid concentration on anthocyanin extraction

考察醋酸浓度对花青素提取量的影响,醋酸浓度在0%~11%(v/v)范围内变化。从图1中可以

看出,在酸化水的情况下,与纯水相比花青素的提取程度更高,提取量随醋酸浓度的增加而增加,随着pH值降低花青素稳定性的提高。虽然花青素提取量随着醋酸浓度的增加而增加,但是在实际提取中选择了较低浓度的醋酸(7%, v/v),因为较高浓度的醋酸不利于食品工业大规模的应用。

2.1.2 乙醇浓度对花青素提取量的影响

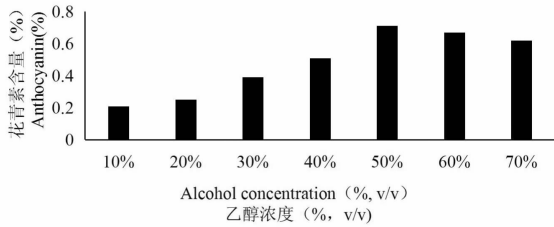


图2 乙醇浓度对提取量的影响

Fig. 2 Effect of ethanol concentration on anthocyanin extraction.

考察乙醇浓度对花青素提取量的影响,将乙醇浓度从10%~70%(v/v)范围内变化。从图2中可以看出,花青素的提取量随着乙醇浓度的增加而增加,达到50%(v/v)浓度时提取量最高,随后提取量随浓度的增加而降低,可能是由于提取液中的水的浓度随乙醇含量的增加而降低。有研究报道^[15],少量水的存在是花青素提取的必要条件,本实验与研究报道相符。同时纯乙醇作为萃取溶剂是不可取的,既不利于食品工业,也存在安全隐患。

2.1.3 利用组合提取介质对花青素提取量的影响

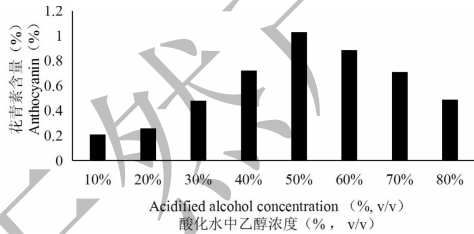


图3 酸化水中乙醇浓度对提取量的影响

Fig. 3 Effect of acidified aqueous ethanol concentration on anthocyanin extraction.

为探讨醋酸与乙醇的协同作用,采用乙醇与酸化水混合提取,酸化水中醋酸浓度保持在7%(v/v),乙醇在酸化水中的浓度从20%~80%(v/v)变化。从图3可以看出,在乙醇浓度50%(v/v)之前,花青素提取量随着酸化水中乙醇浓度的增加而增加,随着乙醇浓度的逐渐增大,花青素的提取量逐渐

降低,花青素提取量最高时乙醇的浓度为50%(v/v)。同时数据表明,结合图2数据相比醋酸和乙醇的协同作用有一定提高,同时表明花青素提取中水介质的存在及水的含量对于提取量有很大的作用。

2.1.4 其他提取试剂对花青素提取量的影响

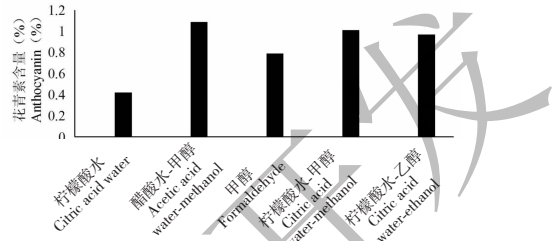


图4 不同提取试剂对提取量的影响

Fig. 4 Effect of different solvents on anthocyanin extraction.

考察柠檬酸水、醋酸水-甲醇、甲醇、柠檬酸水-甲醇、柠檬酸水-乙醇的提取效果。从图4可以看出,醋酸水-甲醇的提取效果最好,综合考虑食品工业的安全性,后续工艺中除去试剂的难易程度,花青素品质等几个方面因素,认为醋酸水-乙醇组合提取介质效果最好,可以实现紫苏花青素的规模化提取。

2.2 不同叶色紫苏花青素含量分析

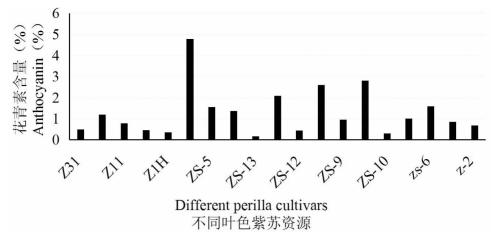


图5 不同叶色紫苏花青素含量分析

Fig. 5 Analysis of anthocyanin content in the leaves of different perilla cultivars

收获不同叶色紫苏叶片,按照优化的提取方法,在相同的条件下测试花青素的含量,实验数据结果如图5所示。如图所示,利用上述提取工艺对不同叶色紫苏资源的花青素进行含量分析,实验数据表明不同叶色紫苏的花青素含量差异性较大,经过对紫苏栽培的条件综合研究及栽培地区气候条件的综合考虑,其中ZS-8紫苏资源的花青素含量最高,且该资源的种子与叶片产量最多,种子产量2835 kg/hm²,风干叶片产量为3795.1 kg/hm²。

2.3 花青素纯化工艺研究

2.3.1 大孔吸附树脂选择

花青素的吸附和解吸性能与吸附剂的性质和溶

质的化学特性有关,与花青素极性相似的吸附剂具有更好的吸附能力,同时吸附剂物理特性在吸附和

解吸过程中也发挥着重要作用。

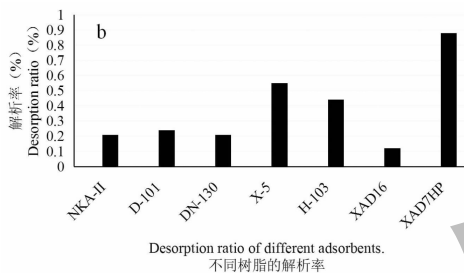
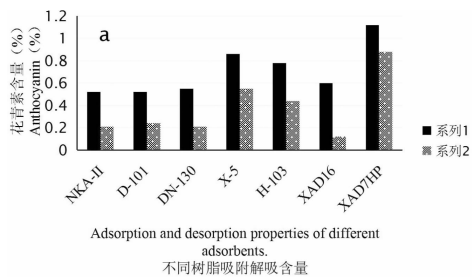


图6 (a)不同树脂的吸附解析能力和(b)不同树脂的解析率

Fig. 6(a) Adsorption and desorption capacities of anthocyanins on different resins and (b) desorption ratio of adsorbents.

由图6(a)所示,系列1为树脂的吸附量,系列2为树脂的解析量,由图可以得出XAD-7HP的吸附能力和解析能力相比于其他树脂较好,不仅是因为XAD-7HP的极性与花青素相似,同时该树脂具有很高的比表面积,树脂本身的物理性质在提高树脂的吸附能力和吸附选择性方面也起到了非常重要的作用。

2.3.2 树脂洗脱条件选择

花青素在工业生产过程中,不仅要考虑吸附剂的吸附和解析能力,还要考虑洗脱溶剂及其浓度条件。为了解乙醇浓度对花色素洗脱的影响,用酸化水乙醇(7%, v/v, 醋酸, 乙醇浓度从10% ~ 100%, v/v)进行了研究。

表1 乙醇浓度对洗脱效果的影响

Table 1 Effect of ethanol concentration on the elution of anthocyanin

乙醇浓度 Concentration of ethanol (%)	洗脱液中花青素含量 Anthocyanin eluted (mg/L)	洗脱率 Elution (%)
10	9.01	8.12
20	20.86	17.25
30	33.12	22.12
40	51.25	42.08
50	68.74	58.12
60	80.54	71.15
70	85.32	73.42
80	90.15	80.13
90	101.15	89.23
100	115.45	97.54

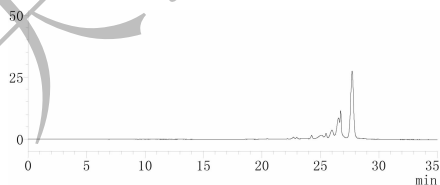


图7 紫苏花青素提取液液相分析

Fig. 7 HPLC chromatograms at 520 nm of anthocyanins of perilla sample

由表1所示,酸化乙醇浓度高于60% (v/v)时可以有效地洗脱花色苷的吸附剂,乙醇的浓度越高

花青素的回收率越高。此外由于共沸原因,低浓度的含水乙醇沸点较高,比浓度较高的水醇溶液更难浓缩,因此可以使用浓度高于60% (v/v)的酸化乙醇溶液进行洗脱。

2.4 花青素成分分析

紫苏花青素液相色谱图如图7所示,使用上述色谱条对ZS-8提取液中花青素成分进行分析,可以看出醋酸水-乙醇组合提取介质效果较好,杂质相对较少。

使用上述的色谱条件,可以使各峰较好分离,同

时本文采用 LCMS 对化合物进行鉴定,结合文献报道、数据库(如 Sci Finder、Reaxys、Pub-Chem 等)及质谱信息,初步分析样品中花色苷类物质的化学组成,具体结果如图 8 所示。

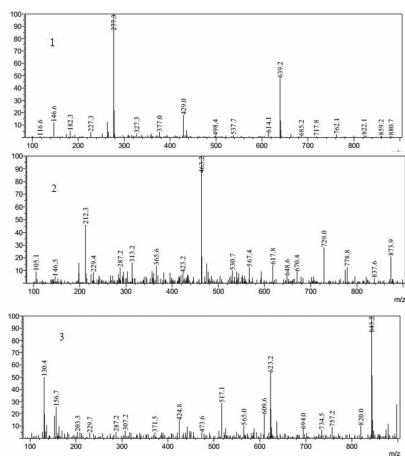


图 8 花色苷主要成分分析

Fig. 8 Chemical structures of major anthocyanins compounds in perilla leaves

由图 8 所示 ZS-8 紫苏资源中 3 种花色苷含量较多,峰 1 分子量为 639 D,为木犀草素-7-O-二葡萄糖醛酸酐(luteolin-7-O-diglucuronide);峰 2 分子量为 463 D,为野黄芩素(scutellarin);峰 3 分子量为 843 D,为矢车菊-3-(E)对香豆酸葡萄糖甙-5-丙二酰葡萄糖苷(Malonyl shisonin),均为花色苷类物质,其中峰 3 的含量最高。

3 结论

本研究中通过对不同叶色紫苏资源进行考察,筛选出一种花青素含量高的紫苏资源,其中 ZS-8 紫苏资源中花青素含量达到 4.7% 且种子及叶片产量均比较高,种子产量 2835 kg/hm²,风干叶片产量为 3795.1 kg/hm²。利用大孔吸附树脂进行花青素吸附纯化工艺研究,结果表明 XAD-7HP 树脂的吸附和洗脱能力较强,并对洗脱液的条件进行了考察,得出酸化乙醇(60%, v/v)溶液可以很好的洗脱花青素且洗脱率大于 70%,并对花青素的主要成分进行分析,纯化后的花青素溶液中糖、有机酸、蛋白质等杂质含量很少,花青素含量提高,可延长花青素的保质期。通过质谱分析得出分子量为 843 D(矢车菊-3-(E)对香豆酸葡萄糖甙-5-丙二酰葡萄糖苷)含量最高,其他含量较高的花色苷类物质为木犀草素-7-O-二葡萄糖醛酸酐和野黄芩素。

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(上接第 553 页)

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