文章编号:1001-6880(2015)8-1501-09

天然植物多糖分离纯化技术研究现状和进展

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摘　要:植物多糖因具有抗肿瘤、增强免疫、抗氧化等功效而备受关注,多糖的分离纯化是多糖结构和生物活性研究的首要前提。本文系统介绍了传统的水提醇沉法以及酶提取法、超声波辅助提取法、超临界流体萃取法、超高压提取技术微波辅助提取法和双水相萃取等新技术,对其分离原理、处理方式和效果进行分析比较和综述;从物理分离方式、分子间作用力及化学性质分离的角度,综述植物粗多糖分离和纯化方法及途径,为天然植物中多糖的分离纯化和综合利用提供参考。

关键词:天然植物;多糖;提取;纯化

中图分类号:R931.71　　**文献标识码**:A　　DOI:10.16333/j.1001-6880.2015.08.031

Review on Extraction and Purification Technology of Polysaccharides from Natural Plants

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Abstract:Increasing attentions have been paid to the natural polysaccharides because of their numerous bioactivities, such as anti-tumor, enhance immunity and antioxidant. The isolation and purification were the primary process for the further study on the structure and bioactivity of natural polysaccharides. In this paper, the isolation of natural polysaccharides in ways of traditional hot water extraction, enzymatic extraction, ultrasonic assisted extraction, supercritical fluid extraction, ultrahigh pressure extraction technology, microwave assisted extraction and aqueous two-phase extraction were comprehensively introduced. In addition, the further separation and purification methods were also reviewed based on mechanism of the purify process, including physical separation, intermolecular force and chemical purification. It provided foundational and valuable reference information for further investigation and efficient utilization of natural polysaccharides.

Key words:natural plants;polysaccharides;extraction;purification

多糖(polysaccharide)是生物体内除蛋白质和核酸之外的另一类具有重要作用的生物大分子,也是机体内不可缺少的组成部分。多糖在自然界分布广泛,与许多疾病病理过程以及衰老过程密切相关。探索多糖先导药物并加以改造、合成已成为当今生命科学界、医药学界的热点[1]。植物多糖具有多羟基醛的结构,由10个以上的单糖通过糖苷键聚合而成,分子量高、结构复杂、提取难度大、合成难度大,植物多糖的有效分离纯化是进行多糖结构和生物活性研究的首要前提。因此,为对植物多糖的结构及药理药效等进行深入研究,选择效率高的分离纯化方法具有重要的意义。

1　天然植物多糖的提取方法

植物多糖提取的宗旨是在不破坏多糖基本性质的基础上实现有效提取,多糖是极性大分子,一般不溶于有机溶剂,难溶于冷水,易溶于热水,多糖的传统提取方法多为热水提取。对于有些酸性多糖,或分子量较大的多糖,通常先用热水法提取,然后残渣再用稀碱溶液提取,这样可将大部分多种类型的多糖提取出来。热水提取法在多数植物多糖的提取中

收稿日期:2014-12-15　　接受日期:2015-05-07

基金项目:国家科技部"创新药物孵化基地课题"中药活性成分分离纯化平台项目(2010ZX09401-306-1-5);重庆市科委科技计划项目攻关项目(CSTC,2010AC5050);重庆市科委自然基金一般项目(CSTC,2013jcyjA80027)

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得到应用,如穿龙薯蓣多糖、香菇多糖及佛手多糖等的提取[2-4]。为提高多糖的提取效率,在传统的水提法基础上引入一些辅助手段如添加特异性的酶、超声、微波、高压等方法的使用,为不同环境下多糖的获取提供了可靠支持。

1.1 微波辅助提取法

多糖是一类亲水性大分子,与其他亲脂性物质在微波场中吸收微波能力存在差异,利用微波能的加热效应来加速溶剂对物料中多糖组分的溶解,可提高萃取效率并具有较好的选择性[5]。微波辅助法能缩短提取时间,减少溶剂用量、促进有效物质溶出,显著地提高提取效率,广泛应用于香椿叶多糖、山茱萸多糖和卷丹多糖的提取[6-8]。

1.2 超高压提取技术

天然植物中获取胞内多糖时,细胞壁在一定程度上阻碍了胞内多糖的提取效率,在常温下可利用超高压力作用于物料,迫使细胞壁破裂,释放出胞内多糖并溶于提取溶剂中达到提取的目的[9]。敬思群等[10]运用超高压技术提取金鸡菊多糖,极大的缩短提取时间,降低提取温度,保护多糖免受高温的影响。Chen[11]等采用超高压提取技术提取冬虫夏草多糖,通过响应面分析法(RSM)获得最佳的提取条件,保证多糖的生理活性的前提下最大限度获取冬虫夏草的多糖成分。

1.3 酶提取法

酶提取法是一种较温和并能有效提高多糖提取效率的提取方法,用酶处理天然植物的细胞壁组织,破坏其细胞壁结构,或切断多糖与组织中蛋白质的结合,可以促进多糖的有效提取。Chen 等[12]应用葡萄糖氧化酶法提取黄芪多糖,响应面分析法确定最佳的条件下的提取效率为 29.96 ± 0.14%。You 等[13]优化了酶法提取山茱萸多糖的条件使得多糖提取率为 9.29 ± 0.31%,与传统方法比较提取效率更高。

1.4 超声波辅助提取法

超声波辅助提取法是一种通过机械破壁而提高多糖的提取效率的方法,该法有别于酶辅助破壁的方法,其操作条件更简单且易控制,在当归多糖、红参果多糖和黄芪多糖的提取中都得到了充分的应用,提取时能耗明显降低[14-16]。但值得提出的是,多糖的活性很大程度上依赖其结构性质[17],超声提取时,功率过大、超声时间过长均可引起多糖结构的改变,多糖的生理活性也随之改变。

1.5 超临界流体萃取法

超临界流体萃取法以其温和的提取操作条件,提高多糖提取效率的同时保持多糖原始结构方面显示出优势。超临界流体萃取技术应用于天然多糖的提取,没有有机溶剂残留,且对提取出的多糖本身的结构没有破坏[18]。Chen 等[19]运用超临界萃取方法提取艾草种子的多糖组分,在设计的最佳提取条件下:提取压力为 45 MPa、温度为 45℃、二氧化碳流速为 20 L/h、提取时间为 2 h,多糖的提取效率为 18.59%(w/w)。

1.6 双水相萃取

双水相萃取技术在多糖的提取中应用相对较少,但其分离操作简单、条件温和,可在接近生理环境的条件下进行提取和分离,在萃取多糖时不会引起活性多糖性质的改变,目前多用于实验室研究,在工业化生产中该方法在也具有一定的实用前景。如在螺旋藻多糖和香菇多糖的分离时运用乙醇/硫酸铵双水相体系、聚乙二醇(PEG)-硫酸铵双水相体系进行萃取分离,结果对应多糖的产率分别达到 84.5% 和 57.42%,相较于传统方法具有很大的提高[20,21]。

实现天然植物多糖的有效提取是多糖分析的首要步骤,综述当前多糖的提取方法如表 1 所示。

表 1 天然植物多糖提取方法比较表

Table 1 Comparison of extraction methods for polysaccharides from natural plants

	方法 Method	原理 Principle	优缺点 Advantage and disadvantage	应用示例 Example	文献 Reference
传统法 Traditional method	水提醇沉 Water extraction and alcohol precipitation	不同醇浓度的条件下提取具有不同分子量的多糖组分 Different molecular weight of the polysaccharide components were extracted under the condition of different concentration of alcohol	适于提取粗多糖或是不同分子量段的粗多糖;提取率低,操作复杂 Suitable for extract of crude polysaccharide or crude polysaccharide with different molecular, extraction rate was low, operation was complex	穿龙薯蓣多糖 *Dioscorea nipponica* Makino Polysaccharide、香菇多糖 *Lentinus edodes* Polysaccharide、佛手多糖 *Finger citron* Polysaccharide	[2-4]

现代提取技术 Modern extraction technology	微波辅助提取法 Microwave assisted extraction	利用物质在微波场中吸收微波能力差异,使得基体物质的某些区域或某些组分被选择性溶出 Different materials had different abilities of absorption of microwave in the microwave field, which made some areas and components extracted or dissoluted selectively	选择性高、操作时间短、溶剂消耗量少、有效成分得率高 Selectivity was high, operation time was short, solvent consumption was less, the yield of effective components were high	香椿叶多糖 *Toona sinensis* Polysaccharide、山茱萸多糖 Corni Fructus Polysaccharide、卷丹多糖 *Tiger lily* Polysaccharide	[6-8]
	超高压提取技术 High pressure extraction	超高压力使细胞壁破裂内部有效成分释放和溶出 Active ingredients inside the cell wall were released and dissoluted by ultra-high pressure	提取率高,提取时间短,工艺操作简单,能耗低,提取液杂质少,容易分离纯化 Extraction efficiency was high, extraction time was short, operation was simple, energy consumption was low, impurity was less and purification was easy	金鸡菊多糖 *Coreopsis basalis* Polysaccharide、冬虫夏草多糖 *Cordyceps militaris* Polysaccharide	[10,11]
	酶提取法 Enzyme extraction	具有一定的选择性和催化活性,在较温和条件下破坏细胞壁结构 The method had certain selectivity and catalytic activity, cell wall structure was destroyed in relatively mild condition	反应条件温和、产物活性高、提取时间短、易控制且提取率较高 Reaction condition was mild, product activity was high, extraction time was short, and it was easy controlled and had higher extraction yield	黄芪多糖 *Astragalus membranaceus* Polysaccharide、山茱萸多糖 *Cornus officinalis* Polysaccharide	[12,13]
	超声波辅助法 Ultrasonic assisted extraction	超声波空化作用使有效成分溶出 Effective ingredients were dissoluted by ultrasonic cavitation effect	提取时间短,收率高 Extraction time was short and yield was high	当归多糖 Angelica Polysaccharide、红果参多糖 Hong Guo Ginseng Polysaccharide、黄芪多糖 Astragalus Polysaccharide	[14-16]
	超临界流体萃取法 Supercritical fluid extraction	超临界流体萃取 Extracted by supercritical fluid	特别适合不稳定天然产物和生理活性物质的分离与精制;有效成分纯度高、杂质少 Particularly suitable for unstable separation and refined of natural products and biological active substances, active ingredients were of high purity, impurities was less	艾草多糖 *Artemisia sphaerocephala* Krasch Polysaccharide	[19]
	双水相萃取 Aqueous two-phase extraction	不同两相系统间分配系数的差异 Different distribution coefficient between two phase system	萃取时间短,条件温和,操作方便易行 Extraction time was less and extraction condition was mild, operation was convenient	螺旋藻多糖 *Spirulina platensis* polysaccharide 香菇多糖 *Lentinan*	[20,21]

上述多种提取法在提取效率上优于传统提取方法,但必须注意的是在多糖提取时必须保证多糖的基本性质不发生改变,如微波、超声、酶解都有可能造成多糖结构的改变,而糖类成分结构复杂,性质各异,相应的提取方法也就很难整齐划一。因此,需了解物种特性、依据实际情况并结合文献调研来设计提取方案。

2　天然植物多糖的纯化技术

天然植物有效提取后获得的粗多糖尚难以满足多糖结构和活性研究的需要,必须采用适当的手段除去非糖类杂质,并将混合多糖分离纯化成化学组成、聚合度和分子形状相同的均一多糖。多糖的纯化方法可按纯化机制和过程大致可分为三类:一是基于分子量大小和溶解度差异的物理分离过程,二是基于分子间作用力的柱层析分离纯化法,第三即化学沉淀法。

2.1　物理分离过程

2.1.1　膜分离法

膜分离技术是以压力差、浓度差、电位差等为动

力，依据相对分子量的不同进行的分离过程，具有分子筛作用，常用于除杂和分离纯化大分子物质。如采用无机陶瓷微滤膜处理虫草菌丝体的粗多糖溶液可以很好的实现去除溶液中的无机盐和小分子肽等物质[22]。分离丹皮多糖、灵芝多糖、白术多糖及青钱柳多糖等[23-26]选用不同截留分子量的超滤膜可以实现多糖的分级，获得不同分子量段的多糖。截留范围更低的纳滤膜则可用于低聚糖的精制和分离，如六味地黄方药液活性多糖[27]的分离。膜分离代替柱层析法对混合多糖按照分子量进行分离具有明显的优点：一是膜分离设备简单，能耗低，在良性使用环境下，可以连续使用，并保持较好的分离效果；二是不损坏多糖的活性，分离过程不引入化学试剂，对多糖本身无污染。然而，活性多糖属于结构复杂的大分子化合物，有黏性，在膜分离过程中加重浓差极化和膜污染等造成的膜渗透通量下降，更易产生膜污染和堵塞，在实际应用中其分离提纯难度较大，此为膜分离应用必须解决的关键问题[28-30]。

此外，利用分子大小差异的分离方法还有基于多糖在超速离心的条件下沉积比率不同的规律而得以分离的超速离心法，虽然该方法对多糖本身的破坏小，分离效果好，但是仪器要求高，操作困难。

2.1.2 分步沉淀和盐析纯化法

分步沉淀通常指根据不同分子量多糖在不同浓度乙醇溶液中的溶解度不同，依此增加醇浓度，从而将多糖按分子量从大到小的沉淀出来的分离过程。分步沉淀实际上可视为一种同时利用分子量大小和溶解度差异的分离过程。该分离方法在多糖的研究中使用最为广泛，同时，利用该方法还可以脱除一些脂溶性的色素等杂质[31,32]。

盐析是另一种常见的利用溶解度差异的分离方法，根据不同多糖在不同浓度盐溶液中具有不同溶解度的性质，加入不同盐析剂使不同多糖逐步析出。该方法也属于分步沉淀，不同之处在于该法使用的是不同浓度的盐溶液而非醇溶液，该法通常仅限于酸性多糖的分离。常用的盐有 NaCl、KCl、$(NH_4)_2SO_4$，其中以$(NH_4)_2SO_4$最佳[33]。

2.2 层析（色谱）分离法

在天然产物的分离纯化研究中，柱层析是常见的分离纯化方法，多糖纯化所使用的柱层析固定相有别于其他诸如黄酮、蒽醌、萜类等小分子成分，根据目标物的理化性质，选择最合适的固定相和流动相以实现目标物的高效分离纯化。在多糖的柱层析分离纯化中常见的固定相有纤维素、DEAE-纤维素、交联葡聚糖、琼脂糖、亲和葡聚糖凝胶等。根据待分离多糖与使用的固定相填料之间的作用力不同可将柱层析分为纤维素柱层析、离子交换柱层析、凝胶柱层析、亲和柱层析，其中离子交换柱层析和凝胶柱层析应用最为广泛[34-41]。

纤维素柱层析法适于分离各种酸性多糖和中性多糖，在多糖的除色素中也常常使用[42]。纯化时常选用不同浓度的洗脱液阶段洗脱，首先是小分子被洗脱，然后是大分子的多糖。此法效果好，多糖纯度高，溶剂消耗量大，但分离耗时，尤其分离粘性多糖时易粘附流速更慢。离子交换柱层析是目前多糖纯化中应用最广的一种方法，适合于分离各种酸性、中性粘多糖，如带负电荷的多糖可在阴离子型 DEAE-cellulose 柱或 DEAE-Sephadex 柱上达到分级[43]。特别是对于体积较大的多糖，大多采取阴离子交换柱层析纯化，甚至可以分离到均一组分，如穿龙薯蓣粗多糖[2]、巴豆多糖[44]、白桦茸水溶性多糖[45]、白毛藤多糖[46]、四角蛤蜊多糖[47]及龙眼多糖[48]。使用较为普遍的交换剂有 DEAE-纤维素（DEAE-cellulose）、DEAE-葡聚糖（DEAE-Sephadex）和 DEAE-琼脂糖（DEAE-Sepharose）。凝胶柱层析是目前植物多糖最常用的纯化方法，实际上起分子筛作用，不同质量的多糖按分子量从大到小的顺序被洗脱出来，达到纯化的目的。其优点是简单、条件温和、快速，但不适宜粘多糖的分离。亲和层析需要将具有一定结构的亲和分子制成固定相吸附剂来对被分离物质进行吸附分离，因此具有特定结构的亲和分子“配基”显得尤为重要，由于植物多糖的复杂性，有些粘性多糖的粘性大，容易在层析柱上产生吸附，配基的选择也比较困难，因此这种层析方法的应用不是很多。

2.3 化学沉淀法

多糖作为一类大分子物质，在某些多糖的长链结构中可能会包含一些特殊取代基或基团如羧基、硫酸基等，使这些多糖具有特殊的化学性质，利用这些特性使多糖发生简单的化学反应并从溶剂中沉淀出来以实现多糖的有效分离。

长链季铵盐能与酸性多糖成盐形成水不溶性多糖季铵盐，以分离酸性及中性多糖的季铵盐沉淀法即为常见的化学沉淀法之一。在季铵盐沉淀法中，酸性强或分子量大的酸性多糖首先沉淀出来，控制季铵盐的浓度，也能分离各种不同的酸性多糖。常用的季铵盐是十六烷基三甲基铵的溴化物（CTAB）

及其碱(CTA-OH)和十六烷基吡啶(CPC),如张锐等[49]对柚子皮果胶多糖提取时运用十二烷基伯胺乙酸盐盐析法。

金属络合法则是另一种较为常见的用于多糖分离的方法,其分离过程是根据某些多糖能与金属离子如铜、钡、钙和铅等形成配合物而沉淀,将配合物沉淀充分洗涤后,用无机酸乙醇液或硫化氢处理,重新获得游离的多糖。常用的络合剂有含有 Cu^{2+} 的斐林试剂、$CuCl_2$、$Ba(OH)_2$ 等。这一类基于简单化学反应的分离方法在分离化学性质差异明显的多糖时效果显著,但分离过程中需要严格控制反应程度,避免多糖结构的不可逆改变而影响其生理活性。

2.4　其他纯化方法

电泳法(制备型区域电泳)也可用于酸性多糖的分离、鉴定或提纯,其分离效果较好。但目前电泳技术应用于多糖研究存在缺陷,在多糖领域的应用上多是用来鉴定纯度,用于多糖分离时时间较长,分离量少[50]。

综上所述,中药多糖的纯化技术较多,利用多糖的理化性质进行物理的及化学的纯化方法,各纯化方法各有优缺点,文中将各种纯化技术总结汇总于表2。从各分离纯化方法的相关应用中可以看到,在对多糖实际样本进行纯化时往往不是上述几种方法的简单应用,一种方法往往不能一次性地获得均一性的多糖组分,只有综合利用几种方法才能达到理想的纯化效果。多糖的纯化应根据不同种类中药材的性质选择合适的纯化方法,尽量避免一种纯化方法劣势的同时充分联合应用两种或多种纯化方法。

表2　植物多糖的分离纯化方法比较表

Table 2　Comparison of purification methods for polysaccharides from natural plants

方法 Method	机制 Mechanism	适用范围 Range of application	效果 Effect	优缺点 Advantage and disadvantage	实例 Example	文献 Reference
膜分离 Membrane separation	筛分机制 Screening mechanism	不同分子量多糖 Different molecular weight of polysaccharide	获得不同分子量多糖 Different molecular weight of polysaccharides	获得不同分子量段的多糖;膜孔易堵塞、模型号的选择困难 Different molecular weight of polysaccharides were got, but the membrane was easily to be jammed and the selection of membrane was hard	虫草菌丝体粗多糖 *Cordyceps mycelia* Polysaccharides、丹皮多糖 *Moutan cortex* Polysaccharide、灵芝多糖 *Ganoderma lucidum* Polysaccharide、白术多糖 *Atractylodes macrocephala* Koidz Polysaccharide、青钱柳多糖 *Cyclocarya paliurus* Polysaccharide、六味地黄多糖 Liuwei Dihuang Polysaccharide	[22-27]
溶剂沉出 Solvent settling out	利用多糖在某些溶剂中具有不同溶解性的性质 Solubility of polysaccharide was different in some solvents	不同分子量的粗多糖 Different molecular weight of crude polysaccharide	得到不同分子量多糖 Different molecular weight of polysaccharide	方法简单,获得不同分子量段多糖,分子量范围不明确 The method was simple and different molecular weight polysaccharide could be got, but the range of molecular weight was not clear	佛手多糖 *Finger citron* Polysaccharide、牛肝菌多糖 *Boletus edulis* Polysaccharide	[31, 32]
盐析法 Salt fractionation	分子量不同的多糖在一定浓度的盐溶液中具有不同溶解度 Different molecular weight of polysaccharide with different solubility in a certain concentration of salt solution	适用大多数粗多糖 For most of the crude polysaccharide	分子量不同的多糖 Different molecular weight of polysaccharides	盐析法的优点是成本较低;缺点是效率不高,易产生共沉淀 Salting out method had the advantage of lower costsThe disadvantage was that efficiency was not high and it was easy to produce coprecipitation	猴头菌多糖 *H. caputmedusae* (Bull. Fr.) Pets. Polysaccharide	[33]

纤维素柱层析 Cellulose column chromatography	吸附并有分子筛作用 Adsorption and molecular sieves	酸性、中性多糖 Acidic and neutral polysaccharide	获得不同分子量范围的均一多糖 Different molecular weight of uniform polysaccharide	多糖纯度高;缺点是流速慢,纯化周期长,特别是对于粘度较大的酸性多糖 Purity was high, but the flow rate was slow, purification cycle was long, especially for larger viscosity acidic polysaccharides	丹参多糖 *Salviam iltiorrhizaby* Polysaccharide	[34]
凝胶柱层析 Gel column chromatography	根据多糖分子的大小和形状的不同即按分子筛的原理进行 Depending on the size and shape of the polysaccharide molecules that according to the principle of molecular sieve	适用大多数粗多糖,按被分析混合物不同组分分子大小的不同进行分离 For most of the crude polysaccharide, the different constituents had been separated according to different molecular size	不同分子量范围的均一多糖 Different molecular weight range of uniform polysaccharide	快速、简便、分离效果好;缺点是分离条件苛刻,受洗脱剂等因素影响,不适宜粘多糖 Quick, easy and separation effect was good but separation condition was rigorous, easily been influenced by such factors as the eluent and not suitable for the separation of mucopolysaccharide	车前子多糖 *Plantago depressa* polysaccharide、绞股蓝多糖 *Gynostemma pentaphyllum* Makino Polysaccharide、榴莲多糖 *Durian rinds* Polysaccharide、苦瓜多糖 Momordica Polysaccharide	[36-39]
离子交换柱层析 Ion exchange chromatography	离子交换原理、可逆交换吸附和解吸 The principle of ion exchange, exchange of reversible adsorption and desorption	酸性、中性多糖及粘多糖等不同多糖尤其是多糖与蛋白质结合在一起的复合多糖 Acidic and neutral polysaccharide and mucopolysaccharide, especially compound polysaccharide of polysaccharide and protein combined	均一性多糖 Uniform polysaccharide	交换容量更大,分离效果更好;缺点是价格昂贵,且会因洗脱液 PH 值或离子强度变化而造成体积变化较大,因而影响流速 The exchange capacity and separation effect was better but the velocity could be impacted for the eluent changes because of strength change of PH and ionic	穿龙薯蓣多糖 *Dioscorea nipponica* Makino Polysaccharide、巴豆多糖 *Croton urucurana* Polysaccharide、白桦茸多糖 *Inonotus obliquus* Polysaccharide、白毛藤多糖 *Solanum lyratum* Polysaccharide、四角蛤蜊多糖 *Mactra veneriformis* Polysaccharide、龙眼多糖 Longyan Polysaccharide	[2][44-48]
亲和层析 Affinity chromatography	分子间的亲和作用 The affinity effect between molecules	和配基具有亲和力的多糖分子 And polysaccharide molecular ligands with affinity	不同性质均一性多糖 Homogeneity polysaccharides with different properties	效率高,操作简单,分离含量较少的多糖,一次可以浓缩几百倍,甚至几千倍;缺点是配基寻找困难 Efficiency was high, operation was simple, the content of polysaccharide was less, concentration could be hundreds even thousands of times and disadvantage was that the ligands were hard to find	–	–
季铵盐沉淀法 Quaternary ammonium saltprecipitation	长链季胺盐能与酸性多糖或长链高分子量多糖形成络合物,在低离子强度的水溶液中沉出 Precipitation was settling out in low ionic strength solution, for complex formation could appear between long chain quaternary ammonium salt and acidic polysaccharide or high molecular weight polysaccharide	适用大多数粗多糖 For most of the crude polysaccharide	酸性及中性粗多糖 Acidic and neutral polysaccharide	方法简便易行;缺点是损失较大,得率低 The method was simple and easy, disadvantage was that loss was bigger, the yield was low	柚子果皮多糖 Grapefruit peel Polysaccharide	[49]

金属络合法 Metal complexing method	多糖能与各种铜、钡、钙和铅离子形成络合物而沉淀 Polysaccharide with all kinds of copper, barium, calcium and lead ions to form complex and precipitation	适用大多数粗多糖 For most of the crude polysaccharide	不同性质的游离多糖 Different properties of the free polysaccharide	方法简单;缺点多糖得率低 The method was simple, yield was low	–	–
凝胶电泳法 Gel electrophoresis	带点离子在电场中定向迁移 Ion moves with directional migration in electric field	分离不同物理性质(如大小、形状、等电点等)的分子 The molecules were separated based on different physical properties (such as size, shape, isoelectric point, etc.)	均一性多糖 Uniform polysaccharide	分离效果好、重现性好、方法简单;缺点是产量低,设备要求高 Separation effect and reproducibility were good, method was simple, but the product was less, equipment requirement was high	螺旋藻多糖 *Spirulina plantensis* Polysaccharide	[50]

3 总结及展望

随着人们对糖类的认识不再局限于能源和细胞结构材料,研究者们对多糖研究的无限可能充满期待。多糖所蕴藏的结构的多样性和复杂性远远超过了核酸或蛋白质的结构,一方面使其成为最有魅力的信息载体,另一方面也给我们的研究带来了巨大的困难,包括结构解析、构效关系等。但所有的研究难点都需要一个突破口——即采用高效的提取、分离纯化手段从天然产物中有效获取结构稳定、化学组成、聚合度均一的多糖。尽管本文对近年来多种提取及纯化方法被用于中药多糖成分的提取分离纯化进行了介绍,但由于多糖的复杂性,选择合适的方法得到具有较好均一性的多糖仍然是多糖研究领域的难题。未来天然多糖研究中,可根据不同来源、不同类别的多糖,兼顾其生物活性,建立起与之相应的一种高效稳定的分离纯化方法,避免在其后续的结构和生物活性研究中因分离纯化的差异而导致的不确定性。

参考文献

1 Finkelstein J. Glycochemistry and glycobiology. *Nature*, 2007, 446:999.

2 Luo DH. Structural investigation of a polysaccharide (DMB) purified from *Dioscorea nipponica Makino*. *Carbo Poly*, 2014, 103:261-266.

3 Wang KP, *et al*. Structural differences and conformational characterization offive bioactive polysaccharides from *Lentinus edodes*. *Food Res Int*, 2014, 62:223-232.

4 Heng ZC, *et al*. Water-soluble polysaccharides from finger citron fruits (*Citrus medica L. var. sarcodactylis*). *Carbo Res*, 2014, 388:100-104.

5 Kratchanova M, *et al*. The effect of microwaveheating of fresh orange peels tissue and quality of extracted pectin. *Carbo poly*, 2004, 56:181-185.

6 Chen M (陈丛瑾), *et al*. Study on the hot water extraction process of polysaccharide from *Toona sinensis* (*A. Juss.*) *Roem.* leaves assisted by microwave pretreatment. *Food Sci Technol* (食品科技), 2010, 35:178-182.

7 Wu YF (吴艳芳), *et al*. Study on microwave-assisted extraction of polysaccharides from *Corni Fructus*. *Hubei Agric Sci* (湖北农业科学), 2011, 50:570- 572.

8 Chen ZG, *et al*. Purification, preliminary characterization and in vitro immunomodulatory activity of *Tiger lily* polysaccharide. *Carbo Poly*, 2014, 106:217-222.

9 Huang HW, *et al*. Advances in the extraction of natural ingredients by high pressure extraction technology. *Trends Food Sci Technol*, 2013, 33:54-62.

10 Jing SQ (敬思群), *et al*. Optimization of the technology condition of ultra-high pressure on extraction polysaccharides from *Coreopsis basalis*. *Food Sci Technol* (食品科技), 2013, 38:168-172.

11 Chen RZ, *et al*. Ultrahigh pressure extraction of polysaccharides from *Cordyceps militaris* and evaluation of antioxidant activity. *Sep Purific Technol*, 2014, 134:90-99.

12 Chen HG, *et al*. Optimization of enzyme assisted extraction of polysaccharides from *Astragalus membranaceus*. *Carbo Poly*, 2014, 111:567-575.

13 You QH, *et al*. Enzyme assisted extraction of polysaccharides from the fruit of *Cornus officinalis*. *Carbo Poly*, 2013, 98:607-

610.
14 Han RS（韩荣生）,*et al*. Study of the extraction technical conditions of *Angelica* polysaccharide. *World J Integ Tradit Western Med*（世界中西医结合杂志）,2011,6:234-237.
15 Chen LH（陈莉华）,*et al*. Extraction and purification of polysaccharides from *Hong Guo Ginseng* and the comparison of antioxidant activity. *Nat Prod Res Dev*（天然药物研究与开发）,2012,25:170-173.
16 Xu Y（徐艳）,*et al*. Extract *Astragalus* polysaccharides by ultrasonic cell disrupter. *Chin J Inf TCM*（中国中医药信息杂志）,2007,14(3):44-45.
17 Peng YF,*et al*. Structure and antitumor activities of the water-soluble polysaccharides from *Ganoderma tsugae mycelium*. *Carbo Poly*,2005,59:385-392.
18 Sahena F,*et al*. Application of supercritical CO_2 in lipid extraction - A review. *J Food Eng*,2009,95:240-253.
19 Chen J,*et al*. Supercritical CO_2 extraction and pre-column derivatization of polysaccharides from *Artemisia sphaerocephala Krasch*. Seeds via gas chromatography. *Indus Crops Prod*,2014,60:138-143.
20 Liu Y（刘杨）,*et al*. Extraction of *spirulina platensis* polysaccharide by ethanol /$(NH)_2SO_4$. *Chem Res Appl*（化学研究与应用）,2012,24:1781-1785.
21 Xu HJ（徐海军）,*et al*. Extraction of lentinan by aqueous two-phase system. *Nat Prod Res Dev*（天然产物研究与开发）,2013,25:982-985.
22 Han YP（韩永萍）,*et al*. Research on inorganic ceramic micro-filtration membrane treating with rough *Cordyceps mycelia* polysaccharides solution. *Food Ferment Ind*（食品与发酵工业）,2008,34(2):90-93.
23 Wang ZY（王子尧）,*et al*. Study on separation of polysaccharides from *moutan cortex* by ultrafiltration technique. *Mem Sci Technol*（膜科学与技术）,2009,29:99-100.
24 Yang ZJ（杨祖金）,*et al*. Purification of *Ganoderma lucidum* polysaccharide by ultrafiltration technology. *J Chin Med Mater*（中药材）,2009,32:126-129.
25 Zhou JR（周家容）,*et al*. Isolation of *Atractylodes macrocephala Koidz* polysaccharides with ultrafiltration membrane and study of their antioxidant activity. *J Southwest Univ*,*Nat Sci Ed*（西南大学学报）,2009,31(4):79-82.
26 Jian HX,*et al*. Separation of water-soluble polysaccharides from *Cyclocarya paliurus* by ultrafiltration process. *Carbo Poly*,2014,101:479-483.
27 Zhong L（钟蕾）,*et al*. Effect analysis of the concentration of *Liuwei Dihuang* polysaccharides by the membrane separation technique and the traditional method. *J Pharm Prac*（药学实践杂志）,2007,25:386-388.
28 Alain M,*et al*. Extraction and fractionation of polysaccharides and B-phycoerythrin from the microalga *Porphyridium cruentum* by membrane technology. *Algal Res*,2014,5:258-263.
29 Mi JZ（米吉造）. Studies on Application of Membrane separation technology production of Vitamin C. Tianjin: Tianjin University（天津大学）,MSc. 2003.
30 Enrico D,*et al*. Membrane engineering in process intensification—An overview. *J Mem Sci*,2011,380:1-8.
31 Wu Z,*et al*. Extraction optimization,preliminary characterization,and in vitro antioxidant activities of crude polysaccharides from *finger citron*. *Ind Crops Prod*,2013,44:145-151.
32 Wang D,*et al*. Characterization of a water-soluble polysaccharide from *Boletus edulis* and its antitumor and immunomodulatory activities on renal cancer in mice. *Carbo Poly*,2014,105:127-134.
33 Sun WY（孙文怡）,*et al*. Optimization of purification technique conditions of fruiting body polysaccharide of *H. caput-medusae*（*Bull. Fr.*）*Pets.* using salting-out method. *J Jilin Norm Univ*,*Nat Sci*（吉林师范大学学报,自科版）,2011,3:88-91.
34 Tang W（汤伟）,*et al*. Study on purification of polysaccharides in root of *Salviam iltiorrhizaby* macroporous adsorption resin and ion-exchange resin methods. *J Chin Med Mater*（中药材）,2010,33:1937-1941.
35 Xie MY（谢明勇）,*et al*. A Review about the research of structure and function of polysaccharides from natural products. *J Chin Insti Food Sci Technol*（中国食品学报）,2010,10(2):1-11.
36 Zhao H,*et al*. Purification,characterization and immunomodulatory effects of *Plantago depressa* polysaccharides. *Carbo Poly*,2014,112:63-72.
37 Chi AP,*et al*. Morphological and structural characterization of a polysaccharide from *Gynostemma pentaphyllum Makino* and its anti-exercise fatigue activity. *Carbo Poly*,2008,74:868-874.
38 Sanya H,*et al*. Water-soluble polysaccharides with pharmaceutical importance from Durian rinds（*Durio zibethinus Murr.*）:isolation,fractionation and bioactivities. *Carbo Poly*,2004,56:471-481.
39 Zhou R（周锐）,*et al*. Purification and hepatoprotective effect of *Momordica* polysaccharides. *Food Sci Technol*（食品科技）,2013,38:173-176.
40 Fang JN（方积年）,*et al*. Bioactivities,isolation and purification methods of polysaccharide. *Chin J Nat Med*（中国天然药物）,2007,5:338-347.
41 Halina L,*et al*. Affinity chromatography for the purification of lectins（a review）. *J Chromatogr A*,1981,215:361-372.

42 Shen J (沈洁), *et al*. Research progress in extraction, chemical modification and anticancer activity of fungi polysaccharides. *Nat Prod Res Dev* (天然产物研究与开发), 2014, 26:1723-1727.

43 Zhang L, *et al*. Purification, antioxidant and immunological activities of polysaccharides from *Actinidia Chinensis roots*. *Inter J Bio Macro*, 2015, 72:975-983.

44 Begona M, *et al*. Characterization of a fucoarabinogalactan, the main polysaccharide from the *Gum Exudate of Croton urucurana*. *J Nat Prod*, 2002, 65:1143-1146.

45 Fan LP, *et al*. Antitumor and immunomodulatory activity of water-soluble polysaccharide from *Inonotus obliques*. *Carbo Poly*, 2012, 90:870-874.

46 Mao JS (毛建山), *et al*. Isolation, purification, and identification of polysaccharide of *Solanum lyratum*. *Chin Trad Herb Drugs* (中草药), 2005, 36:654-656.

47 Ling CW, *et al*. Isolation and structural elucidation of novel homogenous polysaccharide from *Mactra veneriformis*. *Carbo Poly*, 2011, 86:982-987.

48 Zhang MW, *et al*. A method of separation of neutral polysaccharide from *longyan* (一种中性龙眼多糖级分的分离方法). CN 201010603686.5, 2010-12-24.

49 Zhang R (张锐), *et al*. Extraction of polysaccharides from grapefruit peel by salting-out with dodecylamine acetate salt. *Sci Tech Food Ind* (食品工业科技), 2006, 27:120-122.

50 Zheng ZH (郑震寰), *et al*. Studies on the electrophoretic methods of acidic polysaccharide from *Spirulina plantensis*. *Pharm Biol* (药物生物技术), 2004, 11:104-107.

(上接第 1415 页)

9 Liu YH (刘一衡), Yang L (杨玲). Optimization ultrasonic extraction of total alkaloids from Xinjiang black mulberry leafs based on orthogonal design. *Sci Technol Food Ind* (食品工业科技), 2013, 34:306-308.

10 Tian YL (田义龙), Zhao J (赵静), Ren YQ (任艳青), *et al*. Effect and mechanism of gardeniae and sojae praeparati decoction on improving insulin resistance. *Pharmacol Clin Chin Mater Med* (中药药理与临床), 2010, 26(6):5-7.

11 Hanley AJ, Williams K, Gonzalez C, *et al*. Prediction of type 2 diabetes using simple measures of insulin resistance: combined results from the san Antonio heart study, the mexico city diabetes study, and the insulin resistance atherosclerosis study. *Diabetes*, 2003, 52:463-469.

12 World Health Organization. Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications. Genera: World Health Organization, 1999.

13 Maimaiti YM (买买提依明), Lu H (卢红), Muhe DE (木合达尔), *et al*. Study on the determination of effective components and pharmacological activity of *Morus nigra*. *North Sericul* (北方蚕业), 2006, 27(4):16-18.

14 Meng QH (孟庆海), Yin QY (殷秋忆), Guo J (郭静), *et al*. A screening study on blood glucose in diabetic mice of four mulberry extracts. *Chin Tradit Pat Med* (中成药), 2014, 36:1288-1291.

15 Chen FJ (陈福君), Lu J (卢军), Zhang YY (张永煜). Pharmacological studies on *Morus* (I): Effect of total polysaccharide of *Morus* (TPM) on carbohydrate metabolism in diabetic mice. *J Shenyang Pharm Univ* (沈阳药科大学学报), 1996, 13:24-27.

(上接第 1431 页)

11 Sun J(孙杰), Wang YJ(孙艳杰), Zhu LY(朱路英). Study on antifungal and antioxidant activities of alcohol axtract of *Gelidium amansii*. *J Food Sci*(食品科学), 2007, 28(10): 53-56.

12 Wang X(王晓), Jiang Y(姜艳), Su B(苏波), *et al*. Study on seaweed effective extraction using solvent reflux method. *Ocean Technol* (海洋技术), 2007, 26:58-60.

13 Liu WB(刘文波). Establishment of animal hyperuricemia model and selection of Chinese traditional medicine for anti-gouts. Jinan: Shandong University(山东大学), PhD. 2008.

14 Zhou HX(周宏星), Chen YS(陈玉胜). Activity and mechanism research of bergenin anti-hyperuricemic. *J Anhui Pharm Univ*(安徽医科大学学报), 2014, 49(1):63-66.

15 Sánchez-Lozada LG, Tapia E, Avila-Casado C, *et al*. Mild hyperuricemia induces glomerular hypertension in normal rats. *Am J Phy*, 2002, 283:1105-1110.

16 Cui HH(崔海辉). The process technology of *Gelidium amansii* health drink. *J Zhejiang Agric Sci*(浙江农业科学), 2011, 3:590-592.

17 Huang JQ, Wang SW, Zhu MZ, *et al*. Effects of genistein, apigenin, quercetin, rutin and astilbin on serum uric acid levels and xanthine oxidase activities in normal and hyperuricemic mice. *Food Chem Toxicol*, 2011, 49:1943-1947.

18 Lin ZJ, Zhang B, Liu XQ, *et al*. Abdominal fat accumulation with hyperuricemia and hypercholesterolemia quail model induced by high fat diet. *Chin Med Sci J*, 2009, 24:191-194.