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· 专题综述 ·

纳米甲壳素的制备及在食品领域中应用的研究进展

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摘 要: 纳米甲壳素作为一种资源丰富且可再生的生物聚合物, 因优异的物理和化学特性在食品领域备受关注。本文首先详述纳米甲壳素的来源、制备方法及其结构特性, 重点对近年来国内外关于其在食品活性包装、乳化稳定剂、功能因子载体等方面的应用进行梳理总结。同时借助文献计量学方法深入解析纳米甲壳素在食品领域的研究热点, 对其未来的发展方向进行了展望, 旨在为纳米甲壳素的综合开发利用提供参考。

关键词: 纳米甲壳素, 制备方法, 结构性质, 食品领域, CiteSpace 可视化分析

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Advances in the Preparation and Application of Nanochitin in the Food Industry

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Abstract: Nanochitin, a plentiful and renewable biopolymer, has attracted considerable interest in the food industry due to its excellent physical and chemical properties. This paper initially outlines the sources, preparation methods, and structural properties of nanochitin, then focuses on summarizing its recent applications in food active packaging, emulsification stabilizers, functional factor carriers, other fields both domestically and internationally. Additionally, the paper utilizes bibliometric methods to conduct an in-depth analysis of the research hotspots pertaining to nanochitin within the food sector, projecting its future development trajectories. This paper aims to furnish insights for the comprehensive development and utilization of nanochitin.

Key words: nanochitin (NCh); preparation method; structural properties; food field; CiteSpace visualization analysis

甲壳素(Chitin)是仅次于纤维素的第二大天然高分子多糖, 是由 2-乙酰氨基葡萄糖通过 β -1,4 糖苷键连接而成的线性聚合物多糖, 广泛地存在于虾蟹等甲壳动物及乌贼、贝类等软体动物的骨骼中^[1-4]。甲壳素具有可再生性^[5]、生物相容性^[6]、环境友好^[7]、生物降解性^[8]、无毒^[9]、抗菌活性^[10] 等特性, 已被广泛用

于乳化稳定剂^[11]、食品营养添加剂^[12]、活性包装膜^[13] 和凝胶^[14-16] 等方面。但是, 由于甲壳素分子链间存在大量分子间和分子内氢键相互作用导致其溶解性和加工性差, 限制了其在食品领域中的广泛应用。随着纳米技术的发展, 其在食品领域中的应用备受关注。纳米甲壳素(Nanochitin, NCh)是一类用物

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理或化学方法打破甲壳素的长链结构而形成的纳米级甲壳素材料。NCh 不仅具有甲壳素的性质, 而且能够在水溶液中形成均匀分散液, 同时还具有纳米材料的特性, 包括小尺寸、低密度、高表面积、优异的生物相容性和力学性能、可再生性等^[17], 已被研究应用于复合材料^[18]、医药^[19] 和食品^[20] 等领域。同时, NCh 表面含有羟基、氨基等活性官能团易于进行化学修饰, 从而进一步提高 NCh 的应用潜力。不同的制备方式对 NCh 的形态特征、物理化学性质产生显著的影响, 使其呈现出不同的特性。根据结构和形态可以将 NCh 分成甲壳素纳米晶须(Chitin nano-whiskers, CNWs)和甲壳素纳米纤维(Chitin nanofibers, ChNFs)两大类。在食品领域中, NCh 因其优越的力学性能、阻隔性、两亲性和多羟基官能团等特点^[21], 使其在新型食品活性包装、乳化稳定剂、功能因子载体、品质改良剂等方面的应用受到广泛的关注。

本文对近年国内外 NCh 在食品领域方面的研究进行梳理, 重点阐述其在新型食品活性包装、乳化稳定剂、食品功能因子递送体系等方面的应用进展, 结合 CiteSpace 可视化分析当前 NCh 领域的研究热点, 同时对 NCh 未来发展进行展望, 以期为 NCh 的进一步应用研究提供参考。

1 纳米甲壳素的分类及制备方法

1.1 纳米甲壳素的分类

纳米甲壳素根据结构和形态可以分成甲壳素纳米晶须(Chitin nano-whiskers, CNWs)和甲壳素纳米纤维(Chitin nanofibers, ChNFs), NCh 的结构与分类的外貌形态如图 1 所示。

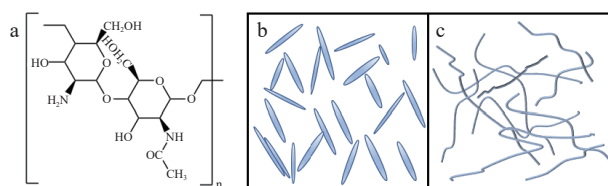


图 1 纳米甲壳素的制备结构式(a)、甲壳素纳米晶须(b)和甲壳素纳米纤维(c)形态图^[29]

Fig.1 Preparation structure formula of NCh (a), schematic of CNWs (b) and ChNFs (c) morphologies^[29]

CNWs 是通过酸解工艺除去甲壳素纤维中无序部分而形成具有高结晶度的棒状或针状纳米晶体, 通常长度在 150~2200 nm 之间, 宽度为 10~50 nm, 长径比在 5.0~122.2 之间^[22]。CNWs 含有丰富的活性基团, 采用酸解法可使其表面富含羟基、乙酰氨基, 还有少量的氨基^[23], 氨基的存在可使甲壳素表面带正电。CNWs 具有高的比表面积^[24]、良好的机械强度和优异的生物相容性^[25]。

ChNFs 主要是通过机械处理获得的一种纳米纤维素类型, 但由于机械处理的局限性, ChNFs 中仍然存在非结晶区, 相对于 CNWs 的结晶度较低, 尺寸更大^[26]。由甲壳素分子有序堆积而成的 ChNFs, 具备

甲壳素所有良好的性能, 如可生物降解性^[8]、生物相容性^[27]、独特的抗菌性^[10] 和优良的吸湿、透湿性^[28] 等。由于 ChNFs 原子排列高度有序, 而且直径极小, 不含普通材料所具有的晶界、位错、空穴等缺陷, 因而具有高强高模的特点, 是一种性能优异、环境友好、天然的增强材料^[26]。

1.2 纳米甲壳素的制备方法

甲壳素作为重要的海洋废弃物综合利用加工产物, 是一类环境友好型绿色可再生资源^[30]。制备纳米甲壳素的一般流程是首先从天然生物组织中提取粗甲壳素粉, 再将粗甲壳素粉进行提纯, 即去除蛋白质、矿物质、脂质以及色素等, 并以一定的物理化学方法将甲壳素纳米化, 从而制备出纳米甲壳素^[31-33]。

根据生物纤维材料的制备工艺的不同, 可将 NCh 的制备方法概括为两大类: “自上而下”(“Top-Down”)和“自下而上”(“Bottom-Up”)^[34], 如图 2 所示。“自上而下”的制备原理通常是利用伯胺质子化和强烈的机械作用, 将大分子的甲壳素通过外力作用制备产生纳米级的 ChNFs, 但不会破坏分子长链^[29,32,35]。而“自下而上”的制备原理则是在甲壳素溶液加工过程中破坏强氢键作用, 可将分子链破坏, 使其完全溶解, 再通过一定的条件将溶液中的小分子甲壳素自组装为纳米级的甲壳素^[36]。近年来, 采用有机溶剂将甲壳素完全溶解为小分子后, 通过静电纺丝和溶解再生组装为纳米纤维的方法也备受关注^[37]。采用“自上而下”的传统纳米化的制备方法更加广泛, 包括机械处理法、TEMPO 氧化法等。

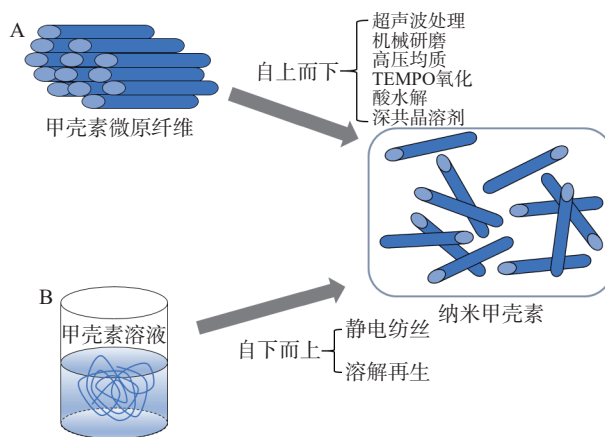


图 2 纳米甲壳素的制备方法^[32]

Fig.2 Preparation method of NCh^[32]

1.2.1 基于“自上而下”的纳米甲壳素的制备方法

在传统的“自上而下”制备方法中, 机械处理方法制备原理简单、操作便利、安全无毒、对于产品的尺寸也可通过处理工艺进行控制, 因此是运用最广泛的方法^[38]。酸碱提纯后的纯甲壳素在酸水解反应下非结晶区将被裂解, 同时甲壳素的伯胺基团在酸溶液中被质子化产生阳离子, 强烈的氢键作用被阳离子产生的静电相互作用破坏, 从而获得更小的纳米纤维束, 再利用强机械力将紧密的甲壳素纤维束分裂开, 从而制

备产生更小的纳米甲壳素^[39-41]。目前,有大量文献报道了利用不同机械力来制备纳米级甲壳素纤维,比如超声波、研磨机、高压均质等。

1.2.1.1 超声波处理法 近年来,超声波处理法被广泛应用于纳米材料的合成,尤其是在基质包裹纳米纤维的组合体生物资源中,主要原因在于超声波所产生的空穴效应,它能通过强大的拉应力使得液体产生局部的高温、高压环境,破坏分子间氢键作用力,从而将结合紧密的甲壳素纤维束分裂,产生更细小的纳米纤维结构^[42]。Lu等^[43]以虾壳为原料,在中性条件下采用高强度超声处理可将天然甲壳素分解成宽度均匀的高纵横比的ChNFs。同时通过调节超声时间,可实现不同粒径的ChNFs的可控制备(20~200 nm间)。Hsueh等^[42]以 β -甲壳素为原料,在冰水浴条件下,使用超声处理器分别超声1和2 h,结果发现超声1 h的纳米纤维表现出柔韧的毛状纤维外观,平均直径为17.24 nm,纤维长度为1725.05 nm,长径比为100:35,而延长处理后的纳米纤维平均直径为15.67 nm。

1.2.1.2 研磨法 研磨法通常是借助超微粉碎机的强大机械力将甲壳素悬浮液中的甲壳素纤维束打开,从而获得更小的单体纤维,并且能够依据磨盘种类、磨盘间隙与回转数来调整纤维尺寸大小^[44-45]。Ifuku等^[44]以干蟹壳为原料,去除蛋白质和矿物质后,溶解于醋酸水溶液中,通过研磨机研磨之后,得到的晶体状的ChNFs具有均匀的宽度(约为10~20 nm)和高长径比。同时,表征结果显示N-乙酰基没有被去除, α -甲壳素晶体结构也保持不变,进一步证实了纳米纤维是从天然甲壳素/蛋白质/矿物复合材料的原始状态提取的。Siahkamari等^[46]将甲壳素微纤维原粉制备成浓度为1%wt的悬浮液,搅拌均匀后倒入机器中进行循环研磨。经过剪切力和压缩力的作用,原纤维束发生了纳米级原纤化过程,从而产生的ChNFs呈网络形状,其中大多数ChNFs的直径尺寸约为60 nm^[32]。Fazli等^[47]摒弃传统酸处理脱盐的方法,以一种基于热水、碱液提取和低能量混合的温和提取法以及简单机械研磨从蘑菇干粉中制备了ChNFs,同时保留了大量葡聚糖(50%~65%),该研究表明葡聚糖的存在能够大大提高ChNFs的机械性能,强度是现已报道的ChNFs的最高值。

1.2.1.3 高压均质法 高压均质的原理是对于注入高压均质腔内的具有一定的流速的液体悬浮液,在腔室循环的过程中,受到高压剪切力、对流碰撞力等机械力作用,使得悬浮液的物质细化,以达到均质的效果^[48-49]。Gao等^[40]通过机械高压均质的方法分别处理了添加与未添加壳聚糖的甲壳素悬浮液,成功制备了ChNFs。表征结果显示在不添加壳聚糖的情况下,通过机械均质可以得到宽度约为4.6 nm的ChNFs。但是,样品中也发现了较大的ChNFs或甲壳素/蛋白质束的聚集物,其宽度约为20~100 nm;而对比在

pH3的高压均质过程中,壳聚糖的加入显著降低了ChNFs的团聚,均质后的ChNFs主要呈现单体约为4.6 nm等宽的纤维,未见较大直径的纤维。研究表明在机械均质过程中适量添加壳聚糖能够促进甲壳素的原纤化过程,防止甲壳素聚集。Salaberria等^[48]提出动态高压均质这一概念,通过对流体施加非常高的压力,将分散的颗粒细分成非常小的尺寸,甚至达到纳米级别。利用动态高压均质的方法,以黄龙虾废弃物为原料,成功地制备了直径100 nm以下的均匀的ChNFs。

1.2.1.4 TEMPO氧化法 TEMPO氧化法是通过TEMPO/NaBr/NaClO氧化体系对甲壳素中的C6位羟甲基进行选择氧化,然后结合机械处理的方式可以制备出羧基化的纳米甲壳素^[50-51]。Ye等^[52]采用机械粉碎结合TEMPO介导氧化的方法,制备出了氧化纳米纤维,研究表明先采用水相碰撞粉碎甲壳素胶束可以提高氧化效率,在所需的氧化剂量相同的条件下,氧化后的ACC-纳米纤维的羧酸含量比报道的直接TEMPO氧化法增加了一倍。Ye等^[53]分别采用TEMPO/NaClO/NaClO₂以及TEMPO/NaBr/NaClO两种不同氧化体系制备了尺寸存在差异的氧化NCh,前者制备出平均宽度为16.67 $\pm$ 7.9 nm,平均长度为770 $\pm$ 170 nm的纤维,后者制备的纤维宽度大多为20~24 nm,平均长度为1 μ m,表明不同氧化过程对纳米纤维材料产生了尺寸效应。

1.2.1.5 酸水解 在酸水解过程中,甲壳素的无序和弱横向结构部分优先被水解并溶解在酸溶液中,而不溶于水的、具有较强耐酸性的高结晶残基则保持完整^[45]。因此,NCh是酸水解的结果,它消除了无序和低横向有序的晶体缺陷^[54]。用3 mol/L HCl在煮沸(104 $^{\circ}$ C)条件下水解纯甲壳素90 min;酸水解后用去离子水稀释悬浮液,离心,取上清;这个过程重复多次,直到悬浮液自发地变成胶体。所形成的晶体为棒状颗粒,平均长200 $\pm$ 20 nm,宽8 $\pm$ 1 nm,可浓缩成液晶相,在一定浓度下可自组装成液晶相^[27]。除了在HCl溶液中水解外,H₂SO₄也被用于制备纳米甲壳素^[55-56]。因强酸运输、设备和成本高以及污染环境等问题阻碍了使用强酸处理的纳米甲壳素的商业化和工业化^[57]。近年来,一直都在尝试用便捷环保的方法制备甲壳素纳米纤维。Liu等^[58]利用固体马来酸水解从贝壳中分离出NCh,所得的NCh为棒状,产率为1.59%~10.42%,粒径为397.8~170.6 nm,表面羧基含量为0.04~0.173 mmol/g。与HCl和H₂SO₄相比,马来酸具有高存储安全性、低运输成本、环境友好性和最小的设备腐蚀、更高的沸点等优点。

1.2.1.6 深共晶溶剂(DES) DES是一种绿色溶剂,由氢键供体(HBD)和氢键受体(HBA)在特定温度下组合而成,具有良好的溶剂容量和低蒸汽压^[59],同时能够有效地与碳水化合物和生物质的氢键系统相互作用,因此已被认为是多糖绿色加工的可持续和可配

置的化学物质^[60]。Mushi 等^[61]进一步研究发现, DESs(氯化胆碱-硫脲比例 1:2)可以制备宽 25~45 nm, 长 162~450 nm 的 ChNFs。同样, 使用氯化胆碱和五种不同有机酸(乳酸、草酸、柠檬酸、丙二酸和 dl-苹果酸)制备的酸性 DESs, 生产收率高达 100% CNWs^[62]。

1.2.2 基于“自下而上”的纳米甲壳素制备方法 静电纺丝法是指能够在高压电场的作用下, 利用高分子材料制备出连续纳米纤维的技术, 当电场力足以克服注射器尖端液滴的表面张力时, 带电聚合物射流就会从泰勒锥中喷射出来, 然后以膨胀螺旋的形式拉长, 获得纳米纤维^[63-64]。Shamshina 等^[65]将虾壳废弃物溶解在离子溶液中, 再通过纯化处理去除蛋白等杂质后, 直接采用静电纺丝技术制备 ChNFs, 结果表明整个制备流程简单、成本低, 并能制备出性能良好的纳米纤维。溶解再生法是将甲壳素先溶解在特殊的溶剂中, 然后通过加入其他试剂诱导再生出 NCh 的方法^[66-67]。

超声、研磨等机械处理法是通过物理的手段对甲壳素进行处理, 不能完全地将甲壳素纳米纤维化, 会存在一定数量的纤维束或未纤维化部分, 但是具有生产能力高、污染小等优点, 有利于工业化。TEMPO 氧化法和酸水解法所制备的机理都是靠静电斥力破坏甲壳素分子间的氢键, TEMPO 氧化法是靠羧基阴离子, 而酸水解法是胺基阳离子。由于 TEMPO 氧化法会产生更多静电斥力, 因此这种方法基本能完全使甲壳素纳米化, 而酸水解法能够观察到聚集现象, 只能部分使甲壳素纳米化, 为了提高纳米化程度, 酸水解后需要进行超声或其它机械处理, 同时还存在产率低的缺点。深共晶溶剂能代替对环境有害的化学试剂, 同时还具有热稳定性好、价格低、毒性低和可制定性的优点, 但由于其极强的亲水性会降低 NCh 的产率。静电纺丝法需要对甲壳素降解, 采用的溶剂大多有毒。溶解再生法制备 NCh 不需要特殊的化学设备, 也不需要甲壳素进行化学改性, 同时这些溶剂可以回收重复使用, 但是成本高, 不利于工业化。

2 纳米甲壳素在食品领域中的应用

2.1 作为生物活性材料用于食品活性包装材料

NCh 具有较高的机械强度、较大长径比、抗菌活性、生物可降解及天然无毒等特点, 可以作为食品包装中最为广泛的生物活性材料(图 3)。Wu 等^[68]将天然红甘蓝提取物和乳酸链球菌素固定在用 ChNFs 增强的普鲁兰多糖/壳聚糖复合基质上制备了一种可动态监测食品新鲜度的新型多功能纳米复合膜。Jiang 等^[69]将魔芋葡甘聚糖和 ChNFs 与柠檬酸结合制备得到具有优良的机械强度和抗菌活性的生物纳米复合薄膜。Mushi 等^[61]在壳聚糖中加入体积分数为 8% 的纳米甲壳素使其复合材料表现出模量、强度和应变破坏的独特组合, 同时也使得断裂功

率高达 35 mJ/m³。Duan 等^[70]报道了一种可作食品包装应用的含有姜黄素和花青素的普鲁兰/ChNFs 的静电纺纳米纤维, 发现纳米纤维可作为监测食品品质变化的 pH 指示剂。另外, Salaberria 等^[71]在聚乳酸中加入 CNWs 制备了可持续包装的功能性纳米薄膜, 这种薄膜对霉菌黑曲霉等微生物具有良好的抑制作用。Xie 等^[72]将 ChNFs 与葱花提取物结合制备的复合薄膜能有效地保存新鲜切好的香蕉, 同时, 与阿魏酸复合涂层结合的 ChNFs 能通过降低呼吸和水分流失以及抑制微生物生长来延长草莓和鲜切苹果的保质期。

2.2 作为界面稳定剂用于 Pickering 乳液

Pickering 乳液是以超细固体颗粒作为乳化剂而得到的稳定的乳液。由于 Pickering 乳液能被糖、蛋白质等固体颗粒所稳定, 而且具有良好的结构稳定性和生物相容性, 在食品领域得到广泛应用^[73]。NCh 因其具有足够的界面活性和天然安全等特性, 常作为稳定剂加入到食品级 Pickering 乳液中^[11]。Sun 等^[74]将 ChNFs 加入到胶体玉米蛋白形成的 Pickering 乳液中, 提高了乳液的稳定性和功能特性。另一方面, 油脂含量较多的食品在贮藏期间容易发生油脂氧化, 导致营养成分损失, 而通过 NCh 稳定的 Pickering 乳液能延缓油脂氧化。Wang 等^[75]制备得到含有 NCh 和单宁酸复合的 Pickering 乳液, 发现添加 0.3% NCh 和 0.2% 单宁酸所形成的复合物能有效延缓乳液中的油脂氧化。Zhong 等^[76]采用 ChNFs 制备含有柑橘精油的 Pickering 乳液, 发现 ChNFs 能有效稳定 CEO Pickering 乳液并使其具有 pH 可逆响应特性及提高了 CEO 的抗菌活性。

2.3 作为载体应用于食品功能因子递送体系

基于 NCh 的纳米粒子^[77]、凝胶^[78]和 Pickering 乳液^[79]等载体在封装食品功能因子方面的研发应用逐渐增多。Dhanasekaran 等^[77]采用 NCh 作为纳米粒子递送姜黄素和胰岛素, 实现了姜黄素和胰岛素的高效包封和缓释。Petrova 等^[78]用部分脱乙酰化的 CNWs 填充藻酸盐水凝胶, 用于四环素的封装与缓释, 延长了四环素在体内释放的时间。Jia 等^[79]用 TEMPO 氧化的 CNWs 稳定 Pickering 乳液, 用于槲皮素封装与递送, 实现了对槲皮素的释放有效控制。

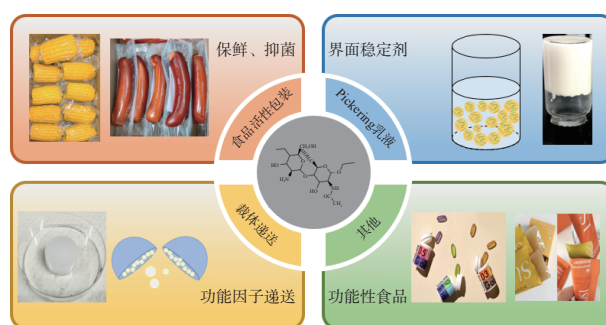


图 3 纳米甲壳素在食品领域中的应用

Fig.3 Application of NCh in the food

films(增强塑料薄膜)、biodegradable(可生物降解)等主题为研究热点。其中 chitin(甲壳素)、chitin nanofiber(甲壳素纳米纤维)、chitin nanowhiskers(甲壳素纳米晶须)等关键词强调了甲壳素和 NCh 是食品领域中所研究的热点材料。

关键词突现分析能够展现短时间内关键词的变化情况,把握不同时期各个阶段的研究热点,反映该主题研究的前沿议题。因此,通过分析 NCh 相关文献关键词的突现情况,可以辨别和探测该主题的研究前沿主题,为之后的研究提供一些可能的发展方向。如图 6 所示,检索出突变词一共有 18 个,突变词的时间跨度不一,最短为 1 年,最长为 3 年。2014~2018 年为早期前沿阶段,研究内容包括 Inflammatory bowel disease(炎症性肠病)、Metabolic syndrome(代谢综合征)、Composites(复合物)、Deacetylation(脱乙酰)等;2018~2022 年为中期前沿阶段,研究内容包括 Tempo mediated oxidation chitinnanocrystals(TEMPO 氧化甲壳素纳米晶)、Chitin nanofiber(甲壳素纳米纤维)等;2022~2024 年为近期前沿阶段,主要的研究内容为 Food packaging(食品包装)、Emulsion(乳液)、Antioxidant(抗氧化)、Chitosan films(壳聚糖薄膜)等。这些突现词反映了近几年 NCh 研究的前沿热点。

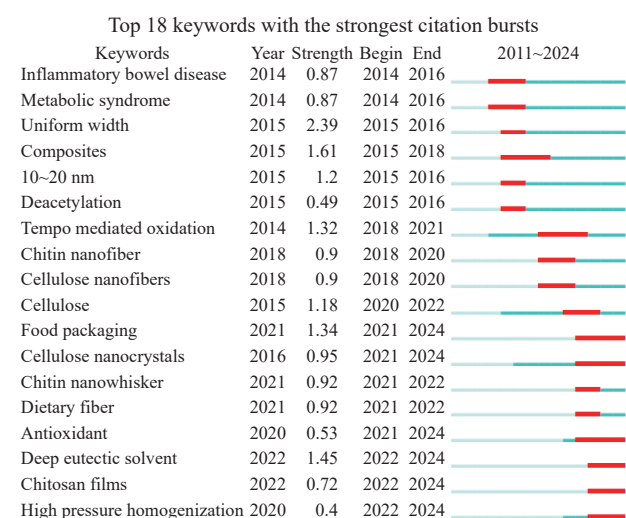


图 6 WOS 数据库中有关纳米甲壳素研究的关键词突现图谱

Fig.6 Keyword emergence atlas of NCh research in the WOS database

4 结语

纳米甲壳素(NCh)在食品领域中具有可持续应用的巨大潜力,但 NCh 的制备以及在食品中的应用研究仍在探索中。文中所讨论的 NCh 可以采用“自上而下”和“自下而上”的方法从天然甲壳素中所提取,但这些方法大多数依赖于化学品及能源的消耗,容易对环境造成负面影响,而且成本高。现阶段 NCh 的制备仍处于实验室研究,各种制备方式都具有一定的优缺点,仍然无法进行大规模的生产,原因

包括搭建合适的生产环境需要极其精细的条件,而且可能存在产量低等缺陷。因此,在开发新的简单有效的成本合理的制备方法来分离 NCh 方面仍有大量的研究需要探索。该综述还讨论了 NCh 作为生物活性材料用于食品活性包装材料、Pickering 乳液的稳定剂、功能性食品中的主要成分和食品功能因子递送载体。同时借助文献计量学的方法,对 NCh 的研究文献进行数据可视化分析,结果显示 NCh、CNWs 和 ChNFs 等是食品领域中所研究的热点材料,近几年的研究热点包括食品包装、纤维素纳米晶、抗氧化、壳聚糖薄膜等,为 NCh 在食品领域中的进一步开发利用提供参考。

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