

糖基化肽的制备、结构表征及生物活性研究进展

Advances in the preparation, structural characterization and biological activity of glycosylated peptides

吴 颖 屈婷敏 文诗雨 吴 昊 程云辉 文 李

WU Ying QU Tingmin WEN Shiyu WU Hao CHENG Yunhui WEN Li

(长沙理工大学食品与生物工程学院, 湖南 长沙 410014)

(School of Food Science and Bioengineering, Changsha University of
Science & Technology, Changsha, Hunan 410014, China)

摘要:肽的结构和功能易受温度、pH 值和离子强度改变等环境因素的影响,而糖基团可与肽的氨基酸侧链基团形成共价键进而发生结构修饰,糖基化修饰能有效提高肽分子的稳定性和生物活性。文章综述了糖基化肽的制备方法与结构表征策略,重点阐述了糖基化肽生理活性影响的最新研究进展;并分析了糖基化修饰肽所面临的挑战及未来研究方向。

关键词:糖基化肽;制备方法;结构表征;生物活性

Abstract: The structure and function of peptides are affected by the changes of environmental factors, including temperature, pH value and ionic strength. Sugar groups can form covalent bonds with amino acid side chain groups of peptides to undergo structural modification, and then the glycosylation modification can effectively improve the stability and biological activity of peptide molecules. In this review, the preparation methods and structural characterization strategies of glycosylated peptides were summarized, and the recent research progress on their physiological activities were highlighted. The challenges and future research directions of glycosylated peptides were also discussed.

Keywords: glycosylated peptide; preparation method; structure characterization; biological activity

肽通常具有高生物利用率和生物活性等优势^[1]。然而,肽活性易受 pH 值、温度、离子强度和溶剂极性等因素影响^[2]。因此,通过基团修饰改善肽的稳定性和生物

活性对于肽资源的深度加工应用具有重大意义。

糖基化修饰是改善肽结构稳定性及生物活性的主要手段之一,将肽分子与糖基团进行共价结合,其反应条件温和,无有害试剂添加,制备过程安全、环保^[3]。糖基化肽来源广泛,小麦^[4]、玉米^[5]、大豆^[6]、蛋^[7]、乳制品^[8]等均为优质的糖基化肽制备原材料。此外,糖基化肽因具有抗氧化、抗菌、免疫调节和抗癌等多种生物活性备受科研人员的关注^[9]。因此,文章拟综述糖基化肽的制备、结构表征、生物活性及其在食品和制药工业的应用,以期为食品及生物工业中资源加工利用提供依据。

1 糖基化肽的制备

糖基化肽的制备方法较多,其中美拉德反应与转谷氨酰胺酶反应是工业中被广泛使用的两种方法。美拉德反应通过控制一定的温度、时间、pH 将糖与肽结合,而转谷氨酰胺酶反应是通过谷氨酰胺转移酶特异反应制备糖基化肽。

1.1 美拉德反应制备法

美拉德反应亦称非酶褐变,是糖类物质的羰基化合物和多肽氨基(主要是赖氨酸中的 $\epsilon\text{-NH}_2$) 之间发生反应,该反应经历缩合、聚合等复杂的化学过程最终生成棕色甚至黑色的大分子物质类黑精^[10]。美拉德反应主要分为 3 个阶段:第 1 阶段为初级阶段风味前体物质形成,如席夫碱等;第 2 阶段为中间阶段风味物质形成;第 3 阶段为高级阶段,以类黑素的形成为核心,生成有害物质。因此,在美拉德反应过程中应适当控制反应程度,以减少有害物质的生成。

由于工艺不同,制备糖基化多肽所消耗的能量及获得的糖肽产率均不同。此外,糖的种类^[6]、pH^[11]、反应温度^[12]、反应时间^[13]、亚硫酸盐^[14]等均能不同程度影响美拉德产物的生物活性。根据制备条件不同,可将美拉德

基金项目:国家自然科学基金面上项目(编号:31972077);湖南省自然科学基金面上项目(编号:2021JJ2024)

作者简介:吴颖,女,长沙理工大学在读硕士研究生。

通信作者:文李(1971—),女,长沙理工大学教授,博士生导师,博士。E-mail: wl@csust.edu.cn

收稿日期:2023-03-10 **改回日期:**2023-08-18

法分为干热法及湿热法。干热法美拉德反应时间长,通常长达几天甚至数周^[15],蛋白质变性和聚合程度更小,褐变程度更低。湿热法具有反应时间短^[16]、反应易控制和产物性能稳定等优点^[17]。但是,湿热法反应物浓度低,回收产品时需要将缓冲溶液或者水分去除,产物回收率低;并且在反应过程中,蛋白质酶解物易于在高温溶液中变性并发生聚合^[18]。为了降低这些不良影响,美拉德湿热法中通常联用离子液体^[19]、高压和高温^[20]、超声^[21]、超临界二氧化碳处理^[22]和高静水压力^[23]等其他物理辅助手段优化美拉德湿热法反应。总而言之,美拉德反应过程影响因素多,需要通过控制反应条件进而经济、高效地获得活性高的美拉德产物。

1.2 转谷氨酰胺酶反应制备法

酰基转移反应又称酶法糖基化反应,可以催化多肽分子中Gln残基的 γ -酰胺基(酰基供体)和不同化合物的 ϵ -氨基(酰基受体)之间形成异肽键。糖基化反应中酰基供体由多肽分子中Gln残基的 γ -酰胺基提供,酰基受体多为葡萄糖胺^[24]。转谷氨酰胺酶(TGase)属于一种转移酶,广泛存在于哺乳动物组织^[25]、无脊椎动物及微生物^[26]、植物组织^[27]中。与动植物来源的TGase相比,微生物来源的TGase具有活性高、不依赖 Ca^{2+} 、不需要特殊辅助因子等特点,且具有良好的pH稳定性、热稳定性、底物特异性^[25],可广泛用作理想的酶制剂^[24]。通常转谷氨酰胺酶在40℃、pH 5.5条件下具有较好的催化活性,由细菌生物合成的转谷氨酰胺酶pH稳定性更好,可在pH值4.5~8.0范围内保持活性^[28]。

在酶促反应体系中添加碳水化合物,如麦芽糊精、甘露糖、海藻糖和还原性谷胱甘肽,可以显著提高转谷氨酰胺酶的热稳定性^[29]。此外,金属离子也能影响谷氨酰胺转移酶活性^[30]。由于TGase对6个碳的直链脂肪胺显示出高度亲和力^[31],因此D-氨基葡萄糖是糖基化反应的首选酰基受体。总之,不同来源转谷氨酰胺酶和不同种类糖对转谷氨酰胺酶法制备生物活性糖肽具有较大影响。

美拉德法和转谷氨酰胺酶反应法均可有效获得糖基化肽,美拉德法易受反应条件影响,但无需酶的参与就可获得大量糖基化肽,制备成本较低,而转谷氨酰胺酶反应可以控制糖基团与特定氨基酸进行连接,具有一定的特异性。因此,工业上常用酶解法与美拉德法、转谷氨酰胺法联用制备糖基化肽。

2 糖基化肽的结构表征

对肽与糖所形成的共价复合物的分子量和分子结构进行表征,对比两者共价结合前后结构的改变,可证明糖基化肽成功制备^[32]。最常见的方法为分析分子量的变化如体积排阻色谱(SEC)、聚丙烯酰胺凝胶电泳(SDS-PAGE);分析分子结构的变化如紫外分光光度计法、紫外

光谱(UV)、傅里叶变换红外光谱(FTIR)、圆二色谱(CD)和荧光光谱(FS)等;以及深入探究肽的氨基酸组成及糖分子组成的变化如高效液相色谱串联质谱(HPLC-MS/MS)。

2.1 分子量测定

利用聚丙烯酰胺凝胶电泳和体积排阻色谱法可测定蛋白酶解物/肽糖基化前后分子量变化从而判定复合物连接程度。糖的加入会影响蛋白酶解物/肽的分子量分布,He等^[5]通过SDS-PAGE试验测定了玉米醇溶蛋白水解物与葡聚糖美拉德反应产物的分子量,相较于反应前的玉米醇溶蛋白水解物分子量,美拉德反应产物的低分子量条带减少,高分子量条带增加且条带强度随美拉德反应时间的延长而增加。Jiang等^[33]选择不同糖并控制不同加热时间获得了不同分子量分布的美拉德产物,随着加热时间的延长,核糖-酪蛋白肽、半乳糖-酪蛋白肽和乳糖-酪蛋白肽分子量逐渐增加。

2.2 结构测定

2.2.1 肽一级结构变化 从蛋白质酶解物的一级结构层面上可通过表征其氨基酸及其共价键变化是鉴定糖肽生成的重要方法。蛋白酶解物/肽经过糖的修饰,尤其美拉德反应产物末端氨基减少,吸光值在一定范围内发生变化,在此基础上可通过紫外分光光度法探究糖基化肽的生成^[34]。根据紫外光谱中吸收峰的位置、强度和形状,获得分子中不同电子结构的信息,判断共轭分子、组分及平衡常数探究糖基化肽的结构变化,证明糖基化肽制备成功。Chen等^[35]于紫外波长190~400 nm处表征鱼鳞肽-木糖,其美拉德反应产物的最大吸收峰出现红移,证明鱼鳞肽和木糖发生交联;在250~350 nm处出现的吸收峰可能为糖裂解产生酮、醛和其他无色无荧光的小分子中间产物;此外,在200~350 nm处有一个较宽的类黑素特征吸收峰^[21]。傅里叶变换红外光谱也常被用于分析蛋白酶解物/肽与糖结合的复合物的共价键。本质上,美拉德反应引起了蛋白质一级结构的变化,蛋白质氨基残基的氨基和还原糖的碳基之间形成共价键相连。通常,吸收光谱在1700~1600 cm^{-1} 酰胺I区($\text{C}=\text{O}$ 拉伸振动肽链接)和1600~1500 cm^{-1} 酰胺II区会发生变化。美拉德产物中含有希夫碱($\text{C}=\text{N}$ 的形成),会造成酰胺II区的变化,糖的加入会增加羟基,并且糖可能会与氨基反应,因此由于羟基的O—H拉伸和N—H的拉伸可能会在3338 cm^{-1} 左右出现特征吸收带,酰胺III区(1450~1230 cm^{-1})也会因芳香结构中C—H基团而发生变化^[12]。不同糖对吸收峰的出现会有不同的影响,壳聚糖在1022 cm^{-1} 处的吸收带出现—C—O—C—的吸收峰^[36],而木糖会在1041 cm^{-1} 处增加峰值^[21]。综上,测定氨基酸残基变化、肽分子基团变化、美拉德中间分子的形成等均可作为表征糖基化肽的有效手段。

2.2.2 肽二级和三级结构变化 圆二色谱常用于探究蛋白质二级结构的变化,Dong 等^[37]利用该法表征乳清蛋白水解物与糊精的美拉德反应产物结构变化,结果表明糊精共价后水解物中 β -折叠含量增加, α -螺旋和无规卷曲减少,其原因为美拉德反应过程中涉及羰基和 ϵ -氨基之间的缩合,导致 α -螺旋结构的降低。荧光光谱主要依据荧光强度和最大波长位移的变化说明氨基酸微环境的改变,进而表征蛋白水解物三级结构的变化,大米蛋白水解物与葡聚糖共价后最大发射波长由 422 nm 转变为 347 nm,荧光强度增加,大米蛋白水解物的三级结构因美拉德反应而改变^[38]。因此,检测反应产物的二级结构及三级结构的特定变化可证明糖基化肽成功制备。

2.3 糖分子及氨基酸组成测定

高效液相色谱串联质谱法是糖分子及氨基酸组成鉴定常用的方法,糖肽分子的高能碰撞解离使碎片解离产生氧离子,氧离子携带着碎片中糖类结构的信息;电喷雾电离质谱和基质辅助激光解吸电离质谱可以准确地鉴定糖基化产物的分子质量并分析其交联程度,在蛋白质结构表征方面发挥了主导作用,这些技术能够在分子水平上提供有关蛋白质修饰的性质和程度的详细信息以及构象信息^[39]。Song 等^[40]通过液相色谱电喷雾电离/多级质谱法从半鲮凤尾鱼水解物/葡萄糖中鉴定出抗菌肽。金属离子辅助分析为通过电喷雾电离质谱法对美拉德中间产物 Amadori 结构和端基异构分析提供了重要信息,该方法可用于确定 Amadori 异构体的糖苷构型,为进一步的研究提供佐证^[41]。肽糖基化常见的类型包括 N-和 O-连接的糖基化,不同修饰的糖肽在机体内的功能不同,测定肽的糖基化类型在医药中非常必要,MS/MS 能识别完整糖肽的位点特异性 N-和 O-糖基化^[42]。HPLC-MS/MS 高效准确,能深入探究糖基化的结构特征,为糖肽的进一步安全应用提供基础。

3 糖基化肽生物活性研究及应用

蛋白质及糖类是许多生物生长代谢过程中所必需的营养物质,生物活性肽及功能性糖可调节生物体的生理功能^[43]。机体内各种生物过程相互关联,如体内的氧化应激状态可导致自由基调节失衡,同时产生大量炎症因子,影响机体免疫调节功能^[44]。此外,血糖调节受炎症因子的影响,严重时可产生对胰岛素受体的不耐受,因而导致血糖升高^[45]。多项研究^[46]证明,糖基化肽的多种生物活性可以改善生物体整体健康状态,降低患特定慢性病的风险,如癌症、糖尿病和心脏病。由此可见,在未来发展中糖基化肽在保健食品和药品领域具有较大应用潜力。

3.1 抗氧化活性

氧化应激是机体氧化和还原失衡导致大量活性氧(ROS)无法清除而引发,绝大部分的慢性疾病都受氧化

影响,机体自由基紊乱将导致炎症的发生,以及血压血糖的失衡^[47]。因此,清除 ROS 对维持内环境稳态具有重要作用。DPPH $\cdot$ 、ABTS $\cdot$ 、 $\cdot$ OH 和 $O_2^{\cdot-}$ 是常见的自由基,因此可作为检测体外抗氧化能力的指标,此外 mRNA 和抗氧化相关酶表达等也是检测糖基化肽在体内外抗氧化能力的重要指标^[4]。玉米谷蛋白水解物与葡萄糖胺通过转谷氨酰胺酶制备的蛋白水解物—葡萄糖胺偶联物中有两种类型的糖肽,在乙醇溶液氧化刺激作用下,糖基化肽可清除 LO₂ 细胞内的 ROS,对细胞具有保护作用^[48]。

抗氧化肽天然无毒,可平衡自由基^[49],抗氧化肽经糖基化修饰后活性显著提升。糖基化抗氧化肽对比反应前的抗氧化肽具有更强的螯合或钝化 Cu^{+} 、 Fe^{2+} 等金属离子的能力,降低体内循环中金属离子的含量,减少自由基生成过程中所需催化剂的量,从而阻止自由基的形成^[50];此外,糖基化抗氧化肽还可提高疏水性氨基酸与疏水性多不饱和脂肪酸的结合能力,抑制脂质中氢的释放或抢先与氧气结合,从而减缓脂质过氧化反应的进行。糖基化修饰可改变肽空间构象,暴露更多具有供电子能力的氨基酸,供电子体与自由基反应,阻断自由基链反应,提高抗氧化能力,从本质上增强抗氧化活性^[51]。糖的种类及肽的分子量大小均能影响糖基化多肽的抗氧化能力,例如,果糖修饰有助于提高蟹壳生物活性肽的抗氧化活性,美拉德反应产物中活性组分的分子量通常为 2~5 kDa^[52]。Liu 等^[53]利用丝素肽与羧甲基壳聚糖在转谷氨酰胺酶的作用下,制备出具有良好自由基清除能力的糖肽。此外,糖基化多肽在胃肠道环境中稳定性好,经过胃肠道消化仍有较好的抗氧化性。Chen 等^[35]通过美拉德反应制备的鱼鳞肽—木糖糖肽,经胃肠道消化后,可显著降低肝脏 MDA 水平,抑制酒精性肝损伤引起的肝脏抗氧化酶 SOD、CAT 和 GSH-Px 活性的降低。Yang 等^[16]制备的糖肽可增强谷胱甘肽的合成从而保护肝脏细胞免受 TBHP 诱导的氧化应激,并且可通过 Nrf2 的核易位和 ERK 的磷酸化,诱导 II 期酶的表达上调。糖基化肽的抗氧化机制如图 1 所示。以上体外和体内试验结果表明,糖基化蛋白酶物/肽具有清除自由基的能力,可被广泛应用于食品与生物医药领域。

3.2 免疫调节活性

为了抑制炎症性疾病,非甾体抗炎药物和皮质类固醇等化学药物被广泛使用。然而,这些合成药物具有潜在的副作用,如血小板功能障碍和胃肠道溃疡^[54]。因此,寻找具有抗炎活性的天然成分来治疗炎症性疾病具有实际意义。

在天然功能成分中,糖基化肽因其高安全性和多功能的生物活性受到广泛关注。大量研究^[55]表明,糖肽还可以与 LPS 直接结合,抑制 LPS 与 TLR4/MD2 复合物

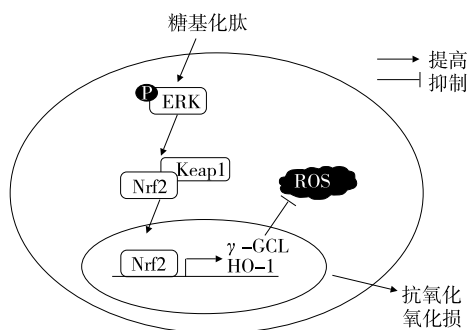


图1 糖基化肽抗氧化机制

Figure 1 Antioxidative mechanism of glycosylated peptides

接触,改善炎症问题,此外,糖基化肽能通过 MAPK 和 NF- κ B 信号通路抑制炎症发生。牛 κ -酪蛋白中获得的糖巨肽(GMP)是一种含有 64 个氨基酸的糖肽,对大鼠脾细胞的体内外作用,在肠道炎症的临床前模型中具有治疗价值^[56]。GMP 可以缓解气道炎症和结肠炎^[57],显著抑制肺组织中嗜酸性粒细胞浸润、杯状细胞增生和胶原蛋白沉积,有效改善哮喘疾病,并能通过抑制脾淋巴细胞过敏反应调节免疫系统^[56]。与天然 GMP 相比,糖巨肽水解物(GMPH)通过阻断 NF- κ B 信号通路的激活,对 LPS 诱导的巨噬细胞一氧化氮的产生和炎症细胞因子 mRNA 的表达有更强的抑制作用^[58]。此外,GMPH 通过抑制 MAPK 和 Akt 对 RAW264.7 巨噬细胞中 NF- κ B 的激活,发挥抗炎作用^[59]。糖基丙肽还能减少过敏原致敏,减轻皮肤过敏,预防机体的过敏反应^[60]。糖肽在调节免疫活性过程中还可提高生物体的抗氧化活性及抗癌活性。Karnjanapratum 等^[61]研究了半乳糖与独角兽皮肤的明胶水解物的美拉德反应产物,对体外细胞抗氧化活性、免疫调节特性和抗癌活性的影响,糖基化肽除了在体外抑制人结肠癌细胞的增殖外,还能抑制氧化应激和炎症反应。糖基化肽的免疫调节机制如图 2 所示。

3.3 降血糖活性

糖尿病是一种常见的慢性代谢性疾病,其特征是外周组织的胰岛素敏感性降低(即外周胰岛素抵抗)和胰腺 β 细胞的胰岛素分泌相对不足,胰岛素抵抗抗胰岛素靶器官对胰岛素敏感下降的病理状态。胰岛素抵抗是各种慢性非传染性疾病如 2 型糖尿病、慢性心血管疾病、慢性非酒精性脂肪等发病的重要前期病症^[62]。二肽基肽酶 IV(DPP-IV)是一种与 Xaa-Pro-(其中 Xaa 代表一种氨基酸)肽裂解相关的酶,并已广泛存在于内皮细胞、上皮细胞、T 细胞、肝细胞和体液中^[45]。抑制 DPP-IV 能调节肠促胰岛素依赖性胰岛素多肽和胰高血糖素样肽-1,是治疗 2 型糖尿病的新疗法^[63]。糖肽类的抗糖尿病功能与 DPP-IV 和血管紧张素转换酶的抑制活性、抗氧化和抗炎活性密切

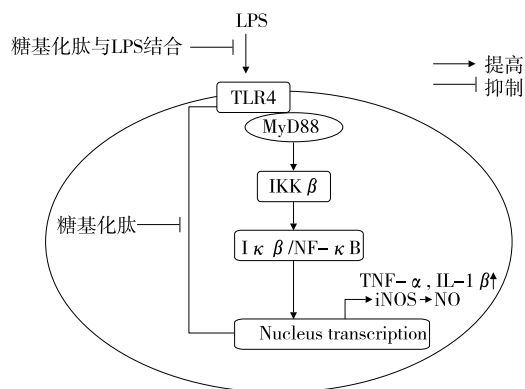


图2 糖基化肽免疫调节机制

Figure 2 Immunomodulatory mechanisms of glycosylated peptides

相关。血管紧张素 II 由血管紧张素 I 在血管紧张素转换酶的作用下产生,通过激活 NADPH 氧化酶诱导氧化应激,并激活 NF- κ B 上调炎症,抑制胰岛素 PI3K/Akt 信号^[64]。

植物源糖肽、动物源糖肽及微生物源糖肽均有降血糖效果。酪蛋白的木瓜蛋白酶水解物糖丙肽可显著降低高脂饮食小鼠的空腹血糖水平、血清胰岛素水平及胰岛素抵抗指数,并且可通过 Akt 磷酸化和 AMPK 磷酸化增加高脂饮食小鼠的糖耐量和肝糖原含量,改善高脂饮食小鼠的肝脏脂肪变性和巨噬细胞浸润^[65]。Yuan 等^[66]也证明酪蛋白糖肽水解物的抗糖尿病作用与骨骼肌胰岛素敏感性的恢复和肠道微生物群的调节有关。此外,CGMP 的衍生肽 IPPKKNQDKTE 可通过激活 AMP 进而激活 AMPK,调节 IRS-1/PI3K/Akt 信号通路,并抑制 HepG2 细胞中高糖诱导的胰岛素抵抗,在预防和治疗肝胰岛素抵抗和 2 型糖尿病中发挥潜在作用^[67]。在多种抗糖尿病的机制中,糖基化肽主要通过 IRS-1/PI3K/Akt 信号通路、MAPK 通路调节血糖,从而达到抗糖尿病的目的。

3.4 抗菌与抗病毒活性

细菌与肠上皮细胞的黏附是人体细菌感染的重要诱因^[68]。四元化壳聚糖衍生物和抗菌肽通过 TGase 交联对大肠杆菌具有抗菌活性^[69]。此外,从酪蛋白糖巨肽中获得的糖肽可作为抗菌饲料添加剂,减少仔猪肠道内容物中大肠杆菌 K88 的数量,缓解致病菌引起的炎症^[70]。Song 等^[40]从半鳍凤尾鱼水解物/葡萄糖的美拉德反应产物中通过液相色谱电喷雾电离/多级质谱法鉴定出抗菌肽。Jiang 等^[71]研究发现,蟹壳生物活性肽与果糖的美拉德产物兼具抗氧化活性和抗菌活性。

人流感病毒有两种膜蛋白:血凝素和神经氨酸酶。前者负责有毒颗粒与细胞外表面的接触,而后者负责在被入侵的细胞中释放新形成的病毒粒子^[72]。当宿主细胞被感染时,病毒首先通过血凝素结合宿主细胞表面糖链

的 α -(2-6)-*N*-唾液酰-*N*-乙酰乳糖胺残基^[73]。GMP 可结合霍乱和大肠杆菌肠毒素、抑制细菌和病毒黏附、抑制胃分泌物、促进双歧杆菌生长和调节免疫系统反应^[74]。酪蛋白糖肽衍生肽的唾液寡糖链与人流感病毒的血凝素竞争性强相互作用^[75], 表现抗病毒能力。唾液糖肽 (SGP) 是蛋黄中的糖肽, 具有复杂型的二唾液酰双触角低聚糖, 具有 α -(2→6)-唾液酰-*N*-乙酰乳糖胺残基。该残基被称为 α 型人流感病毒血凝素的结合配体。其能抑制流感病毒血凝素的活性, 因此具有多价唾液寡糖的唾液寡糖共聚物在预防流感病毒感染方面具有潜在应用价值^[76]。Tsuiji 等^[75] 利用含 *N*-羟基亚砷琥珀酰亚胺酯的水溶性聚合物主链合成了含七肽的唾液酰-*N*-聚糖的糖共聚物, 即唾液酰聚肽 (SGPs); SGP 接枝的糖共聚物与凝集素琥珀色凝集素和人甲型流感病毒血凝素具有较强的相互作用, 其结合关联常数高于游离糖, 具有抗病毒功能。糖基化肽具有较好的抗菌及抗病毒能力, 主要因为糖与肽共价结合后, 具有更多的负电荷, 同时, 糖还能与病毒接触, 从而发挥抗菌与抗病毒作用。

4 展望

近年来, 随着对糖基化肽结构表征的深入研究, 其生物活性研究取得了较大进展, 同时也存在较多亟待解决的问题。计算机能高效、经济预测肽的生物活性, 而计算机模拟项目对糖肽的生物活性开发较少, 需广泛研究以促进糖基化肽生物活性经济、高效的筛选。目前糖基化肽研究主要为混合物, 糖基化肽分离纯化困难, 且化学/生物合成纯糖肽昂贵, 尽管对纯糖肽的作用机制研究不充足, 但随着研究的深入, 纯糖肽有望得到更广泛的应用。机体中抗氧化性、免疫调节活性、降糖活性、抗菌和抗病毒活性相互关联, 深入研究糖基化肽在机体内的生物活性及其作用的量效关系, 可开拓糖基化生物活性肽在食品与医药领域的广阔应用前景。

参考文献

[1] 陈月华, 程云辉, 许宙, 等. 食源性生物活性肽免疫调节功能研究进展[J]. 食品与机械, 2016, 32(5): 209-213.
CHEN Y H, CHENG Y H, XU Z, et al. Research progress in immunomodulatory function of food-derived bioactive peptides[J]. Food & Machinery, 2016, 32(5): 209-213.

[2] SHI J, ZHAO X H. In vitro immuno-modulatory ability of tryptic caseinate hydrolysate affected by prior caseinate glycation using the Maillard reaction or transglutaminase[J]. Food and Agricultural Immunology, 2017, 28(6): 1 029-1 045.

[3] LI M, MCCLEMENTS D J, LIU X, et al. Design principles of oil-in-water emulsions with functionalized interfaces: Mixed, multilayer, and covalent complex structures [J]. Comprehensive Reviews in Food Science and Food Safety, 2020, 19(6): 3 159-

3 190.

[4] ZHANG X X, LI X D, LIU L, et al. Covalent conjugation of whey protein isolate hydrolysates and galactose through Maillard reaction to improve the functional properties and antioxidant activity[J]. International Dairy Journal, 2020, 102: 104584.

[5] HE W Y, TIAN L, FANG F, et al. Heat-induced glycosylation with dextran to enhance solubility and interfacial properties of enzymatically hydrolyzed zein [J]. Journal of Food Engineering, 2022, 321: 110946.

[6] YU M, HE S D, TANG M M, et al. Antioxidant activity and sensory characteristics of Maillard reaction products derived from different peptide fractions of soybean meal hydrolysate[J]. Food Chemistry, 2018, 243: 249-257.

[7] ZHAO M G, MA A M, HE H, et al. Desalted duck egg white peptides-chitosan oligosaccharide copolymers as calcium delivery systems: Preparation, characterization and calcium release evaluation in vitro and vivo[J]. Food Research International, 2020, 131: 108974.

[8] ZHU B, HE H, GUO D, et al. Two novel calcium delivery systems fabricated by casein phosphopeptides and chitosan oligosaccharides: Preparation, characterization, and bioactive studies [J]. Food Hydrocolloids, 2020, 102: 105567.

[9] WANG C H, SHI S S, CHEN Q, et al. Antitumor and immunomodulatory activities of ganoderma lucidum polysaccharides in glioma-bearing rats [J]. Integrative Cancer Therapies, 2018, 17(3): 674-683.

[10] CONG H H, WU Q M, ZHANG Z R, et al. Improvement of functional characteristics of hypophthalmichthys molitrix protein by modification with chitosan oligosaccharide [J]. Frontiers in Nutrition, 2023, 10: 1140191.

[11] HAN Y, WANG L X, JIANG W P, et al. An enhanced stability nanoparticle preparation by corn protein hydrolysate-carboxymethyl chitosan Maillard conjugates loaded with rutin[J]. Journal of Food Science, 2019, 84(7): 1 829-1 835.

[12] CHEN K, YANG Q, HONG H, et al. Physicochemical and functional properties of Maillard reaction products derived from cod (*Gadus morhua* L.) skin collagen peptides and xylose[J]. Food Chemistry, 2020, 333: 127489.

[13] KCHAOU H, BENBETTAIEB N, JRIDI M, et al. Influence of Maillard reaction and temperature on functional, structure and bioactive properties of fish gelatin films[J]. Food Hydrocolloids, 2019, 97: 105196.

[14] DALEFIELD R R, MUELLER U. Gastric mucosal irritation following oral exposure to sodium metabisulphite: A reproducible effect? [J]. Regulatory Toxicology and Pharmacology, 2016, 80: 277-282.

[15] HOU C, WU S, XIA Y, et al. A novel emulsifier prepared from Acacia seyal polysaccharide through Maillard reaction with casein peptides[J]. Food Hydrocolloids, 2017, 69: 236-241.

[16] YANG S Y, LEE S, PYO M C, et al. Improved physicochemical

- properties and hepatic protection of Maillard reaction products derived from fish protein hydrolysates and ribose [J]. *Food Chemistry*, 2017, 221: 1 979-1 988.
- [17] PIRESTANI S, NASIRPOUR A, KERAMAT J, et al. Preparation of chemically modified canola protein isolate with gum Arabic by means of Maillard reaction under wet-heating conditions [J]. *Carbohydrate Polymers*, 2017, 155: 201-207.
- [18] CHEN X, ZOU Y, WANG D, et al. Effects of ultrasound pretreatment on the extent of Maillard reaction and the structure, taste and volatile compounds of chicken liver protein [J]. *Food Chemistry*, 2020, 331: 127369.
- [19] XU W, ZHAO X H. Structure and property changes of the soy protein isolate glycosylated with maltose in an ionic liquid through the Maillard reaction [J]. *Food & Function*, 2019, 10(4): 1 948-1 957.
- [20] RUIZ G A, XI B, MINOR M, et al. High-Pressure-High-Temperature processing reduces Maillard reaction and viscosity in whey protein-sugar solutions [J]. *Journal of Agricultural and Food Chemistry*, 2016, 64(38): 7 208-7 215.
- [21] CHEN X, JIANG D, XU P, et al. Structural and antimicrobial properties of Maillard reaction products in chicken liver protein hydrolysate after sonication [J]. *Food Chemistry*, 2021, 343: 128417.
- [22] LIU S, CAO W, WANG Y, et al. Characteristics and mechanisms of nitrogen transformation during chicken manure gasification in supercritical water [J]. *Waste Management*, 2022, 153: 240-248.
- [23] MA X J, GAO J Y, TONG P, et al. Tracking the behavior of Maillard browning in lysine/arginine-sugar model systems under high hydrostatic pressure [J]. *Journal of the Science of Food and Agriculture*, 2017, 97(15): 5 168-5 175.
- [24] DUARTE L, MATTE C R, BIZARRO C V, et al. Review transglutaminases: Part II-industrial applications in food, biotechnology, textiles and leather products [J]. *World Journal of Microbiology & Biotechnology*, 2019, 36(1): 11.
- [25] DUARTE L, MATTE C R, BIZARRO C V, et al. Transglutaminases: Part I-origins, sources, and biotechnological characteristics [J]. *World Journal of Microbiology & Biotechnology*, 2020, 36(1): 15.
- [26] SHEN M L, CIOU J Y, HSIEH L S, et al. Recombinant *Streptomyces netropsis* transglutaminase expressed in *Komagataella phaffii* (*Pichia pastoris*) and applied in plant-based chicken nugget [J]. *World Journal of Microbiology & Biotechnology*, 2023, 39(8): 200.
- [27] PARROTTA L, TANWAR U K, ALOISI I, et al. Plant transglutaminases: New insights in biochemistry, genetics, and physiology [J]. *Cells*, 2022, 11(9): 1 529.
- [28] KIELISZEK M, MISIEWICZ A. Microbial transglutaminase and its application in the food industry: A review [J]. *Folia Microbiologica*, 2014, 59(3): 241-250.
- [29] CHEN H R, WU D, MA W C, et al. Strong fish gelatin hydrogels double crosslinked by transglutaminase and carrageenan [J]. *Food Chemistry*, 2022, 376: 131873.
- [30] LIU J P, ZHANG Y Q, HE S Z, et al. Microbial transglutaminase-induced cross-linking of sodium caseinate as the coating stabilizer of zein nanoparticles [J]. *LWT-Food Science and Technology*, 2021, 138: 110624.
- [31] FUCHSBAUER H L. Approaching transglutaminase from streptomyces bacteria over three decades [J]. *Febs Journal*, 2022, 289(16): 4 680-4 703.
- [32] YANG R, ZUO P, ZHANG M, et al. Transglutaminase induced oligochitosan glycosylation of ferritin as a novel nanocarrier for food bioactive molecules [J]. *Food Hydrocolloids*, 2019, 94: 500-509.
- [33] JIANG Z, WANG L, WU W, et al. Biological activities and physicochemical properties of Maillard reaction products in sugar-bovine casein peptide model systems [J]. *Food Chemistry*, 2013, 141(4): 3 837-3 845.
- [34] BI B W, YANG H, FANG Y P, et al. Characterization and emulsifying properties of beta-lactoglobulin-gum Acacia Seyal conjugates prepared via the Maillard reaction [J]. *Food Chemistry*, 2017, 214: 614-621.
- [35] CHEN X, FANG F, WANG S. Physicochemical properties and hepatoprotective effects of glycosylated Snapper fish scale peptides conjugated with xylose via Maillard reaction [J]. *Food and Chemical Toxicology*, 2020, 137: 111115.
- [36] ZHANG C, WANG Z G, LI Y, et al. The preparation and physicochemical characterization of rapeseed protein hydrolysate-chitosan composite films [J]. *Food Chemistry*, 2019, 272: 694-701.
- [37] DONG S Y, PAN Y A, ZENG M Y, et al. Characteristics and antioxidant activity of hydrolyzed beta-lactoglobulin-glucose Maillard reaction products [J]. *Food Research International*, 2013, 51(2): 992.
- [38] LI Y, ZHONG F, JI W, et al. Functional properties of Maillard reaction products of rice protein hydrolysates with mono-, oligo- and polysaccharides [J]. *Food Hydrocolloids*, 2013, 30(1): 53-60.
- [39] GAZI I, FRANC V, TAMARA S, et al. Identifying glycation hot-spots in bovine milk proteins during production and storage of skim milk powder [J]. *International Dairy Journal*, 2022, 129: 105340.
- [40] SONG R, SHI Q, YANG P, et al. Identification of antibacterial peptides from Maillard reaction products of half-fin anchovy hydrolysates/glucose via LC-ESI-QTOF-MS analysis [J]. *Journal of Functional Foods*, 2017, 36: 387-395.
- [41] BAI Y, LIANG Y, LI G, et al. Metal-ion-assisted structural and anomeric analysis of Amadori compounds by electrospray ionization mass spectrometry [J]. *Rapid Communications in Mass Spectrometry*, 2021, 35(1): e8960.
- [42] ESHGHI S T, YANG W, HU Y, et al. Classification of tandem mass spectra for identification of N- and O-linked glycopeptides [J]. *Scientific Reports*, 2016, 6: 37189.
- [43] LIU X L, WANG J T, LIU Y, et al. Conjugation of the glutelin hydrolysates-glucosamine by transglutaminase and functional

- properties and antioxidant activity of the products [J]. Food Chemistry, 2022, 380: 132210.
- [44] QIAO G H, WENXIM D, ZHIGANG X, et al. Antioxidant and anti-inflammatory capacities of pepper tissue[J]. Italian Journal of Food Science, 2020, 32(2): 265-274.
- [45] SHARIPOV A, TURSUNOV K, FAZLIEV S, et al. Hypoglycemic and anti-inflammatory effects of triterpene glycoside fractions from aeculus hippocastanum seeds[J]. Molecules, 2021, 26(13): 3 784.
- [46] LIU L, LI S, ZHENG J, et al. Safety considerations on food protein-derived bioactive peptides[J]. Trends in Food Science & Technology, 2020, 96: 199-207.
- [47] CHEN J Y, SUN Y J, HUANG S, et al. Grub polypeptide extracts protect against oxidative stress through the NRF2-ARE signaling pathway[J]. Animal Cells and Systems, 2021, 25(6): 405-415.
- [48] LIU X L, WANG J T, LIU Y, et al. Conjugation of the glutelin hydrolysates-glucosamine by transglutaminase and functional properties and antioxidant activity of the products [J]. Food Chemistry, 2022, 380: 132210.
- [49] NGOH Y Y, GAN C Y. Enzyme-assisted extraction and identification of antioxidative and alpha-amylase inhibitory peptides from Pinto beans (*Phaseolus vulgaris* cv. Pinto)[J]. Food Chemistry, 2016, 190: 331-337.
- [50] CUI H, HAYAT K, ZHANG X. Antioxidant activity in vitro of N-(1-deoxy-alpha-d-xylulos-1-yl)-Phenylalanine: Comparison among Maillard reaction intermediate, end-products and xylose-phenylalanine[J]. Journal of Food Science, 2019, 84(5): 1 060-1 067.
- [51] NOOSHKAM M, VARIDI M, BASHASH M. The Maillard reaction products as food-born antioxidant and antibrowning agents in model and real food systems[J]. Food Chemistry, 2019, 275: 644-660.
- [52] JIANG W, LIU Y, YANG X Q, et al. Antioxidant and antibacterial activities of modified crab shell bioactive peptides by Maillard reaction[J]. International Journal of Food Properties, 2018, 21(1): 2 730-2 743.
- [53] LIU M, MIN L, ZHU C, et al. Preparation, characterization and antioxidant activity of silk peptides grafted carboxymethyl chitosan[J]. International Journal of Biological Macromolecules, 2017, 104: 732-738.
- [54] WANG Q H, KUANG H X, SU Y, et al. Naturally derived anti-inflammatory compounds from Chinese medicinal plants [J]. Journal of Ethnopharmacology, 2013, 146(1): 9-39.
- [55] CHENG X, GAO D, CHEN B, et al. Endotoxin-Binding peptides derived from casein glycomacropeptide inhibit lipopolysaccharide-stimulated inflammatory responses via blockade of NF-kappa B activation in macrophages[J]. Nutrients, 2015, 7(5): 3 119-3 137.
- [56] ROLDAN N R, JIMENEZ M, CERVANTES-GARCIA D, et al. Glycomacropeptide administration attenuates airway inflammation and remodeling associated to allergic asthma in rat [J]. Inflammation Research, 2016, 65(4): 273-283.
- [57] ORTEGA-GONZALEZ M, CAPITAN-CANADAS F, REQUENA P, et al. Validation of bovine glycomacropeptide as an intestinal anti-inflammatory nutraceutical in the lymphocyte-transfer model of colitis[J]. British Journal of Nutrition, 2014, 111(7): 1 202-1 212.
- [58] CHENG X, GAO D X, CHEN B, et al. Endotoxin-binding peptides derived from casein glycomacropeptide inhibit lipopolysaccharide-stimulated inflammatory responses via blockade of NF-kappa B activation in macrophages[J]. Nutrients, 2015, 7(5): 3 119-3 137.
- [59] LI T G, GAO D X, DU M, et al. Casein glycomacropeptide hydrolysates inhibit PGE2 production and COX2 expression in LPS-stimulated RAW 264.7 macrophage cells via Akt mediated NF-kappa B and MAPK pathways[J]. Food & Function, 2018, 9(4): 2 524-2 532.
- [60] REYES-PAVON D, CERVANTES-GARCIA D, BERMUDEZ-HUMARAN L G, et al. Protective effect of glycomacropeptide on food allergy with gastrointestinal manifestations in a rat model through down-regulation of type 2 immune response[J]. Nutrients, 2020, 12(10): 2 942.
- [61] KARNJANAPRATUM S, O'CALLAGHAN Y C, BENJAKUL S, et al. In vitro cellular bioactivities of Maillard reaction products from sugar-gelatin hydrolysate of unicorn leatherjacket skin system[J]. Journal of Functional Foods, 2016, 23: 87-94.
- [62] ORLIAGUET L, EIJLALMANESH T, ALZAID F. Metabolic and molecular mechanisms of macrophage polarisation and adipose tissue insulin resistance [J]. International Journal of Molecular Sciences, 2020, 21(16): 5 731.
- [63] BRUNTON S. GLP-1 receptor agonists vs. DPP-4 inhibitors for type 2 diabetes: Is one approach more successful or preferable than the other? [J]. International Journal of Clinical Practice, 2014, 68(5): 557-567.
- [64] WANG X, SON M, MERAM C, et al. Mechanism and potential of egg consumption and egg bioactive components on type-2 diabetes [J]. Nutrients, 2019, 11(2): 357.
- [65] SONG J J, GAO J, DU M, et al. Casein glycomacropeptide hydrolysates ameliorate hepatic insulin resistance of C57BL/6J mice challenged with high-fat diet[J]. Journal of Functional Foods, 2018, 45: 190-198.
- [66] YUAN Q C, ZHAN B Y, CHANG R, et al. Antidiabetic effect of casein glycomacropeptide hydrolysates on high-fat diet and STZ-induced diabetic mice via regulating insulin signaling in skeletal muscle and modulating gut microbiota [J]. Nutrients, 2020, 12(1): 220.
- [67] SONG J J, WANG Q, DU M, et al. Casein glycomacropeptide-derived peptide IPPKKNQDKTE ameliorates high glucose-induced insulin resistance in HepG2 cells via activation of AMPK signaling [J]. Molecular Nutrition & Food Research, 2017, 61(2): 1600301.

(下转第 217 页)

- Transactions of the Chinese Society of Agricultural Engineering, 2016, 32(7): 19-27.
- [41] 孙小永, 李滨, 潘荣晴, 等. 油茶果脱壳分选一体机设计研究[J]. 科技创新与生产力, 2021(7): 102-104.
- SUN X Y, LI B, PAN R Q, et al. Design and research on the integrated machine of camellia oleifera fruit shelling and sorting[J]. Sci-tech Innovation and Productivity, 2021(7): 102-104.
- [42] 杜鑫. 一种油茶果装置高效脱壳分离装置: CN107822153B[P]. 2019-12-13.
- DU X. An efficient hulling and separating device for camellia oleifera: CN107822153B[P]. 2019-12-13.
- [43] KIM S Y. Nut cracking mechanism for variable-sized nuts: US7717033[P]. 2010-05-18.
- [44] IQBAL Z, JOWOWASITO G, LUTFI M, et al. Designing small-medium scale groundnut (*Arachis hypogaea* L.) shelling machine for local merchant in Tuban, East Java[J]. IOP Conference Series: Earth and Environmental Science, 2019, 230(1): 012013.
- [45] 樊涛, 吴兆迁, 曲振兴, 等. 油茶果脱青皮机的设计[J]. 林业机械与木工设备, 2011, 39(10): 35-36.
- FAN T, WU Z Q, QU Z X, et al. Design of green camellia fruit peeling machines [J]. Forestry Machinery & Woodworking Equipment, 2011, 39(10): 35-36.
- [46] 刘亮亮. 压剥式油茶果破壳机的设计与试验[D]. 武汉: 湖北工业大学, 2017: 1.
- LIU L L. Design and study on pressure stripping machine for camellia shelling [D]. Wuhan: Hubei University of Technology, 2017: 1.
- [47] 马君. 多通道气动刨削式油茶果破壳机设计及试验研究[D]. 武汉: 湖北工业大学, 2018: 15.
- MA J. Design and test of multi-channel pneumatic planer for camellia oleifera shelling [D]. Wuhan: Hubei University of Technology, 2018: 15.
- [48] UCHIYAMA N, HO MINH P, YAMANAKA H, et al. Force control for automatic cashew shelling considering size variance[J]. Journal of Advanced Mechanical Design, Systems, and Manufacturing, 2014, 8(3): 0018.
- [49] 熊平原, 王毅, 吴卓葵, 等. 油茶青果脱壳装置研究与设计[J]. 中国农机化学报, 2016, 37(5): 126-129.
- XIONG P Y, WANG Y, WU Z K, et al. Research and design on shelling machine of green camellia fruit[J]. Journal of Chinese Agricultural Mechanization, 2016, 37(5): 126-129.
- [50] 李善森. 油茶青果脱皮机及皮籽分离装置的设计研究[D]. 武汉: 武汉轻工大学, 2016: 21.
- LI S M. Design and research on dehulling machine and separating equipment of camellia oleifera [D]. Wuhan: Wuhan Polytechnic University, 2016: 21.
- [51] 陈礼东. 油茶果脱蒲与分离结构设计及试验研究[D]. 北京: 中国农业机械化科学研究院, 2021: 61.
- CHEN L D. Design and experimental research on shelling and separation structure of camellia oleifera [D]. Beijing: Chinese Academy of Agricultural Mechanization Sciences, 2021: 61.
- [52] 肖本贵, 胡淑芬, 邓勇杰, 等. 油茶果划线破壳装置设计[J]. 南方农机, 2021, 52(16): 17-19.
- XIAO B G, HU S F, DENG Y J, et al. Design of marking and breaking shell device for camellia oleifera fruit[J]. China Southern Agricultural Machinery, 2021, 52(16): 17-19.
- (上接第 198 页)
- [68] SANKARGANESH M, RAJESH J, KUMAR G G V, et al. Synthesis, spectral characterization, theoretical, antimicrobial, DNA interaction and in vitro anticancer studies of Cu(II) and Zn (II) complexes with pyrimidine-morpholine based Schiff base ligand[J]. Journal of Saudi Chemical Societ, 2018, 22(4): 416-426.
- [69] CHUDINOVA Y V, SHAGDAROVA B T, IL'INA A V, et al. Antibacterial effect of peptide conjugates with a quaternized chitosan derivative and its estimation by the method of atomic force microscopy [J]. Applied Biochemistry and Microbiology, 2016, 52(5): 496-501.
- [70] RONG Y, LU Z, ZHANG H, et al. Effects of casein glycomacropeptide supplementation on growth performance, intestinal morphology, intestinal barrier permeability and inflammatory responses in *Escherichia coli* K88 challenged piglets [J]. Animal Nutrition (Zhongguo Xu Mu Shou Yi Xue Hui), 2015, 1(2): 54-59.
- [71] JIANG W, LIU Y, YANG X, et al. Antioxidant and antibacterial activities of modified crab shell bioactive peptides by Maillard reaction[J]. International Journal of Food Properties, 2018, 21(1): 2 730-2 743.
- [72] BATOOL S, CHOKKAKULA S, SONG M S. Influenza treatment: Limitations of antiviral therapy and advantages of drug combination therapy[J]. Microorganisms, 2023, 11(1): 183.
- [73] YAN J, LI J H, HU Z F, et al. Enzymatic synthesis of sialyl lactosamine grafted chitoooligosaccharides [J]. Chinese Journal of Chemistry, 2023, 41(11): 1 299-1 304.
- [74] SAUVE M F, SPAHIS S, DELVIN E, et al. Glycomacropeptide: A bioactive milk derivative to alleviate metabolic syndrome outcomes [J]. Antioxidants & Redox Signaling, 2021, 34(3): 201-222.
- [75] TSUJI S, ASO Y, OHARA H, et al. Aqueous synthesis of sialylglycopeptide-grafted glycopolymers with high affinity for the lectin and the influenza virus hemagglutinin[J]. Journal of Polymer Science, 2020, 58(4): 548-556.
- [76] STADTMUELLER M N, BHATIA S, KIRAN P, et al. Evaluation of multivalent sialylated polyglycerols for resistance induction in and broad antiviral activity against influenza viruses[J]. Journal of Medicinal Chemistry, 2021, 64(17): 12 774-12 789.