大豆蛋白的甘露聚糖糖基化研究

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摘 要:将大豆分离蛋白与甘露聚糖糖基化生成大豆糖蛋白,力求为工业化生产大豆糖肽作为原料。采用SDS-PAGE电泳技术,OPA法测定大豆蛋白的接枝度,结果大豆分离蛋白的7S和11S中各亚基均参与了糖基化反应,70℃和80℃的蛋白接枝度在30%左右;糖蛋白的溶解性测定结果显示经过糖基化之后的大豆糖蛋白溶解性有显著提高并且等电点向酸性方向偏移,糖基化之后的蛋白含糖量随糖基化温度升高而增加。

关键词:大豆分离蛋白;糖蛋白;甘露聚糖;糖基化

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Study on Glycosylation of Soy Protein Isolated and Mannose

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Abstract: In recent years, studies have shown that the soybean glycopeptide digested by β-conglycinin has the potential function of inhibiting the adhesion of pathogens and the maintenance of intestinal health. Therefore, the soybean glycoprotein glycosylated by soy protein isolated and mannose can provide raw material for the industrial production of soybean glycopeptide. The research investigated the soybean glycoprotein and the grafting degree with the method of SDS-PAGE and OPA. Results showed that the subunits of soy protein isolated all involved in the glycosylation reaction and the grafting degree is about 30% in 70℃ and 80℃. After glycosylation, the solubility of glycoprotein significantly improved and isoelectric point shift to the acid direction, and the sugar content of glycosylated protein increased with the increment of glycosylation temperature.

Key words: Soy protein isolated; Glycoprotein; Mannose; Glycosylation

大豆中的蛋白质主要是由大豆球蛋白(glycinin)和β-伴大豆球蛋白(β-conglycinin)构成的,研究表明,β-伴大豆球蛋白不同于大豆球蛋白,是含有3.8%甘露糖和1.2%葡糖胺的糖蛋白[1],糖链以N-糖基化的方式与肽链相连[2]。对大豆糖蛋白的N-聚糖结构研究发现,糖链构型主要是高甘露糖型(96.6%)和含有木糖的N-乙酰葡糖胺(3.4%)[3]。近年来的研究表明,其中的甘露糖亚结构在抑制致病性大肠杆菌O114,O26,O111和鼠伤寒沙门氏菌、肠炎沙门氏菌粘附LoVo细胞方面起关键作用[4]。所以甘露糖糖基化的大豆蛋白具有新的开发价值。

但是由于大豆中的天然甘露糖蛋白含量很低,仅7S中含少量,提取率不高,难以工业化生产,因此,采用甘露聚糖与大豆蛋白进行干热反应,其目的是制备甘露聚糖与蛋白的复合物,提高大豆糖蛋白的产量。

1 材料与方法

1.1 材料与设备

低温脱脂豆粕,蛋白质(干基)%≥50,安阳漫天雪食品制造有限公司;大豆分离蛋白,自制;甘露低聚糖,成都协力魔芋科学种植加工园有限公司;考马斯亮蓝G-250、溴化钾、氢氧化钠、盐酸、三氯乙酸、浓硫酸、溴酚蓝、十二烷基硫酸钠(SDS)、偏亚硫酸氢钾、碱性品红、偏重亚硫酸钠、甲醇、脲等均为北京化学试剂公司分析纯产品;牛血清白蛋白(BSA)购自北京天来生物医学科技有限公司;β-巯基乙醇(2-ME)为Sigma公司产品;N, N′-甲叉双丙烯酰胺、四甲基乙二胺(TEMED)为Fluka公司产品;邻苯二甲醛(OPA)赖氨酸、硼酸,国药化学试剂有限公司。

紫外可见分光光度计,上海光谱仪器有限公司;电泳槽BIO-CRAFT, JAPAN MODEL BE-210;稳压稳流定时电泳仪,北京六一仪器厂;脱色摇床,江苏海门市麒麟医用仪器厂;喷雾干燥塔,JAPAN EYELA。

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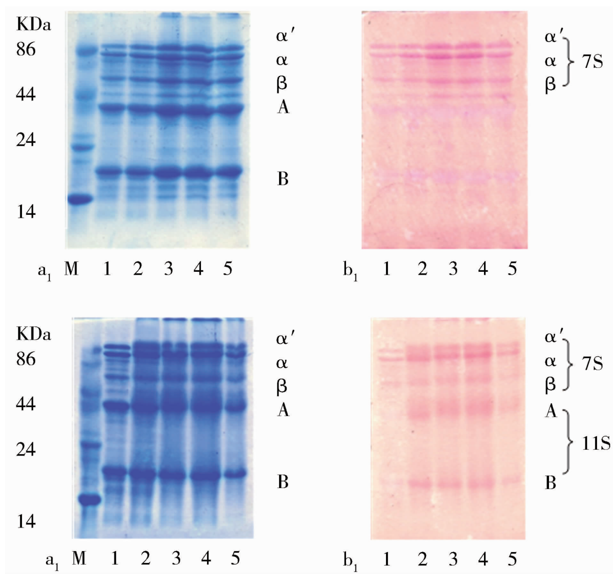

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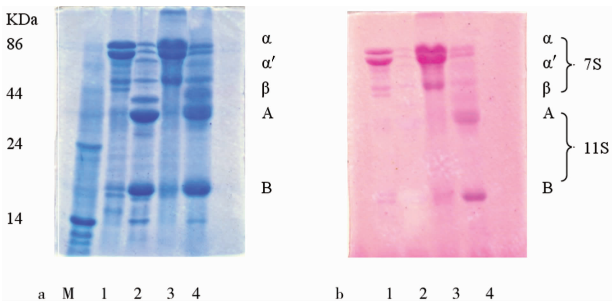

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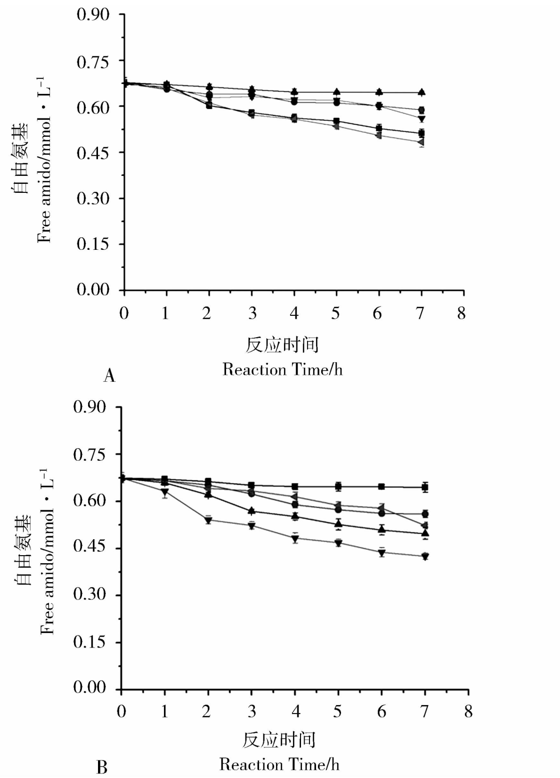

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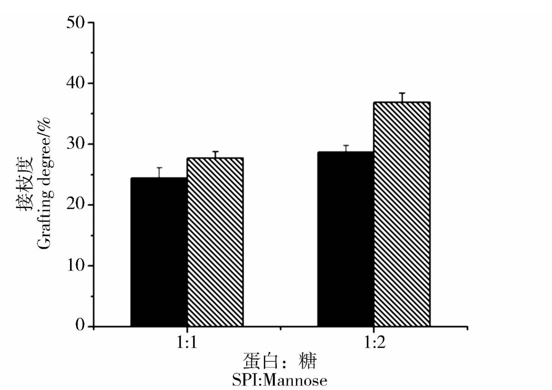

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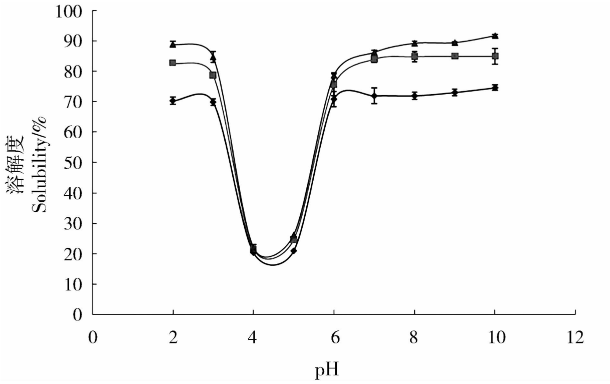

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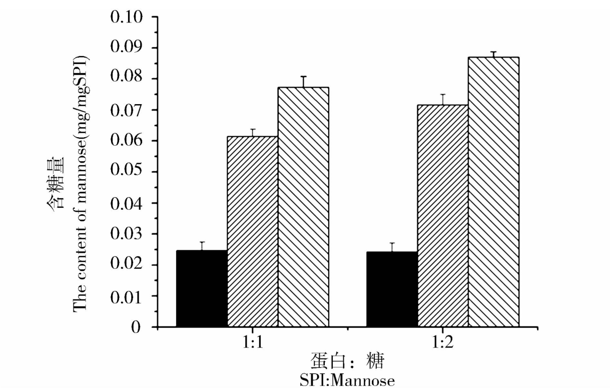

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