Purification, chemical analysis and antioxidative activity of polysaccharides from pH-modified citrus pectin after dialyzation

Tao Zhang a b 1, Ming Shuai a b 1, Pengcheng Ma a b, Jian Huang a b, Chengxin Sun c, Xiaodong Yao c, Zehui Chen a b, Xun Min a b, Shengkai Yan a b

Abstract

Oxidative stress is a major risk factor implicated in the pathogenesis of many human diseases ranged from inflammation to cancer. Modified citrus pectin (MCP) is a commercially available dietary supplement produced from citrus pectin. However, the antioxidative activity of MCP is currently unknown. In this study, MCP was fractionated by a combination of DEAE-cellulose and Sephadex G-75 chromatographies into one neutral fraction (MCPN) and four acidic fractions (MCP02a, MCP02b, MCP04a, and MCP04b). Among these fractions, MCP02b and MCP04a exhibited good DPPH•-scavenging effect with a concentration-dependent manner. Structural analysis results showed that MCP02b mainly contained the HG domain as well as some RG-I domains with β-(1 → 4)-galactan side chains and 4-O-Me-β-GlcA attached to the nonreducing terminals of the side chains. These findings could extend the applications of MCP as an antioxidant agent for functional foods or food additives.

Introduction

Antioxidants support the antioxidative defense network in our bodies against the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that induce damage to biomolecules in vivo, such as DNA, proteins, and lipids, and to reduce the risk of many diseases, such as diabetes, neurological diseases, cardiovascular diseases, aging, and inflammation (Pham-Huy, He, & Pham-Huy, 2008; Poprac et al., 2017; Raha & Robinson, 2000; Totter, 1980; Wiseman & Halliwell, 1996). Antioxidants are very important for human health, but synthetic antioxidants may have serious side effects, such as liver damage and carcinogenesis (Silva & Lidon, 2016; Soubra, Sarkis, Hilan, & Verger, 2007). Thus, it is necessary to find new natural antioxidants without toxicity or side effects to protect the human body from ROS and RNS.

Recently, it has been discovered that pectin has positive effects on human health and multiple biomedical activities, including immunostimulatory, antitumor, anti-inflammatory and antiviral activities, in addition to serving as a gelling and stabilizing polymer in diverse food and specialty products (Maxwell, Belshaw, Waldron, & Morris, 2012; Minzanova et al., 2018; Mohnen, 2008; Naqash, Masoodi, Rather, Wani, & Gani, 2017; Zhang, Xu, & Zhang, 2015). Pectin is in a family of galacturonic acid-rich complex heteropolysaccharides containing several domains, such as homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (Mohnen, 2008; Perez, 2003; Yapo, 2011). Modified citrus pectin (MCP) includes acid/alkali hydrolysis products named GBC-590/GCS-100 (Chan et al., 2018; Eliaz & Raz, 2019; Platt & Raz, 1992), high-temperature modification products named HTCP (Hao et al., 2013) and enzymatic degradation products named PectaSol-C (Hossein et al., 2019; Merheb, Abdel-Massih, & Karam, 2019), which have been registered as the dietary supplements in the United States (Eliaz & Raz, 2019; Yan & Katz, 2010). A number of studies have demonstrated that MCP is effective against cancers such as breast carcinoma, prostate carcinoma and colon carcinoma, exhibiting strong antitumor activities both in vitro and in vivo (Eliaz & Raz, 2019; Glinsky & Raz, 2009). Although many of the biological activities of MCP have been studied extensively, its active fractions and chemical structures related to antioxidative effects have rarely been addressed. Thus, in this study, MCP was fractionated by a combination of DEAE-cellulose and Sephadex G-75 chromatography, and the antioxidative effects and chemical structures of the purified polysaccharide fractions were studied. Our results provided insights into the deep understanding of MCP and its potential use in functional foods or food additives.

Section snippets

Materials

Citrus pectin (CAS: 9000-69-5) was purchased from Kuerhuaxue Company Limited (Beijing, China). Dextrans (2000 kDa, 800 kDa, 100 kDa, 50 kDa, 10 kDa, and 0.18 kDa), galacturonic acid (GalA), glucuronic acid (GlcA), galactose (Gal), arabinose (Ara), rhamnose (Rha), glucose (Glc), mannose (Man), xylose (Xyl), fucose (Fuc), 1-phenyl-3-methyl-5-pyrazolone (PMP), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma

Monosaccharide composition of MCP fractions

MCP was separated and purified into three homogeneous subfractions, MCP02a (yield 2.9% MCP), MCP02b (yield 1.4% MCP), and MCP04b (yield 1.1% MCP), and one heterogeneous subfractions, MCP04a (yield 5.4% MCP), as shown in Fig. 1A & B. Pectin is composed of several types of monosaccharide residues, and the monosaccharide compositions were analyzed by the PMP precolumn derivatization HPLC method in order to determine how many types of monosaccharide residues were present in the MCP fractions. As

Conclusions

In this study, MCP was fractionated by a combination of DEAE-cellulose and Sephadex G-75 chromatographies into five fractions named MCPN, MCP02a, MCP02b, MCP04a and MCP04b. The structural characterization and in vitro antioxidative activities of these fractions were investigated, and one fraction, MCP02b, exhibited strong DPPH•-scavenging activity with a concentration-dependent manner. MCP02b was mainly composed of GalA (70.0%), GlcA (3.4%), Rha (1.0%) and Gal (25.6%), with an average molecular

CRediT authorship contribution statement

Tao Zhang: Conceptualization, Methodology, Writing - review & editing. Ming Shuai: Methodology, Data curation, Writing - original draft. Pengcheng Ma: Methodology, Data curation. Jian Huang: Software, Visualization. Chengxin Sun: Validation, Investigation. Xiaodong Yao: Software. Zehui Chen: Supervision. Xun Min: Methodology. Shengkai Yan: Writing - review & editing. Declaration of competing interest The authors declare that they have no competing interests.

Acknowledgments

This work was supported by the Technology Research and Development Program of Guizhou (No. qiankehezhicheng [2018]2803), the Foundation for Young Scientists Growth Project of Guizhou Province (No. qianjiaoheKYzi2017192), the Program for Excellent Young Talents of Zunyi Medical University (No. 18zy-006).