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Preparation and characterization of low methoxyl pectin by high hydrostatic pressure-assisted enzymatic treatment compared with enzymatic method under atmospheric pressure

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Citric acid (PubChem CID: 311)
Sodium citrate (PubChem CID: 6224)
Sodium azide (PubChem CID: 33557)
Sodium hydroxide (PubChem CID: 14798)
Sucrose (PubChem CID: 5988)
Calcium dichloride (PubChem CID: 5284359)
Sodium chloride (PubChem CID: 5234)

ABSTRACT

A commercial pectin was de-esterified by using the HHP-assisted enzymatic method (E-HHP), and the effects of pressure, temperature and pressure-holding time on the degree of esterification (DE) of pectin de-esterified by E-HHP were investigated. A single factor experiment and an orthogonal test were performed to optimize the de-esterification condition of E-HHP. The optimal conditions of E-HHP consisted of a pressure of 400 MPa, a temperature of 45 °C and a pressure-holding time of 15 min. Under the optimal conditions, the DE was decreased from 65.32 ± 0.64% to 28.08 ± 1.39% within 15 min, whereas 120 min was required to obtain pectin with a similar DE (26.64 ± 1.00%) using the enzymatic method under atmospheric pressure (E-AP). The physicochemical properties, rheological and gelling characteristics of pectin de-esterified by E-HHP and E-AP were also compared. The results showed that the pectin prepared by E-HHP had much higher viscosity and induced a rapid and homogeneous gelation, leading to the formation of gel with better viscoelastic properties, whereas the other characteristics showed no significant difference. E-HHP had no degradation action on the pectin molecule as demonstrated by the viscosity-average molecular weight and molecular weight distribution. From these results, it could be concluded that E-HHP is a highly efficient and novel method to prepare low methoxyl pectin.

1. Introduction

Commercial pectin is widely applied in the preparation of jams, jellies and other food gels and is divided into two general groups: high methoxyl pectin (HMP) and low methoxyl pectin (LMP) depending on the degree of esterification (DE) (Thakur, Singh, & Handa, 1997).

Gelation of HMP occurs in the presence of a high concentration of soluble solids (typically ~60–65 wt%) such as sugars under pH < 3.5 (Evageliou, Richardson, & Morris, 2000; Thibault & Ralet, 2003); LMP forms gel in the presence of a bivalent cation (such as calcium) in a wide pH range regardless of sugar concentration (Endress, Mattes, & Norz, 2006; Thibault & Ralet, 2003). This property makes LMP useful for many applications in which HMP

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cannot be used, such as low calorie or dietetic foods (Lopes da Silva & Rao, 2006; Endress et al., 2006).

Pectin obtained from fruits and vegetables is mostly HMP with a DE ranging from approximately 60%–90% (Van Buren, 1991) and therefore LMP is usually produced through the de-esterification of HMP. The main methods reported for the preparation of LMP from HMP have used four types of agents: acids, alkali, ammonia in alcohol or concentrated aqueous ammonia, and pectin methyl esterase (Renard & Thibault, 1996). The first three methods are disadvantageous in that they produce chemical waste that can cause environmental damage and the de-esterification process is random and can result in a decreased molecular weight due to the depolymerization of pectin (Ishii & Yokotshuka, 1971; Renard, 1996). Therefore, it is desirable to establish a new environmentally friendly method to prepare LMP in which the molecular weight is preserved. Undoubtedly, enzymatic de-esterification with pectin methyl esterase (PME) represents an attractive alternative to chemical de-esterification considering that it could catalyze pectin de-esterification and have no effect on the molecular weight of pectin (Körner, Limberg, Christensen, Mikkelsen, & Roepstorff, 1999; Willats et al., 2001) as this method releases methanol and protons and also creates blocks of free carboxyl groups (Sila et al., 2007). Nevertheless, enzymatic de-esterification is time-consuming and undergoes a relatively long period of 50–240 min (Zykwinska et al., 2008; Kim, Yoo, Kim, Park, & Yoo, 2008; Limberg et al., 2000). Therefore, there is a need to explore new methods to enhance enzyme catalysis or elevate the reaction rate to obtain an LMP with the desired DE more efficiently.

High hydrostatic pressure (HHP) can be used to inactivate microorganisms and enzymes as an alternative or complementary method to conventional heat treatment in the food industry such as in fresh fruit or vegetable juice (Cao et al., 2012; Rao et al., 2014). HHP can also alter the conformation of starch or protein (Liu, Zhang, Shen, Hu, & Wu, 2012; Qin et al., 2013) and extract bioactive ingredients from natural products (Corrales, Garcia, Butz, & Tauscher, 2009). Recently, several studies utilizing HHP for pectin extraction produced pectin with higher viscosity and better emulsifying stability (Guo et al., 2012, 2014; Naghshineh, Olsen, & Georgiou, 2013), indicating that HHP is a more efficient, time saving, and eco-friendly alternative for pectin extraction. Furthermore, there is evidence that HHP can induce the stimulation and enhancement of pectin de-esterification reaction catalyzed by pectin methyl esterase (PME) in the range of 200–400 MPa, depending on the PME origin, pH, or systems (buffer, juice, tissue) (Bordenave, 1996; Hsu, 2008; Sila et al., 2007). Therefore, HHP assisted PME treatment could be a novel and promising technology for pectin de-esterification with high efficiency and contamination-free. However, little information has been published that the combined effects of HHP and PME treatment on the properties of pectin, namely the substrate of the PME catalyzed reaction.

The aim of this study was to investigate the effect of an HHP-assisted enzymatic treatment (E-HHP) on the de-esterification of a commercial HMP from citrus and to optimize the de-esterification condition. In addition, the characteristics of the pectin de-esterified by E-HHP were compared with that of the pectin produced by enzymatic de-esterification under atmospheric pressure (E-AP).

2. Materials and methods

2.1. Materials and apparatus

A commercial pectin methyl esterase (PME) named Novoshape, with a declared activity of 10 PEU/mL, was kindly supplied to us by Novozymes China. It is a gene-encoding enzyme derived from the fungus *Aspergillus aculeatus* and transferred into a strain of the food grade organism *Aspergillus oryzae*.

Esterified citrus pectin (DE = 65.31 ± 0.64%, galacturonic acid content = 71.23 ± 3.5% on dry basis) was purchased from Quzhou pectin CO., LTD (Zhejiang, China) and used as a "parent pectin" to be de-esterified by different methods.

Chemical reagents, including ethanol, citric acid, sodium citrate, sodium azide, etc., were analytical grade and purchased from Lanyi reagent company (Beijing, China). Galacturonic acid monohydrate was purchased from Sigma–Aldrich (Shanghai, China).

HHP-assisted enzyme de-esterification was performed using a pressure-assisted thermal high hydrostatic pressurization unit (CAU-HHP-700-6, Baotou Kefa High Pressure Food Processing Inc., Inner Mongolia, China) with a cylindrical pressure chamber capacity of 7 L. The pressure-transmitting medium was distilled water. Pressure was built up at a rate of 130 MPa/min and the pressure release was immediate.

2.2. De-esterification and purification of pectin

2.2.1. Enzymatic de-esterification under atmospheric pressure

Parent pectin was dissolved in distilled water (1 g/100 mL). 0.5 mL of the PME solution that had been diluted 10 times was added to the pectin solution and subsequently the pH value was adjusted to 4.5 by the addition of 1.0 M NaOH following a preheating process at 45 °C for 5 min, and then the de-esterification was performed at 45 °C for 2 h in a reciprocating shaker bath. The solution was heated in 100 °C water for 5 min to inactivate the enzyme and then cooled to below 40 °C. One volume of pectin solution was precipitated using two volumes of 95% (v/v) ethanol and was then kept overnight without stirring at 4 °C.

The precipitated pectin was filtered off and washed three times using 95% ethanol to remove monosaccharides and disaccharides (Minkov, Minchev, & Paev, 1996). After purification, the wet pectin was dried at 40 °C in a vacuum drying oven until its weight was constant.

2.2.2. HHP-assisted enzymatic de-esterification

Parent pectin was dissolved in distilled water (1 g/100 mL). The pH of the solution was adjusted to 4.5 with NaOH (1.0 M) followed by the addition of 0.5 mL of the PME solution that had been diluted 10 times. The mixture underwent a preheating process at 45 °C for 5 min and then was vacuum-packaged in a polyethylene bag and placed in the high hydrostatic pressure vessel. After HHP treatment, the solution was heated in 100 °C water for 5 min to inactivate the enzyme and then cooled to below 40 °C.

Precipitation, purification and drying of the de-esterified pectin were the same as the procedure described in Section 2.2.1.

2.3. Optimization of HHP-assisted enzyme de-esterification

2.3.1. Single factor test

Based on the method described in Section 2.2.2, de-esterification was performed under pressure levels ranging from 100 to 400 MPa, pressure-holding times ranging from 5 to 15 min and temperatures ranging from 35 to 65 °C; the effects of these factors on the DE of pectin were observed.

2.3.2. Orthogonal test

It was shown in the single factor experiments that the three factors, pressure (A), pressure-holding time (B), and temperature (C) each affected the DE of pectin. Therefore, an orthogonal L9 (3^3) test design was used to optimize the de-esterification condition and a DE was chosen as the determination index. The three factors at