ISSN:3007-9640 DOI: https://doi.org/10.70114/acmsr.2025.3.1.P90

# Effect of High-Pressure Homogenization on Physicochemical Properties and Antioxidant Activity of Hawthorn Pectin

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**Abstract.** This study aimed to investigate the effect of high-pressure homogenization (HPH) on hawthorn pectin's physicochemical properties and antioxidant activity. The results of the study showed that the galacturonic acid (Gal A) content of hawthorn pectin increased by 12%, esterification decreased by 12%, and the molecular weight and its distribution decreased at a high-pressure homogenization pressure of 150 MPa and several times. FT-IR showed that the measurements of pectin were degraded. According to the results of four in vitro antioxidant activity experiments used in this study, the antioxidant activity of hawthorn pectin was effectively enhanced. Therefore, high-pressure homogenization is a practical modification method that can enhance pectin's antioxidant activity by changing its relevant physicochemical properties.

**Keywords:** Pectin, High-pressure homogenization, Antioxidant activity.

## 1. Instruction

Pectin, an acidic polysaccharide with a galacturonic backbone, consists of three polysaccharide structural domains based on common characteristics: homogalacturonan (HG), rhamnogalacturonan I (RG I), and rhamnogalacturonan II (RG II) [1,2]. As a natural polysaccharide, pectin has excellent antioxidant and anti-inflammatory activities [3], which can be intensively studied as a novel antioxidant. The biological activity of pectin depends on its physicochemical and structural properties. Some studies have shown that the antioxidant activity of pectin is closely related to the degree of esterification, the content of galacturonic acid, and the degree of branching [5]; Li et al. believe that the reduction of the molecular weight and the simplification of the structure of pectin is conducive to the improvement of its bioactivity and bioavailability [5]. Some studies have shown that hawthorn pectin has a significant molecular weight, and its biological activity depends on molecular weight size, structure, and physical and chemical properties [6,7]. Therefore, it is necessary to modify pectin to obtain pectin with higher antioxidant activity.

Traditional modification methods such as acid hydrolysis and alkaline hydrolysis are demanding for experimental conditions and are not environmentally friendly [8]. High-pressure homogenization (HPH), which is widely used in industry for fluid material processing, can affect emulsion properties by inducing different stability mechanisms [9]; on the other hand, HPH can change the structure and physicochemical properties of biomolecules in solution. Zhou et al. successfully improved the antioxidant activity of large-leaf yellow tea polysaccharides using HPH [10].

To the best of our knowledge, most current studies on modifying the antioxidant activity of hawthorn pectin have focused on ultrasonic treatment, heating treatment, and so on. Very few studies have examined the properties and antioxidant activity of hawthorn pectin by high-pressure homogenization treatment. Therefore, this study aims to discuss the effects of high-pressure homogenization and pressure changes on the antioxidant activity of hawthorn pectin.

ISSN:3007-9640 DOI: https://doi.org/10.70114/acmsr.2025.3.1.P90

# 2. Results and Discussion

## 2.1 Physicochemical Properties

It can be seen that the main component of hawthorn pectin is galacturonic acid (Gal A), followed by other neutral sugars, mainly rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glu), xylose (Xly) (Table 1). The ratio of galacturonic acid to rhamnose, Gal A/Rha, can be used to assess the primary type of pectin [16], which indicates that hawthorn pectin is of HG type. Rha, Ara and Xly, as the main components of pectin's neutral sugar side chain, tended to decrease with the increase of treatment pressure, indicating that high-pressure homogenization destroyed pectin's side chain. According to the experimental results, DE decreased from 76.64% to 64.53% as the HPH pressure increased from 0 to 150 MPa. It has been shown that low-esterification pectin has excellent thermal stability and higher biological activity [11]. Therefore, high-pressure homogenization reduced the esterification degree of hawthorn pectin, which positively affected the enhancement of the antioxidant activity of hawthorn pectin. Dong et al. found that Gal A content played a crucial role in the antioxidant capacity of polysaccharides in their study of polysaccharides [12]. In this experiment, the Gal A content of hawthorn pectin increased significantly after undergoing high-pressure homogenization treatment. However, there was no significant difference in Gal A between different homogenization pressures, which was the same as the findings of Xie et al. [13]. The possible reasons for this are that HPH treatment degraded some covalent ester cross-links between the homogalacturonan acid region of pectin and the hydroxyl groups of other cell-wall polysaccharides [14] and that Gal A was more tolerant to the action of high-pressure homogenization than other neutral sugars.

Table 2 shows the Mw (weight-averaged molecular weight) and Mn (number-averaged molecular weight) of hawthorn pectin before and after high-pressure homogenization, as well as the ratio PDI (dispersion index) of the two. Both Mw and Mn of hawthorn pectin decreased significantly after undergoing a high-pressure homogenization process; with increasing homogenization pressure, Mw decreased from 311.20 KDa to 220.82 KDa, and Mn decreased from 105.11 KDa to 85.47 KDa. It has been demonstrated that pectin with low molecular weight has more potent antioxidant activity [14]; therefore, the enhancement of pectin antioxidant activity caused by high-pressure homogenization may be related to the decrease in molecular weight. From Table 2, we can see that the dispersion index of hawthorn pectin decreased from 2.96 to 2.58 with the increase of high-pressure homogenization pressure, which represents that the pectin long and short chains of pectin changed from a mixed state to a separated state after undergoing the process of high-pressure homogenization; we also noticed that the tendency of the PDI value to decrease with the increase of the pressure was not fluctuating, which indicated that the process of the high-molecular-weight pectin being degraded to low-molecular-weight pectin and uniformly distributed in the system was rapid.

Table1. Carbohydrate composition of pectin under diffident HPH treatment.

| Sample | Rha (mol%) | Ara (mol%) | Xly (mol%) | Gal (mol%) | Glu (mol%) |
|---|---|---|---|---|---|
| CK | $1.21\pm0.02^{c}$ | $1.17\pm0.05^{a}$ | $0.98\pm0.04^{a}$ | $0.59\pm0.52^{a}$ | $1.17\pm0.01^{b}$ |
| 30 MPa | $0.94\pm0.03^{b}$ | $1.19\pm0.01^{c}$ | $0.92\pm0.01^{b}$ | $1.08\pm0.01^{a}$ | $1.27\pm0.04^{c}$ |
| 60 MPa | $0.87\pm0.01^{a}$ | $1.29\pm0.01^{b}$ | $0.93\pm0.01^{ab}$ | $1.01\pm0.02^{a}$ | $1.07\pm0.09^{a}$ |
| 90 MPa | $0.85\pm0.28^{b}$ | $1.08\pm0.21^{a}$ | $0.96\pm0.11^{a}$ | $0.96\pm0.02^{b}$ | $1.15\pm0.02^{a}$ |
| 120 MPa | $0.85\pm0.31^{a}$ | $0.84\pm0.13^{a}$ | $0.95\pm0.13^{b}$ | $0.94\pm0.01^{a}$ | $1.20\pm0.04^{b}$ |
| 150 MPa | $0.84\pm0.03^{b}$ | $0.73\pm0.06^{a}$ | $0.94\pm0.02^{a}$ | $0.92\pm0.03^{a}$ | $1.18\pm0.01^{a}$ |

Table2. Gal A content, DE value and molecular weight distribution of pectin under diffident HPH treatment.

| Sample | Gal A(%) | DE(%) | Mw(KDa) | Mn(KDa) | Mw/Mn |
|---|---|---|---|---|---|
| CK | $70.79\pm0.11^{e}$ | $76.64\pm0.47^{a}$ | $311.20\pm0.13^{a}$ | $105.11\pm0.21^{a}$ | $2.96\pm0.11^{a}$ |

ISSN:3007-9640 DOI: https://doi.org/10.70114/acmsr.2025.3.1.P90

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|---|---|---|---|---|---|
| 30 MPa | 75.63±0.16$^{d}$ | 68.23±0.09$^{b}$ | 266.29±0.13$^{b}$ | 96.25±0.14$^{b}$ | 2.76±0.34$^{b}$ |
| 60 MPa | 76.6a5±0.23$^{d}$ | 66.29±0.12$^{c}$ | 261.96±0.56$^{c}$ | 95.33±0.13$^{b}$ | 2.74±0.25$^{c}$ |
| 90 MPa | 78.55±0.29$^{c}$ | 65.22±0.14$^{d}$ | 248.90±0.22$^{d}$ | 93.98±0.35$^{c}$ | 2.64±0.13$^{d}$ |
| 120 MPa | 80.32±0.21$^{b}$ | 64.77±0.10$^{e}$ | 235.53±0.39$^{e}$ | 92.76±0.28$^{d}$ | 2.53±0.33$^{e}$ |
| 150 MPa | 82.43±0.15$^{a}$ | 64.53±0.13$^{e}$ | 220.82±0.42$^{f}$ | 85.47±0.19$^{e}$ | 2.58±0.16$^{e}$ |

## 2.2 FT-IR analysis

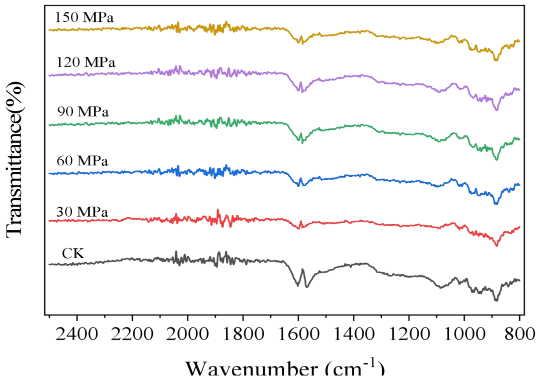


Fig. 1. FTIR spectra (800 cm−2400 cm−1) of pectin under different HPH treatment.

The FT-IR spectra of hawthorn pectin subjected to different pressure high-pressure homogenization processes were shown in Fig. 1. The pectin samples subjected to different HPH pressures differed in the intensity of some bands, and the rest were the same. The two peaks at wavelengths of 1678 cm-1 and 1603 cm-1 are the characteristic peaks of pectin, which represent the stretching vibration of methylated carboxyl group C-O and the asymmetric stretching vibration of free carboxyl group C=O, and the area ratio of these two peaks can be calculated as the DE value of the pectin [15]. From the figure, it can be inferred that all the sample pectin is high ester pectin, and the pectin esterification decreases with increasing pressure, consistent with the titration measurement of esterification. The absorption peaks at 1090 cm-1 and 1010 cm-1 indicate the presence of pyranose and furanose in the pectin [16]. The peaks near 890 cm-1 represent asymmetric C=O stretching vibrations [17]. Meanwhile, the absorption band in the 1300-800 cm-1 range is referred to as the “fingerprint region” of pectin [18]. The results showed that the side chains of hawthorn pectin were degraded by high-pressure homogenization.

## 2.3 Antioxidant Activity

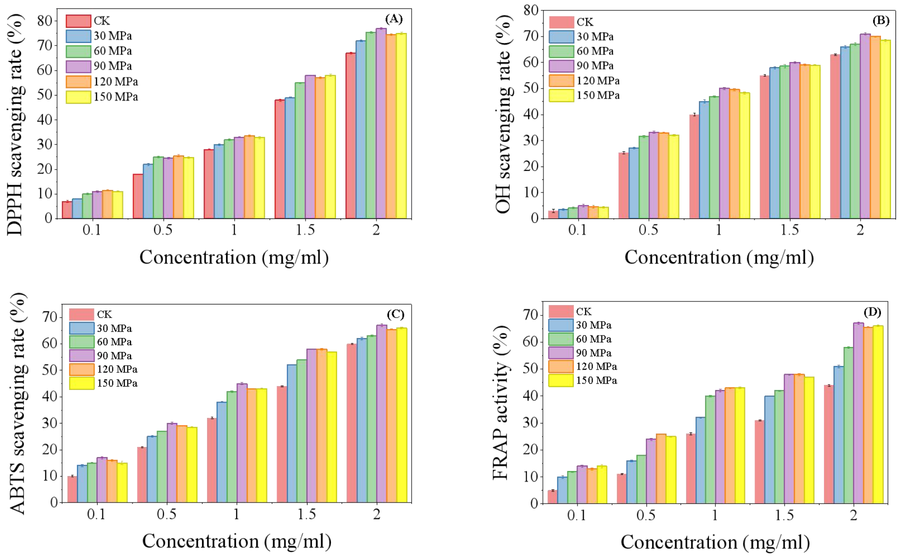


Fig. 2. Scavenging activity of hawthorn pectin on (A) DPPH radical, (B) OH radical (C)ABTS radical, (D)FRAP radical

ISSN:3007-9640 DOI: https://doi.org/10.70114/acmsr.2025.3.1.P90

According to the experimental results of Fig. 2(A)(B)(C)(D), The antioxidant activity of hawthorn pectin was positively correlated with the concentration of pectin solution in all four antioxidant activity assay experiments selected for this experiment. The DPPH scavenging rate of pectin samples was lower at a solution concentration of 0.1 mg/ml; at a pectin concentration of 2 mg/ml and a pressure of 30 MPa, the DPPH scavenging rate was 72%, which was 5% higher than the control (67%). With the increase of homogenization pressure, the antioxidant activity of DPPH firstly increased and then slightly decreased but still higher than that of control (CK). This indicated that high-pressure homogenization can increase the DPPH radical scavenging rate of hawthorn pectin within a specific pressure range. From the Fig 2(B), the scavenging rate increased from 63% to 71% when the pectin concentration was 2 mg/ml, and the pressure increased from 0 to 90 MPa; the scavenging rate decreased to 68.5% when the pressure continued to increase to 150 MPa. Therefore, high-pressure homogenization can increase the hydroxyl radical scavenging rate of pectin, and this phenomenon may be attributed to the decrease in the molecular weight of pectin, which has been shown to possess more potent antioxidant activity for pectin with small molecular weights within a specific molecular weight range [18]. As shown in Fig. 2(C), all the experimental groups at different concentrations had a specific scavenging ability for ABTS radicals, and the ABTS scavenging rate was positively correlated with the concentration of pectin solution. High-pressure homogenization positively affected the increase of ABTS scavenging rate of hawthorn pectin. The scavenging rate increased significantly when the pressure was increased from 0 to 90 MPa, and all the groups had the highest scavenging rate at 90 MPa; as the pressure continued to be increased to 150 MPa, the scavenging rate decreased slightly or remained unchanged, but it was always higher than that of the untreated group. The possible reason for this is that high-pressure homogenization reduces the molecular weight of pectin, and low-molecular-weight pectin contains more exposed reactive groups that provide more hydrogen to scavenge ABTS radicals [19]. The FPAR values of pectin are shown in Fig. 2(D). The concentration pectin solution had higher FPAR activity; high-pressure homogenization-treated hawthorn pectin had more vigorous FPAR antioxidant activity and increased with increasing pressure, which agreed with the experimental results of the three antioxidant activity experiments discussed previously. In addition, high-pressure homogenization leads to pectin depolymerization, which may generate new functional groups within the formed low molecular weight pectin, thereby reducing free radical species [20]. In addition, according to the previous discussion, the increase in pectin Gal A and the decrease in esterification caused by high-pressure homogenization also positively affected the increase in antioxidant activity.

## 3. Conclusion

The experimentally determined fundamental indexes showed that DPPH radical scavenging , hydroxyl radical scavenging , ABTS scavenging rate and FPAR activity were increased in pectin modified by high-pressure homogenization (HPH) compared to natural hawthorn pectin. The galacturonic acid (Gal A) content of pectin was increased, the degree of esterification (DE) and molecular weight (including Mw and Mn) were decreased, and the pectin side chains were degraded. In conclusion, high-pressure homogenization is a green and efficient modification method to improve the antioxidant activity of pectin.

## Acknowledgments

This research was financially supported by Key R&D Program of Shandong Province, China (2024TZXD004, 2023TZXD030)

ISSN:3007-9640 DOI: https://doi.org/10.70114/acmsr.2025.3.1.P90

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