

Ethanol graded extraction and physicochemical properties of pectin from blacken Hawthorn

Huiyao Zhang ^{1, a}, Zhixin Li ^{1, b}, and Chuanhe Zhu ^{1, c *}

¹ College of Food Science and Engineering, Shandong Agricultural University, Taian 271018, China

^a 2465727776@qq.com, ^b 1094123974@qq.com, ^c chhzhu@sdau.edu.cn

Abstract: In this study, four pectin fractions (BHP20, BHP40, BHP60, BHP80) were successfully extracted and separated from blacken hawthorn using hot water extraction and ethanol graded precipitation. The physicochemical properties of BHP fractions were analyzed using various characterization methods. The results revealed that the color of pectin extracted after blackening process was obviously deepened, and the pectin fractions were degraded. The molecular weight, esterification degree and galacturonic acid content of BHP fractions decreased with the increase of ethanol concentration. Furthermore, the composition and proportion of monosaccharides differed significantly. Our findings offer a theoretical foundation for characterizing and utilizing blacken hawthorn pectin, thereby facilitating its high-value utilization.

Keywords: Blacken hawthorn pectin; Ethanol grading; Physicochemical properties

1. Introduction

Hawthorn is a plant of the genus Hawthorn in the family Rosaceae, widely distributed in northern China [1]. Since fresh hawthorn is sour and astringent and not easily accepted by consumers, hawthorn is usually processed appropriately before facing consumers. Blackening processing of hawthorn refers to the process in which raw materials use their own substances to undergo the Melad reaction and other physicochemical changes under high temperature and high humidity conditions, gradually turning black [2]. Black dates, black wolfberries [3], black garlic and other products made through blackening processing have already appeared, while there are fewer research reports on blacken hawthorn.

Hawthorn is rich in pectin (9.94%) and is considered a good source of pectin. In recent years, hawthorn pectin has attracted extensive attention from researchers for its diverse functional properties. Some studies have found that blackening processing decreases the molecular weight of pectin in hawthorn [4]. In addition, due to the polydispersity of pectin polysaccharides, different components may have different properties [5]. In order to better study these properties, researchers have isolated pectic polysaccharides from plants by various methods, among which the ethanol graded precipitation method has been widely used in laboratory preparation and industrial production due to its economic and easy application [6]. Therefore, in this study, the effect of ethanol grading on the physicochemical properties of blacken hawthorn pectin was investigated with blacken hawthorn as the raw material to provide theoretical basis for the application of blacken hawthorn pectin.

2. Materials and methods

2.1 Materials

Fresh hawthorn purchased from Laiwu Wanbang Foods Co. and kept at 4°C.

2.2 Hawthorn blackening

Blacken hawthorn was processed with following to the method of Li et al. [4].

2.3 Ethanol graded extraction of blacken hawthorn pectin

According to the method of Li et al. [7], hot water extraction method and ethanol graded precipitation method were used to extract and separate the blacken hawthorn pectin, and different ethanol graded blacken hawthorn pectin fractions were obtained, which were named as BHP20, BHP40, BHP60 and BHP80.

2.4 Basic physicochemical indicators

2.4.1 Chemical composition

The yield of the BHP fraction was calculated following the method outlined by Li et al [4]. The degree of esterification (DE) was determined using the Fourier infrared spectroscopy (FT-IR) method [7]. Protein content in pectin was determined using the Coomassie Brilliant Blue method [8]. The GalA content in pectin was determined using the m-hydroxybiphenyl method [9]. Gas chromatography was employed to analyze the monosaccharide composition of BHP fractions[4].

2.4.2 Molecular weight distribution

The molecular weight of pectin was determined by high performance gel permeation chromatography (HPGPC) with reference to the method of Li et al. [4].

2.4.3 Colorimetric analysis

The L^* , a^* , and b^* values of the blacken hawthorn pectin samples were determined by a colorimeter.

2.5 Structural characterization of BHP fractions

The surface structure of the BHP fractions was characterized using scanning electron microscopy following the method described by Li et al. [7] The functional group structures of the BHP fractions were analyzed using FT-IR spectroscopy, covering the range of 4000-400 cm^{-1} .

2.6 Thermogravimetric analysis

The freeze-dried pectin samples (5-10 mg) were weighed and measured using a thermogravimetric analyzer. The temperature range was set at 30-600°C, the temperature was gradually increased at a rate of 10°C/min under nitrogen purge.

2.7 Data analysis

The experimental data underwent analysis and processing via SPSS 21.0. One-way analysis of variance (ANOVA, $P < 0.05$) was conducted, employing Duncan and LSD tests. The results were presented as mean and standard deviation, and graphical representations were generated using Origin 2019.

3. Results

3.1 Basic Physicochemical Indicators of BHP

Table 1 Basic physicochemical properties of BHP components

	BHP20	BHP40	BHP60	BHP80
Yield (%)	3.16±0.01a	1.62±0.02b	0.51±0.02c	0.27±0.03d
DE (%)	56.15±0.59a	55.04±0.08b	22.99±0.37c	10.46±0.58d
Protein (%)	2.75±0.14a	1.89±0.15b	1.41±0.18c	3.85±0.26d
Mw (kDa)	61.90±0.41a	34.68±0.70b	24.50±0.28c	4.96±0.17d
Mn (kDa)	36.11±0.30a	20.93±0.54b	12.78±0.47c	1.92±0.09d
Mw/Mn	1.71±0.03a	1.66±0.08ab	1.92±0.09a	1.41±0.27b
GalA (%)	72.96±0.26a	60.77±0.72b	45.19±0.18c	37.77±0.14d

Rha (%)	1.58±0.03a	1.47±0.03b	1.60±0.01a	1.48±0.03b
Ara (%)	1.03±0.03c	1.05±0.02c	1.83±0.04b	2.50±0.09a
Xyl (%)	0.92±0.01d	1.08±0.04c	1.58±0.02a	1.18±0.03b
Man (%)	0.77±0.03c	0.76±0.04c	0.87±0.01b	1.31±0.03a
Gal (%)	1.29±0.02c	1.66±0.03b	7.42±0.15a	7.30±0.06a
Glu (%)	1.06±0.01d	1.31±0.07c	2.37±0.11b	3.34±0.05a
HG (%)	97.44±0.05a	97.13±0.08b	95.82±0.05c	95.36±0.09d
RGI (%)	2.56±0.05d	2.87±0.08c	4.18±0.05b	4.64±0.09a
(Gal + Ara)/Rha	1.48±0.03d	1.83±0.04c	5.79±0.11b	6.61±0.12a

As can be seen from the table, the yield of BHP fractions gradually decreased as the ethanol concentration increased, and BHP20 was the main precipitation product. The decrease in pectin fraction yield may be attributed to the solution's changing polarity, whereby high ethanol concentration impacts the hydration of pectin chains, consequently affecting the yield [10]. The DE of the pectin fraction also decreased from 56.15% to 10.46% with increasing ethanol concentration. In addition, all four BHP fractions contained small amounts of protein (3.85%-1.41%). Hence, controlling ethanol concentration during pectin precipitation is crucial for the chemical composition of pectin fractions.

In terms of molecular weight distribution, the molecular weight range of BHP (56.15-10.46 kDa) was lower compared to the molecular weights of hawthorn pectin (159-213 kDa) determined by Roman et al. [11]. This suggests that degradation of the pectin components occurs during blackening processing. The molecular weight of the pectin fraction gradually decreased with the increase of ethanol concentration, consistent with previous studies on astragalus polysaccharides [12]. In addition, studies have reported that a smaller Mw/Mn ratio corresponds to a narrower molecular weight distribution. Among the four BHP fractions, BHP80 had the narrowest molecular weight distribution, while BHP60 had the widest molecular weight distribution. The results confirmed that ethanol graded precipitation enables the isolation of pectin fractions with varying molecular weights, thus achieving the preliminary isolation of hawthorn pectin polysaccharides.

As can be seen from the table, the main component of the BHP fraction is GalA, followed by Rha, Ara, Gal and Glu. With the increase of ethanol concentration, the content of GalA in the pectin fractions gradually decreased, and the content of Gal and Glu gradually increased, suggesting that the blackening process and the grading of ethanol affects the pectin main chain. In addition, the proportion of the RG-I region increased with the increase of ethanol concentration and it has also been suggested that during the heat treatment process a series of reactions such as hydrolysis and β -elimination occur, which increase the content of monosaccharides such as Gal, Ara and Glu, leading to an increase in the ratio [4].

3.2 Apparent morphology and Colorimetric analysis of BHP

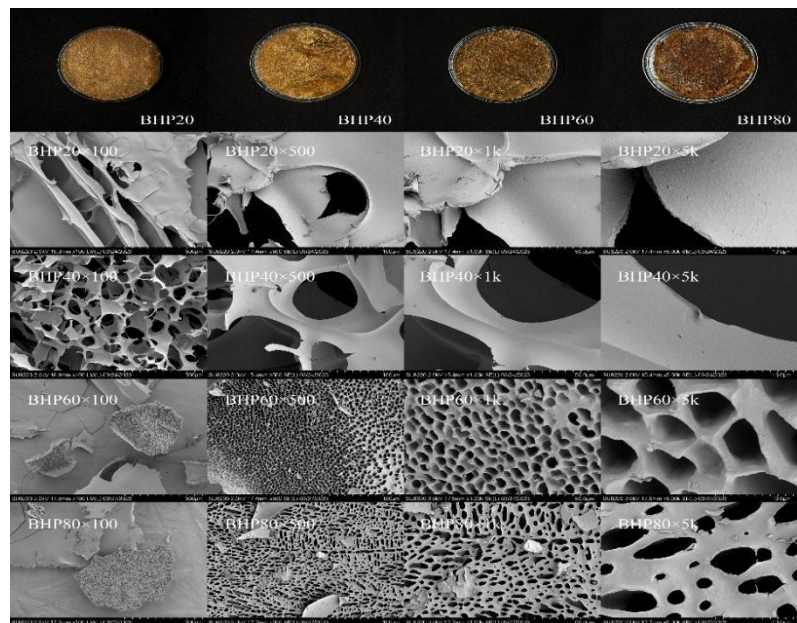


Fig. 1 Visual photographs and scanning electron microscope images of BHP fractions

Table 2 Colorimetric analysis of BHP fractions

	L*	a*	b*
BHP20	34.23±0.12c	16.10±0.36a	26.23±0.55b
BHP40	34.50±0.10c	16.43±0.06a	28.40±0.10a
BHP60	41.67±0.15a	14.27±0.06b	22.67±0.15c
BHP80	35.90±0.20b	11.50±0.10c	18.13±0.25d

Fig.1 reflect that the ethanol concentration has an effect on the color and state of the pectin fractions. As shown in Table 2, the brightness of the BHP fraction gradually decreased with the increase of ethanol concentration, and the changes of a^* and b^* values also reflected the deepening of the color of the samples, corresponding to the intuitive photographs. This may be due to the fact that fruit and vegetable raw materials undergo melard and browning reactions during the blackening process, which produces substances such as nigrosine-like substances, thus leading to changes in color. In addition, the ethanol concentration affects the microscopic morphology of the BHP fractions, with the increase of ethanol concentration, the vacuoles and pores in the lamellar structure of the BHP fractions increase and become looser and more fragmented, and the pores gradually show densification under higher magnification.

3.3 Fourier Infrared Spectroscopy and Thermal Stability of the BHP

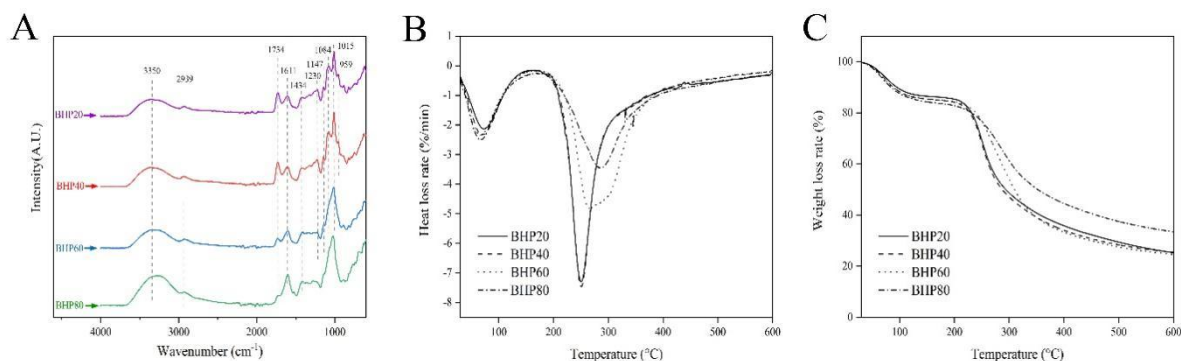


Fig. 3 FT-IR spectra of BHP fractions (A); Thermogravimetric analysis of BHP fractions (B, C)

As can be seen from the figure, the four BHP fractions have similar FT-IR spectra, indicating that they have similar structural features, similar to the FT-IR spectra of ungraded ethanol-graded BHP reported by Li et al. [4]. The absorption peaks at 3353 cm^{-1} and 2936 cm^{-1} correspond to the stretching vibrations of O-H and C-H, respectively. Peaks around 1734 cm^{-1} and 1611 cm^{-1} result from C-O and C=O stretching vibrations, respectively, with peak area ratios correlating with pectin's esterification degree [7]. The stretching and vibration of C-OH and $-\text{CH}_3\text{CO}$ lead to absorption peaks appearing around 1434 cm^{-1} and 1230 cm^{-1} [13]. In addition, the fingerprint region of BHP60 and BHP80 changed mainly in the range of 1200-900 cm^{-1} , which may be related to the monosaccharide composition of the BHP fractions, suggesting that high ethanol concentrations may alter the type of glycosidic bonding in this region [14].

As can be seen from the figure, the four BHP fractions have similar thermogravimetric curves, showing three stages of decomposition in Fig. 3C. The initial stage, 30-200°C, shows a small mass loss, probably due to the loss of free and bound water within the pectin chains, while the second stage occurs at 200-340°C, where the maximum mass loss of the pectin fractions may be because of the increase in degradation of pectin polysaccharides as well as the decomposition and breakage of the long chains at elevated temperatures [15]. The last stage occurs at 340-600°C, witnesses a deceleration in the rate of mass loss, likely attributable to the carbonization of pectin polysaccharides, rendering the residues more thermally stable and consequently leading to a reduced rate of pyrolysis [16]. The highest temperatures and mass residuals at the time of occurrence of the maximum rate of pyrolysis of the BHP80 indicate that the BHP80 is the most thermally stable.

4. Summary

A series of physicochemical reactions occurring during the blackening process would cause the blackening hawthorn pectin to appear brown and have a significant effect on the physicochemical properties of BHP fractions. As the ethanol concentration increased, the esterification degree, molecular weight, GalA content and the proportion of HG region of BHP fractions decreased. Simultaneously, the pores on the surface of pectin became denser, the microscopic morphology was more looser and fragment. Moreover, the thermal stability gradually increased, and the thermal stability of BHP80 was the best. The monosaccharide composition of the four fractions was the same but the contents were significantly different, and the FT-IR spectra showed similar functional group compositions. Overall, hawthorn pectin fractions with small molecular weight, low esterification and good thermal stability could be obtained by black change processing and ethanol grading.

Acknowledgments

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

References

- [1] Cui Meng, Cheng Lei, Zhou Zhongyu, et al. Traditional uses, phytochemistry, pharmacology, and safety concerns of hawthorn (*Crataegus* genus): A comprehensive review. *Journal of Ethnopharmacology*, 2024, 319: 117229.
- [2] L. Martínez-Casas, M. Lage-Yusty, J. López-Hernández, Changes in the Aromatic Profile, Sugars, and Bioactive Compounds When Purple Garlic Is Transformed into Black Garlic. *Journal of Agricultural and Food Chemistry*, 2017, 65(49): 10804-10811.
- [3] Sun Xin, Gu Duanyin, Fu Quanbin et al. Content variations in compositions and volatile component in jujube fruits during the blacking process. *Food Science & Nutrition*, 2019, 7(4): 1387-1395.

- [4] Li Zhixin, Zhang Jiarui, Zhang Hao et al. Effect of different processing methods of hawthorn on the properties and emulsification performance of hawthorn pectin. *Carbohydrate Polymers*, 2022, 298: 120121.
- [5] Wang Ying, Li Xia, Chen Xuetao et al. Effect of stir-frying time during *Angelica Sinensis Radix* processing with wine on physicochemical, structure properties and bioactivities of polysaccharides. *Process Biochemistry*, 2019, 81: 188-196.
- [6] Zhang Ke, Yuan Dan, Li Chao et al. Physicochemical properties and bioactivity of polysaccharides from *Sargassum pallidum* by fractional ethanol precipitation. *International Journal of Food Science & Technology*, 2021, 56(7): 3536-3545.
- [7] Li Zhixin, Zhang Xiaoyan, Zhu Chuanhe. Physicochemical properties and Pb(2+) adsorption capacity of freeze-dried hawthorn pectin fractions by gradient ethanol precipitation. *International Journal of Biological Macromolecules*, 2023, 245: 125581.
- [8] Bradford M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 1976, 72(1): 248-254.
- [9] J.P.V.B. PAUL K. KINTNER III. Carbohydrate Interference and Its Correction in Pectin Analysis Using the m-Hydroxydiphenyl Method. *Journal of Food Science*, 1982, 47(3): 756–759.
- [10] Guo Xiaoming, Meng, Hecheng, Zhu Siming et al. Stepwise ethanolic precipitation of sugar beet pectins from the acidic extract. *Carbohydrate Polymers*, 2016, 136: 316-321.
- [11] Roman L., Guo Mengmeng, Terekhov A. et al. Extraction and isolation of pectin rich in homogalacturonan domains from two cultivars of hawthorn berry (*Crataegus pinnatifida*). *Food Hydrocolloids*, 2021, 113: 106476.
- [12] Jiang Yiping, Qi Xiaohui, Gao Kai et al. Relationship between molecular weight, monosaccharide composition and immunobiologic activity of *Astragalus* polysaccharides, *Glycoconjugate Journal*, 2016, 33(5): 755-761.
- [13] Chen Tingting, Zhang Zhihong, Wang Ziwei et al. Effects of ultrasound modification at different frequency modes on physicochemical, structural, functional, and biological properties of citrus pectin, *Food Hydrocolloids*, 2021, 113: 106484.
- [14] Kpodo F.M., Agbenorhevi J.K., Alba K. et al. Pectin isolation and characterization from six okra genotypes. *Food Hydrocolloids*, 2017, 72: 323-330.
- [15] Ding Man, Liu Yong, Ye Yun-Fang et al. Polysaccharides from the lignified okra: Physicochemical properties and rheological properties. *Bioactive Carbohydrates and Dietary Fibre*, 2021, 26: 100274.
- [16] Tang, Xiaomin, Zhang, Yaqiong, Li, Feiyang et al. Effects of traditional and advanced drying techniques on the physicochemical properties of *Lycium barbarum* L. polysaccharides and the formation of Maillard reaction products in its dried berries. *Food Chemistry*, 2023, 409: 135268.