NEW METHOD FOR PECTIN EXTRACTION FROM SUNFLOWER HEAD RESIDUE

Zayniddin K. Muhidinov, Khurshed Ikromi, Abduvaly S. Jonmurodov, Abubakr S.

Nasriddinov, Jamshed T. Bobokalonov, LinShu Liu*

Chemistry Institute of the Academy of Sciences of the Tajikistan Republic, Tajikistan

e-mail: muhidinovzayniddin@gmail.com

**Eastern Regional Research Center ARS USDA, USA*

The world consumption of pectin is rising to about 55.000 metric tons with annual growth of 6.7% and is important for both the food and pharmaceutical industries. For industrial production, the prime sources are apple pomace, citrus peels, and sugar beet chips. With increased awareness of obesity, cancer and an aging population, low-calorie foods and low-sugar foods are preferred. This has promoted the use of additional sources of pectin, such as sunflower head residue (SHR). The traditional extraction methods are not suitable for sunflower sources due to the low density and oil content of these raw materials, thus new approaches are required. SHR pectins were usually isolated from raw materials by a static method using sodium hexametaphosphate (SHMP) as the quarantine reagent [1]. A gravity flow dynamic method was applied for pectin extraction from SHR. Sodium salts were used as an ion exchange reagent to decouple the divalent metal ions in sunflower cell wall tissues [2]. In comparison with traditional static approaches that are popularly used for sunflower pectin extraction, sodium ion in gravity flow mode can make this new extraction procedure simpler, more efficient, and cost-effective. Mathematical modeling of ion exchange was used to provide a scientific knowledge base concerning the mass transport mechanisms, which are involved in the calcium exchange from the plant cell wall in dynamic and static mode. Pectin obtained by the new method had higher galacturonic acid content and higher molecular weight.

1. INTRODUCTION

The pectin production is the first technologically unique industrial object in the domestic as well as CIC countries industry. The world consumption of pectin is 55.000-60.000 metric tons (with grows rate of 7.1%) and is important in the food and pharmaceutical industries. Pectin is generally extracted from plant tissue, such as citrus, apple pulps, sugar beet, and sunflower heads. Tajikistan has a large resource of such biological raw materials. Food or food ingredients that can enhance health, so-called 'functional foods', are without a doubt one of the leading trends in the food industry today. Pectin from sugar beets and sunflower heads are potential sources, but there are not, at the moment, commercially available technologies for extraction from these materials. However, to dispose of these byproducts in an environmentally acceptable way will add processing costs.

Pectin in native form is a network of the linear and branched polymers of covalently linked galacturonic acid-rich plant cell wall polysaccharides. Its main chain consists of three types of pectic polymers, which are randomly connected to each other: homogalacturonan (HG), rhamnogalacturonan I (RG I), rhamnogalacturonan II (RG II) and xylogalacturonan (XGA) [3-5]. In the aspects of the chemical structure and composition, pectin from citrus, apples, and sugar beets of various locations have been extensively conducted. In comparison with this, pectin from sunflower head residue (SHR) is less studied. Compared to other pectins, sunflower pectins have, at natural state, high HG moieties with a low degree of methylation (DM), which makes them gel at lower sugar concentration and they may thus find application in low-calorie foodstuffs.

With increased awareness of obesity, cancer and an aging population, low-calorie foods and low-sugar foods are preferred. This has promoted the use of other sources of pectin like sunflower head residue (SHR) pectins in food industries. The low methoxyl (LM) nature of SHR pectin extends its applications into the business of health foods and functional foods, such as in the manufacture of low sugar jellies, low-calorie yogurt, and confectionery jelly products. Also, gels from LM-pectin are thermally reversible.

SHR pectins were usually isolated from raw materials by a static method using sodium hexametaphosphate (SHMP) as the quarantine reagent [2,6] and by acid catalysts [7,8].

The objective of this study was to compare the dynamic, kinetically extraction process with the conventional batch process, with emphasis on the SHR pretreatment effect of calcium displacement by sodium, salt forms, concentration, and hydrolysis pH on pectin recovery and quality. To develop an economically effective and environmentally friendly method to extract SHF pectin, we used aqueous sodium chloride from rock salt, instead of SHMP, to remove Ca^{2+} and Mg^{2+} divalent ions from raw SFH residues that was followed by acidic pectin extraction. The capability of sodium chloride to remove the divalent ions from SFH was compared with that of using SHMP. Furthermore, a gravity-flow dynamic method was applied for the extraction of SFH pectin. The efficacy of the dynamic method was compared with that of the static method. Pectin thus obtained was evaluated for galacturonic acid content and other molecular characteristics.

2. MATERIALS AND METHODS

2.1. *Sunflower heads and chemicals*

Sunflower (*Helianthus annuus*, Avangard) harvested in Dushanbe, Tajikistan. The de-seeded heads were sun-dried to a moisture content of approximately 105 g/kg. The dried raw SHR was ground using a laboratory mill to a diameter of 0.6 -1.0 mm, stored in sealed plastic bags at room temperature. The moisture content of SHR was determined by drying samples in an air-circulated oven at 110 °C, for 16 h. All values were calculated on a dry-weight basis.

Sodium Chloride was obtained from a local market and purified by re-crystallization before use. Sodium hexametaphosphate (SHMP) and other chemicals were purchased from Sigma-Aldrich. Deionized (D.I.) water was prepared by ion-exchange columns (Millipore).

2.2. *SHR pretreatment & Pectic Polysaccharides (PP) extraction*

Before extraction, dried SHR pectin powders were washed to remove pigments, fats, and waxes. The washed SHR powders were filled into glass columns (d, 3.5 cm), in sequence treated with sodium chloride of various concentrations (solid/liquid, 20g/600ml; flow rate, 2.0 ml/min), deionized water, and solution of various pH ranging from 1.8 to 5.5 adjusted with diluted HCl. The temperature of hydrolysis and flow rate (v) through the column was constant for all experiments, 85°C and 6ml/min consequently. All these treatments were performed at room temperature.

After extraction, the PP solutions were cooled to room temperature, then centrifuged at 6000 rpm for 60 minutes. This step separated microgel (MG) from soluble pectin further named pectin substances (PS) that were pumped to a reservoir and diluted with 2-fold D.I. water. The diluted pectin solution was then passed through a hollow fiber membrane module (MiniKross Sampler Hollow Fiber Module 100KD PS, KrossFlow, USA). The concentrated pectin solution thus obtained was dried by a commercially available spray drier to form pectin powder (100-500 μm). For the control experiments, SHMP was used to extract calcium ions by kinetic pectin and static methods under the same conditions.

2.3. *Analytical methods*

2.3.1. Ash content and $\text{Ca}^{++}/\text{Mg}^{++}$ content. Ash content in raw SHR and treated SHR was measured by the method described in the Official Methods of Analysis of the Association of Official Analytical Chemistry [9]. Briefly, 10 g pectin sample was placed in a furnace, incinerated at 525 °C for 4 h; cooled to room temperature and stored in a desiccator over calcium oxide until weighting. The contents of calcium ions and magnesium ions in both of the untreated SHR and treated powders were determined by titration with EDTA.

2.3.2 Swelling behavior. The swelling properties of treated and untreated SHR were determined by immersing a sample (1.0 g) in 50 ml D.I. water at room temperature for XX h. The weight of the swelled samples was recorded after removing the surface water by tapping with Tissue Wiper.

The swelling degree was then calculated according to $(W_w - W_d)/W_d \times 100\%$, where W_w and W_d were the weights of wet samples and dry samples, respectively.

2.3.3 Determination of the anhydrogalacturonic acid (AGA) and degree of methyl esterification (DM). Both the dry pectin samples and washing solutions were assayed for AGA content. AGA content was determined by the Sulfamate/3-phenylphenol colorimetric method with a UV/VIS spectrophotometer (Thermo Spectronic, UK) at 520 nm [10]. The DM of pectin was analyzed by the method provided by CP Kelco [11].

3. RESULTS AND DISCUSSION

3.1. SHR ash, calcium and magnesium content. SHR residues had an ash level of 11.5 %, from which the amount of calcium and magnesium was found 1.95% and 0.77%, respectively (Table 1). Washing the SHR powders with water or water/ethanol solution could remove water-soluble minerals, but could not remove Ca^{2+} and Mg^{2+} ions. Washing with 8% sodium chloride reduced mineral content by 22%, and removed 64% of Ca^{2+} and 82% of Mg^{2+} from the SHR.

Table 1. Ash, Ca^{2+} and Mg^{2+} contents in sunflower head residues

Treatment method	Ash content, %	Ca^{2+} , %	Mg^{2+} , %	Swelling degree, g/g
Crude SHR	11.47	1.95	0.77	2.7
SHR washed by water/alcohol	7.22	2.02	0.58	3.0
SHR washed by water	6.10	1.61 (1.84)*	0.42(0.44)*	2.8
SHRW & treated by 8,0 % NaCl	8.90	0.72 0.62	0.14 0.11	3.2
SHRW & treatedby 0,5% SHMP	8.17	- 0.46	- 0.01	3.1
SHRW & treatedby 2,5% SHMP	8.29			3.4
SHRW & treated by 8.0NaCl and Hydrolyzed at pH 2.0	2.41	0.75	0.09	3.0

*Determined by the capillary electrophoresis method.

The treatment with SHMP reduced mineral content by about 25%. Although the treatment with SHMP removed Ca^{2+} and Mg^{2+} from SHR powders, there was no quantitative data available due to the quarantine nature of SHMP. Table 1 also showed that the treatment of SHR with water/ethanol or salt solutions enhanced the swelling degree of the powders. By removing Ca^{++} and Mg^{2+} , and other minerals, the structure of SHR became a loser, facilitating water penetration and absorption. The SHR swelled more after treating with SHMP than with NaCl, indicating more inorganic ions were removed.

3.2. Hydrolysis and Extraction in dynamic mode. Washed SHR, free of Ca^{2+} obtained by sodium salts pretreatment in dynamic mode, subsequently has been subjected to hydrolysis and pectin extraction by acidic solutions of various pH values in the same column as described in the experimental section. Extracted PP solutions in every 50 ml were collected from the column, cooled to ambient temperature. Then the solution separated into two subfractions by centrifugation: pectin gel (PG) and supernatant solution (PS). Pectin gel was dried and pectin from supernatant solution was isolated by alcohol precipitation. As an example, Table 2 summarizes PP fraction and subfraction yields, AGA contents, DM and alcohol soluble fractions (ASF) obtained from SHR preliminary treated with 8%NaCl and hydrolysate at pH=2,0. From this data, it is visible that the gel fraction yields pass through the maximum, while the amount of pectin

precipitated from ethanol increases and remains constant. At the same time the number of impurities soluble in alcohol ASF increase. The quality of PP (% AGA and its DM) in gel form were higher than that in solution.

Table.2. Pectin subfracfraction yields, AGA content, DE and ASF obtained from SHR preliminary treated with 2M NaCl (8%) and hydrolyzed by dynamics mode (in the column) at pH=2,0; t=85⁰C; v=4 ml/min

Fraction volume, ml	Pectin subfraction yields and its characteristics							
	Pectin Gel, %			Pectin, %			ASF, %	Sum of Fraction
	PG	AGA	DM	PS	AGA	DM		
50	0.65	77.2	32.5	0.41	40.8	30.8	0.9	1.95
100	0.70	81.1	37.5	0.62	52.4	36.6	1.2	2.51
150	3.15	87.3	48.9	0.75	60.5	40.5	2.6	6.50
200	1.10	86.6	46.1	1.82	71.1	52.2	3.2	6.10
250	0.50	86.3	36.3	2.51	76.5	53.5	4.1	7.11
300	0.30	78.5	31.6	3.43	76.3	52.3	5.4	9.12
350	0.20	68.2	31.7	3.54	74.8	45.2	6.9	10.61
400	0.10	67.6	31.7	3.60	70.0	44.6	8.8	12.52
Total	6.70	78.9	37.6	16.68	65.25	44.5	33.1	56.48

In summary, there were extracted 6.7 % PG,16.68% PS and 33,1% ASF presenting the sum of neutral sugar, oligosaccharides, and other impurities. Pectin constituents were 67.6–86.6% galacturonic acid with DE of carboxyl group ranging from 31.6-48.9; 40.8-76.3% and AGA with DM range from 30.8-53.5% accordingly for PG and PS forms.

3.2.1. Influence of acid flow rate on PP fractions (PS and PG) yields, AGA content and DM obtained from SHR hydrolysis at pH 2.0. One of the aims of this development was to reduce the

acid contact time with plant raw material. For this purpose, we study the influence of acid flow rate on PP fraction (PS and PG) yields, AGA content and DE obtained from SHR hydrolysis at pH 2.0. AGA content of PS decreased from 78.2 to 58.6 with an increasing flow rate from 1.0 ml/min to 5.0ml/min.

Fig 1. Influence of acid flow rate on PP fractions obtained from SHR hydrolysis, pH 2.0: (■) PS; and (▲) PG yields.

Fig 1 shows the influence of acid flow rate on PP fraction (PS and PG) yields, obtained from SHR hydrolysis in a dynamic regime. Increase in flow rate decreased PS yield, while PG amount increased, probably through three parallel processes occurring at SHR hydrolysis: the first is the diffusion of HG released from junction zone, the second is the hydrolysis of covalent bond holding PP with cell wall polymers and the last is the degradation of PS under acid condition. Thus from this hypothesis for further study, we choose the optimal rate equal to 3.0 ml/ml. Less flow rate may produce more depredated PS, which will be discussed in the last chapter.

3.2.2. Influence of hydrolysis pH on PP fractions (PS and MG) yields obtained from SHR.

Besides, the intensive parameters in pectin production like plant raw sources, maturity, milling condition (particle size) raw material pretreatments, the acid pH is extensive parameters in the process of PP hydrolysis as that effect on the extraction yields, PS degradation and its quality.

Fig.2. Influence of pH on pectin yields from sunflower head residue washed by water and treated by different concentrations of NaCl (Δ) 4% NaCl, (●) 8% NaCl, (X) 12% NaCl, the elution rate is 3.0 ml/min.

Therefore the effect of initial pH on both PP yields and it constitutes was studied using SHR consequently washed by water and treated by NaCl of different concentrations (Fig. 2). pH was adjusted with hydrochloric acid, the solution was applied to a column containing pretreated SHR and hydrolysis extraction processes in different pH was compared in a series of SHR pretreated with NaCl of different concentrations. It is visible from fig.2 that at the beginning, at pH values in the range of 1.6-4.0, PS yields were lowered slowly before a sharp decrease. At low pH value, PP degraded into PS, PG and low molecular fraction, which dissolved in the alcohol that decreases total PS yield. From these results, the optimal pH was chosen equal to 2.0.

These differences likely occurred because of diffusion properties of washed SHR, which arise after different washing. Total Ca^{2+} , Mg^{2+} contents in the SHR and its swelling degree have no large differences, 2.6% and 3.0 against 2.03% and 2.8. The same feature observed in the case of hydrolysis of the SHR washed by water, treated by SHMP (the date not included here). However, the PS produced was less in the last treatment than with NaCl. The reason for low PS yield is the extraction of pectin among with chelating complexes, as stated above.

3.2.3. Influence of salt concentration on PP fractions (PS and PG) yields obtained from SHR

Figs 3 clearly illustrates a mechanism of Na⁺ ion action in different salt forms for calcium removal and the pectin extraction process. Na⁺ ion in the form of chelating salt was more powerful (at a lower concentration) for Ca²⁺ ion exchange, but chelating anion favored pectin extraction in the first process (fig 3 left side), which is undesirable. Salt concentration has a negligible effect on PS yield, but it increases the sharp reduction in PG amounts. On the contrary, Na⁺ ion in labile chlorine anion form shows the proactive effect for both calcium ion exchange in the first process than for PS extraction in kinetically favorable mode. The increase of salt concentration at the first process hold particular promise for further PS extraction progress and reduce pectin aggregates (fig 3 right side).

Fig. 3. Influence of Na⁺ concentration in a different form on PP yields from sunflower head residue washed by water treated by different concentrations of NaCl (left) and SHMP (right): (Δ) PS yield and (●) PG yield, the elution rate is 3/0 ml/min.

3.2.4. Influence of pH, salt form and concentration on AGA content and DE of PS. The combined influence of pH, salt form and concentration are given in Table 3. The AUA content and degree of methyl esterification in the pectin samples obtained from SHR pretreated by SHMP and hydrolyzed in dynamic mode demonstrate negligible changes on salts concentration change, but it was a visible minor increase in SHR pretreated with NaCl. Increase of acidity in the range of pH 5.6 to 1.06 improved pectin quality by increasing AUG content in 8-10 % for those samples treated with NaCl, as well as PS yield showed before.

Tab.3. Dependent of PS constituents (AUA and DM) on sodium salt form concentration and pH hydrolysis at 85°C in dynamic mode

SHMP, %	0.5		2.5		5.0	
pH	AGA	DM	AGA	DM	AGA	DM
1.06	68.8	46.4	66.0	48.2	62.0	45.0
1.21	68.2	45.7	66.8	49.4	64.6	45.5
1.60	66.4	44.9	68.6	50.0	66.4	48.7
2.00	65.0	43.4	66.4	48.6	68.0	50.0
3.50	62.0	41.5	65.2	46.9	67.2	48.1
5.60	58.2	40.8	64.6	45.0	66.0	47.5
NaCl, %	4.0		8.0		12.0	
1.06	69.1	47.1	70.4	47.1	78.0	51.2
1.21	68.4	45.9	70.0	47.0	69.6	50.0
1.60	67.4	44.1	69.2	46.4	69.4	48.8
2.00	64.2	42.9	68.2	45.2	66.6	47.6
3.50	63.2	42.4	65.4	44.7	66.5	47.0
5.60	60.8	42.3	62.6	44.7	66.0	46.5

Comparison of the molecular characterization between two methods (a new-dynamic and traditional - static) [1] showed that SFH pectin obtained by the new method had higher galacturonic acid content and higher molecular weight.

Thus we have developed a combination of environmentally friendly processes for producing low-cost refined pectin. The main technological stages (3,6 and 8) are patented [12-14]. The process lines (fig.4) include cross flash hydrolysis-extraction of pectin from raw materials [13,14], flow membrane filtration technology with dia-ultrafiltration systems [12] that make it possible to simultaneously purify and concentrate the pectin hydrolyzed solution. The flexibility of technological schemes in addition permits to process various types of raw materials and derive a wide range of semi-products (crude, human consumable peptic materials containing fiber, and pectin gel, neutral sugars and anticancer phenolic compounds).

Figure 4: Process line of Sunflower Pectin Production. 1, capacity for solutions; 2, pump; 3, Hydrolysis columns for; 4, tank for CaCl_2 solutions; 5, Filter press; 6, Centrifuge; 7, capacity for pectin solution; 8, membrane modules; 9, spray drier; 10, tank for pectin powders.

REFERENCES

1. Muhidinov Z.K., Khalikov D.Kh., Fishman M.L., Liu LS. (2017). Gravity flow dynamic method for pectin extraction from sunflower head residue. *Biopolymers Res* 1: 101.
2. Kim, W.J., Sosulski, F., Campbell, S.J. (1978a). Formulation and characteristics of low-ester gels from sunflower pectin. *Journal of Food Science*, 43, 746-749.
3. Albersheim P, Darvill AG, O'Neill MA, Schols HA, Voragen AGJ (1996). An hypothesis: the same six polysaccharides are components of the primary cell walls of all higher plants. In *Pectins and Pectinases*. Edited by Visser J, Voragen AGJ. Amsterdam: Elsevier Sciences B.V.; 1996:47-53.
4. Voragen, A.G.J., Pilnik, W., Thibault, J.F., Axelos, M.A.V., Renard, C.M.G.C. (1995). Pectin. In A.M. Stephen (Ed.), *Food polysaccharides* (pp.287-339). New York: Marcel Dekker
5. Ridley, B.L., O'Neill, M.A., Mohnen, D. (2001). Pectins: Structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry*, 57(6), 929-967.
6. Chang, K.C., Dhuranhar, N., You, X., Miyamoto, A. (1994). Sunflower head residue pectin extraction as affected by physical conditions. *Journal of Food Science*, 59(6), 1207-1210.
7. Khalikov D.Kh., Mukhiddinov Z.K., Avloev Kh.Kh. et al. The influences of Electrolyte on Sunflower Protopectin Hydrolysis. *Chem. of Natural Compounds*. 2002, No 1. C.5-10.

8. Khalikov D.Kh., Muhidinov Z.K. Physicochemical fundamentals of the plant cell walls protopectin degradation under the action of acid catalysts, a review- Chem. of Natural Compounds. 2003, No 2.
9. Official Methods of Analysis of the Association of Official Analytical Chemistry (1970), W. Horwitz editor, 11 edition, Washington, DC.
10. Filisetti-cozzi T.M.C., Carpita N.C. (1991). Measurement of Uronic Acids without interference from Neutral Sugars. *Anal. Biochem.* 197, p.157-162.
11. CP Kelco Control methods – March 7, 2001, p. 3.
12. TJ Patent 197. Method for purification of pectin hydrolysate solution and pectin gel production, 25.03.1996. Muhiddinov Z.K.
13. EA Patent 016871. Process for sunflower pectin production. 30, 2012. Muhidinov Z.K., Fishman M.L., Liu LS.
14. UA Patent 70047 “Processing Line for Pectin Production from sunflower head residue”, 25.05.2012. Muhidinov Z.K., Liu LS., et al.