

EXTRACTION AND EVALUATION OF PECTIN FROM SUGAR-BEET PULP

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ABSTRACT: *Chemical composition of dried sugar-beet pulp was determined. The yield of sugar-beet pulp pectin was evaluated using hydrochloric acid (HCl) and ammonium oxalate. Acid extracted pectin was determined at different levels of pH and heating temperatures for different periods of extraction. Two levels of ammonium oxalate concentration (0.25 and 0.50%) were used for pectin extraction, and extraction was carried out at three levels of temperature (65, 75 and 85 °C). The highest yield of acid extracted pectin was 24.6% when extracted at 75 °C for 4 hours at pH 1.5, while the highest yield of ammonium oxalate extracted pectin was 22.75% when extracted by 0.5% ammonium oxalate at 75 °C for 1 hour. Chemical and physical characteristics of the obtained beet-pulp pectin were also evaluated.*

Key Words: *Sugar-beet – pectin – extraction – Hydrochloric acid – ammonium oxalate - Yield.*

INTRODUCTION

In Egypt, the first factory for extracting sugar from sugar beet was built in 1981 in Kafr El-Sheik. Since this date, sugar beet culture spread at a commercial level. In the same time, the production of beet sugar increased from 16933 ton in 1982 to 95752 ton in 1993 (Ministry of Agriculture, 1993). Sugar-beet pulp is the main by-product of sugar industry. It is used as an animal feed at relatively low price. Sugar beet pulp could be used as a source of pectin. Nutritionally, pectin functions as a soluble dietary fibre; also, Bonder and Golubev, 1992; Andersson (1995); and Zhemerichkin and Ptitchkina, 1995 reported that the pectin of sugar beet could be used medicinally for removal of heavy metals and radioactive elements from the body and reduce cholesterol levels. It might also be noticed that the beet pectin could be used for manufacture of jams, jellies, marmalades and sweets, where such low ester pectin form gels with or without sugar in the presence of divalent cation such as calcium (El-Nawawi and Heikal, 1995). Also, pectin could be used in dairy product, as an effective synergistic antioxidant (Macfadyen, 1993), as an additive in some drying method or meat products (Rede *et al.*, 1993). These indicate that the sugar-beet pectin is important as food components.

In contrast to pectins from apple and citrus residues, pectins in beet pulp are strongly bonded to proteins, cellulose, lignin and other components of cell walls (Yuldasheva *et al.*, 1994a). In order to understand the role played by

each of the components of sugar beet roots, it is known to differ in the chemical composition according to the varieties and some factors affecting its growth, a previous study (Kamil *et al.*, 2000) was done to evaluate ten varieties of sugar beet which were planted recently in Egypt for producing sugar from sugar-beet. Our attention was then focused to evaluate dried sugar beet pulp (by-product), which was obtained from Kafr El-Sheik factory for sugar industry.

Baker (1948) indicated that the efficiency of pectin extracted from lemon albedo was related to the conditions of temperature, time and pH. Several investigators (El-Sherbiny and El-Manawaty, 1982; Michel *et al.* 1985; Phatak *et al.*, 1988; Bertin *et al.*, 1988) studied the possibility of using HCl, tartaric acid, HNO₃ and potassium or ammonium oxalate for extracting pectin from sugar-beet pulp. Also, Yuldasheva *et al.* (1994b) studied the suitability of HCl, HNO₃ and oxalic acid to release the pectin of beet pulp.

Furthermore, improving the quality of the obtained pectin of sugar beet was studied by Suprunchuk *et al.* (1991). They found that, washing raw materials with water at 15-25°C for 10-20 min., then carrying out acid hydrolysis (pH 1.5 at 70-78°C) with continuous removal of the hydrolysate improved the quality and increase the yield of the obtained pectin and also reduced the amount of reagent used. Also, Bonder and Golubev (1992) added that, the optimal extraction conditions are 65°C, water excess (1 : 6 for fresh sugar beet, 1 : 7-1 : 8 for dried sugar beet), for 4 hours and mineral acids adjusted to pH 1.5.

So, the aim of this investigation is to study some possible modifications of pectin extraction through a wide range of pH, temperature and heating time to maximize the yield and improve the quality of pectin of dried sugar-beet pulp. Moreover, the chemical and physical properties of the isolated pectin were also evaluated.

MATERIALS AND METHODS:

Materials:

Dried sugar-beet pulp was obtained from Delta Company for sugar production, Kafr El-Sheikh Governorate, Egypt.

Methods:

1. Technological processes:

1.1 . Preparation of raw materials:

The dried sugar-beet pulp was taken for purification to remove all possible interfering compounds. The samples (20 gm) were soaked in a mixture of chloroform : methanol at the ratio of 3:1 v/v at room temperature for 6 hours, as suggested by Phatak *et al.* (1988), followed by filtration. The sediment layer was then washed by water at 25°C for 20 min., then filtered and dried.

Extraction and evaluation of pectin from sugar- beet pulp

1.2. Extraction of pectin

Hydrochloric acid and ammonium oxalate were used for pectin extraction. Extraction conditions were selected according to the method of Phatak *et al.* (1988). To maximize the yield of pectin, pectin was extracted from sugar-beet pulp at different conditions of HCl, i.e. different levels of pH (2, 1.5 and 1), different extraction periods (1, 2, 3, 4, and 5 hours) and different temperatures (65, 75, 85 and 95°C). Ammonium oxalate was evaluated for pectin extraction of the dried sugar-beet pulp at two levels of ammonium oxalate concentrations (0.25 and 0.5 %), and the samples were extracted under a wide range of temperatures levels (65, 75, and 85°C) and periods (1, 2, 3, and 4 hours).

1.3. Isolation and precipitation of pectin

The clear layer of pectin extract was decanted and filtered through 20-micron nylon cloth. The obtained filtrate was centrifuged at 4000 r.p.m for 10 mins. The pectin extract was concentrated under vacuum using rotary evaporator at 50°C to remove about 80% of the initial water of the extract as described by Michel *et al.* (1985).

The pectic substances were precipitated using ethyl alcohol according to Siliha (1989) as follows: Acidified ethyl alcohol (80% ethanol + 1% HCl) was added at the rate of 1 ml acidified alcohol to 3ml. pectin extract. The precipitate was allowed to stand for about 2 hr. to complete precipitation and aggregation. The precipitated pectin was isolated from alcoholic solution by filtration using two layers of nylon cloth.

1.4. Purification and dehydration of the isolated pectin:

The remnant chlorides and ammonium ions and other material were removed from isolated pectin by washing with ethyl alcohol as mentioned by Wang and Chang (1994). Then the obtained purified pectin was dried at 50°C in vacuum oven for 12 hours. The dried pectin was then ground to fine powder (80 mesh).

2. Analytical methods:

2.1. Chemical methods:

The moisture, total and reducing sugars, crude fiber and ash of sugar-beet pulp were determined according to the methods of A.O.A.C. (1990). Total nitrogen was determined by micro-Kjeldahl method according to Ranganna (1978). The pH values were measured using a pH- meter (Hanna model H I. 9021).

Total Pectic substances content (water, ammonium oxalate and acid extracted fractions) were determined by the method of Carre and Hayness, as described by Pearson (1976). The equivalent weight of pectin, the methoxyl groups content, the acetyl content and the anhydrogalacturonic acid were determined as mentioned by Ranganna (1978). While, the degree of methylation was calculated as reported by Phatak *et al.* (1988).

2. Physical Methods

Physical properties of the obtained pectin of dried sugar beet pulp as viscosity were determined, according to Phatak *et al.* (1988), molecular weight, according to the method mentioned by Christensen (1954), jelly grade, according to the method of the U.S. Department of Agriculture, mentioned by Ranganna (1978).

Statistical Analysis

The obtained results were expressed as a mean values plus or minus standard deviation. Statistical analysis were carried out as described by McClave and Benson (1991).

RESULTS AND DISCUSSION

Chemical constituent of sugar-beet pulp play a major role in the yield of extracted beet pectin. Pectins in beet pulp are strongly bonded to proteins, cellulose, lignin and other components of cell walls (Yuldasheva *et al.*, 1994a). Some chemical constituents of dried sugar beet pulp, obtained from the waste of sugar industry were determined and tabulated in Table (1). As shown in this table total sugars were found in a lowest concentration, i.e. 0.06%, while ash and protein reached to 3.02 and 4.17%, respectively. Crude fibres were found to represent the second main constituent of sugar-beet pulp, where it was 34.07%. The total pectic substances represent the main constituent of sugar-beet pulp (44.71%). Also, acid extract was found to contain the highest percentage (37.15%) of pectic substances if compared with those obtained by both fractions of water-extract (5.09%) and ammonium-oxalate (2.74%). These findings are in agreement to those obtained by Gomanjuk (1993). However, total pectic substances in our sample were higher than those obtained by Ghanem *et al.* (1991), who found that total pectic substances of beet pulp reached 34%.

Table (1): Chemical composition of the sugar-beet pulp.

Chemical Composition	Fresh weight	Dry weight
Moisture (%)	6.13 ± 0.117	-
Ash (%)	3.02 ± 0.060	3.22 ± 0.065
Total sugars (%)	0.06 ± 0.021	0.07 ± 0.031
Total protein (%)	4.17 ± 0.155	4.44 ± 0.158
Crude fibres (%)	34.07 ± 0.104	36.29 ± 0.086
Water-extract fraction of pectin (%)	5.09 ± 0.081	5.42 ± 0.083
Ammonium oxalate extract fraction of pectin (%)	2.47 ± 0.110	2.63 ± 0.118
Acid - extract fraction of pectin (%)	37.15 ± 0.162	39.58 ± 0.198
Total pectic substances (%)	44.71 ± 0.103	47.63 ± 0.149

Values are average of three representative samples ± standard deviation.

Extraction and evaluation of pectin from sugar- beet pulp

In this study, all favorable conditions were used to hydrolyze protopectin with hot acid and dissolves the pectin. Pectin extraction was carried out using mineral acid (HCl) at different levels of pH (2, 1.5 and 1) and heating temperatures (65°, 75°, 85° and 95°C) for different times (1-5 hrs). The obtained results are shown in Table (2).

As shown in the table, the yield of pectin in case of acid extraction (HCl) was affected by the pH, heating temperature and heating time. In case of extraction at pH 2 and heating at 65°C, the highest pectin yield reached to 14.40% after heating for 5 hrs. Increasing heating temperature to 75 and 85°C for 5 hrs at the same level of pH, increased the yield of pectin to 18.51 and 18.85% respectively. The highest yield of pectin under this condition of pH was 20.03% after heating for 3 hrs at 95°C.

Furthermore, Table (2) showed also that, extraction of beet-pectin at a lower level of pH (1.5) and heating at 75°C for 4 hrs gave higher pectin yield reached to 24.60%. At the same pH level and increasing heating temperature to 85°C, the yield of pectin reached 22.55% after heating for 3 hrs, while increasing heating temperature to 95°C at the same condition of pH, the yield of pectin reached to 21.57% after 2 hrs of heating.

The efficiency of pH 1 to release the pectin of beet pulp was also studied. As shown in the same table, when the pH level decreased to 1, the yield of beet pectin increased rapidly during the first 2 hrs of heating at 75°, 85° and 95°C to 21.61, 22.26 and 21.15%, respectively.

Table (2): Effect of time and temperature on the yield of pectin (%), extracted by HCl at three levels of pH.

Temperature	Time in hours				
	1	2	3	4	5
Extraction at pH 2:					
65°C	5.11 ± 0.193	7.48 ± 0.184	12.75 ± 0.101	13.68 ± 0.083	14.40 ± 0.104
75°C	8.12 ± 0.154	12.75 ± 0.085	15.18 ± 0.070	16.45 ± 0.135	18.51 ± 0.090
85°C	10.8 ± 0.180	13.40 ± 0.132	16.55 ± 0.087	17.00 ± 0.175	18.85 ± 0.110
95°C	12.81 ± 0.173	17.37 ± 0.046	20.03 ± 0.081	19.04 ± 0.091	18.14 ± 0.085
Extraction at pH 1.5:					
65°C	7.96 ± 0.100	10.04 ± 0.060	13.02 ± 0.092	14.53 ± 0.085	15.90 ± 0.125
75°C	9.83 ± 0.176	15.67 ± 0.065	18.49 ± 0.107	24.60 ± 0.101	19.51 ± 0.066
85°C	12.53 ± 0.112	16.34 ± 0.0057	22.55 ± 0.110	17.67 ± 0.085	16.58 ± 0.103
95°C	14.38 ± 0.153	21.57 ± 0.093	19.62 ± 0.107	17.62 ± 0.093	15.63 ± 0.099
Extraction at pH 1:					
65°C	10.09 ± 0.142	11.31 ± 0.066	12.98 ± 0.086	13.92 ± 0.097	16.47 ± 0.060
75°C	15.37 ± 0.104	21.61 ± 0.059	21.91 ± 0.080	20.51 ± 0.097	18.25 ± 0.072
85°C	16.68 ± 0.136	22.26 ± 0.060	21.50 ± 0.138	21.05 ± 0.075	16.49 ± 0.072
95°C	18.37 ± 0.176	21.15 ± 0.081	18.78 ± 0.120	17.29 ± 0.101	13.89 ± 0.067

Values are average of three representative samples ± standard deviation.

From the above-mentioned table, it could be concluded that the pectin yield of pH 2 was found to be lower than those obtained at 1.5 or 1 at any temperature for any time of extraction. The pectin yield of pH 1 was found to be higher than those extracted at pH 1.5 at all temperatures used for one or two hours. The optimum condition was found in case of pH 1.5 and extraction at 75°C for 4 hrs, where the yield of pectin reached to 24.60%. These results are in agreement with those found by Phatak *et al.* (1988), who reported that the optimum conditions of pectin extract were at pH 1.5 and heating temperature at 80°C for 4 hours. The yield of pectin reached about 19.53%. Arslan (1995) obtained the optimum yield of pectin at the same previous conditions, but the yield reached 17.71%.

On the other hand, ammonium oxalate was used to release the pectin of beet pulp at pH 3.5 using ammonium oxalate solution at two levels of concentrations namely 0.25 and 0.50%. Extraction was carried out at three temperatures 65, 75 and 85°C for several periods 1, 2, 3 and 4 hours. Table (3) shows that the yield of pectin in case of pH 3.5 and 0.25% ammonium oxalate solution increased gradually to reach 15.70% during heating at 65°C for 4 hrs. While, in case of heating at 75°C, the yield of pectin increased during the first two hours, where it was 16.5 and 18.97% after one and two hours of heating, respectively. Then the yield of pectin showed a rapid decline to reach 12.07% after 4 hrs of heating. The yield of pectin was maximized in case of heating at 85°C for one hour only, where it reached 21.90%, then declined to 20.11, 14.67 and 11.05% after 2, 3 and 4 hours of heating, respectively. So, it could be concluded that the optimum conditions for pectin extraction is by using 0.25% ammonium oxalate solution at 85°C for one hour. While, Phatak *et al.* (1988) found that the optimum yield of sugar-beet pulp pectin was 19.33% using 0.25% ammonium oxalate at pH 3.5 and heating at 75°C for one hour. Also, Arslan (1995) recommended the same previous conditions, where the optimum yield reached 21.75%.

Table (3): Effect of time, temperature and concentration of ammonium oxalate at pH 3.5 on the yield (%) of pectin.

Temperature	Time in hours			
	1	2	3	4
Ammonium oxalate (0.25%):				
65°C	10.97 ± 0.126	11.37 ± 0.156	12.97 ± 0.254	15.70 ± 0.307
75°C	16.50 ± 0.120	18.97 ± 0.257	14.15 ± 0.246	12.07 ± 0.188
85°C	21.90 ± 0.200	20.11 ± 0.220	14.67 ± 0.305	11.05 ± 0.205
Ammonium oxalate (0.50%):				
65°C	13.16 ± 0.185	15.26 ± 0.317	19.44 ± 0.399	19.03 ± 0.193
75°C	22.75 ± 0.279	22.04 ± 0.136	17.75 ± 0.380	15.03 ± 0.221
85°C	22.25 ± 0.300	21.92 ± 0.271	15.87 ± 0.372	13.59 ± 0.381

Values are average of three representative samples ± standard deviation.

Extraction and evaluation of pectin from sugar-beet pulp

Furthermore, the yield of pectin at pH 3.5 and 0.5% ammonium oxalate solution was also studied. As shown in Table (3) the yield of pectin at heating temperature of 65°C was gradually increased during the first 3 hrs to reach 19.44%. Heating at 75°C or 85°C gave the maximum yield just after one hour of heating, where it reached to 22.75 and 22.25%, respectively. Then the yield of pectin decreased gradually to 15.03 and 13.59% during heating at 75° and 85°C for 4 hrs, respectively.

It is clear from Table (3) that the maximum yield of pectin was reached during the first and second hours of heating at 75° and 85°C in both cases of 0.25 and 0.5% ammonium oxalate solutions. Table (4) clarified the optimum conditions of the maximum yield of sugar beet pulp pectin in both cases of ammonium oxalate concentration. As shown in table (4) it could be concluded that the optimum conditions of pectin extract were found at 75°C for one hour of heating in case of 0.5% ammonium oxalate, or at 85°C for two hours of heating in case of 0.25% ammonium oxalate, where the pectin yield reached to 22.75% and 21.9%, respectively.

Table (4): Effect of temperature and concentration of ammonium oxalate at pH 3.5 on the yield (%) of sugar-beet pulp pectin at the optimum time of heating.

Ammonium oxalate concentration	Heating time					
	1 hour			2 hours		
	65°C	75°C	85°C	65°C	75°C	85°C
0.25 %	10.97	16.5	21.9	11.37	18.97	20.11
	± 0.126	± 0.120	± 0.200	± 0.156	± 0.257	± 0.220
0.50 %	13.6	22.75	22.25	15.26	22.4	21.97
	± 0.185	± 0.279	± 0.300	± 0.317	± 0.136	± 0.271

Values are average of three representative samples ± standard deviation.

The chemical and physical characteristics of the previous two pectin extracts were studied and the results obtained were tabulated in Table (5). As shown in the table, pectin of ammonium oxalate extracted was characterized by its high percentage of ash content, where it reached to 8.85%. In contrast, ash content of acid extract reached to 1.97%. The percentage of anhydrogalacturonic acid content of acid extract and ammonium oxalate extract reached to 63.57 and 56.73%, respectively. This result indicated that the purity of pectin in case of acid extract was higher than those obtained by ammonium oxalate. Furthermore, beet pectin of the previous two extracts was characterized by its low content of methoxyl, where it was lower than 7%. This means that, the gelling capacity of sugar-beet pectin was poor. Also, the acetyl content of beet pectin extract varied as a result of using acid or ammonium oxalate extractors, where acid extract gave a low percentage of acetyl content (1.74%) compared with those obtained by ammonium oxalate (5.23%). So, the gelling capacity of pectin of ammonium oxalate extract was lower than that obtained in case of acid extract. This poor gelling ability of pectin could be attributed to the presence of acetyl groups. The

degree of methylation of acid extract pectin reached to 46.17%, while, it was 38.91 in case of ammonium oxalate extract. Acid and ammonium oxalate extracts were found to contain 2.95 and 3.22% protein, respectively. The pH value of acid and ammonium oxalate extracts reached to 3.65 and 4.03, respectively. In addition, the viscosity of acid extract reached to 386 cp., while it was decreased to 202 cp. in case of ammonium oxalate. It could be also noticed that, the pectin of sugar-beet pulp has a relatively low molecular weight, it was 47000 and 32000 dalton in cases of acid extract and ammonium extract, respectively. The poor gelling ability of beet pectin could be also attributed to the low molecular weight. The present findings and reached conclusion agree to a great extent, with those reported by El-Sherbiny and El-Manawy, 1982; Phatak et al., 1988; Arslan, 1995; and Zhemerichkin and Ptitchkina, 1995.

To maximize the utilization of beet pulp, which is a by-product of sugar industry, it could be recommended to use the previous optimum conditions of pectin extraction to obtain the maximum yield of beet pectin from varieties of sugar-beet pulp cultivated in Egypt. The remained waste of beet-pulp could be used again as a feeding material.

Table (5): Some chemical and physical characteristics of sugar beet pectin.

Chemical and physical characteristics	Pectin extracted by Acid	Pectin extracted by ammonium Oxalate
Yield of pectin (%)	24.60 ± 0.101	22.75 ± 0.279
Moisture (%)	5.63 ± 0.055	6.17 ± 0.046
Ash (%)	1.97 ± 0.040	8.85 ± 0.087
Anhydrogalacturonic acid (%)	63.57 ± 0.091	56.73 ± 0.106
Methoxyl content (%)	6.75 ± 0.060	5.02 ± 0.042
Acetyl content (%)	1.74 ± 0.045	5.23 ± 0.050
Degree of methylation	46.17 ± 0.055	38.91 ± 0.074
Protein content (%)	2.95 ± 0.138	3.22 ± 0.091
PH (1 % Solution)	3.65 ± 0.010	4.03 ± 0.006
Viscosity (CP)	386 ± 4.583	202 ± 3.606
Molecular weight	47000	32000
Jely Grade	10	---

Values are average of three representative samples ± standard deviation.

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إستخلاص وتقييم البكتين من لب بنجر السكر

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الملخص العربي

تم تقدير التركيب الكيماوي لللب بنجر السكر الجاف الذي يعتبر من النواتج الثانوية لصناعة السكر. أوضحت النتائج أن المواد البكتينية تمثل المكون الرئيسي لللب بنجر السكر (44,71%). وتم تقييم إنتاج البكتين من لب بنجر السكر باستخدام طرق فصل مختلفة كالحمض المعدني (حمض الهيدروكلوريك) وأكسالات الأمونيوم. هذا وقد قدر الناتج من البكتين في حالة الاستخلاص الحامضي وذلك على عدة مستويات كل من: pH - درجات حرارة - زمن للتسخين. هذا وقد قدر أيضاً الناتج من البكتين باستخدام أكسالات الامونيوم على تركيزي 25، 50، 75% وذلك على ثلاث مستويات من درجات حرارة الاستخلاص (65، 75، 85°م) وذلك لفترات زمنية مختلفة. وقد وجد أن أعلى ناتج للبكتين في حالة الاستخلاص الحامضي قد وصل إلى 24,6% وذلك عند الاستخلاص على 75°م لمدة 4 ساعات على pH 1.5. بينما وجد أن أعلى ناتج من البكتين عند الاستخلاص بأكسالات الامونيوم قد وصل إلى 22,75% وذلك في حالة الاستخلاص على تركيز 0,5% من أكسالات الامونيوم على 75°م لمدة ساعة واحدة. وكذلك درست الخواص الكيماوية والطبيعية للبكتين المنتج من لب بنجر السكر.