RESEARCH NOTE

A STUDY ON PLANT POLYMER: PECTIN PRODUCTION AND MODIFICATION

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Abstract Pectin from two different plant sources: apple and sugar beet was studied. Apple pectin was modified by chemical treatments such as: acid, alkali and ammonia, so as to obtain an ester level equal to or less than 50 %, which is described as low methoxyl (LM) pectin. LM pectin form gels with significant characteristic. In this study, de-esterification of apple pectin with acid treatment resulted in gel specification with higher molecular weight and viscosity. Acid treatment resulted in pectin with average molecular weight of 32000 Dalton. Pectin was extracted from sugar beet pulp by acid and alkali treatments. The quality of gel formation and water uptake was improved using sugar source (glucose), $CaCl_2$ and peroxidase enzymes. The optimal levels of materials in formation of gel with high water absorption capacity were: Glucose 5 %, Pectin 15 %, $CaCl_2$ 80mg/g, and enzyme unit 170 U/g pectin. The level of ingredients responsible for gel hardness was related to sugar content (glucose) of 15 %.

Keywords Pectin, Enzymes, De-esterification, Peroxidase

چکیده پکتین از دو منبع مختلف گیاهی، سیب و چغندرقند مورد بررسی قرار گرفت. پکتین تجاری سیب توسط روش شیمیایی با استفاده از اسید، سود و آمونیاک جهت کاهش میزان استر تا حد زیر ۵۰ درصد که به نام پکتین نوع (LM) با متیل پایین مطالعه شد. پکتین LM ایجاد ژل با کیفیت بالا می نماید. در این مطالعه رد استریفیه شدن پکتین سیب به روش اسیدی منتج به ایجاد پکیتن با وزن ملکولی و ویسکوزیته بالا گردید. روش اسیدی به تولید پکتین با وزن ملکولی متوسط ۳۲۰۰۰ دالتون منجر گردید. پکیتین از تفاله چغندرقند به روش فرآیند اسیدی و قلیایی استخراج گردید. کیفیت ژل و میزان جذب آب با استفاده از منبع قندی (گلوکز) کلرورکلسیم و آنزیم پراکسیداز بررسی گردید. شرایط بهینه در تشکیل ژل با قدرت جذب آب بالا شامل گلوکز ۵ درصد، پکتین ۱۵ درصد، کلرورکلسیم ۸۰ میلی گرم و ۱۷۰ واحدآنزیم بود. شرایط بهینه در تشکیل ژل با سختی بالا با استفاده از درصد قند ۱۵ درصد گرم حاصل گردید.

1. INTRODUCTION

Polysaccharides produced by a wide variety of plants are generally water soluble gums which have novel and unique physical properties. Because of their wide diversity in structure and physical characteristics, these polysaccharides have found a wide range of applications in the food, pharmaceutical and other industries. The natural polysaccharide pectin, a basic building material of the higher plants cell wall, possesses a wide spectrum of functional properties. It can act as a thickener, gelling agent, stabilizer, emulsifier, film former, lubricant, coagulant, and caption binding agent [1]. Pectin is an important polysaccharide widely explored as the matrix for drug delivery systems. Pectin is able to resist against protease and amylase which are active in the upper gastrointestinal tract, so pectin is a good choice for these protein drug delivery systems [2].

The world consumption of pectin constantly grows and has already exceeded 20,000 tons a year. Lemon or orange peel and apple pulp are the main raw materials for pectin production. Searching for new pectin-containing raw materials is a significant subject in science and industry.

Table 1 is based on published literature data and shows the estimated US consumption and approximate price of different types of commercial polysaccharides used in food and other industrial applications. These data show that the majority of industrial polysaccharides used in the USA are starch based, comprising about 41% of the total market. Plant-derived polysaccharides, such as Arabic gum and guar gum, constitute about 6.6% of the total market and pectin about 4%, while algae-derived alginates account for about 6.7% of the total market value. Xanthan gum is a microbial exopolysaccharide derived from *Xanthomonas campestris* fermentation and accounts for 4% of the total market value [3]. The data in Table 1 are the industrial polysaccharides concerned in US including percentage of total value [3].

Pectin is a specific type of polymeric carbohydrate which is made of lots of galactronic acid and methyl ester units (Figure 1) [4-10]. Common raw materials for production of pectic materials are different kinds of fruit such as apples, citrus, pear, banana and tomato [11]. During World War II sugar beet wastes were used for pectin production in England and Germany instead of apple [3,4], but obtained products were not qualified from gel formation view point comparing other sources. Availability of sugar beet pulp makes it a good rival for pectin production beside apple and citrus [7]. The quality of agro-food products besides color and taste is determined by the product firmness. After harvest, the product

TABLE 1. Estimated Consumption and Price of Industrial Polymers in the US [3].

Industrial Polysaccharide	Food Usage (Tones)	Industrial Usage (Tones)	Total Usage (Tones)	Price ($ kg^{-1})	Approx. Total Value (million $)	% of Total Value
Corn Starch	203 000	1 013 000	1 216000	0.20	243.2	40.77
Carboxymethyl Cellulose	6100	40000	46100	2.00	92.2	15.46
Methylcellulose	820	21500	22320	3.30	73.7	12.36
Alginate	3700	3600	7300	5.50	40.2	6.74
Pectin	4900	0	4900	4.85	23.8	3.99
Xanthan	950	2500	3450	6.90	23.8	3.99
Gum Arabic	9340	2850	12190	1.65	19.4	3.36
Guar Gum	6070	14180	20250	0.96	19.4	3.20
Carrageenan	3700	90	3790	4.40	16.7	2.80
Tragacanth	526	81	607	26.50	16.1	2.77
Locust Bean Gum	3650	1620	5270	2.00	10.5	1.76
Karaya	410	2900	3310	2.10	6.9	1.15
Ghatti	4050	410	4460	1.15	5.1	0.85
Agar	125	165	290	16.6	4.8	0.80

quality starts to decrease; part of this decrease is due to the loss in firmness. Breakdown of pectin moiety of the cell walls by the enzymes could be related to a part of this quality decrease [12,13]. The main problem is obtaining a high molecular weight (MW) pectin and formation of a qualified gel with high sugar content which is difficult, either if modification of the process is effected by changing the degree of etherification and decrease in acetyl groups, which inhibits gel formation. Chemical methods, for example, process in acidic methanol can dismiss acetyl groups and increase ester group's ratio, but it can lead to further decrease in MW which reduces the quality of pectin in gel formation. Sugar beet pectin is famous for the feruloyl groups in its chain groups (Figure 2), which have the ability to make bonds between excess and free frolic acid in order to make gels, forming stable side covalent bonds, which could be dehydrated and hydrated again.

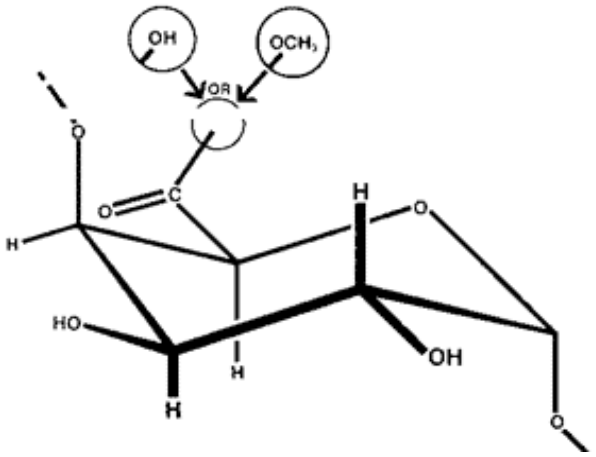

Figure 1. Galactronic acid and methyl ester units in pectin [1].

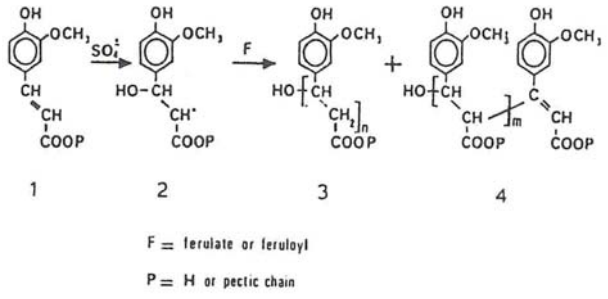

Figure 2. Sugar beet pectin with feruloyl group [16].

Peroxides in the presence of hydrogen peroxide cause side bonds with the help of ferroalloy groups of pectin [7,14-20]. This condition may bear applications which are different with common pectin applications. These applications may include items such as optimum usage of water in agriculture, optimum usage of chemical fertilizer and pesticides, possibility of cultivation in desert and steep surfaces, better soil aeration, swell able rubbers in water, application in controlled drug delivery systems (DDS), improving concrete quality and absorbing humidity from coal [7,21].

Pectins with an ester level over 50% based on the total level of carboxylic acid and ester are described as high ester pectin (HM). They are used to form jams and jellies having soluble solid levels near 65%. Formation of the gelled state requires high levels of dissolved solids and acidic pH. High methoxyl pectin does not contain sufficient acid groups to form gel or precipitate with calcium ions, as the pH is gradually reduced; the pectin is capable of forming gel at first, at high sugar content of around 80%. Pectin, modified so as to have an ester level equal to or less than 50% are described as low methoxyl or low ester pectin (LM), which are used to form gels with or without sugar in the presence of divalent cations. They are, therefore, used in low calorie or dietetic foods [2]. Low methoxyl pectin is produced by further de-ester fiction to a point where less than 50% of the total carboxyl groups are esterified. If this process is carried out using acid or alkali, the balance exists as free acid groups. Low mehoxyl pectin forms gel in the presence of calcium and gel formations are believed by the egg box mechanism [3].

There are four different methods for the preparation of low ester pectin from high ester pectin, and HM pectin demethylation could be affected by: (a) acid (b) alkali; (c) enzyme and (d) ammonia in alcohol or concentrated aqueous ammonia demethylation and amidation [4,5].

Low methoxyl pectin produced by enzyme demethylation have been found to be inferior in quality to those produced by other methods, because of the non random distribution of methyl ester groups among molecules of the pectin and also the removal of very small units, such as non-uronide materials. Acid demethylation removes

special units at a high rate, leading to the production of pectin having a higher percentage of poly galacturonic acid. The main disadvantage of acid treatments is the slow reaction. It may be speeded up using higher temperatures which results in de-polymerization of the pectin chains and the rate of de-polymerization is faster than the rate of demethylation as the temperature increases [8].

The use of ammonia in alcoholic or concentrated aqueous ammonia systems results in a low methoxyl pectin that contains amide groups. It has been found that the percentage of amide groups in the pectin plays a positive role in gel formation and contributes to the texture and strength [9,10].

So far, numerous methods have been implemented for the extraction and improving sugar beet pulp for LM pectin gel production [6-10]. Different extraction modes like extraction with water, oxalate, hot acid, cold alkaline and EDTA were investigated. Also effect of different oxidizing agents like laccasses enzyme, peroxidase enzyme, ammonium persulfate, potassium periodate, potassium permanganate, sodium chloride and hydrogen peroxide were studied [10-21]. Common affecting parameters on LM pectin gel production are: pH, pectin concentration, calcium ion content and the percentage of glucose [7,9].

In this study, attempts were made to determine the experimental procedures required to prepare low ester apple pectin. Due to its high utility as gelling agent and very versatile thickeners, different treatment method using acid, alkali and ammonia were studied. The effect of different parameters on LM pectin production were also studied and compared. Also, sugar beet pectin was extracted by cold alkaline and hot acidic methods. Quality of the gel from the extracted pectin was examined under different condition; also hardness and the percentage of water uptake were determined.

2. MATERIALS AND METHODS

This investigation was studied in laboratory scale and all the data presented in the results section are the mean of three replicates. All the chemical and biochemical compounds studied in these experiments were pure analytical grades.

The polysaccharide studied in this investigation was pectin from two different sources: apple and beet sugar, and various evaluation methods were studied.

2.1. Pectin Extraction Applied steps involved in pectin extraction are: increasing the pectin solution pH using 10% sodium hydroxide to the range of 8 to 8.5, acidification for continuing the extraction using 0.1N HCl, solution to pH 3.7 (pH was adjusted by Metrohm pH meter), heating operation and quenching (100 to 110 min) at 80 to 85˚C, separation of sugar beet pulp from pectin solution by cotton filter, washing with distilled water and drying pectin solution in the incubator (Heraeus model) and evaluation tests were also studied [22-29].

2.1.1. Gel formation 5 ml distilled water, required for starting the experiment is poured in a small beaker and then dry pectin is added to this solution in (2-20) w/v %. In the next step, calcium chloride is added to the mixture until all pectin is solved in the solution and heating with electrical heater, while the mixture is stirring. After the solution starts boiling, stirring continues for 2 min and lost of water would be replaced. After this time, glucose is added and stirring continues until homogeneity of the solution is achieved. Then the fluid is poured in a container and when its temperature reaches ambient condition, 3 ml hydrogen peroxide per each gram of pectin and peroxidase in phosphate buffer at pH 7, is added to the cold media and stirred for 5 min. Finally, the container is kept under stationary condition for gel formation [7].

2.1.2. Gel hardness measuring Equipment needed for measuring the hardness was a thin rod with specified diameter which was entered into the gel and the required force for its entrance was measured (The required entering force) is regarded as a measure of gel hardness, g force. In this investigation, gel hardness defined as the force required for compression of the gel by a 12 mm cylindrical rod with 5 mm diameter [7].

2.1.3. Water absorption capacity of pectin gel The experiment begins with addition of gel after formation in a glass container, which was previously numbered and weighed. Weight of the

container and gel are measured again, and then container is placed in the oven until gel is dried and dehydrated. After this step, weight of dried gel in calculated. With subtraction of the two measured weights, water content could be calculated [7].

Pectin in this research was pure apple pectin also a sample extracted form shah-roud sugar beet pulp with cold alkaline solution and hot acid methods. Peroxides enzyme was provided from Merck Co. with 170 U per milligram, with optimal pH of 7. Hydrogen peroxide, solid calcium chloride and solid glucose and other materials used in this research were pure with analytical grades.

3. RESULTS AND DISCUSSIONS

3.1. Apple Pectin Low ester pectin due to its high utility as gelling agent was produced from high ester pectin (analytical apple pectin). Three different treatments of acid, alkali and ammonia were evaluated for preparing pectin with low methoxyl groups [3].The intrinsic viscosity was measured by an ubbelohde viscometer, which was a glassware viscometer with 5.5 ml inherent volume and 0.5mm capillary diameter. It was tested with 0.155 M of NaCl solution with flow time of 105s. This method is described by Hwang [22]. The average molecular weight (MW) of pectin and intrinsic viscosity was determined by the following Mark Houwink equation in NaCl solution [22,24], which the results are presented in Table 2.

$$[\eta] = 9.55 \times 10^{-2} \times M_w^{0.73}$$

Where $[\eta]$ is the intrinsic viscosity and M_w is the viscosity average molecular weight.

In this investigation, comparisons between LM pectin production methods using acid, alkali and ammonia as de-esterification agent showed that acid treatment under prescribed condition results, LM pectin with high viscosity of 186 (ml/g) and high average molecular weight M_w of 32000 which is close to the commercial LM pectin molecular weight [25]. LM pectin with high molecular weight is selected as it is an ingredient in food product, used as thickener.

3.2. Sugar Beet Pectin Parameters studied in these experiments are: Glucose quantity, pectin amount, $CaCl_2$ content and enzyme unit. Glucose quantity changes from 5-45%, Pectin amount changes from 2-20%, Calcium concentration changes from 15-100 mg/g pectin, and-enzyme (Peroxidase unit) from 85-425 $\frac{U}{g\,Pectin}$.

3.2.1. Statistical study For evaluation of the effect of parameters involved in gel production such as glucose, pectin, $CaCl_2$ and peroxidase on water absorption capacity of the pectin gel from sugar beet pulp, Completely Randomized method was utilized [28,29]. Four parameters under five levels were investigated.

Parameters:

- Glucose%: G5, G15, G25, G35, G45: Glucose levels from 5-45%
- Pectin%: P2, P5, P10, P15, P20: Pectin levels from 2-20%
- $CaCl_2$ mg/g pectin: C15, 30, C40, C80, C100: $CaCl_2$ levels from 15-100 mg/g
- Enzyme Unit: A85, A170, A205, A340, A425: Enzyme unit levels from 85-425

The average of water absorption for each treatment and LSD calculation [28,29] with multiple comparison test mean or test of significance, presents the optimal water absorption under glucose 5%, pectin 10%, $CaCl_2$ 80 mg/g and enzyme 170 U/g pectin which is comparable with the experimental data presented in Table 3.

Gel hardness and water absorption was studied under fixed conditions and changing one of the parameter in each case [5]. The experimental conditions were as follow: in case one, pectin 10%, $CaCl_2$ 80 mg/g and enzyme unit 170; in case two, glucose, 15%, $CaCl_2$ 80 mg/g, and enzyme unit 170; in case three, pectin 15%, sugar 15% and enzyme 170 unit and in case four, pectin 10%, glucose 15% and $CaCl_2$ 80 mg/g. The difference observed in results relates to the changes in different ingredients in gel formation. Increase in the pectin and sugar amount results in the formation of hard gel with low water uptake capacity. If a gel with high water absorption capacity is needed, condition mentioned in case one with low sugar quantity is significant, but if gel hardness is the aim, case two must be selected

TABLE 2. Average M_w and Viscosity of LM Pectin After Various Treatments.

Treatment	MW	Intrinsic Viscosity ml/g
Acid	32000	186
Alkali	28300	170
Ammonia	27400	166

TABLE 3. Optimal Conditions for Gel Hardness and Water Absorption.

Case	Materials	Gel Hardness (g Force)	(%) Water Absorption
1	Glucose 5%	5.2	850
2	Pectin 15%	11.7	768
3	$CaCl_2$ mg/g Pectin 80	8.7	711
4	Enzyme Unit $U/_{g\ pectin}$ 170	8.7	711

with high sugar quantity. Many investigators studied gel formation with different gelling agents such as persulfate, peroxidase, and calcium but none of them optimized overall [16-20]. In these references, ferulated group and effect of oxidative agent on gel formation by enzymatic method using Laccase and chemical agents such as ammonium persulfate are studied. As mentioned, optimal conditions for gel hardness and water uptake capacity is presented in Table 3. This table presents the conditions for preparation of gel with different quality, if agricultural product utilization and superabsorbent is the aim, case one is selected but as food thickeners agent, other cases are preferred.

4. CONCLUSION

In this investigation natural polymers from plant sources were studied. For plant polymers, two types of pectins, apple pectin and sugar beet pectin were evaluated as the source of plant polysaccharide. For apple pectin, analytical apple pectin was selected and the effects of three different chemical treatments, acid, alkali and ammonia on LM pectin production were studied. Acid treatment resulted in pectin with an average molecular weight of 32000 and higher viscosity which is a source of pectin utilizable as food thickeners.

Sugar beet pectin was extracted by chemical

process and gel formation was studied under chemical and biochemical treatments. Effect of parameters such as glucose, pectin, $CaCl_2$ and enzyme (Peroxidase) quantity, on gel quality such as: hardness and water absorption capacity were investigated. The optimal levels of materials in formation of gel with high water absorption capacity were:

Glucose 5 %, Pectin 15 %, $CaCl_2$ 80 mg/g, and enzyme unit 170 U/g pectin. Higher amount of glucose resulted hard gel with low quality in water absorption capacity. For preparation of gel with high quality as super absorbent, glucose content must be in lower amount, but hard gel with low water uptake results from higher amount of glucose.

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