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Research Note

Investigation into Some Parameters Affecting Pectin Gel Quality

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In this research, gel production from the extracted pectin gel of shahroud sugar beet pulp has been investigated. The pectin extractions were performed in a cold alkaline solution and a hot acid solution, respectively. The effects of different parameters, such as percent of sugar, pectin concentration, calcium content and quantity of peroxidase enzyme, hardness and content of water absorbed by the gel, were studied. Optimum conditions for production of favorable gel, from extracted pectin in this research are, as follows: Percent of sugar (glucose): 15%, peroxidase enzyme content: (pectin unit) 170 $\frac{\text{pu}}{\text{g of pectin}}$, pectin concentration (if only gel water uptake is important): 10%, pectin content (if only strength and hardness of gel are important): 15%, calcium content (if only gel water uptake is important): 60 $\frac{\text{mg } CaCl_2}{\text{g of pectin}}$ and calcium content (if only gel uptake strength is important): 80 $\frac{\text{mg } CaCl_2}{\text{g of pectin}}$. Simultaneous application of peroxidase enzyme and hydrogen peroxide as oxidizing agents, besides calcium, glucose and suitable content of LM (Low Methoxyl) pectin, resulted in interesting properties and a decrease in gel formation time from "5 minutes - 24 hours", to "15 - 20 seconds" and a favorable increase in gel strength.

INTRODUCTION

Industrial pectins are specific types of polymeric carbohydrates, which are made of many galactronic acid units. Common raw materials for the production of pectic materials are different kinds of fruit, such as apples, citrus, pears and bananas, which may be extracted under different processes and from different sources. During World War II, sugar beet waste was used for pectin production in England and Germany instead of apple pulp [1,2], but the obtained products were not of good quality from a gel formation viewpoint compared to those from other sources. Availability of sugar beet pulp makes it a good rival for pectin production compared to apple and citrus. The main problem is obtaining a high molecular weight pectin and the formation of a qualified gel with a high sugar content, which is difficult, either by modifying the process by changing the degree of esterification or by decreasing the acetyl groups, which inhibit gel formation, using chemical methods. The process in acidic methanol can dismiss acetyl groups and increase the ratio of the ester group, but, it can lead to a further decrease in MW (Molecular Weight). Sugar beet pectin is famous for the froluyl groups in its chains, and its ability to make bonds between excess and free frolic acid, in order to make gel with thermally stable side covalent bonds, which may be dehydrated and hydrated again. Peroidase, in the presence of hydrogen peroxide, causes side bound with the help of froluyl groups of pectin [3-14]. This condition may include applications which are different from common pectin applications. These applications may include items, such as the optimum usage of water in agriculture, the optimum usage of chemical fertilizer and pesticides, the possibility of cultivation in deserts and on steep surfaces, better soil aeration, swellable rubber in water; application in controlled Drug Delivery Systems (DDS), improving concrete quality and absorbing humidity from coal.

So far, numerous methods have been performed for extracting and improving sugar beet pulp in LM pectin gel production [3-7]. Different extraction modes,

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such as with water, oxallate, hot acid, cold alkaline and EDTA, have been investigated. Also, the effects of different oxidizing agents, such as the laccase enzyme, the peroxidase enzyme, ammonium persulfate, potassium periodate, potassium permanganate, sodium chloride, hydrogen peroxide and other similar materials, have been studied [7-15]. Common affecting parameters in LM pectin gel production are: pH, pectin concentration, calcium ion content and percent of glucose [3-5]. In this research, pectin was extracted using cold alkaline and hot acidic methods. The quality of the gel from extracted pectin was examined under different conditions, and the springiness, hardness and percent of water uptake were also determined.

MATERIALS AND METHODS

Materials

In this research, the required LM pectin was extracted from shahroud sugar beet pulp using a cold alkaline solution and hot acid methods. The peroxidase enzyme was provided by the Merck Co. with 170 U per milligram and an optimal pH of 7. Hydrogen peroxide, solid calcium chloride, solid glucose and other materials used in this research were pure with analytical grades.

Methods

Pectin Extraction

The applied steps necessary for pectin extraction are: Increasing pectin solution pH with 10% sodium hydroxide to the range of 8 to 8.5, acidification for continuing extraction with 0.1N HCl, solution to pH of 3.7, heating operation and quenching (100 to 110 minute at 80 to 85°C), separation of sugar beet pulp from pectin solution by cotton filter, washing with distilled water and drying pectin. Quality control of the extracted pectin was also undertaken [3].

Gel Formation

5 ml of distilled water, which is required for starting the experiment, is poured into a small beaker and then dry pectin is added to this solution in w/v%. In the next step, calcium chloride is added to the mixture until all pectin is solved in the solution and heated with an electrical heater, while the mixture is stirred. After the solution starts boiling, stirring continues for 2 minutes and evaporated water was be replaced. After this time, glucose is added and stirring continues until the homogeneity of the added glucose is reached. Then, the fluid is poured into a container and when its temperature reaches an ambient condition, 3 ml of hydrogen peroxide per each gram of pectin and peroxidase in a phosphate buffer at pH of 7, is added to the cold media and stirred for 5 min. Finally, the container is placed in a stationary position for gel formation.

Gel Hardness Measuring Equipment

The equipment needed for measuring hardness was a thin rod with a specified diameter, which entered the gel. The required force for entering the rod in the gel was measured. In the first step, a specified entrance is regarded as a measure of gel hardness, i.e., g force. In this investigation, gel hardness is defined as the force required for the compression of the gel a cylinder of 12 mm from a rod with a 5 mm diameter.

Measuring Gel Water Content

The experiment begins with the addition of gel in a glass container (which has been previously numbered and weighed). The weight of the container and the gel are measured again. Then, the container is placed in the oven until the gel is dried and dehydrated. After this step, the weight of the dried gel is calculated. By subtraction of the 2 measured weights, water content and water percentage can easily be calculated. The remaining gel in the container is required for continuing experiments. In this way, a certain quantity of water is added, in order that the semi-dry gel can easily move in it. Although, in this condition, gel preserves its structure, this condition leads the dry gel to possible water absorption.

RESULTS AND DISCUSSION

The results of quality control experiments have shown that extracted pectin from sugar beet pulp, includes 9.5% humidity, 3.4% total ash, 0.83% soluble ash in acid, 44.3% esterification degree, 9.7% amid replacement degree, 73.7% galactronid and 21.6 mg sugar content and organic acids.

Effect of Glucose Quantity

Evaluations show that increasing solid materials, including glucose, affects LM pectin gel properties. Norsker et al. showed that sugar has a positive effect on pectin gel LM properties [6]. Others approved that sugar has a positive effect on gel structure, but this effect depends on sugar concentration [1-3,6,9,16]. Conditions: Pectin concentration: 10%, pH: 7, peroxidase unit: 170 pu/g pectin, mg $CaCl_2$/g pectin: 80

This phenomena is approved by Figure 1, which shows the effects of sugar on gel hardness. Gel hardness decreases after its maximum point at 15%, rapidly, until it become linear. This point is 45% of the sugar content. The time of gel formation at 35% and 45% sugar content was more than 5 minutes, whereas, in other treatments, this time was less than 60 seconds.

Figure 2 compares the effect of sugar concentra-

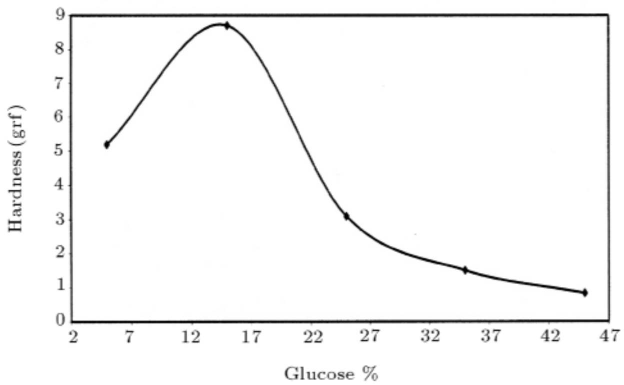

Figure 1. Effect of glucose content on gel hardness.

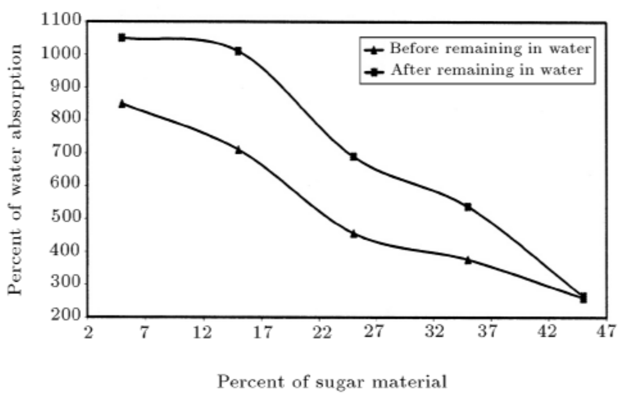

Figure 2. Effect of glucose content on percent of water absorption before and after remaining in water.

tion on water absorption before and after remaining in water. Both curves have an inclination point and a downward movement. This figure shows that increasing sugar content causes a decrease in the water absorption of gel. The decrease in the distance of the two curves approves that an increase in sugar percentage causes a decrease in the gel capacity for water absorption. At the final point, the curves approximately coincide. Thus, utilization of 15% sugar is the best percentage for gel formation.

Effect of Pectin Concentration

The figures of this section are evaluated under conditions where some parameters (percent of sugar materials: 15%, pH: 7, $\frac{\text{pu}}{\text{g of pectin}}$: 170, $\frac{\text{mg CaCl}_2}{\text{g of pectin}}$: 80) are assumed to be constant.

Figure 3 shows the effect of pectin concentration changes in gel hardness. In this figure, initially, gel hardness increases with pectin concentration as a semi logarithmic curve and an increase in gel hardness occurs very fast. However, this increasing trend decreases little by little, as the curve tends to change to a horizontal line. In this figure, the maximum rate of gel hardness is at 20 percent concentration. Gel hardness

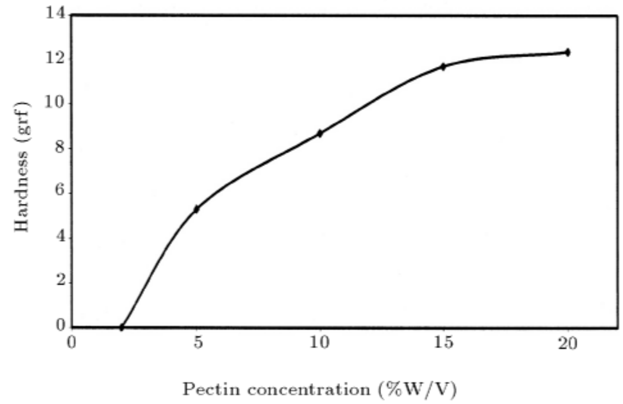

Figure 3. Effect of pectin concentration on gel hardness.

increase partly relates to an increase in gel quality, but, an increase in concentration will cause hardness, which causes a decrease in the percent of water absorption.

Figure 4 shows the changes of water absorption percent by the change in pectin concentration. In this figure, increasing trend is followed by increasing trend and reaches a maximum point. The data, before holding in water, is maximized at 15 percent concentration, but, after holding in water, is maximized at about 10 percent of pectin concentration. This shows that the gel ability for additional water absorption at 10 percent concentration is more than the ability for water absorption at 15 percent concentration and the decreasing distance between the two curves after the 10 percent point, shows that, after this point, the gel network cannot maintain much water and this loss increases by increasing pectin concentration. It should be said that in cases where the objective of gel construction is additional water absorption in the medium or where the gel is used as a super absorber, the best content for pectin concentration is 10 percent. But, if the main objective is gel firmness, 15 percent pectin concentration is preferred. It must also be mentioned that the time for gel formation was from 15 to 60 seconds.

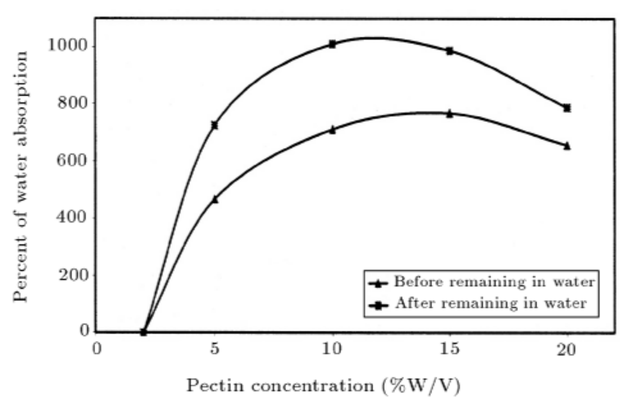

Figure 4. Pectin concentration effect on percent of water absorption before and after remaining in water.

Effect of Calcium

There has been much research on the effect of multivalent ionic cations on the creation of suitable structures in water absorbent gels. Ca^{++} has been found to be the best for LM pectin and calcium linkages have a direct relationship with the hardness of the pectin gel [1,3,15]. Therefore, the amount of calcium is very sensitive. If the amount of calcium is low, the resultant gel would be very weak, such that, during transportation, it would be damaged. On the other hand, too much calcium would result in a brittle gel. The lack of, or too little, calcium will introduce defects in structure, the most important being the release of water by the gel [3,16].

In many papers there have been references to the fact that the role of calcium in forming the gel does not only rely on the first bond between calcium and the chain structure, but also on the second and third bonds between calcium and the hydroxyls, which play a major role. By increasing the amount of calcium, the pectin chain structures will start combining with the calcium bonds and, therefore, will start to cluster [15].

According to the theory of Mr. Jean-Francois Tibault, LM pectin produced from "sugar beet" cannot form gel with calcium on its own, or produce a strong, hard and suitable gel. To generate the figures, the percentage of sugar and the concentration of pectin have been assumed constant; pectin concentration: 10%, pH: 7, $\frac{\text{pu}}{\text{g of pectin}}$: 170 and optimum sugar level: 15%.

Figure 5 shows, first, how the hardness of the gel with a gradual increase in calcium concentration, will increase linearly and how, after a peak, 80 $\frac{\text{mg CaCl}_2}{\text{g of pectin}}$, any increase will result in gel brittleness.

Figure 6 demonstrates this quite well. As can be seen, up to a certain level, the time that the gel spends in water will affect its water absorbency. After this point, gradually, the gel will start losing its absorbency. On the other hand, it can be said that, in the same way that gel absorbs lots of water due to the weakness of its structure caused by low levels of calcium, it will, somehow, lose more water. This is referred to in [3,16].

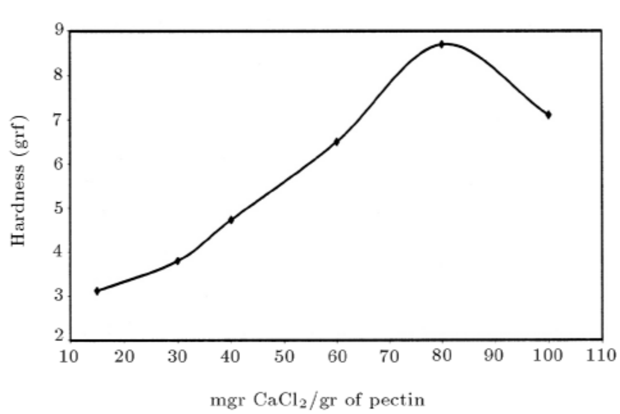

Figure 5. Effect of calcium on hardness.

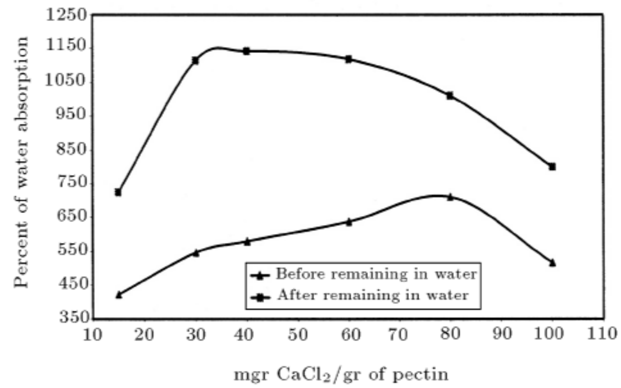

Figure 6. Effect of calcium on rate of water absorption before and after remaining in water.

Considering the observations, where gel water absorbency is required, the best quantity is 60 $\frac{\text{mg CaCl}_2}{\text{g of pectin}}$ and, if strength and hardness are required, 80 $\frac{\text{mg CaCl}_2}{\text{g of pectin}}$ will be considered, which is important to know.

In general, 80 $\frac{\text{mg CaCl}_2}{\text{g of pectin}}$ was selected because this is the optimum level for the gel, prior to being exposed to water, or, after that.

The time period required to get the gel with 100 $\frac{\text{mg CaCl}_2}{\text{g of pectin}}$ was 1 minute; for 60 mg Ca, about 30 seconds; for 80 mg Ca, 15-20 seconds and for 30 mg Ca, more than 60 seconds. Also, the gel was fragile.

Effect of Enzyme

The LM pectin of sugar-beet pulp and sugar-beet was recognized by the group of feruloyl in their original chain [5,6,11,14]. Much research has been executed with different enzymes and chemicals, sush as: Laccase, peroxidase, ammonium per sulfate, potassium periodate, potassium permanganate, sodium chloride, hydrogen peroxide and mixtures of chemicals with enzymes [5-7,11-14]. Between various oxidizing agents, peroxidase, ammonium peroxysulfate and one kind of laccase produced suitable gel, but the processing time was too long [6,11,14]. Studies about reaction mechanisms show that radical polymerization is the mechanism for these reactions [13]. The peroxydase enzyme with hydrogen peroxide caused cross linkage bound with free radicals [14]. The figures in this section are evaluated under conditions where some parameters (pectin concentration: 10%, percent of sugar materials: 15%, pH: 7, $\frac{\text{mg CaCl}_2}{\text{g of pectin}}$: 80) are assumed constant. Figure 7 shows this phenomena and demonstrates that, after maximum increase in gel hardness at 170 $\frac{\text{pu}}{\text{g of pectin}}$, the curve falls slowly, which is the reason for the brittleness that increases slowly.

Figure 8 shows changes in water absorption, due to changes in enzyme amount. The curve of water

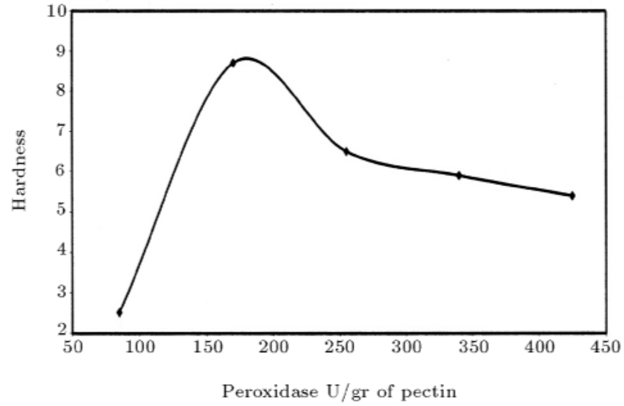

Figure 7. Effect of peroxidase unit on gel hardness.

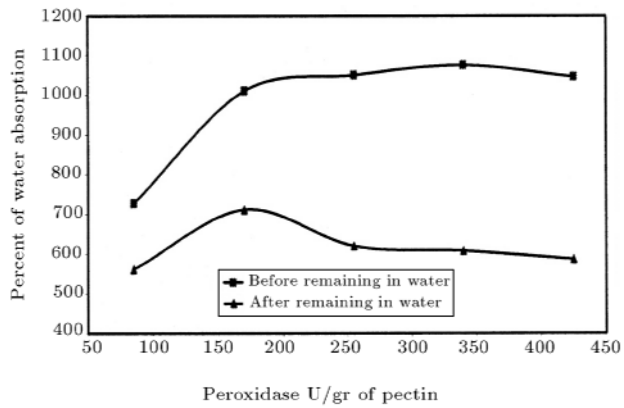

Figure 8. Effect of peroxidase unit on water absorption.

absorption before remaining gel in water condition, after a slow increase, reaches a maximum point and, then, decreases slowly. The curve of water absorption, after gel remains in water shows that, after a short increase there is a little decrease and the distance between the two curves, after a little increase, is almost constant. This shows that enzyme content, after optimization, should not have an influence on the gel water absorption ability. According to Figures 7 and 8 and the cost of the enzyme, the optimum content for the enzyme is 170 $\frac{\text{pu}}{\text{g of pectin}}$. So, the required time of gel formation, under three conditions i.e., 255, 340, and 425 $\frac{\text{pu}}{\text{g of pectin}}$, are almost equal to 60 seconds, but, in the case of 85 $\frac{\text{pu}}{\text{g of pectin}}$, gel formation time has been more than 5 minutes.

GENERAL CONCLUSION

Increasing sugar levels in gel produced from LM pectin up to an optimum level will increase gel hardness, but will reduce its water absorbency. Increasing the concentration of pectin will increase the hardness of the LM pectin gel. An increase in calcium levels up to an optimum level will positively affect the absorbency and the hardness of the gel. The amount of added peroxidase enzymes will hugely affect the hardness of pectin gel. But, there is an optimum level, after which, the degree of gel absorbency is not affected.

The use of peroxidase and hydrogen peroxide as oxidants, in conjunction with calcium, sugar and an optimum level of LM pectin, will produce interesting properties, such as a reduction in the time required for the formation of the gel from 5 minutes to 24 hours, to 15 to 20 seconds, as well as an increase in gel strength.

According to this study, the optimum levels for formation of gel extracted from pectin, are as follows:

1. Optimum glucose levels: 15%,
2. Enzyme peroxidase levels: 170 $\frac{\text{pu}}{\text{g of pectin}}$,
3. Optimum pectin concentration (if only gel water absorbency is considered): 10%,
4. Optimum pectin concentration (if only strength and hardness of gel are considered): 15%,
5. Calcium levels (if only gel water absorbency is considered): 60 $\frac{\text{mg } CaCl_2}{\text{g of pectin}}$,
6. Calcium level concentration (if only strength of gel is considered): 80 $\frac{\text{mg } CaCl_2}{\text{g of pectin}}$.

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