

Effect of Different Acids, Heating Time and Particle Size on Pectin Extraction from Watermelon Rinds

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ABSTRACT :

Watermelon cultivation has widely been expanded in Iraq and industrial by-products like watermelon rinds play an important role in pectin manufacture. The objective of this investigation was to determine a practical follow-up to the extraction of pectin from watermelon rinds and to characterize it in a laboratory, on a small scale, aiming at establishing the optimum conditions for acid extraction. The independent variables were citric acid concentration (0.08 – 5 g/ml) and heating time (20 – 110 min). The highest yields were obtained when watermelon rinds was dried and ground to obtain a watermelon flour to be used as raw material, citric or nitric acids were used and when the citric acid concentration was (5 g/100 ml) and the time of reaction was (110 minutes). The watermelon variety in itself was not significant in pectin yield. The degree of esterification (DE = 44.37 %) of the product obtained, as well as its physical looks, show the success of pectin extraction. Results have shown that the generated model adequately explained the data variation and significantly represented the actual relationship between the independent variables and the responses, with a correlation coefficient of (0.938) and a (44.9454 %) absolute average percent error.

الخلاصة :

يزرع نبات الرقي على نحو واسع في العراق وتوجد في قشور الرقي نواتج عرضية صناعية تلعب دوراً مهماً في صناعة البكتين. يهدف البحث لاستخلاص البكتين من قشور الرقي وتنقيته في المختبر، من خلال توفر الظروف الأفضل للاستخلاص الحامضي. أن المتغيرات المستقلة كانت هي تركيز حامض الستريك (0.08 – 5 غم \ مليلتر) وزمن التسخين (20 – 110 دقيقة). وأظهرت النتائج التي تم الحصول عليها ما يلي:

1. أن قشور الرقي التي جُفّت وطجنت أفضل بالاستعمال كمادة خام.
2. إن أستعمل حامض الستريك أو النتريك هو الأفضل مقارنة بحوامض الكبريتيك والفسفوريك والهيدروكلوريك.
3. عندما يكون تركيز حامض الستريك (5 غم \ 100 مليلتر) وزمن التسخين (زمن التفاعل) (110 دقيقة) يعطي أفضل النتائج. وأظهرت النتائج عدم وجود فروقات معنوية بين الأصناف في إنتاج البكتين. وقد كانت درجة "الاسترة" للمنتج المستحصل عليه هي (DE = 44.37 %) ، بالإضافة إلى نظارته الطبيعية. كما تم التوصل إلى علاقة رياضية فعّالة تربط إنتاج استخلاص البكتين بالمتغيرات المستقلة، حيث بلغ معامل التطابق 0.938 ومعدل نسبة الخطأ المطلقة

1. INTRODUCTION

Pectin substances are a large family of structural elements of primary cell walls and intercellular regions of higher plants where they function as hydrating agent and cementing material of the cellulosic network. Figure (1) is a schematic view of the molecular architecture of the cell wall. They are commonly produced during the initial stages of primary cell growth and make about one – third of the cell-wall dry substances. The highest concentration of pectins in the cell wall is seen in the middle lamella, with a gradual decrease from the primary cell wall toward the plasma membrane (McCann and Roberts 1991).

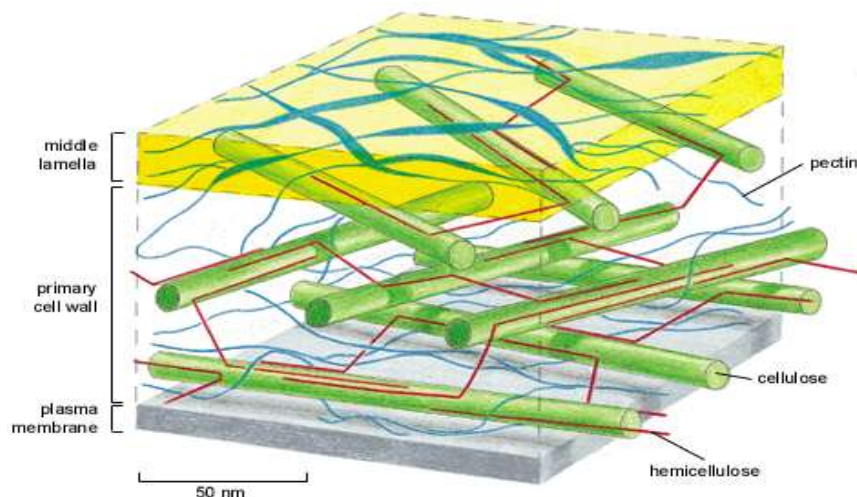


Figure 1. Simplified and schematic representation of the architecture of the cell wall.

Chemically, pectins are a family of complex heteropolysaccharides comprised by a diversity of carbohydrate residues. Like most other plant polysaccharides, pectins are polydisperse in composition and molecular size, that is, they are heterogeneous with respect to both chemical structure and molecular weight. Their composition varies with the source and conditions of extraction, location, and other environmental factors (Chang et al. 1994).

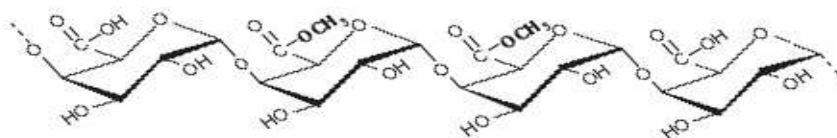


Figure 2. The structure of repeating segment of pectin molecule

Pectin is a polymer of α -galacturonic acid with a variable number of methyl ester groups (Liu et al., 2006). However, pectin also contains α -L-rhamnopyranosyl residues in the backbone chain and branch chains of arabinan and galactan, and their fine structure vary considerably (Shingthong et al., 2004). Some of the carboxylic groups of galacturonic acid molecules in the pectin chains are methyl esterified (Figure.2) and the percentage of esterified groups is expressed as DE (degree of esterification). Depending on the degree of esterification, pectin is divided into two major groups: high-ester pectin, with DE higher than 50%, and low-ester pectin, with DE lower than 50% (Thakur et al., 1997; Boonrod et al., 2006). In high-ester pectin, the junction zones are formed by the cross-linking of homogalacturan through hydrogen bond and the hydrophobic interaction between methoxyl groups, both of which are promoted by high-sugar concentration and low pH. In low-ester pectin, junction zones are formed by calcium cross-linking between free carboxyl groups (Willats et al., 2006). Pectin is a high-value functional food ingredient widely used as gelling agent and stabilizer. Its gelling property makes pectin be of use in food industries e.g. jam, jelly, emulsion stabilizer and thickener in sauces. Pectin can also be used in medicine as a fiber source, a supplement to lower cholesterol and increase immunity or as dentistry adhesive (Vidal-Valverde, et al., 1982). It is also an abundant, ubiquitous and multifunctional component of the cell walls of all land plants (Willats et al., 2006). The peel represents about half of the fruit mass. Because of significant juice production, the peels, as a major waste, have become a substantial burden to the environment. Hence it is necessary to find a feasible way to turn the peels into useful products or to adequately dispose of them, seeking a positive environmental impact (Liu et al., 2006). According to (May, 1999), only a few source materials have been used for commercial production of pectin as food additive. One of the reasons for this is that most of the pectic materials present in nature do not have any functional properties; in particular, the ability to form sugar acid gel systems and this property has been the main requirement of commercial pectin until recently.

Pectin extraction is a multiple-stage physicochemical process in which the hydrolysis and extraction of pectin macromolecules from plant tissue and their solubilisation take place under the influence of different factors, mainly temperature, pH, and time (Paga'n et al., 2001). Studies are available on the extraction of pectins with mineral acids, such as sulfuric acid (Yapo et al., 2007), hydrochloric acid (Iglesias and Lozano, 2004; Boonrod et al., 2006), and nitric acid (Paga'n et al., 2001; Boonrod et al., 2006). However, very little is known about the extraction of pectin with citric acid (Canteri-Schemin et al., 2005; Pinheiro et al., 2008), that could be better than the other extractors from an economic as from an environmental point of view. The objectives of this work were to obtain optimum conditions for pectin extraction from watermelon rinds on a laboratory scale and to characterize it in order to observe the influence of some factors (citric acid concentration, heating time and particle size) on the yield of pectin and to obtain the relationships between the variables and the response (yield of pectin).

2. MATERIALS AND METHODS

2-1. Materials

Samples from two different watermelon (*Citrullus lanatus*, L.) varieties (Charleston Gary and Crimson Sweet) were used as raw material in this work (Splitstoesser, 1992). In the laboratory, after the whole watermelons were harvested from the field, the rind was removed from the flesh. This usually was done with a knife. Approximately, the rind makes up about 30 percent of the weight of the whole watermelon. All The rinds were first washed with water to remove some of the sugars and ground in an electric grater, and then pressed. It dried, initially at room temperature and then at 50°C, to a constant weight with air circulation. This dry watermelon pool was then crushed and mixed and the product was called watermelon flour and it was used as the raw material for all the assays made concerning pectin extraction and characterization. Two watermelon flour varieties were selected (Charleston Gary and Crimson Sweet) in order to establish the influence of watermelon variety on the yield of pectin extraction and the results were compared with a watermelon pool of the watermelon varieties.

2-2. Pectin extraction

The extraction procedure was carried out according to the Canteri-Schemin et al. (2005) and Pinheiro et al. (2008) method. Five replicates for each assay were made. Pectin was extracted with different citric acid concentrations (CAC = 0.08, 0.5, 1.5, 2.5, and 5 g %) and heating times (HT = 20, 30, 60, 90, and 110 min), under reflux in a condensation system at 97° C (solute/solvent 1:50), using water acidified with citric acid to pH 2.5, using watermelon flour (pool) as raw material. Next, the pool was classified with four metallic sieves manufactured by Jayant Scientific Ind., India (300, 100, 120, and 95 µm) in a shaker device in order to separate them in five portions with different particle sizes. Three batches, as well as crude dried watermelon without crushing were used to observe the effect of particle size on pectin yield. To isolate pectin, hot acid extract was pressed in a cheese cloth bag and the concentrated "juice" was cooled to 4° C. The watermelon pectin was precipitated by alcohol-juice treatment 2:1 (v/v). The mixture of solvent and precipitate was stirred for ten minutes and then left to rest for one hour in order to allow pectin flotation (Kalapathy and Proctor, 2001 and Boonrod et al., 2006). With this procedure the pectic substances remain at the surface of the alcohol/water mixture and thus it is easier to remove them in a quantitative way. The floating pectin was filtered through cheesecloth, rinsed with alcohol and then pressed. The pressed pectin was dried to constant weight at 55° C, cooled in a dessicator and the yield calculated on a dry weight basis (initial weight of sample). The hard pectin cake was broken up, ground and sieved in order to obtain powdered pectin. The extraction of pectin was performed with watermelon from selected varieties under equal conditions. Pectin extraction was also performed under reflux at 97° C for 30 min (solute/solvent 1:50), using water acidified with different acids, to investigate the effect of each kind at pH 2.5 and to ratify the use of citric acid. In this assay, the raw material selected was the watermelon flour pool with particles between 120 and

95 µm. The selected acids utilized for extraction were citric, phosphoric, hydrochloric, nitric, and sulfuric acids. The analysis of variance (ANOVA) was used to define the optimum conditions concerning the acid used for pectin extraction, the watermelon variety and the size of particles of the watermelon.

2-3. Determination of degree of esterification

The degree of esterification (DE) of pectin samples were determined by the potentiometric titration method by Bocek et al. (2001) and Pinheiro et al. (2008). Dried pectin (0.2 g) was placed in a weighing bottle for titration and wetted with ethanol. Distilled water, at 40° C (20 ml), was added by stirring. The polymer was dissolved by stirring for 2 h. The resulting solution was titrated with 0.1 N NaOH in the presence of phenolphthalein and the result was recorded as (the initial titration (It)). Then, a 0.1 N NaOH solution (10 ml) was added to a neutralized polygalacturonic acid sample after determination of the free carboxy groups. The weighing bottle was plugged with a stopper. The content was stirred at room temperature for 2 h to saponify the esterified carboxy groups of the polymer. Then 0.1 N HCl (10 ml) was added. Excess HCl was titrated with 0.1 N NaOH. The number of the esterified carboxy groups was calculated from the volume of 0.1 N NaOH solution spent for titration (the final titration – Ft). The DE was calculated by using:

$$\% DE = \left(\frac{Ft}{It + Ft} \right) \times 100 \quad (1)$$

Experimental values obtained for the degree of esterification at the designed points are shown in Table (1).

Table 1. Variables and responses of degree of esterification based on citric acid concentration and heating time.

experimental order	Variables		Degree of esterification (%)
	CAC (g/100 ml)	HT (min)	
1	0.08	110	52.19
2	0.5	110	45.21
3	1.5	110	48.12
4	2.5	110	36.45
5	5	110	44.37

3. RESULTS AND DISCUSSION

3-1. Effect of the size of the particles on pectin extraction

Four watermelon flour fractions were collected during the study of the particle size and use to confirm these results. The flour fractions with particles larger than 300 µm show lower pectin yield while when smaller particles are used the yield is higher (Figure.3). According to the Canteri-Schemin et al. (2005), the smaller particle size was a tendency to higher yield of pectin.

According to the results (Figure.4), the pectin yield is significantly (significant level = 0.05) higher with the use of flour as raw material (14.024 %); when the extraction is made from the particles of rind the yield is (11.925 %). That is agreeing with results of the Canteri-Schemin et al. (2005). There are several explanations for this fact, but protopectin is more available in small particles than in large ones.

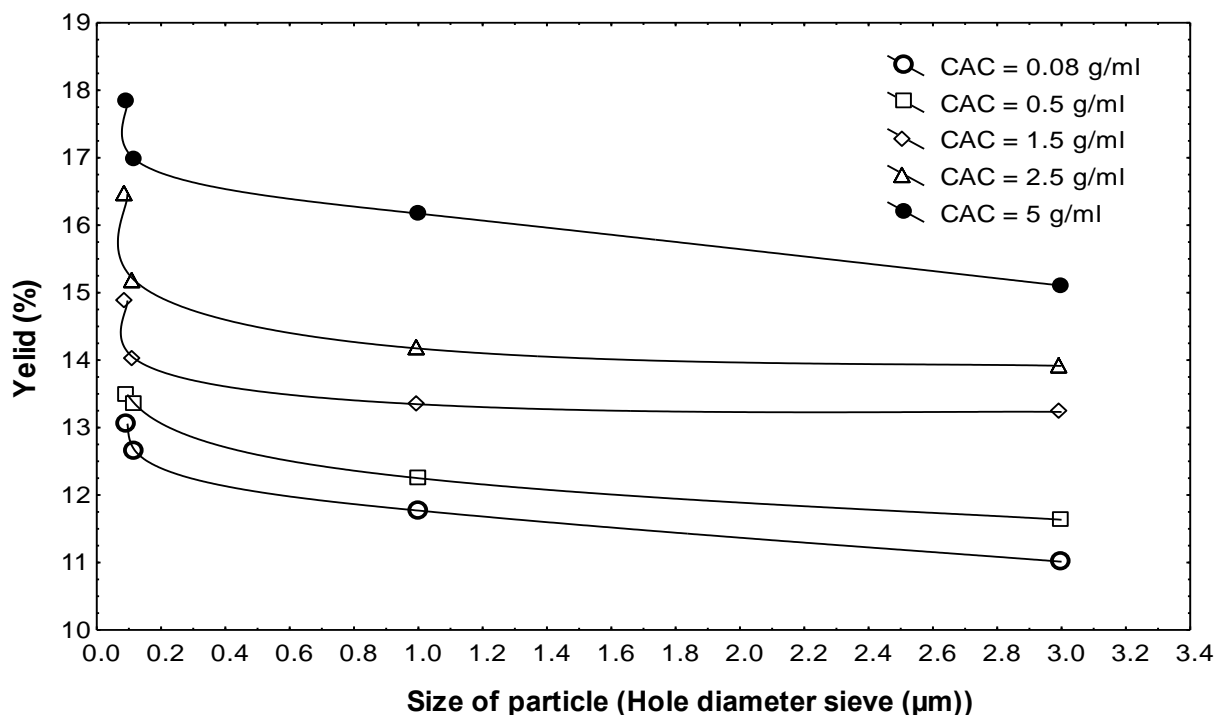


Figure 3. Effect of particle size on the pectin extraction yield.

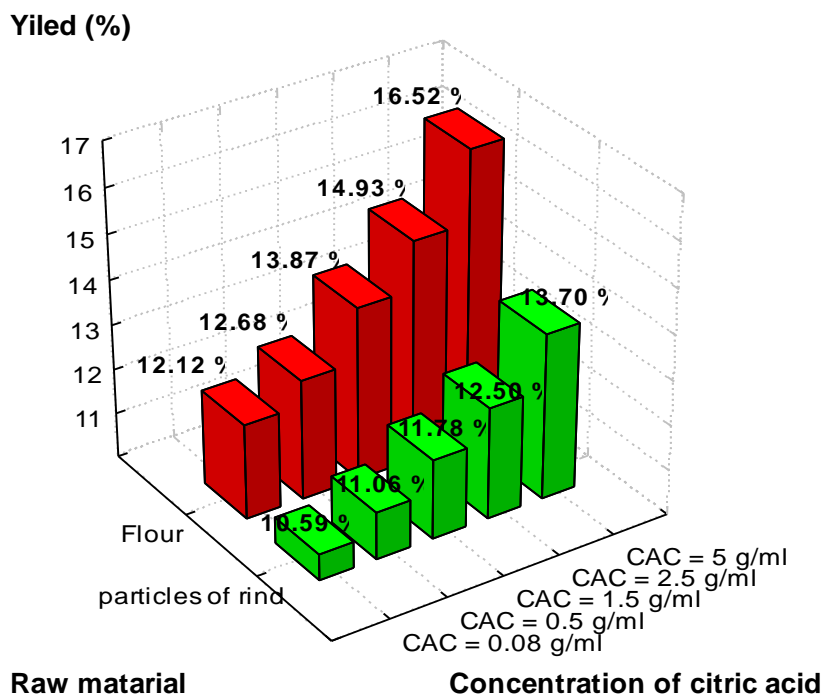


Figure 4. Effect of particle size on the yield of extraction using watermelon flour and particles of rind as a pectin source.

3-2. Effect of watermelon variety on pectin extraction

Although the experimental results show that some varieties (Charleston Gary and Crimson Sweet) do have a high concentration of soluble pectin in the watermelon-rind juice, the pectin extracted from the particles of rind do not show a pronounced variation, although there is a statistical difference amongst the varieties. The blend of varieties results in a raw material more similar to industrial raw particles of rind constitute of various watermelon varieties in indeterminate

proportions and several maturation stages (Figure.5). The results indicated that the watermelon variety was not significant in pectin yield in accordance with Canteri-Schemin et al. (2005).

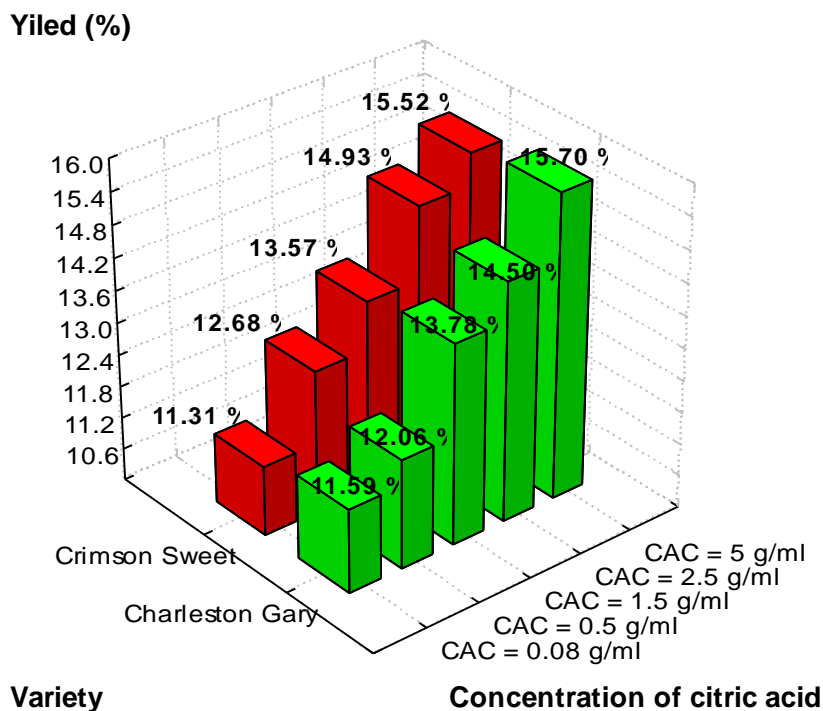


Figure 5. Effect of watermelon variety on the pectin extraction yield.

3-3. Effect of different acids on pectin extraction

Several experimental runs were carried out for the extraction of pectin could be utilized from several acids. According to Canteri-Schemin et al., (2005), the acids used for pectin extraction were the citric and phosphoric acids but there was a tendency to use the cheaper mineral acids, such as sulfuric, hydrochloric and nitric acids. Articles published recently have recommend the use of hydrochloric (Kalapathy and Proctor, 2001; Boonrod et al., 2006) and nitric acids (Pagán et al., 2001; Boonrod et al., 2006). (Figure. 6) shows that the effect of the kind of acid on the pectin extraction yield at different particle size, the lowest yields are obtained when phosphoric, sulfuric and hydrochloric acids are used. They have the average value (9.44 %), (11.27%) and (12.31 %) respectively. Although nitric acid shows the highest yield (14.07 % as the average value), the variation is very large. Citric acid has the highest average value (15.19 %) and it is better than the other acid from an economic as well as from an environmental point of view. The analysis of variance indicates statistical significance in the yield.

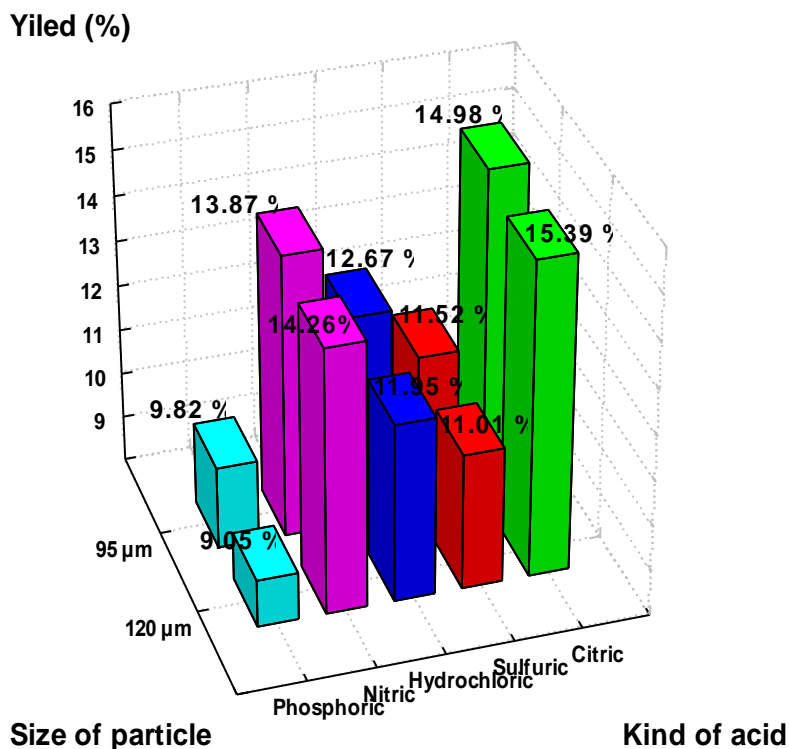


Figure 6. Effect of the kind of acid on the pectin extraction yield.

3-4. Effect of heating time on pectin extraction

A series of pectin extraction yield determinations were made with the watermelon flour fractions under various conditions to investigate the effect of changing the reaction time. It is observed that pectin extraction yield increased with increase in the heating time due to increase in the reaction time, see figure (7). Figure (8) shows the effect of increasing the concentration of citric acid with different heating time on the pectin extraction yield. It is clearly shown that the pectin extraction yield increase with increasing heating time and concentration of citric acid due to decrease in the degree of esterification is illustrated in Table (1). Compared to the data found in literature such as Canteri-Schemin et al., (2005), this degree of esterification was close to decrease with increasing heating time and concentration of citric acid in accordance with Pinheiro et al. (2008).

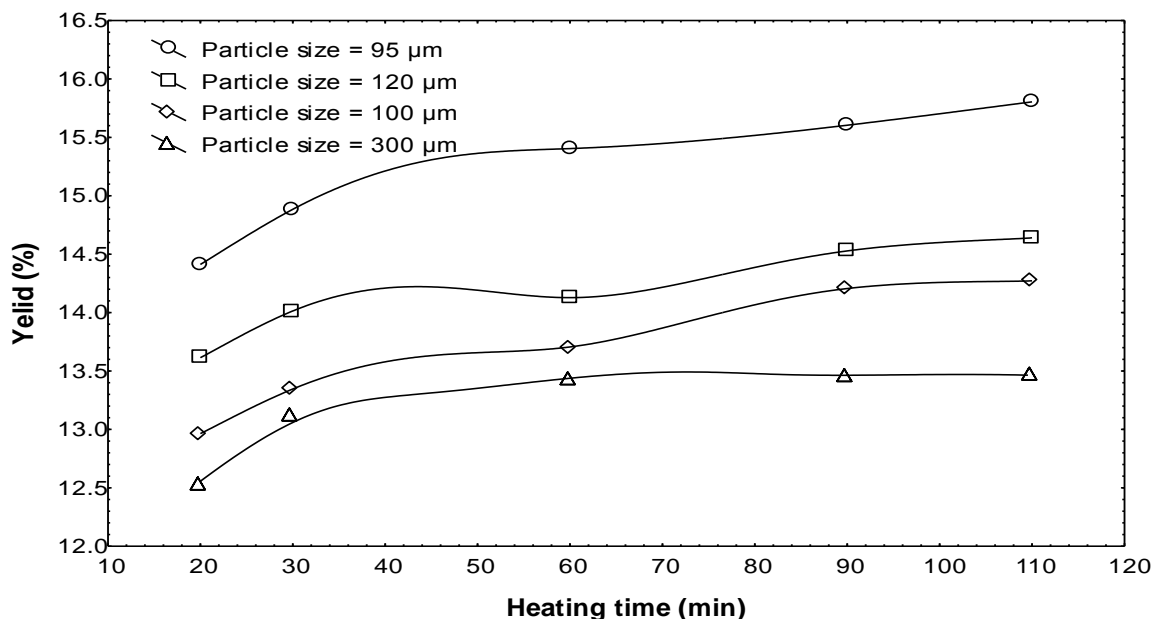


Figure 7. Effect of the heating time on the pectin extraction yield.

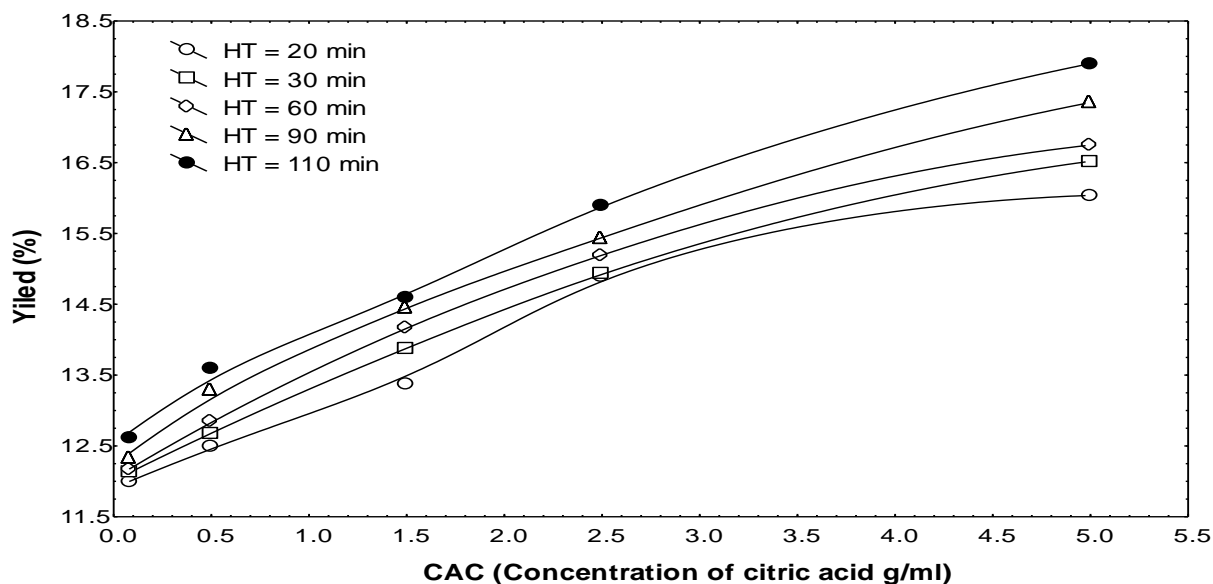


Figure 8. Effect of the citric acid concentrations with different heating time on the pectin extraction yield.

4. Theoretical Investigation

The forgoing discussion showed that the factors affecting the pectin extraction yield are: concentration of acid (CAC), heating time (HT) and particle size (PZ). The qualitative results of the statistical analysis were taken as the basis of the development of a correlation for pectin extraction yield of the form:

$$Y (\%) = f(CAC, HT, PZ) \tag{2}$$

$$Y (\%) = K \times CAC^a \times HT^b \times PZ^c \tag{3}$$

Where Y is pectin extraction yield, K is constant, CAC is concentration of citric acid, HT is heating time, and PZ is particle size. The computer STATISTIC program (99 Edition by StatSoft, Inc.) using to find the constant and the indices in equation (3). For all pectin extraction yield studied, 400 data points were used to obtain the final correlation of the form:

$$Y (\%) = 11.60783 CAC^{0.080097} HT^{0.043392} PZ^{0.039603} \tag{4}$$

The scatter of all the experimental data as compared with values calculated from this correlation are almost within $\pm 32\%$, and with a correlation coefficient of 0.938 and a 44.9454% absolute average percent error. These results are shown in figure (9).

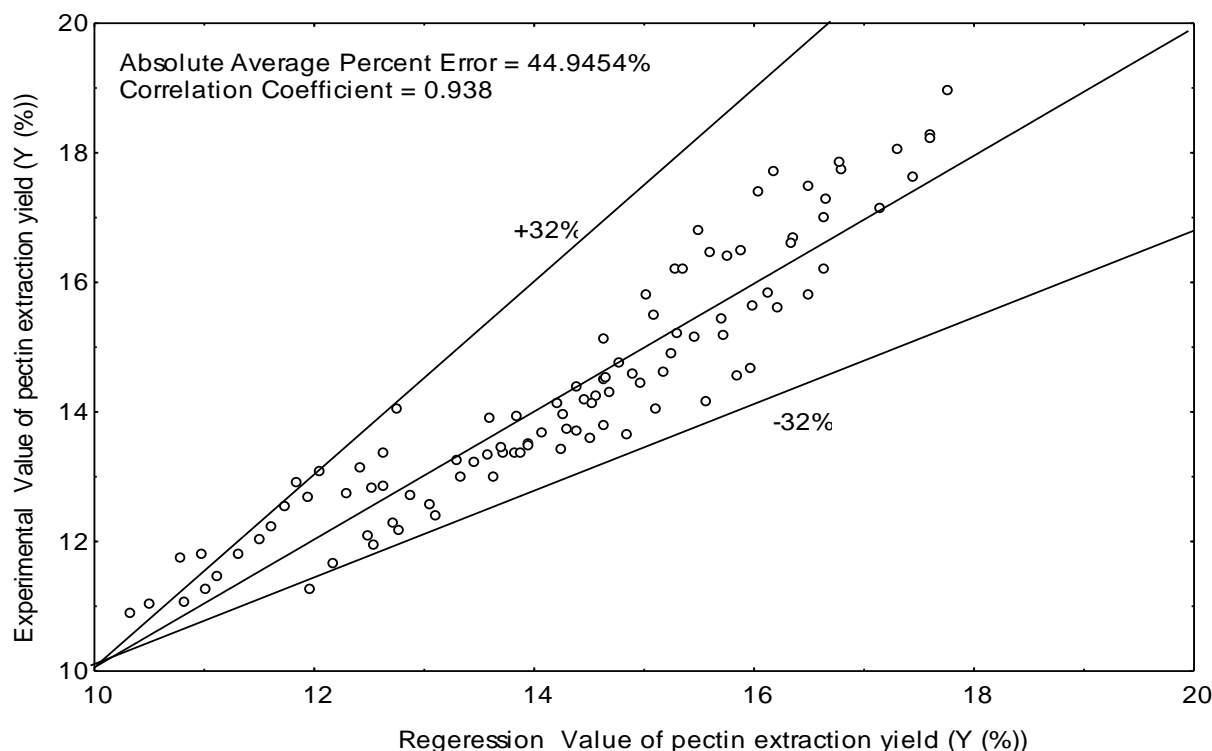


Figure 9. Pectin extraction yield correlation.

5. CONCLUSION

When the effects of particle size were studied it was found that the highest yields of pectin extraction were obtained when the particle was larger than (95 μm) and smaller than (120 μm). Extraction of pectin using particles of rind as raw material produced a lower pectin yield than when watermelon flour was used. This result indicated that it was necessary to produce watermelon flour as an intermediary step in the acid extraction of pectin from particles of rind. Pectin extraction from different varieties of watermelon rinds did not show any significant effect on pectin yield as compared with a pool of watermelon samples. Citric acid and nitric acid showed the highest yield among the organic and mineral acids tested. The highest yields were found to correspond to 5 g/100 ml of citric acid with a reaction time around 110 min. The watermelon pool pectin showed a degree of esterification of approximately 44.37 %.

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