A PROCESS VARIABLE STUDY OF APPLE PECTIN EXTRACTION

by

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E.AYÇA DEREBOYLU

ABSTRACT

Dried Amasya apple pomace was used for extraction of apple pectin in this process variable study. Process variables of the extraction process were chosen as extraction temperature, duration of the extraction and acidity of the extraction medium. The effect of these variables on the yield and quality of pectin obtained were studied. To determine the quality of pectins their equivalent weights and methylester contents were measured by titrating 0.5 per cent water solutions of pectins obtained with dilute alkali. Using the titration results total anhydrouronic acid contents of pectins were calculated. Absolute viscosities of one per cent water solutions of pectins obtained were measured. The results showed that pectin yield increased with time and temperature. It was seen that yield increase was directly related to the extraction time and the temperature effect was similar in all levels studied. The anhydrouronic acid content of pectins increased with temperature. The methylester content of pectins showed similar behaviour as anhydrouronic acid

content. The constant temperature lines crossed each other near 75°C - 45 min point. At lower temperatures pectins with higher degree of esterification were obtained. The results showed also that pectin yield increased with increasing acidity. Anhydrouronic acid content was highest acidity and pectin with lower methylester content was obtained. Degree of esterification of pectins extracted increased with decreasing acidity of extraction medium. Experimental runs showed deviations from the literature as a result of the clarification problem.

ÖZET

Bu çalışmada kurutulmuş Amasya elması posasından elma pektini ekstraksiyonuna süreç değişkenlerinin etkileri incelenmiştir. Ekstraksiyon işleminin süreç değişkenleri olarak seçilen ekstraksiyon sıcaklığı 60 C-90 C arasında, ekstraksiyon süresi 30 dak-60 dak arasında ve ekstraksiyon ortamının pH'sı 2.0-3.0 arasında değiştirilmiş ve bu değişkenlerin elde edilen pektinlerin verimi ve kalitesi üzerindeki etkileri incelenmiştir. Elde edilen pektinlerin kalitesini tesbit etmek için eşdeğer ağırlıkları ve metilester miktarları pektinlerin yüzde 0.5'lik su çözeltilerinin seyreltilmiş alkali çözeltisiyle sabunlaştırma metoduyla ölçülmüştür. Sabunlaştırma sonuçlarından yaralanarak pektinlerin toplam anhidrouronik asit miktarları ve esterleşme dereceleri hesaplanmıştır. Pektinlerin yüzde birlik su çözeltilerinin mutlak viskositeleri ölçülmüştür. Sonuçlar pektin veriminin ekstraksiyon sıcaklığı, zamanı ve pH'sıyla arttığını göstermiştir. Bu çalışmada kuru elmadan elde edilen pektin verimi yüzde 6.0-12.0 arasında değişmiştir. Elde edilen pektinlerin anhidrouronik asit miktarları ekstraksiyon sıcaklığı ve pH'sıyla artma göstermektedir ve esterleşme dereceleri

de ekstraksiyon sıcaklığının ve ortamın asidetisinin düşmesiyle artmaktadır. Bu çalışma sonucunda düşük metilesterli pektinler elde edilmiştir.

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c	Concentration
N	Normality of the acid and alkaline solutions
V, V_1, V_2	Amount of alkaline solutions consumed, in ml
k'	Constant in the equation to determine the intrinsic viscosity
w	Weight of the pectin samples
μ	Absolute viscosity
μ_r	Relative viscosity
μ_{sp}	Specific viscosity

I. INTRODUCTION

Pectin is a complex polysaccharide occuring in higher plants and is an important and powerfull gelling agent used essentially in the manufacture of fruit jellies, jams and marmalades. Besides there are many other suggested applications such as emulsifying and suspending agent for particulate materials and it has also many pharmaceutical applications. Pectin acts as the intracellular cement of cell wall tissue holding the adjacent cells together, maintaining the proper dehydration of the young growing cells, offering winter hardiness to plant and providing the fruit firmness.

Pectin is obtained from plant material by extracting with hot acidulated water. The main industrial raw materials for pectin are apple pomace and citrus peel. It is either recovered from the extract by precipitation and marketed in powder form or the extract is concentrated and used in liquid form.

In Europe and in the United States the manufacture of pectin is well established since the second decade of the twentieth century and it is usually carried out by large apple and citrus juice producers whose by-products are used as raw materials for the manufacture of pectin before they are consumed for cattle feed. In Turkey all of the pectin consumed is imported and it is largely consumed in food and pharmaceutical industries. According to the market survey done in 1983 the average importation of ten years was 18.5 tons pectin/year and the selling price of pectin was 5.8 $/kg in 1983 (Design Project, Chem. Eng. Dept., Bo. Univ, 1984).

In this work pectin was extracted from dry apple pomace in hot water acidified with hydrochloric acid. The aim was to investigate the effects of extraction time, extraction temperature and acidity of the extraction medium on the yield and quality of pectin obtained. Methylester and anhydrouronic acid contents of the dried end-product were determined. The viscosities of the pectin solutions at constant pH and at 25°C were measured in an effort to relate the pectin quality to easily determinable industrial quality control of gelling power.

II. LITERATURE SURVEY

A. CHEMICAL STRUCTURE AND PROPERTIES OF PECTIN

Pectic substances, in combination with cellulose, are responsible for the structural properties of fruits and vegatables. They are located in the middle lamella and in the primary cell wall of the plants and serve to cement cells to each other.

According to the nomenclature published by the Agricultural and Food Chemistry Division of the American Society in 1944 (Kertesz, et. al. 1944) the pectic substances are divided into three groups :

1. Protopectin: Pectic substances occur predominantly in the form of water-insoluble protopection during the early stages of growth in plants. During ripening it is transformed into water-soluble pectin. This transformation leads to the softening of the tissue in many plants.

2. Pectinic acids : They are colloidal water-soluble polygalacturonic acids partially esterified with methanol and are called pectins shortly. Under suitable conditions they can form gels with sugars and acids or with certain metallic ions. This property makes pectinic acid the most useful of pectic substances.

3. Pectic acids : This term is applied to the colloidal polygalacturonic acids containing non-esterified carboxyl groups.

Polygalacturonic acid, the main constituent of pectin molecule, is a polysaccharide and also called galacturonan. It is build up from D-galacturonic acid units connected together by α-1,4 glycosidic linkages forming long, chain-like, linear polymers. Unesterified carboxyl groups are either free acids or neutralized with cations. Fig.1 shows a part of a polygalacturonic acid molecule esterified with methanol and cross-linked through a polyvalent cation (Ca).

Pectin is a complex polysaccharide consisting of two other polysaccharides besides galacturonan :arabinan and galactan (Frank et.al., 1967). Galacturonan methylester is responsible for the gelling property of pectin.

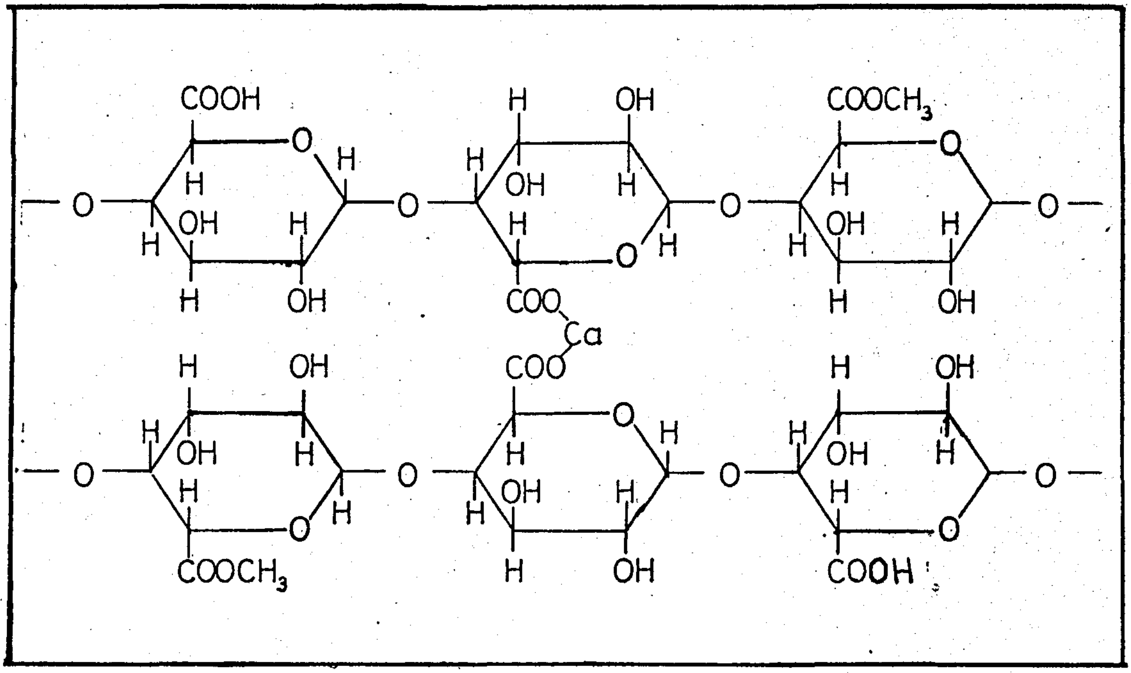

Fig.1. A part of a polygalacturonic acid molecule (Nagy, et. al. 1977).

As an important portion of arabinan and galactan is attached to the galacturonan chain by esterlinkages complete separation of them is very difficult to achieve without degrading the pectin molecule. The composition of pectin varies with its source fruit and the conditions used in its extraction. Besides methylester groups some pectins contain esterified acetic acid units.

Commercially, pectinic acids are classified according to the amount of methylester groups they contain. Degree of esterification, DE, the ratio of methoxylated galacturonic acid groups to total galacturonic acid groups, influences especially the solubility and gelation properties of pectin. The highest DE that can be achieved by extraction of natural raw material is about 75 per cent.

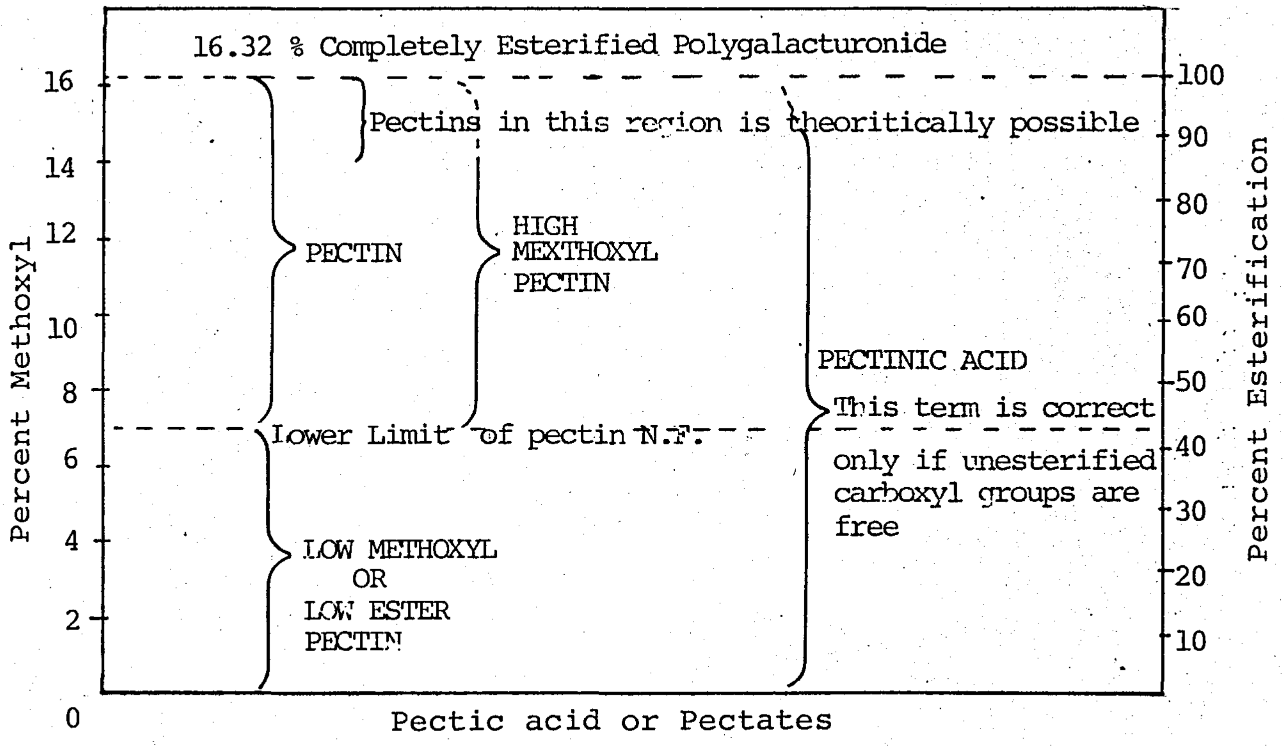

Fig. 2 Classification of pectin (Joseph, 1953)

As shown in Fig.2 pectins may be grouped as high ester or high methoxyl (HM) and low ester or low methoxyl (LM) pectins. The two groups exhibit different gelling mechanisms. HM pectin will from jams and jellies with sugar and acid. LM pectins will form gels with small quantities of polyvalent cations such as calcium without the additional soluble solids and acids (Nagy, et.al,1977)

B. INDUSTRIAL MANUFACTURING PROCESSES OF PECTIN

1. RAW MATERIALS USED IN PECTIN MANUFACTURE

The pectin content of plant materials depends upon the following factors : plant variety, plant tissue type and the growth period. Fruits and vegatables contain varying amounts of pectin. Table 1 shows the pectin consent of some plant materials.

Citrus peel (lemon, lime, orange and grapefruit) and apple pamace are main sources of pectin in the United States and in Western Europe. As sugar-beet pectin contains 4-6 per cent acetly groups whereas lemon and apple pectins contain 0.35-0.45 per cent, it must be processed under strong acid conditions to reduce the acetyl content, because high acetyl content prevents the formation of sugar-acid jellies (Frank, et. al, 1967). Sugar-beet pectin is produced in Sweden and Russia on a limited scale. Sunflower heads contain pectin of high quality and they are employed in Germany, Bulgaria and Romania.

Table 1. Pectin Content of Plant Materials (Frank, et. al., 1967)

Plant material	total pectin as percent of dry substance
potatoes	2.5
carrots	10
horseradish	15
tomatoes	3
apple pomace	15-20
sunflower heads	25
sugar beet pulp	15-20
citrus peel (albedo)	30-35

Flowcharts for preparing citrus fruit and apple or pectin manufacture are presented on Fig. 3a and 3b, respectively, After expression of the juice and extraction of peel oil the white inner rind of the citrus peel, called albedo, is ground to a suitable mesh size. The ground peel is washed to remove soluble solids such as sugars, acids, colloidal materials such as pigments, glycosides and finely divided materials produced during the cutting operation (Graham, et. al, 1953). In this manner the quality of the final pectin product is improved. The washed peel is heated to a temperature around 90°C for a few minutes in order to prevent the pectin from enzymatic action. Prolonged heating above 60°C deterious to pectin

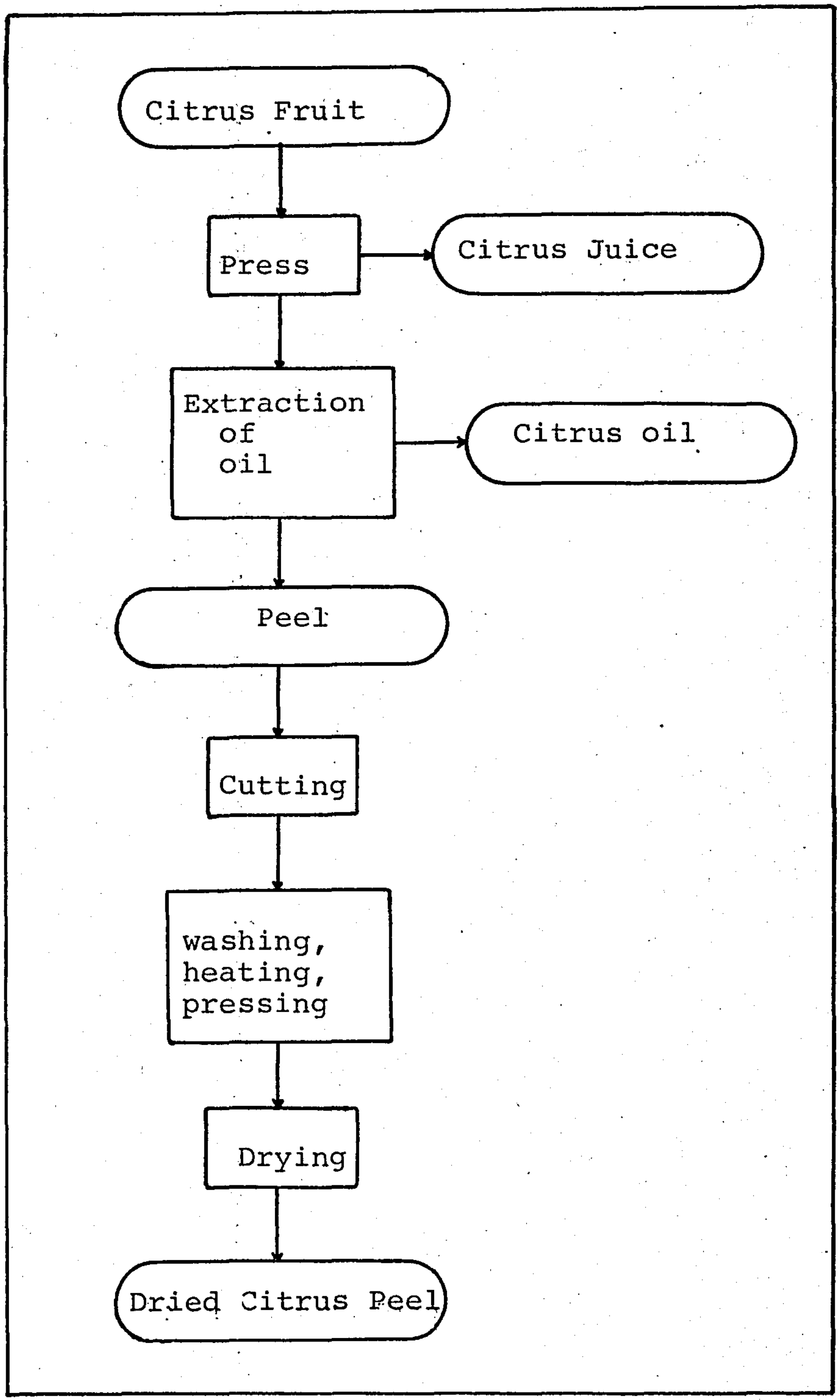

Fig. 3a. Preparation citrus fruit for pectin extraction

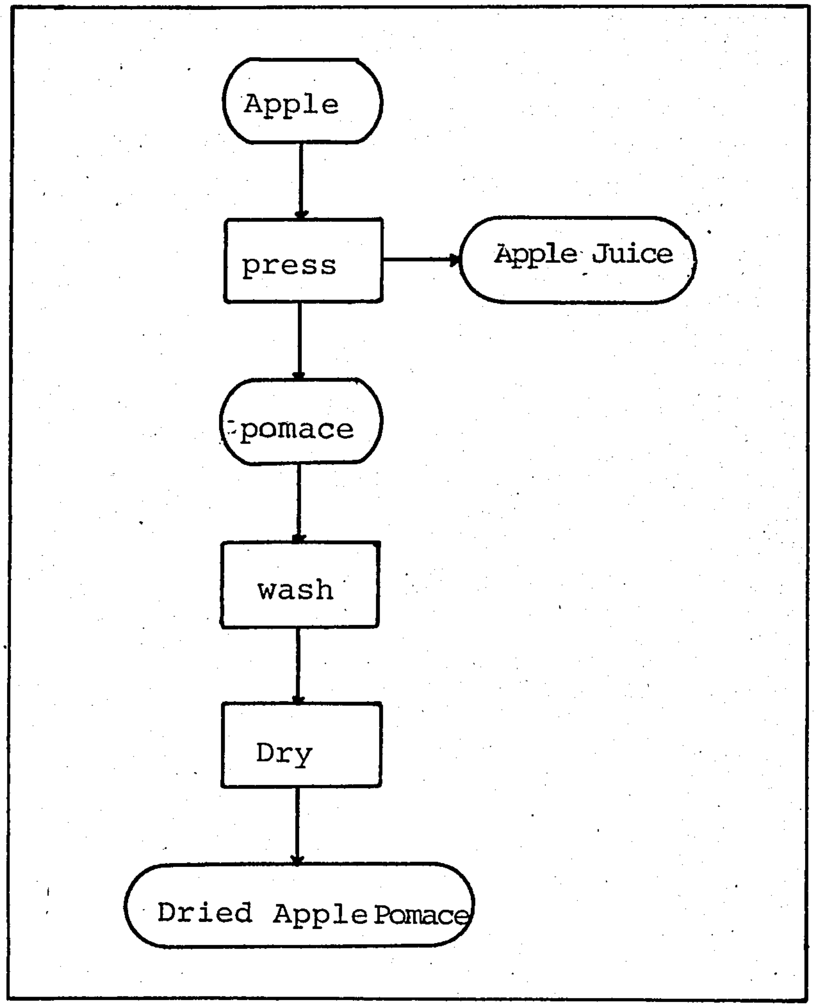

Fig. 3 b. Preparation of apple for pectin extraction

(Nagy, et.al, 1977). Then the peel is pressed and dried for off-season usage. The drying temperature should not be higher than 90°C not to damage pectin. When the average moisture content of peel is reduced to about 30 per cent the temperature should be reduced to 55°C. The peel should not be dried to a moisture content less than 10-12 per cent. (Graham, et. al., 1953)

Apple pomace is prepared in the same manner as citrus peel. The freshly pressed apple pomace is washed some water to purify it, then it is dried. Drying temperature may range between 65-90°C, and it is important to avoid overheating (Frank, et al., 1967). The finished pomace usually contains 4-8 per cent moisture.

2. INDUSTRIAL PROCESS FOR PECTIN MANUFACTURE

The recovery of pectin from the raw material has three essential steps :

a. Solubilization of pectin from raw material
b. Clarification of the pectin solution
c. Isolation of pectin from solution

Even if the methods used at each step will change from one process to the other all of the industrial processes are based on these steps. After describing each step generally four of the industrial process will be briefly surveyed.

a. Solubilization of Pectin from Raw Material

Commercially pectin is isolated from raw material by heating in acidulated water. The important factors in this extraction process are mainly the duration, the acidity and the temperature of the process. The amount of pectin extracted increases as the extraction temperature and acidity increase. High acidities and high extraction temperatures are deterious to pectin. On the other hand, if the temperature is too low, prolonged extraction time is required and excessive deesterification of pectin can occur. So the recommended range of temperature is between 40°C - 100°C, that of acidity is between 1.8-3.0 pH and extraction time should change between 0.5-2.0 hours. Since these three factors are interdependent, the extraction process should be shorter in duration when temperature and acidity are high.

The amount of water used as extraction medium effects the yield of pectin extracted from raw material. Higher amount of water means higher yield, but also it means that a greater amount of extract must be filtered, pumped, concentrated or treated for precipitation. The amount of water depends also upon the water holding capacity of dried raw material.

To acidulate the water, organic and inorganic acids such as tartaric, lactic, citric, phosphoric, sulfuric, sulfurous and hydrochloric acids may be used.

b. Clarification of the Pectin Solution

Immediately after the extraction the extract must be cooled to 60°C to arrest degradation of pectin. Then waste solids are separated from the pectin liquor by centrifuging. The waste solids may be pressed and dried. The dried pulp can be used as an animal feed. The pectin liquor is filtered to remove as much particulate matter as possible.

The turbid pectin liquor is either decolorized with activated carbon, or after reaching about 40°C the acidity is raised to about pH 4.5 and clarifying enzymes are added to hydrolyse starch and dextrines. After they are decomposed as judged by iodine tests, the liquor is quickly heated to 85°C to inactivate the enzymes and then cooled. In both cases the clarified liquor is filtered by using filter aids.

The slightly colored clear pectin liquor is concentrated to between 3-5 percent at 60°C or below to prevent loss of pectin grade. This concentrated product is either processed in third step to produce powder pectin, or it is neutralized to pH 4.0 -4.2 with sodiumcarbonate and filled into sterile bottle to be used in the liquid form. Liquid pectin is produced in small amounts for household use as it is easier to use since it can be directly applied.

c. Isolation of Pectin from Solution

Two ways are mentioned in the literature to recover pectin from the liquor. The widely used one is the precipitation with water miscible organic solvents as isopropanol, ethanol, or isobutanol. By vigorous stirring pectin liquor blended with an amount of solvent which will provide a final alcohol concentration of 50-70 per cent. Pectin precipitates as a gelatinous mass. The precipitate is separated from the solvent mixture by pressing. Then it is washed with alcohol several times.

The second method involves the heavy metal precipitation process using copper sulfate or aluminum chloride as the precipitation agent. More commonly aluminum chloride is used as the heavy metal salt. In the case of added aluminum chloride to pectin solution the pH should be adjusted by sodium carbonate. The pH region for precipitation varies between 3.8-4.2. The aluminum salt hydrolyses to aluminum hydroxide in the pectin liquor. The negative charged pectin coagulates with positively charged aluminum hydroxide to form green aluminum pectinate which floats to the surface. The precipitate is separated from mother liquor, and the pectin cake is washed with acidified dilute alcohol to dissolve the aluminum compound. The acid in pectin washed off with neutral alcohol.

The pressed pectin is dried under vacuum at a temperature not to exceed 60°C. The used alcohol and alcohol from vacuum oven are recovered for further use.

These basic steps are presented on Fig. 4. Low methoxyl pectins are obtained from high methoxyl pectins by deesterification methods.

On Table 2 four industrial processes are summarized. Three of these processes are used by two well-known manufacturers, namely H.P. Bulmer from England and Sunkist Growers from U.S.A. Two prosesses of Sunkist Growers are presented there, one process uses alcohol precipitation, the other one uses heavy metal precipitation. They both produce high methoxyl and low methoxyl pectins. Actually in tiese processes pectin extraction is a batch type process. They use several extraction tanks in series to provide a continous flow throughout the whole process. The fourth process is on pilot scale to produce low methoxyl pectin. This is an entirely continous type process.

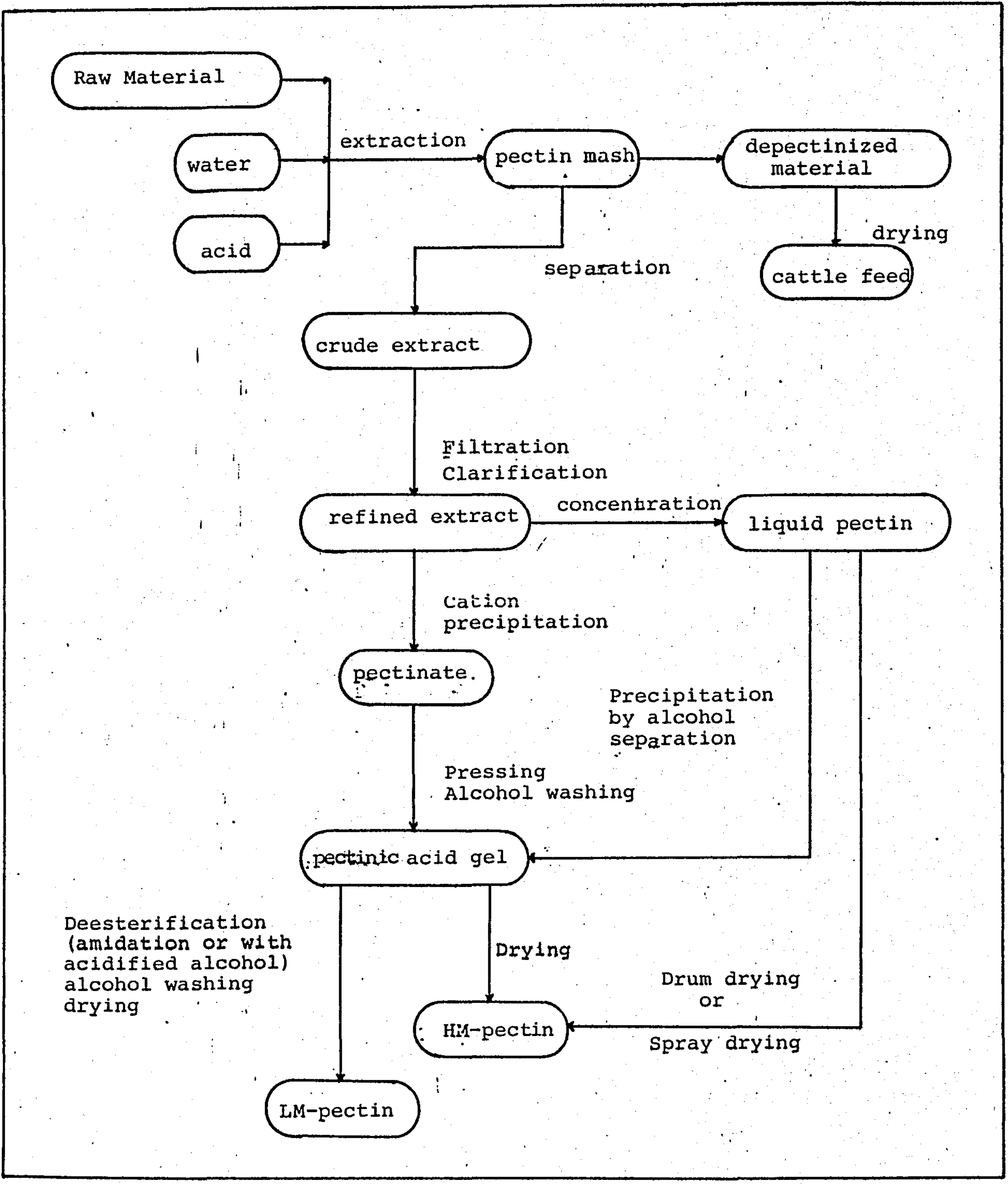

Fig.4. General Flowchart of Pectin Manufacture

Table 2. Summary of Different Industrial Processes for Pectin Manufacture

References	Raw Material	Solubilization of Pectin From Raw Material	Clarification of Pectin Solution	Isolation of Pectin From Solution	End-Product
Pilot Plant Production of Pectin, (Graham, R.P., et.al., 1953) Fig.6	Citrus Peel, cutted into slices,blanched and washed in a continous wooden trough pressed and dried.	A continous process, parallel-flow extraction in a stainless steel steam jacketed trouhg water acidified with sulfuric acid, extraction at 194°F,with retention of peel between 20 min-30 min, a pH of 1.8-1.9, cooled to 90°F in a forced draft evaporative cooler	First solid material is removed in a overflow type centrifuge. Filtration is carried out in a conventional plate and frame filter press precoated with filter aid. Solution is cooled to avoid degradation during deesterification process by vacuum evaporation in a cylindrical stainless steel vacuum body to 43-58°F	Deesterification operation carried out in a batch manner, in stainless steel tanks, provided with agitator to break gels. In these tanks extract is treated with ammonia. Methylester groups are hydrolysed to ionized carboxyl groups. Low methoxyl pectin is precipitated with acid, at pH 1.5. Acidulated water is drained in a loose cloth lined reel and pectin gel is drained in a loose cloth lined reel and pectin gel is washed with water pressed in rack-and-frame hydraulic press, neutralized and dried in thin layers ontrays in a forced draft owen at 130°F	Low-methoxyl pectin powder
Sunkist Growers, USA (Joseph, G.H., et.al., 1952)	lemon peel, macerated and lemon oil and juice are pressed.	Actually a batch type process. Two or more extraction tanks are used to provide continuous flow. Sulfurous acid is used to provide a pH 1.8-2.7. Tanks are heated with steam at 15 psi, injected by perforated cross pipes, temperature upto 90°C, a total 45 min extraction time Peel is extracted two or three times.	Waste solids are separated in the reel-house. Pectin liquor is mixed with diatomaceous earth and filter press. Clarified liquor is cooled in cooling tower to 30°C. So_2 is removed by aeration.	In precipitation tanks clarified liquor is mixed with ammonium chloride solution. With calcium carbonate pH as raised to 4.0. Precipitate is separated from mother liquor in a copper rotary reel, pressed in hydrolic presses, mixed with acidified alcohol to separate pectin from complex, centrifuged, washed with alcohol. After first alcohol wash some pectin is deesterified by heating to 35°C for 15-20 his, in acidified alcohol. Remaining pectin is washed with alcohol for second time. Then deesterified and-non-deesterified pectins are neutralized with alcohol and ammonia mixture. Dried is two-stage vacuum dryer.	Low-methoxyl and high-methoxyl pectins in powder from.
Sunkist Growers, USA (Hull, O.W., et.al, 1953) Fig.7	Orange peel from oil extraction is shredded	Actually a batch type process, 6 fir extraction tanks are used with sweep agitators. Saturated steam is introduced through perforated pipes,with hydrochloric acid pH decreased below 3, mixture is cooked for 30-45 min.	Pulp is separated pectin liquor is a rotary vacuum string filter. Liquor is filtered in four plate and frame filters with diatomaceous earth as filter aid. Clarified liquor is concentrated is vertical tube double effect evaporators.	Pectin is precipitated in precipitation tanks using isopropyl alcohol. Pectin is washed two times, in drain screws pectin is separated from alcohol. Dried in air dryer. If low methoxyl pectin is required before drying deesterified in hydrochloric acid solution.	Low methoxyl and high methoxyl pectins in powder form. Also pectic acid, galacturonic acid produced.
H.P. Bulmer, England Fig.8	Citrus Peel	Location 1-Peel handling Location 2-Peel feeding,washing screening. Location 3-Extraction in multi-stage stirred tanks with acid.	Location 4-Solid liquid separation in plate and frame filter presses. Location 5-Extracted peel washing Location 6-Second solid-liquid separation in plate and frame filter presses. Location 7-Pectin liquor clarifying using filter aids and plate and frame filter presses. Location 8-Pectin liquor concentration in multiple effect evaporators.	Location 9-Two stirred tanks for washing pectin concentrate and precipitation tanks with alcohol/pectin ratio control. Location 10-Centrifuges to separate pectin Location 11-Pecipitate washing and neutralizing with ammonia. Location 12-Centrifuges to separate pectin Location 13-Drying in cabinet dryer and two stage dryer.	Low methoxyl and high methoxyl pectins.

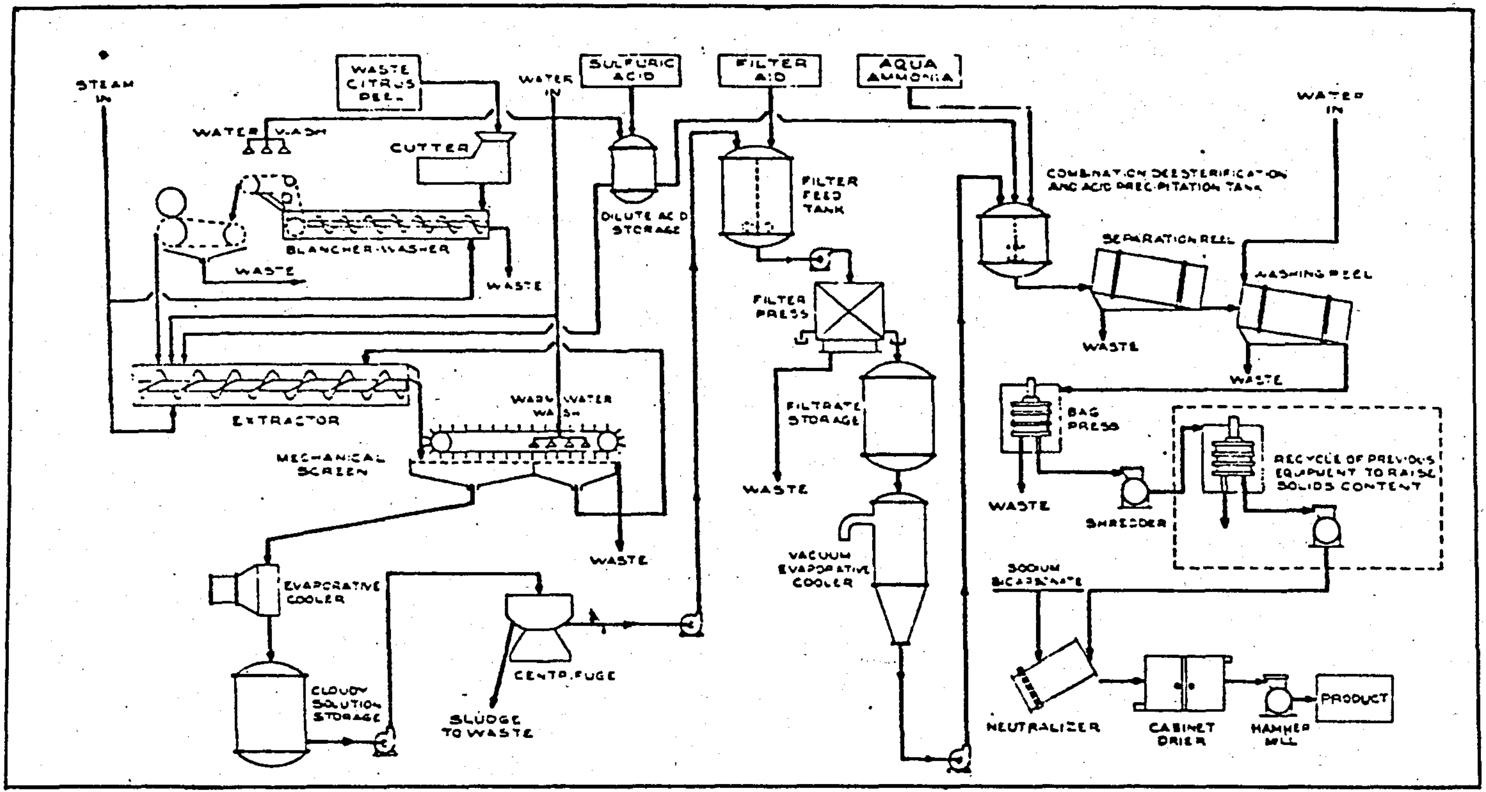

Fig. 5 Flow diagram of pectin manufacture from citrus peel on a pilot plant scale (Graham, et. al., 1953)

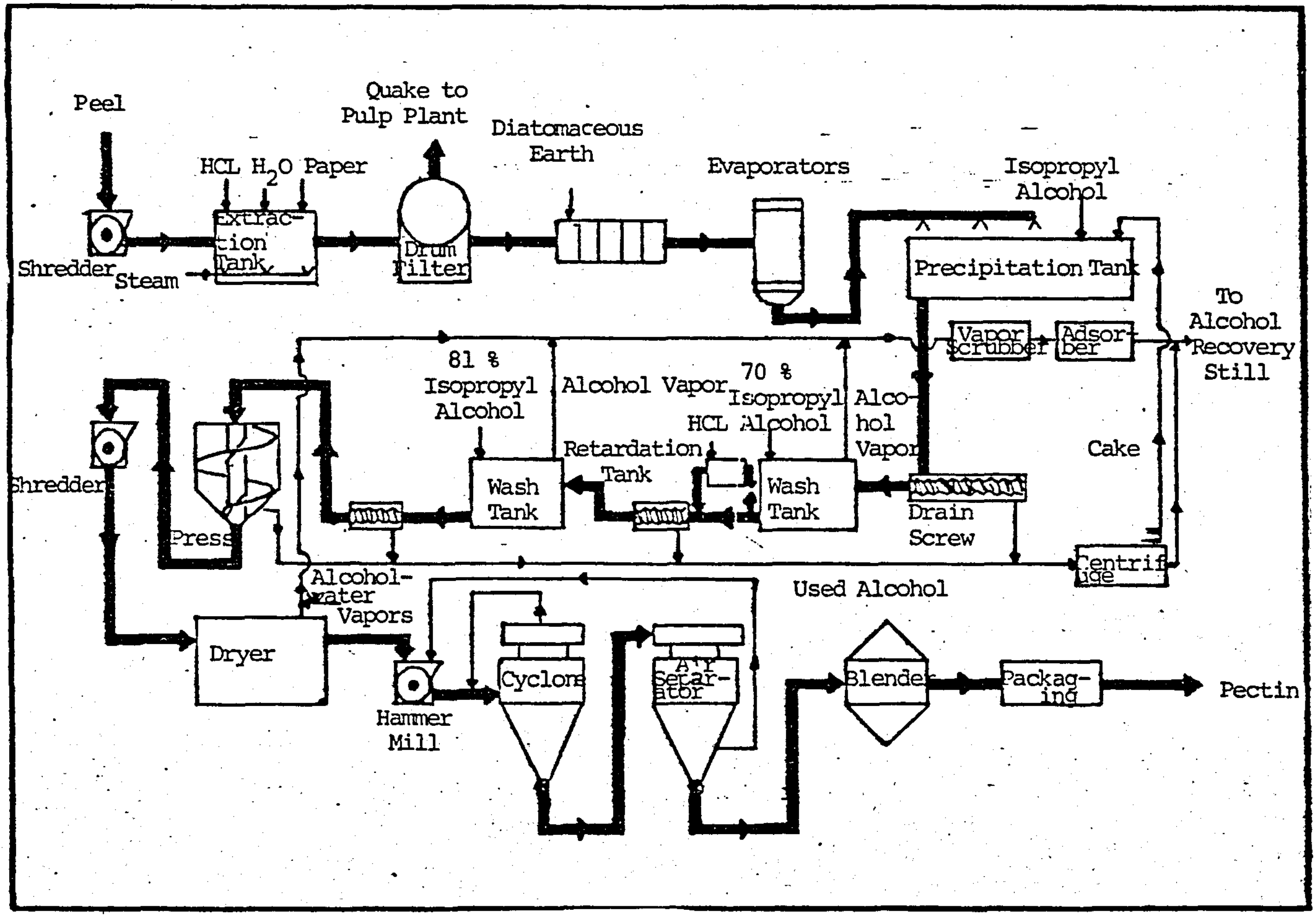

Fig. 6 Flow diagram of pectin manufacture from orange peel in Sunkist Growers Inc. Ontario, Calif. U.S.A (Hull, et. al., 1953)

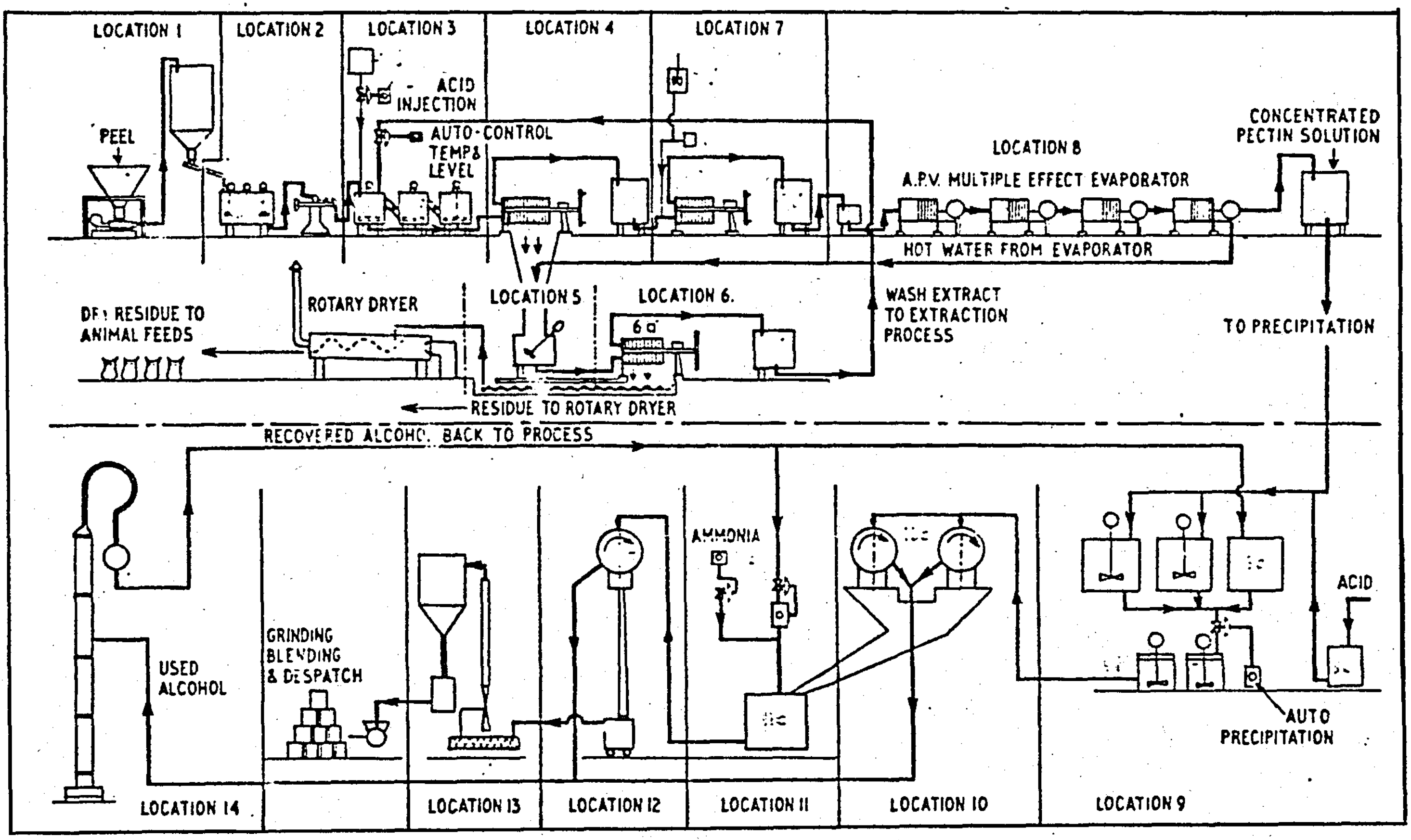

Fig.7 Flow diagram of pectin manufacture from citrus peel in H.P. Bulmer Ltd. Hereford England (Technical Booklet from H.P. Bulmer Ltd.)

C. ANALYTICAL METHODS TO DETERMINE THE QUALITY OF PECTIN

As pectin is a natural product the analytical methods used to determine the pectin quality differ from the methods used for synthetic products. For example as pectin is a polysaccharide it does not have a melting point, because pectin decomposes upon heating. The analysis and characterization of pectin should include some of the following determinations :

1. Moisture content
2. Ash content
3. Amount of free and esterified carboxyl groups
4. Amount of acetyl groups
5. Viscosity measurements
6. Jelly grade
7. Setting time and temperature

1. Moisture Content

An entirely dry pectin product is not desirable. Excessive drying causes degradation of pectin and a completely dry product would absorb moisture from atmosphere. A product containing 10-15 per cent moisture is stable (Graham and Shepherd, 1953). Moisture content of pectin is determined by drying it under vacuum between 5-20 mm. Hg either from four hours at 100°C or for 16 hours at 70°C (Owens, et. al., 1952).

2. Ash Content

Washing pectin with acidified alcohol is desirable before drying, so that little or no correction for ash is necessary. Because volatile impurities such as ammonium salts, some cations such as aluminum, iron and others may give wrong results in ash determination (Owens, et.al. 1952). Usually ash in pectin is determined in the following way: The sample is ignited slowly, heated for three or four hours at 600°C, cooled to room temperature and weighed (Owens, et.al., 1952).

3. Amount of Free and Esterified Carboxyl Groups

Besides partially esterified polygalacturonic acid pectin contains ten per cent or more of organic materials which are composed of arabinans, galactans and other sugars. The information on the percentage of anhydrogalacturonic acid is necessary to determine the purity and the degree of esterification of pectin. For this determination essentially four methods are used in literature :

a. Carbazole Method (Mc Comb and Mc Cready 1952)

This is a colorimetric method. Pectin is deesterified in sodiumhydroxide solution. Deesterified and diluted pectin solution is heated in concentrated sulfuric acid.

Carbozole and ethanol are added to the cooled solution and the mixture is allowed to stand at room temperature. The color intensity of the mixture is determined with light of wave lenght 520 mμ. A standard curve prepared with galacturonic acid hydrate is used to obtain the anhydrouronic acid content of the pectin sample.

b. Decorboxylation Method (Owens, et. al., 1952)

The pectin sample is heated in 19 per cent hydrochloric acid solution under carbondioxide free conditions. Sodiumhydroxide is used in the absorbtion tower to absorb liberated carbondixoide. Excess alkali is titrated with hydrochlorid acid. This amount of acid is substracted from the amount of acid required for titration of a control standardization of the alkali and the net amount of acid in ml, V, is used in the calculations.

$$\% \text{ A.U.A} = \frac{N \times V \times 88}{w \times 1000} \times 100 \qquad (1)$$

where w is amount of the pectin sample in grams.

c. Titration Method

Methos used by Ahmann and Hooker (1926) and by Hills, et.al. (1945) was madified by Owens, et.al.,(1952). Ammonia and ash free pectins should be used in this method.

At first pectin solution titrated with standardized sodiumhydroxide solution. The end point is determined by using pH meter. Hills, et.al (1945) determined the neutralization point of pectinic acids to be pH 7.5. A titration curve of a 0.5 per cent pectin solution is presented on Fig. 22. Using the amount of alkali consumed in the titration (V_1) equivalent weight or the free acid groups of pectin is determined

$$\text{eq.wt} = \frac{1000 \times w}{N \times V_1} \qquad (2)$$

The neutralized solution is used to determine the methylester groups. For this purpose the necessary amount of alkali is added to the neutralized solution for saponification of pectin. Saponification is arrested with acid. The solution is backtitrated with alkali to the same end-point as before (pH 7.5). The amount of alkali consumed (V_2) in ml in the back titration is used to calculate the amount of methylester groups.

$$\%\ \text{MeO} = \frac{N \times V_2 \times 31}{w \times 1000} \times 100 \qquad (3)$$

31 being the molecular weight of the $-OCH_3$ group.

Knowing the equivalent weight, methylester content and alkalinity of ash the amount of anhydrouronic acid (A.U.A.) can be evaluated (Mc Comb and Mc Cready, 1952)

$$Z = \frac{w \times 1000}{N(V_1 + V_2) + \text{milliequivalent of ash}}$$

$$\%\ A.U.A = \frac{176}{Z} \times 100 \qquad (4)$$

176 being the molecular weight of anhydrouronic acid. Then degree of esterification DE is determined by (TÜBTAK Gıda Ünitesi, 1973)

$$\%\ DE = \frac{176 \times (\%\ MeO)}{31 \times (\%\ A.U.A)} \times 100 \qquad (5)$$

d. Zeisel Method (Hills, 1945)

This method is used to determine the methylester groups more precisely. There are some major disadvantages such as time consumed, special apparatus needed and a pretreatment of the pectin sample needed. Glycosidic and ether methoxyl groups are measured in the same manner as estermethoxyl groups.

Mc Comb and Mc Cready (1952) have compared the first three methods, and they have obtained similar results by all methods.

4. Amount of Acetyl Groups (*Owens, et. al., 1952*)

Sugar beet pectin and some other pectins contain acetyl groups. Their determination involves saponification of pectin sample with sodiumhydroxide solution and steam distillation of the diluted saponified solution with magnesium sulfate- sulfuric acid mixture. This distillate contains acetic acid. It is titrated with alkali to a phenol-red end-point using V ml of alkali

$$\% \ (-OCCH_3) = \frac{N \times V \times 43}{w \times 1000} \times 100 \qquad (6)$$

43 being the molecular weight of acetyl $(-OCCH_3)$ group.

5. Viscosity

Viscosity of pectin solution may be determined for the purpose of obtaining a measure of the molecular weight and evaluating the thickening effect of pectin.

Viscosity of a pectin solution mainly depends on concentration, pH, salts added, size of polygalacturonic acid chain and temperature. Viscosity of a pectin solution decreases as the temperature increases. Thin pectin solutions show Newtonian fluid properties, but as pectin concentration increases, or when polyvalent ions are added pectin solutions show pseudoplastic properties (Nagy, et. al., 1977).

Ohn (1926, 1930) investigated the effect of sugar, acid and concentration of pectin on the gelling power of pectin by measuring the relative viscosities of pectin solutions. He showed that a relationship exists between the gelling power and the relative viscocity of pectin solutions.

Owens et.al. (1944, 1946) examined effects of concentration, change of pH, presence of sodium chloride or urea and change of temperature on the relative viscosities of aqeous solutions of purified apple and citrus pectins. They showed that the relative viscosity is uneffected by changes in pH in the range of 1-7 as the concentration of pectin is increased above 0.5 per cent. They stated also that changing the temperature from 0°C to 50°C has little influence on the relative viscosity of pectin solutions at concentrations below 0.05 per cent. At higher concentrations the relative viscosity decreases with the increase in temperature.

They also deduced the shape and size of pectinic acid molecules from intrinsic viscosity measurements. Intrinsic viscosity is defined as

$$\mu = \left[\frac{\ln \mu_r}{c}\right]_{c \to 0} ,$$

where μ_r is the relative viscosity, ratio of solution viscosity to solvent viscosity at the some temperature and a is the concentration of the solution. Intrinsic viscosities are evaluated graphically by extrapolating $\ln \mu_r/C$ vs C data to $C=0$. The intrinsic viscosities of pectin solutions decrease with rise in temperature and are essentially independent of pH and methoxyl content.

Speiser and Eddy (1946) investigated the effect of molecular weight on the gelling behaviour of pectin. They found out that the strengths of hydrogen bonded gels are primarly determined by molecular weight of pectin, gel strength increasing with increasing molecular weight,and independent of degree of esterification. The strengths of ionic bonded gels are less effected by molecular weight.They are strongly dependent on degree of esterification.

Owens, et.al(1952) prepared a curve, presented on Fig.8, using Martin's exponential equation.

$$\mu_{sp}/C = [\mu]\ e^{k'[\mu]C} \qquad (7)$$

where μ is intrinsic viscosity, μ_{sp} specific viscosity $(\mu_r - 1)$ and C the concentration of pectin solution is in g/100 ml. (Owens, et.al., 1946). The constant k' is dependent upon the size and shape of solution molecules. By preparing the Fig.8 they assumed k' to be 0.40. Specific viscosities of 0.1 per cent by weight solutions are measured at 25°C and pH 6.0. Knowing the relative viscosity of a pectin solution intrinsic viscosity can be deduced from this curve.

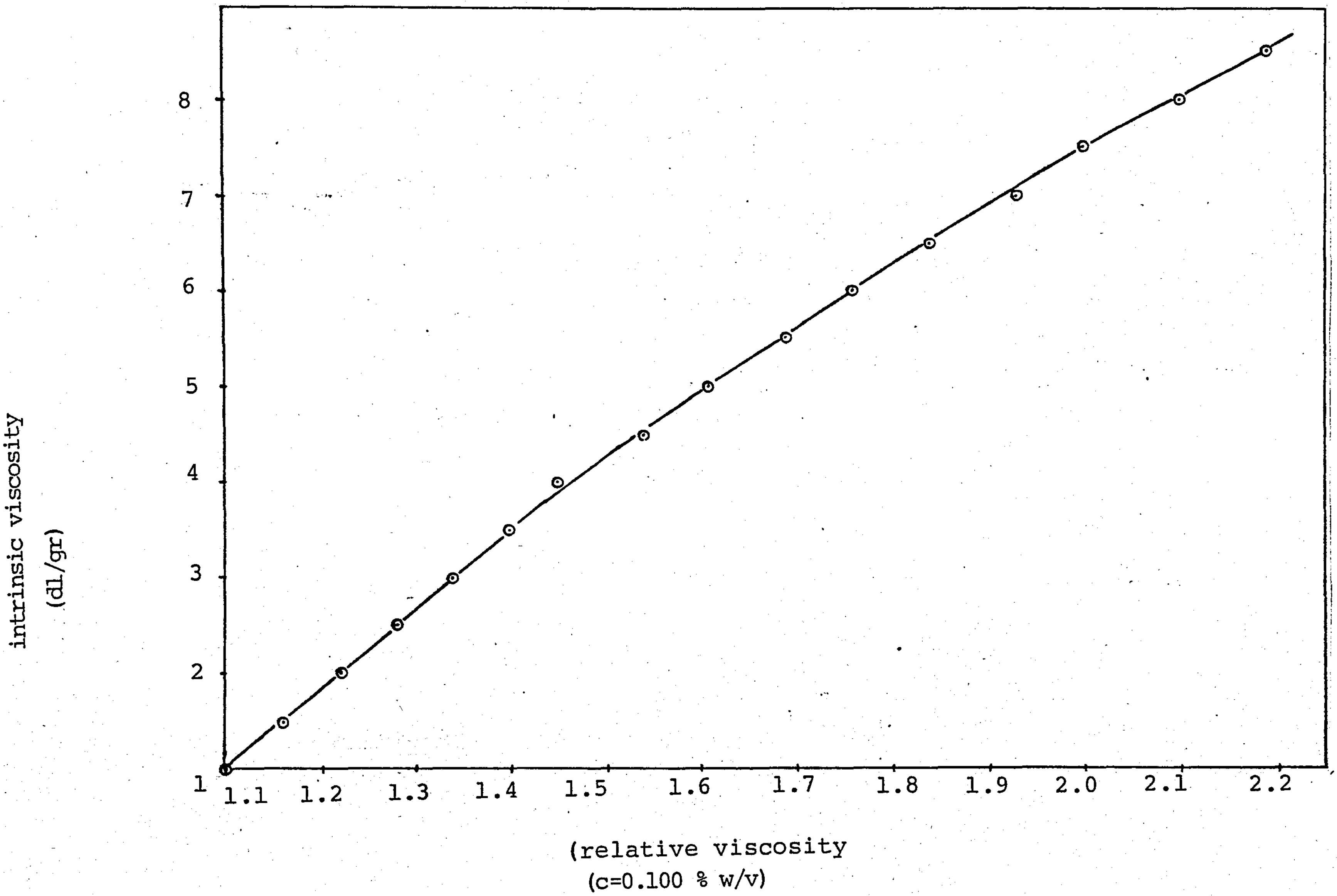

Fig.8 Intrinsic viscosity v.s. relative viscosity for 0.100 per cent pection solution (Owens, et. al., 1952)

6. Jelly Grade

Commercial pectins are evaluated in terms of an unit called jelly grade. This is defined as the number of pounds of sucrose with which one pound of pectin will form a standard jelly containing 65 per cent sugar solid. Many methods are developed for measuring jelly grade of pectin preparations (Bender 1949, Cox and, Highby, 1944, Joseph and Baier, 1949, Owens, et.al, 1947). Today a method accepted by the Committee of Pectin Standardization of the Institute of Food Technologists is used by manufacturers (IFT Commitee, 1959). The usual grade levels of commerical pectins are 100,150,200 (Frank, et. al., 1962)

Owens, et. al (1952) showed the relation between the intrinsic viscosity and jelly grade of pectins. The graphical representation of this relationship is given in Fig. 9

7. Setting Time and Temperature

Various pectin preparations will solidify a batch of jelly mixture into a firm jelly after different periods of standing which is called setting time. There are two kinds of commercial pectins : rapid setting (at 90-95°C, within a few minutes) and slow setting (at 50-60°C in approximately one hour). They are used for different purposes. Rapid setting is desirable for products like

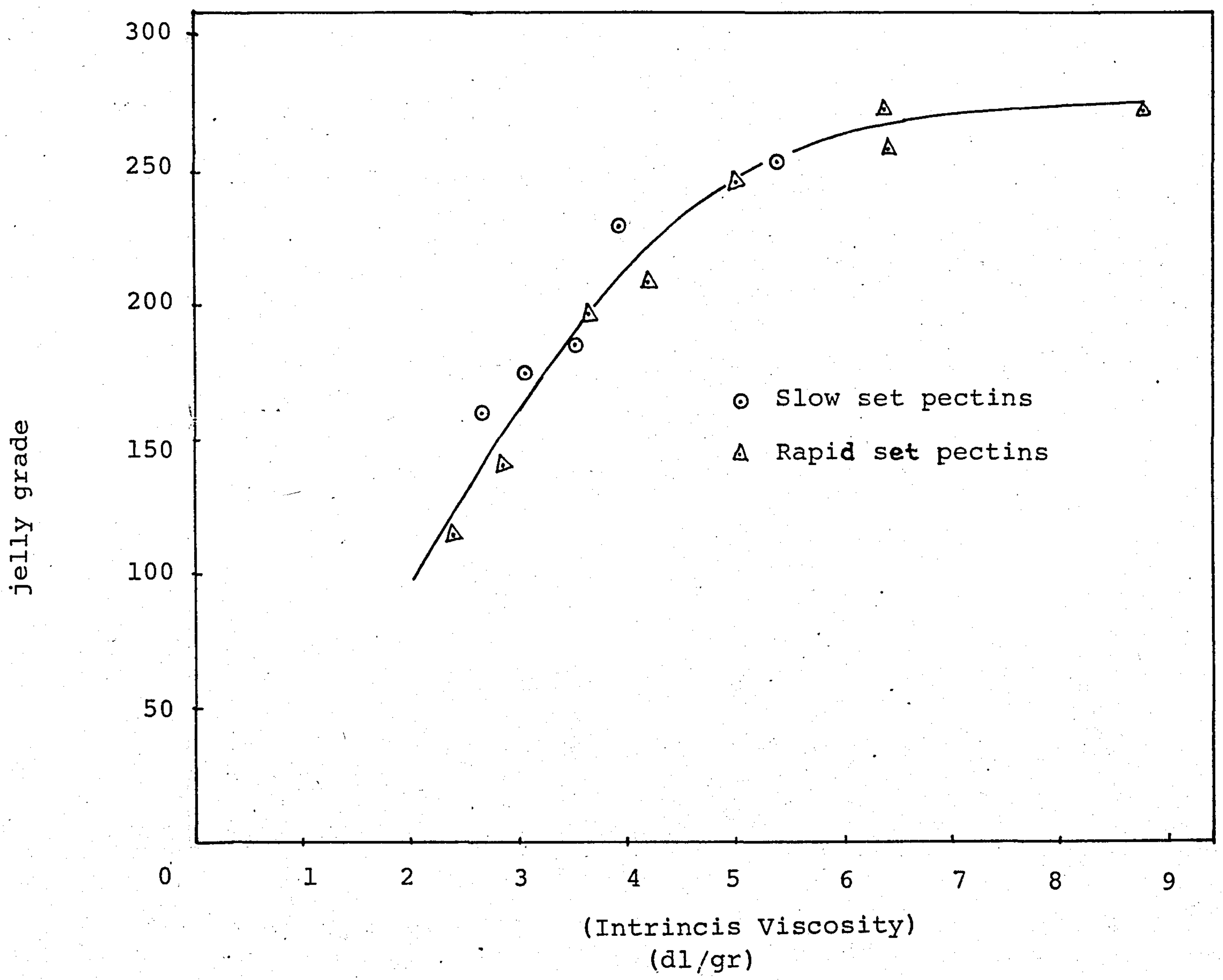

Fig.9 Relationship between jelly grade and intrinsic viscosity of pectins.

(Owens, et. al., 1952)

preserves where solid fruit particle will float to the surface when the mixture does not begin to set quickly. Slow setting is required for jellies which must be filled into containers before setting starts. The setting time is influenced by the temperature and pH of the jelly mixture. Also the decrease in methylester content lengthens the setting time. The addition of certain buffers will also delay setting (Joseph and Baier, 1949).

III. EXPERIMENTAL WORK

The main raw material used in this work was dried apple pomace. Juice of ninety kilograms . Amasya apple was pressed off in the hydraulic press of Tübitak Gıda Ünitesi Pilot Plant. Fresh apple pomace obtained was dried in an oven at a temperature lower than 90°C, yielding five kilograms dried apple pomace.

Each experimental run involved the following four steps :

1. Extraction of pectin from dried apple pomace
2. Clarification of the extract
3. Recovery of pectin from extract
4. Analysis of pectin

The experimental set-up, chemicals and experimental procedures will be considered for each step separately.

A. EXPERIMENTAL SET-UP

1. Extraction of Pectin from Dried Apple Pomace

The extraction apparatus consisted of :

a. Extraction vessel
b. Water bath
c. Mechanical stirrer
d. Heater
e. pH meter
f. Thermometer.

The system is shown on Figure 10. The extraction vessel is made of stainless steel and has a capacity of three liters. It is covered by a lid which has three holes to insert the thermometer, the electrode of the pH meter and the stirrer into the pomace and water mixture. The water bath was heated with a (Haake E52) constant temperature immersion circulator. The temperature of the bath was regulated and measured with a contact thermometer. The pH measurements were made using a Photovolt 112 pH meter equipped with a combined glass electrode (Ingold, U 402-K7). The extraction mixture was stirred with Baird and Tatlock Laboratory stirrer having a 1/100 horsepower motor giving speeds up to 2500 rpm.

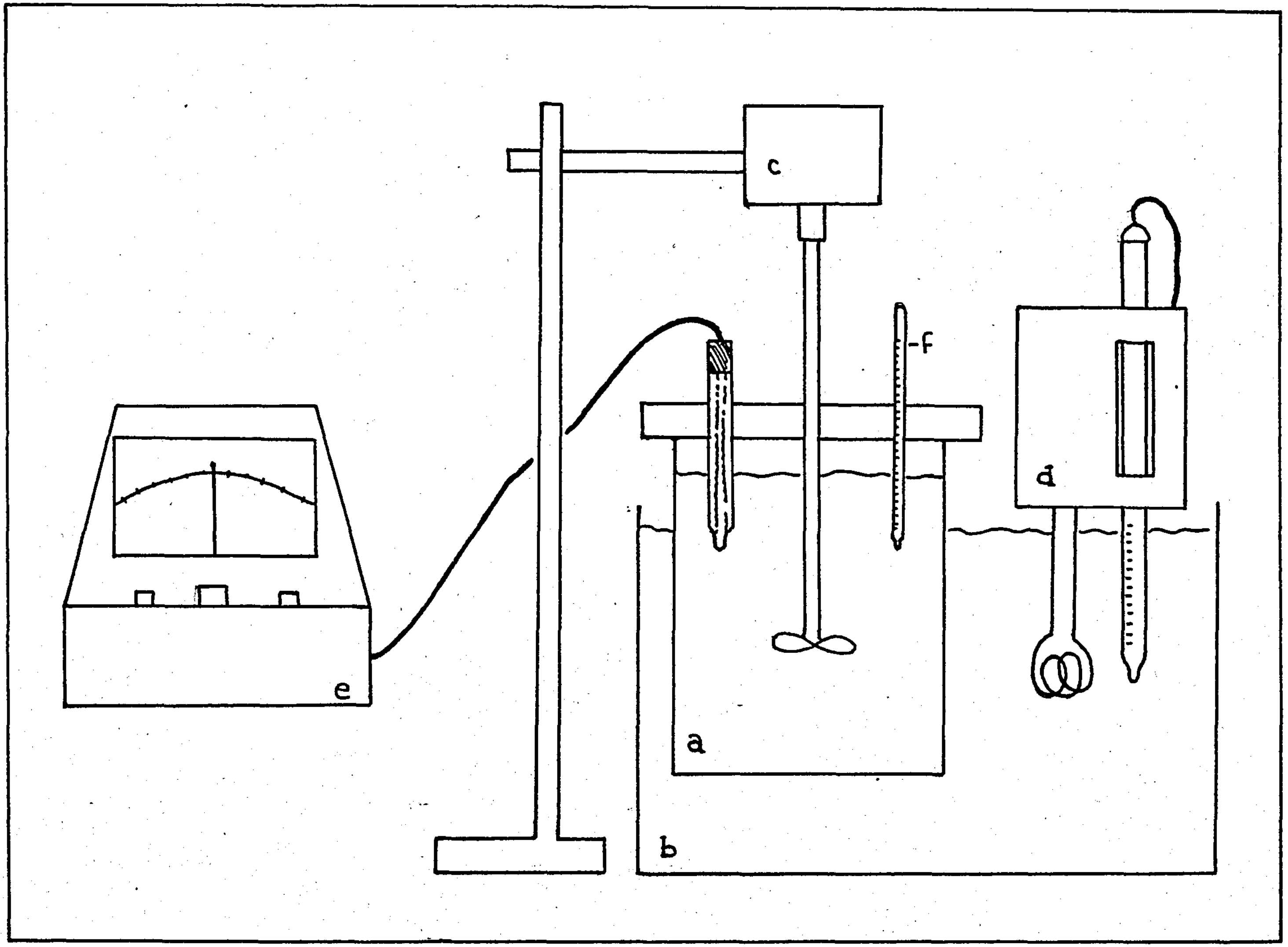

Fig. 10 Experimental Set-up for Pectin Extraction

2. Clarification of the Extract

To clarify the extract the following instruments were used:

a. Centrifuge : International Chemical Cetrifuge (International Equipment Co.) consists of stainless steel draining chamber, sliding cover and five inch diameter basket. The hole size of the basket is too large to hold the waste pomace. At first the inside of the basket was covered with filter paper. When wetted in use the filter paper was torn at the basket holes and could not hold the waste solids. This problem was solved by putting a stainless steel wire mesh inside the basket periphery which was also covered with filter paper. In this manner only very small particles passed through the wire and filter paper which were separeted later easily by filtering.

b. Evaporator : Rotary evaporator (Büchi Rotavapor R 110)

c. Laboratory glasswares and filter papers.

3. Recovery of Pectin from Extract

After precipitated the pectin was separated from the liquor by using the following instruments :

a) Vacuum pump : To drain the precipitate in the Büchner funnel a two-stage vacuum pump (Cenco Hyvac Mechanical Vacuum Pump) with the following specifications was used :

pump speed : 475 rpm
displacement of free air : 20 lt/min
ultimate vacuum : 0.1×10^{-3} mm Hg.

b) Vacuum pump : In connection with the vacuum oven a two-stage, oil sealed, rotary slide-vane pump (Brand RD4) was used with the following specifications

pump revolution :1420 min^{-1}
volume flowrate :4.3 m^3/h
ultimate total pressure with gas ballast :4×10^{-3}mbar
ultimate total pressure without gas ballast:2×10^{-3}mbar

c) Vacuum oven : It is a product of Elektro Metal Koll. Şt. with following specifications :

maximum temperature : 250 ^{o}C
maximum vacuum : 0 mm Hg

d. Laboratory glasswares.

4. Analysis of Pectin

The pectin samples were analysed by using the following instruments :

a. Various laboratory glasswares

b. pH meter (Photovolt 112)

c. Viscosimeter : A rotational viscometer (Haake VT 181) with viscosity sensor system NV, operable at two speeds (181 rpm and 45.2 rpm) and maximum torque appliable being approximately 1 Ncm was used to measure the viscosities of the pectin solutions.

B. CHEMICALS USED

1. Extraction of Pectin from Apple Pomace

The hydrochloric acid, HCl, used to acidify the water was 30 per cent pure and produced by Atabay Kimya Sanayi. Dried apple pomace was prepared as indicated at the beginning of this chapter.

2. Recovery of Pectin from Extract

To precipitated pectin from the extract 99 per cent pure ethyl alcohol was used which was obtained from Tekel.

3. Analysis of Pectin

The solutions used in the analysis of pectin were prepared by using the following chemicals :

Sodium hydroxide pellets, NaOH, extra pure and manufactured by Merck.

Sulphuric acid, H_2SO_4, 98.5 per cent pure and manufactured by Pür Kimya Sanayi.

Distilled water was used in all experiments.

C. EXPERIMENTAL PROCEDURE

1. Extraction of Pectin from Apple Pomace

The extraction medium was acidulated water. Distilled water was used in a ratio of 16 parts per part of dry pomace by volume. The extraction vessel containing three liters of distilled water was put into the water bath. Adding concentrated hydrochloric acid with a 10 ml pipet the acidity of the water was adjusted to the extraction pH as the vessel was being heated to the desired extraction temperature. To measure the pH of the water pH meter should be adjusted to the temperature of the extraction medium at its temperature control button. When the desired pH and temperature were reached 70 g of dry apple pomace was put into the vessel. The vessel was covered, and the electrode of the pH meter, the thermometer and the stirrer were inserted into the vessel. During the extraction pH and temperature of the extraction mixture were controlled and the mixture was stirred continuously. At the end of extraction process the mixture was cooled in cold water.

2. Clarification of the Extract

Pomace was separated by centrifuging the cooled mixture. After the extract was filtered approximate 60 per cent of it was evaporated in the rotary exaporator at a temperature around 55°C

3. Recovery of Pectin from Extract

By vigorously stirring in beakers 80 per cent ethylalcohol, acidified with hydrochloric acid to pH around one, was added to the extract in a ratio of three parts per one part of extract by volume. The mixture was allowed to stay overnight to permit the pectin gel to rise to the surface of the container. The gel was drained in a Büchner funnel by vacuum pumping and washed with alcohol. Drained pectin was put into evaporating dishes and dried in vacuum oven under 400 mm Hg. pressure at a temperature around 50°C The wet pectin was spread in the evaporating dishes so that it dried in the form of thin sheets. These sheets were separated easily from the surface of the evaporating dishes. The dry pectin was weighed, and the yield of each experimental run was obtained on dry pomace basis which are presented on the Table 3.

4. Analysis of Pectin

According to the procedures outlined in the section II.C moisture content, equivalent weight, methoxyl and total anhydrouronic acid contents of pectin were determined for each experimental run. Degrees of esterification of each sample were calculated. Absolute viscosities of one per cent pectin solutions were measured at 25°C.

a. Equivalent weight

Titration method (Owens, et. al, 1952) was used. Approximately 0.5 g pectin was dissolved in 100 ml. carbondioxide free distilled water. The solution was titrated to pH 7.5 by adding 0.1 N NaOH solution from 25 ml. burette. The amount of alkali consumed in the titration was used in equation (2) to calculate the equivalent weight of the sample on moisture free basis.

b. Methoxyl content

The some method as in part (a) was used. To the neutralized pectin solution from part (a) 62.5 ml of 0.1 N

NaOH solution was added. The solution was allowed to stay at room temperature for 30 minutes. Then 62.5 ml of 0.1 N H_2SO_4 solution was added to arrest the saponification. The solution was back titrated to pH 7.5 with NaOH solution. The amount of NaOH consumed in this titration was used in equation (3) to calculate the methoxyl content of the pectin an a moisture free basis.

Titration data for each run are presented on Table 6 in the Appendix.

c. Total Anhydrouronic Acid Content

Assuming that total ash was removed with the acidified alcohol during the precipitation (Owens, et. al, 1952) total anhydrouronic acid content of pectin was calculated according to equation (4) on moisture free basis.

d. Degree of Esterification

Using results of parts (b) anr (c) degree of esterification was calculated from equation (5).

e. Moisture Content

Taking the procedures outlined by Owens, et.al, (1952) and Graham and Shepherd (1953) into consideration the moisture content of pectin samples were determined in the following way :

Pectin samples were put into crucibles and weighed. They were then put into on oven to stay overnight at 50-55°C. The samples were weighed and put again into the oven. When weighed after a couple of hours for the second time there was no change in their weight. So to dry the samples overnight was considered to obtain constant weight. The different between the first weight and the last weight was takes as the moisture content of the pectin. Moisture content data are presented on Table 8 in the Appendix.

f. Viscosity

One gram sample of dry pectin were dissolved in 100 ml of distilled water by stirring vigorously. The pH of the solutions were measured to be around 2.5-2.7. Water at 25°C was circulated in the tempering vessel of Haake Viscometer. Sensor system NV was used in the measurements, and the measurements were made at 181 rpm and 45.2 rpm speeds of the viscometer. Viscosity in terms of centipoise was calculated from the equation (instruction Manual, Haake Viscometer VT 181)

$$u = F \times U \times S \qquad (8)$$

where F is a constant for the sensor system of the

Viscometer, for NV F = 0.24

U is the speed number, 1 for 181 rpm
4 for 45.2 rpm

S is the scale reading of the Viscometer

Viscosity measurement data are presented on Table 9 in the Appendix.

IV. RESULTS AND DISCUSSION

In this work the precision and the accuracy of the analytical methods used to determine the pectin quality were investigated besides the effects of the three process variables studied on the pectin yield and quality. Therefore this chapter is divided into three parts: namely, the results of the extraction experiments, precision and accuracy of the analytical methods used and the discussion of the results.

A. RESULTS OF THE EXTRACTION EXPERIMENTS

In this study the intention was to investigate the effects of three factors, namely the extraction temperature, the duration of the extraction process and the acidity of the extraction medium on the yield and quality of the pectin obtained. The previous studies summarized in Chapter II of this work were taken into consideration in determining the experimental levels of these factors. The levels of these factors were:

extraction temperature: 60 C-90 C
extraction time : 30 min-60 min
extraction pH : 2.0-3.0

These values can be represented as the corners of a cube. The conditions of eight of the nineteen experimental runs performed in this work are represented on Figure 11. The conditions and results of these experiments are summarized on Table 3. The results of rest of the experimental runs had to be

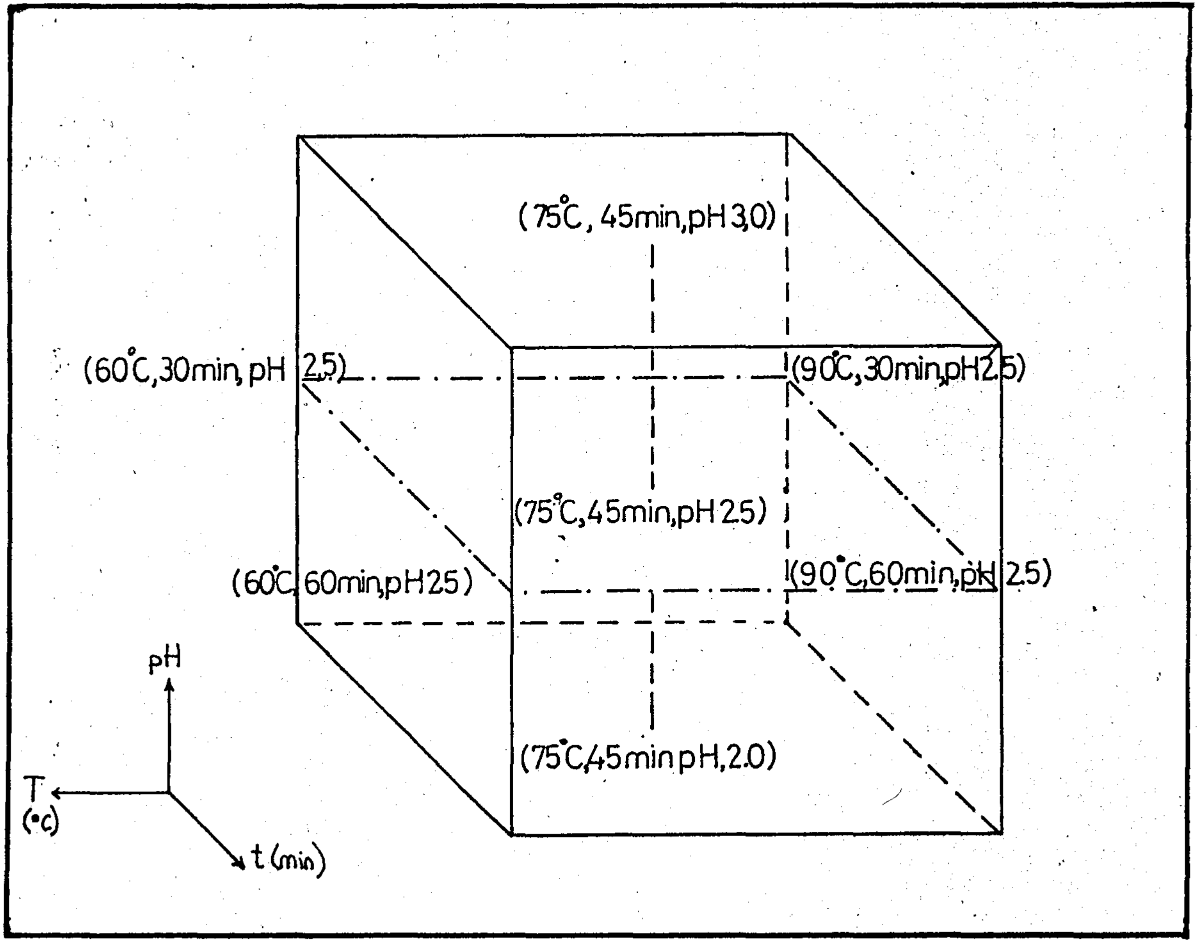

Fig.11. Pictorial representation of the extraction conditions.

Table 3. Results of the Extraction Experiments

Experiment no.	12	15	14	16	17	19	18	13
Amount of HCl added (ml)	6	10	5.75	9	6.5	10.25	5.7	6.5
Extraction pH	2.5	2.5	2.5	2.5	2.5	2.0	3.0	2.5
Extraction Temperature (°C)	60	60	90	90	75	75	75	60
Extraction Time (min)	30	60	30	60	45	45	45	30
Moisture Content (%)	3.1	4.5	4.0	6.2	6.6	14.2	7.2	2.8
Methoxyl Content (%) (moisture and ash free basis)	4.62	4.41	4.15	5.17	4.59	4.98	6.02	4.58
Anhydrouronic acid Content (%) (moisture and ash free basis)	41.62	40.15	43.11	50.31	42.01	60.33	52.02	42.60
Degree of Esterification (%) (moisture and ash free basis)	63.00	62.42	54.59	58.35	57.94	46.86	65.73	61.00
Equivalent weight (moisture and ash free basis)	1063	1164	898	839	930	551	987	1060
Absolute Viscosity (cp)	1.44	1.92	4.08	3.12	3.36	1.70	2.16	1.49
Yield (%)	6.3	8.6	11.7	13.8	9.37	9.46	7.57	6.0

neglected,since it was found out that some of the pH meter readings obtained during these runs probably had unacceptably high errors during these runs due to a bad connection in the pH meter.

Runs 12,15,14 and 16 were performed by keeping the pH of the extraction medium constant at 2.5 in order to see the effects of extraction time and temperature. In the Runs 17, 18 and 19 the extraction time and temperature were kept constant at 75 C and 45 minutes and the effect of pH was observed. The Run 13 is a repetition of the Run 12 performed to test the reproducibility of the extraction experiments.

B. PRECISION AND ACCURACY OF THE ANALYTICAL METHODS USED

In an effort to determine the precision and the accuracy of the analytical methods used in this work two procedures, as outlined below were performed.

Firstly, the pectin sample obtained in Run 16 was analysed for its quality in duplicate. The results of this duplicate analysis are shown on Table 4 below. The detailed data of this analysis are given on Table 10 of the Appendix.

As seen on Table 4 the reproducibility of four different tests range between 0.08-4.14 per cent. Namely the methoxyl content was reproduced with a 2.94 per cent, the

Table 4. Comparision of the Duplicate Analytical Results for Run 16.

Experiment no.	16(1)	16(2)	% Difference
Methoxyl Content (%) (moisture and ash free basis)	5.17	5.02	2.94
Anhydrouronic acid Content (%) (moisture and ash free basis)	50.31	50.35	0.08
Degree of Esterification (%)	58.35	56.60	3.04
Equivalent weight (moisture and ash free basis)	839	805	4.14

anhydrouronic acid content with a 0.08 per cent, degree of esterification with a 3.04 per cent and equivalent weight with a 4.14 per cent.

Secondly, a commercial pectin sample was analysed in the University laboratory. To determine the accuracy of the analytical methods used a portion of the same sample was submitted for analysis in the quality testing laboratory of a pharmaceutical firm, namely İbrahim Ethem Ulagay Kimya Evi T.A.Ş..The comparative results of these tw0 analyses are shown on Table 5 below. Since the two sets of experiments were performed by different analysts and using the same methods but probably using solutions of different concentrations the discrepency between results as shown on Table 5 should be considered acceptable.

Table 5. Comparision of the Analytical Methods for a Commercial Pectin.

	Analysis made in the laboratory	Analysis made in the firm
Methoxyl Content (%) (moisture and ash free basis)	7.79	8.73
Anhydrouronic acid Content (%) (moisture and ash free basis)	77.25	88.26
Degree of Esterification (%)	57.25	56.15

C. DISCUSSION OF THE RESULTS OF THE EXTRACTION EXPERIMENTS

The discussion of the experimental results will be made in accordance with the experimental design shown on Figure 11. Thus, firstly the experimental results at constant pH, i.e. pH 2.5, and varying the extraction temperature and time will be discussed to bring out the the effect of these two variables on pectin yield and quality. Next the effect of varying pH at constant extraction temperature and time, i.e. 75°C and 45 minutes, will be examined.

1. EFFECT OF EXTRACTION TEMPERATURE AND TIME ON PRODUCT YIELD AND QUALITY

The effect of extraction temperature and the duration of the extraction at constant extraction pH on the pectin yield, the methylester and anhydrouronic acid contents and degree of esterification of pectins and on the absolute viscosity of the pectin solutions will be discussed.

a. Effect of Extraction Temperature and Time on Pectin Yield

Figure 12 shows the change of pectin yield with extraction time and temperature. Yield increased with time and temperature. This result was expected since by prolonged heating at high temperature more water-insoluble protopectin

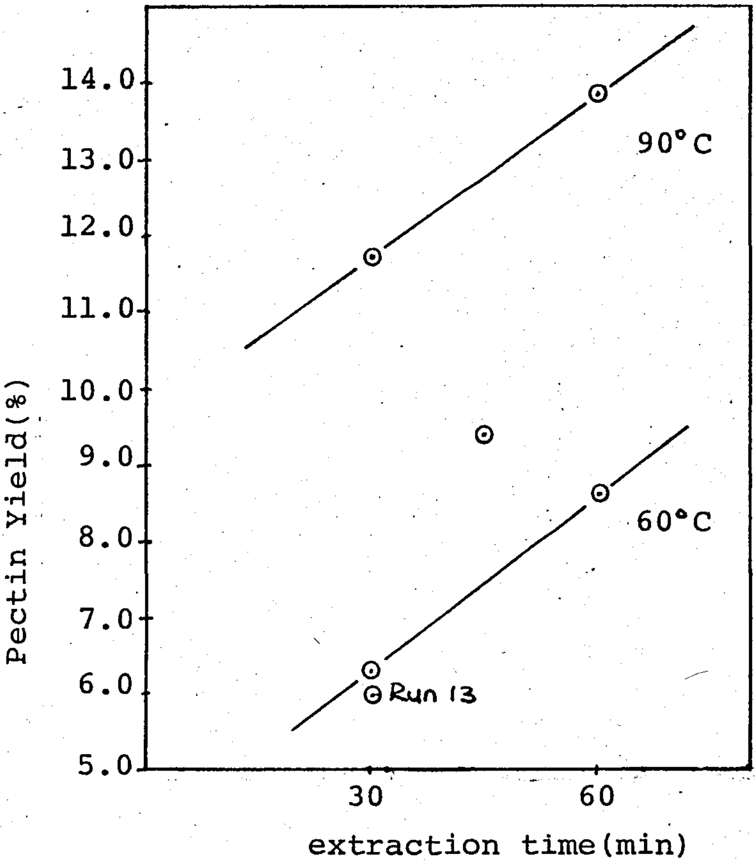

Fig.12. Percentage pectin yield vs extraction time at constant extraction pH(pH=2.5)

is converted into water-soluble pectin. Similar results were obtained for both temperatures studied (60°C and 90°C). Yield vs extraction time lines obtained for each temperature seem to be parallel to each other. Thus, it is seen that yield increase is directly related to the extraction time and the time effect is similar at all levels studied.

b. Effect of Extraction Temperature and Time on the Anhydrouronic Acid Content of Pectin

The purity of pectin extracted is indicated by the amount of total anhydrouronic acid (owens,et.,al.;1952). As shown on Figure 13 the purity of the pectins obtained in the

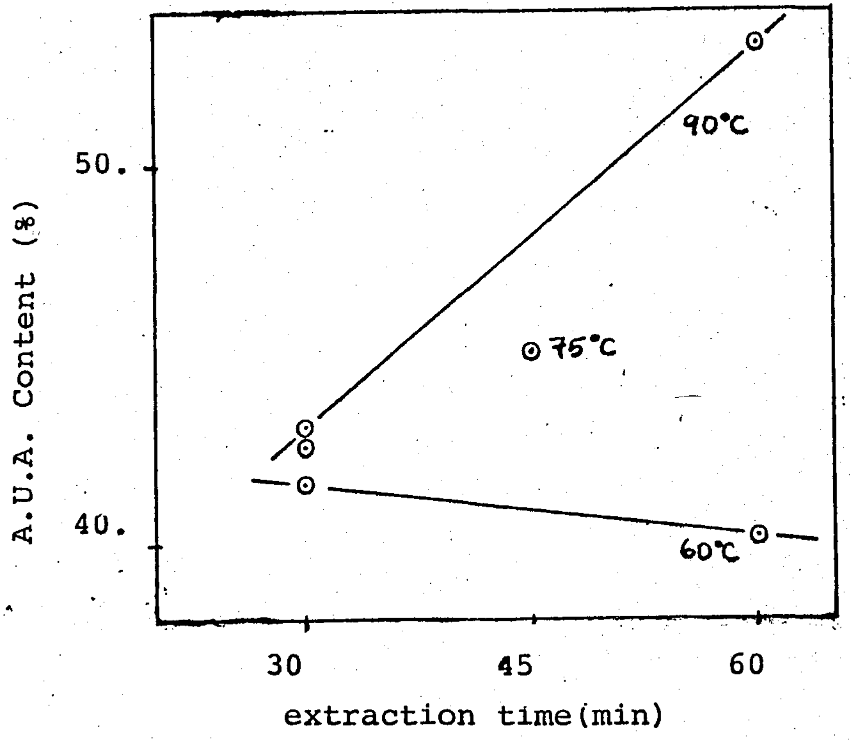

Fig.13. Total anhydrouronic acid content vs extraction time at constant extraction pH(pH=2.5)

experiments increased with temperature. The reason for this may be suggested as being the fact that extraction at high temperatures causes breaking of the ester linkages between polygalacturonic acid and other polysaccharides and sugars. As a result purer polygalacturonic acid was obtained. Furthermore, it is also suggested that prolonged heating strengthens the effect of the temperature so that a steep line is obtained for 90°C. The purity of pectin extracted at 60°C shows a slow decrease which cannot be explained. Maybe it can be deduced from this fact that at low extraction temperatures the extraction time does not effect the purity of pectin obtained. The temperature 75°C lies inbetween which means that the purity increases with extraction temperature.

c. Effect of Extraction Temperature and Time on the Methylester Content of Pectin

As shown on Figure 14 methylester content of pectins shows a similar behaviour as anhydrouronic acid content. On Figure 14 the two constant temperature lines cross each other near 75°C-45 min point. As it was indicated in section II.B.2, prolonged extraction at too low temperatures causes deesterification of pectin, so extracting at 60°C for 60 minutes results in pectin with lower methylester contents as compared to extracting at 60°C for 30 minutes. This suggests that before an optimum point near the extraction time of 45 minutes is reached pectins with higher methyl-

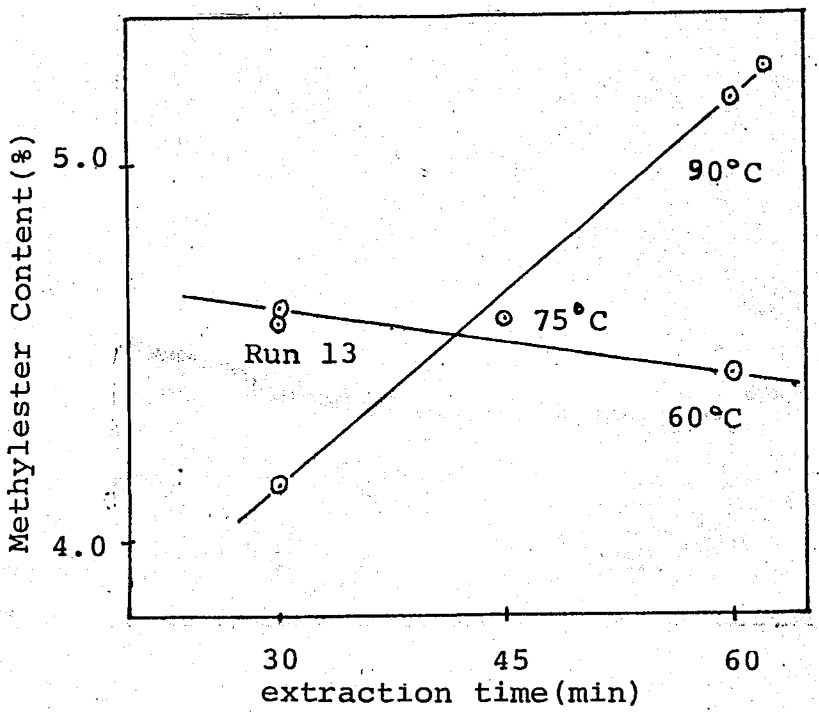

Fig.14. Methylester content vs extraction time at constant extraction pH(pH≈2.5)

ester contents are obtained at low temperatures. When the extraction time exceeds 45 minutes, higher temperature is necessary to extract pectins with higher methylester content which seems unreasonable, because high extraction temperatures are deterious to pectin as indicated in section II.B.2.

d. Effect of Extraction Time and Temperature on Degree of Esterification of Pectins

On Figure 15 the behaviour of degree of esterification as a function of time is presented. At lower temperatures pectins with higher degree of esterification are obtained. Again at 60°C, the time effect is not strong. At high temperatures prolonged extraction seemed to increase the degree of esterification of pectin. This is unexplainable.

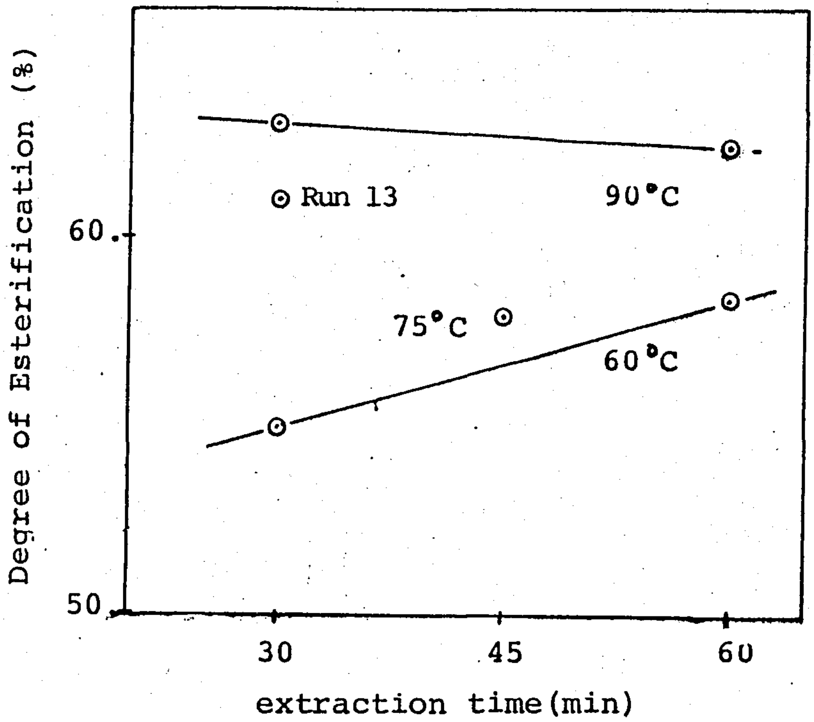

Fig.15. Degree of esterification vs extraction time at constant extraction pH (pH=2.5)

e.Effect of Extraction Time and Temperature on Absolute Viscosity of Pectin Solutions

The relation between absolute viscosity of pectin solution and extraction time and temperature is shown on Figure 16. When compared with Figure it is seen that there is an inverse relation between the viscosity of pectin solutions and the degree of esterification of pectins obtained.

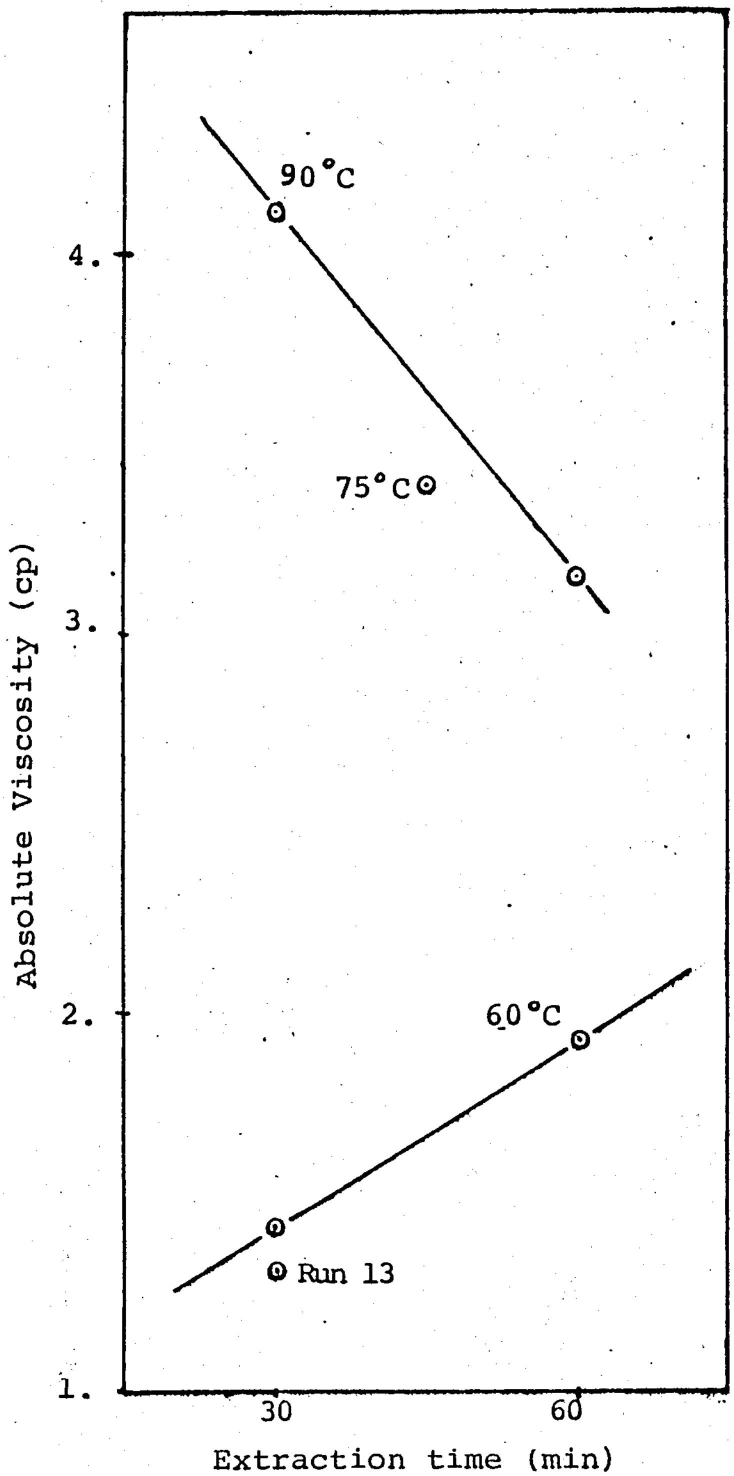

Fig.16. Absolute viscosity of one weight per cent pectin solution vs extraction time at constant extraction pH (pH=2.5)

2. EFFECT OF EXTRACTION pH ON PRODUCT YIELD AND QUALITY

The effect of the acidity of the extraction medium at constant extraction temperature and time on pectin yield, the methylester and anhydrouronic acid contents and degree of esterification of pectins and on the absolute viscosity of the pectin solutions will be discussed.

a. Effect of Extraction pH on Pectin Yield

As shown on Figure 17 the pectin yield increased with increasing acidity as expected(Graham,et. al.,1953). This

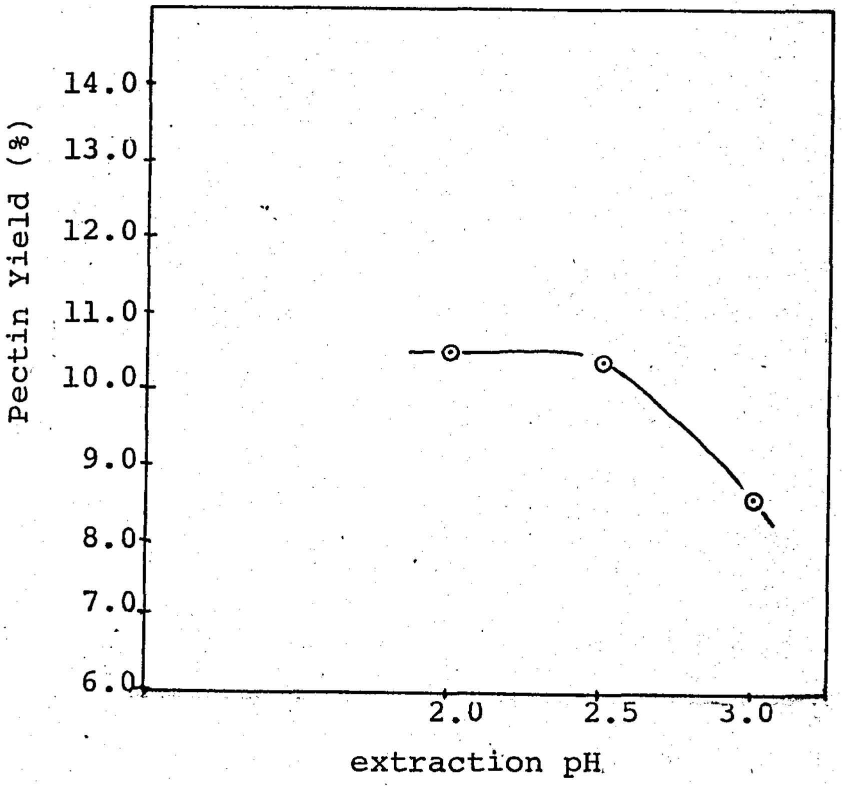

Fig.17. Percentage pectin yield vs extraction pH at constant extraction temperature and time (T=75°C, t=45 min)

is thought to be due to the fact that at high acidities more protopectin is hydrolysed into water-soluble pectin so that the amount of pectin recovered will be higher. No appreciable difference between pH 2.0 and pH 2.5 results was detected.

b. Effect of Extraction pH on Total Anhydrouronic Acid Content of Pectin

Figure 18 shows the relation between the purity of

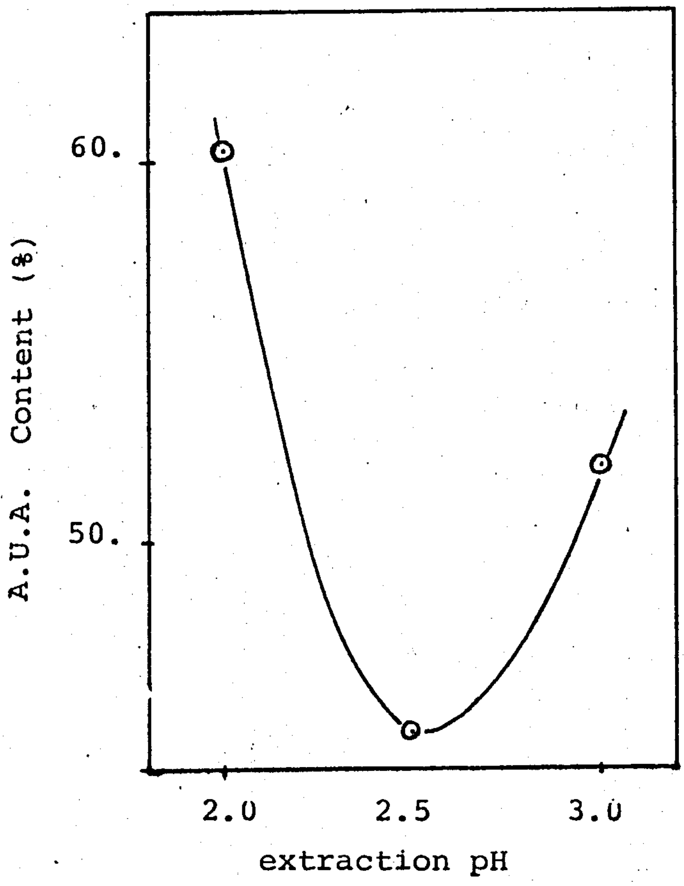

Fig.18. Total anhydrouronic acid content vs extraction pH at constant extraction time and temperature (T=75°C, t 45=min)

extracted pectin and the acidity of the extraction medium. Purity is highest at highest acidity, because the medium is deterious enough to break the ester linkages between the polygalacturonic acid and the other polysaccharides and sugars.

c.Effect of Extraction pH on Methylester Content of Pectin

High acidity is also deterious to methylester bonds of polygalacturonic acids(Graham,et.al.,1953) and pectin with lower methylester content is obtained at high acidities as shown on Figure 19.

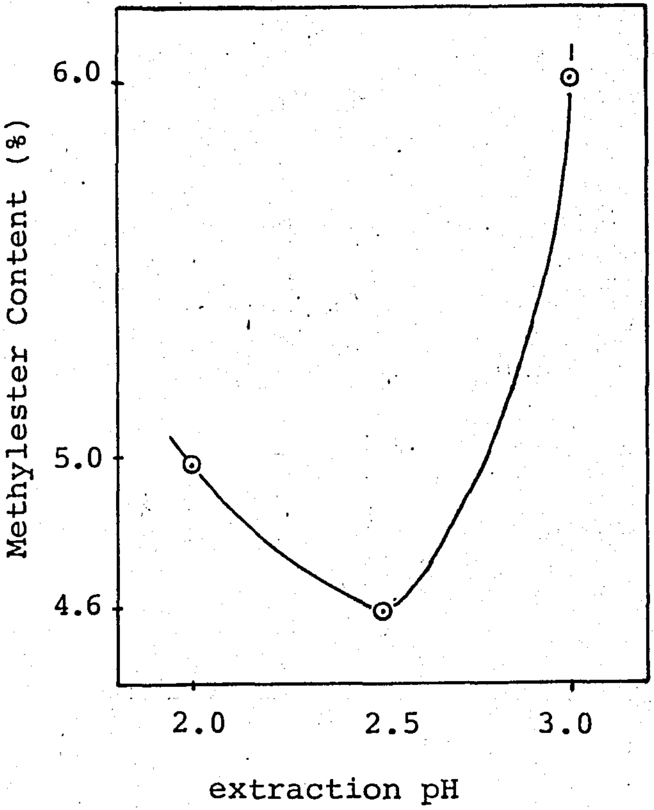

Fig.19. Methylester content vs extraction pH at constant extraction temperature and time(T=75°C, t=45 min)

d. Effect of Extraction pH on Degree of Esterification of Pectin

As shown on Figure 20, the degree of esterification of pectins extracted increased with increasing acidity of the extraction medium. This trend agrees with the literature(Nagy, et.,al.,1977).

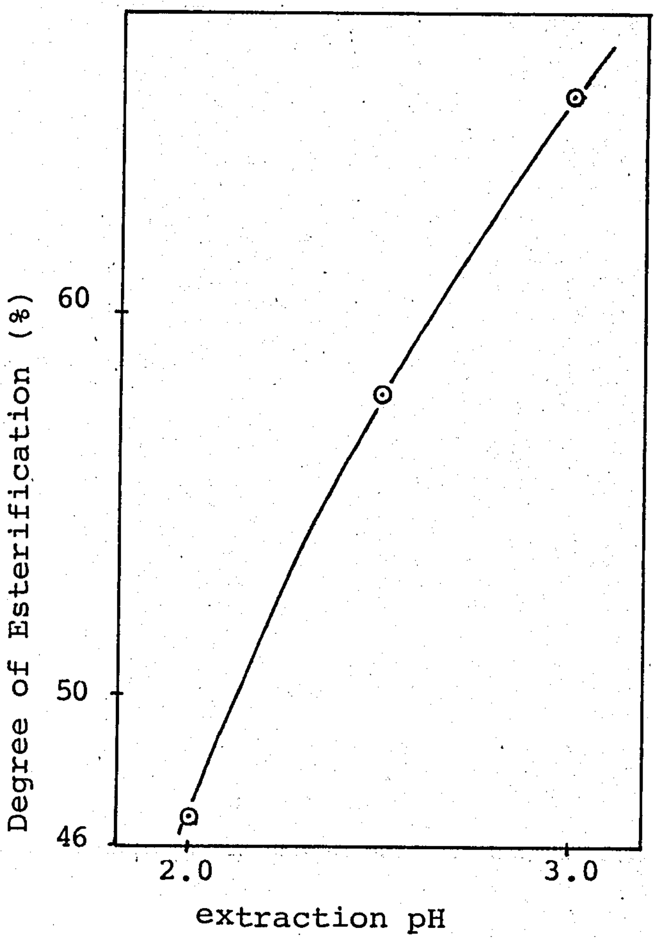

Fig.20. Degree of esterification vs extraction pH at constant temperature and time (T=75°C, t=45 min)

e. Effect of Extraction pH on Absolute Viscosity of Pectin Solutions

Figure 21 shows the relation between absolute viscosity of pectin solution and extraction pH. When compared with Figure 19 there is an inverse relation between the absolute viscosity of pectin solutions and methylester content of pectins obtained.

In general low ester pectins were obtained as the methylester contents of the pectins were under seven per cent (Joseph,1953). The methylester contents and the equivalent weights of the pectins obtained do not agree with the data obtained from the literature as shown on Figures 22 and 23. A pectin sample obtained from a pharmaceutical firm was analysed in the laboratory. The methylester content and the equivalent weight of this pectin were closer to the curves on Figures 22 and 23, which means that they agree with the literature values within the acceptable limits.

Pectins with low methylester content but with high degree of esterification were obtained in this work. This result is in contradiction with the expectations.

These deviations of the results from the literature may be due to impurities present in the pectins prepared in this work. These impurities, if any, may result from the clarification problems which arose in this work. The use of activated carbon and diatomaceous earth to clarify the pectin

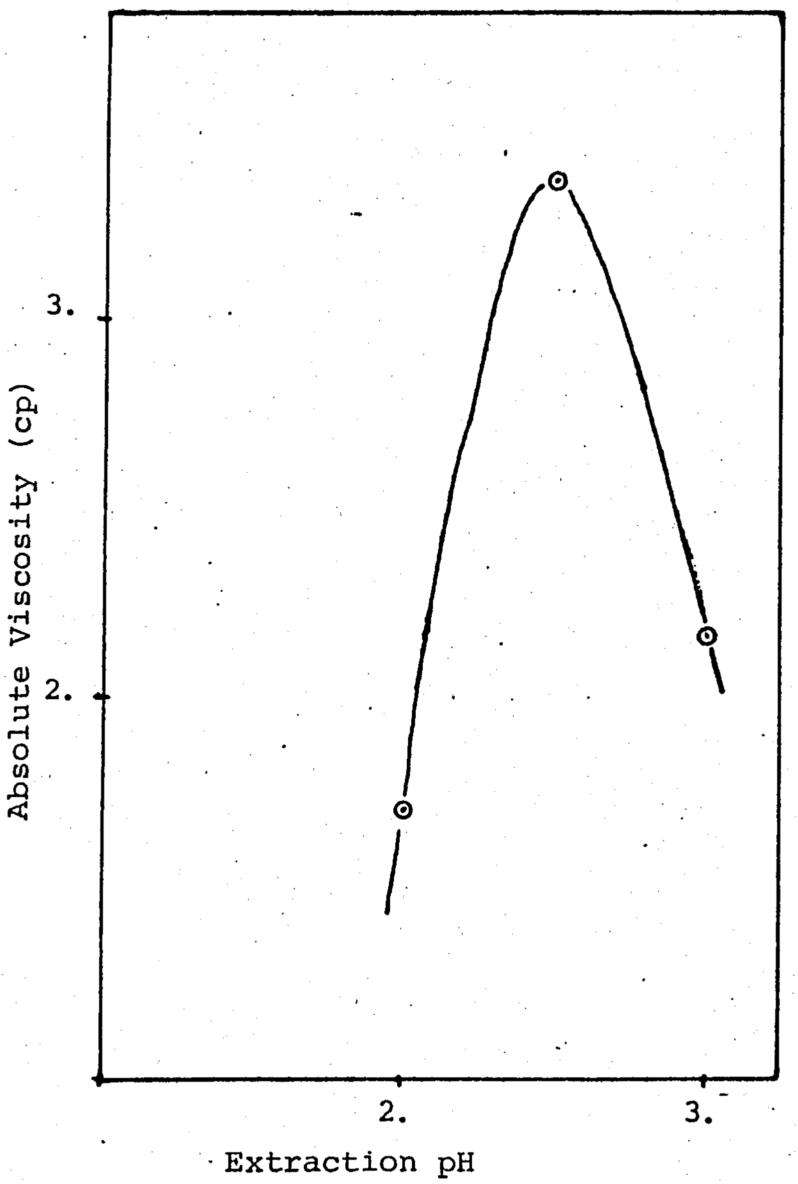

Fig.21. Absolute viscosity of one weight per cent pectin solution vs extraction pH at constant extraction time and temperature (T=75 °C, t=45 min)

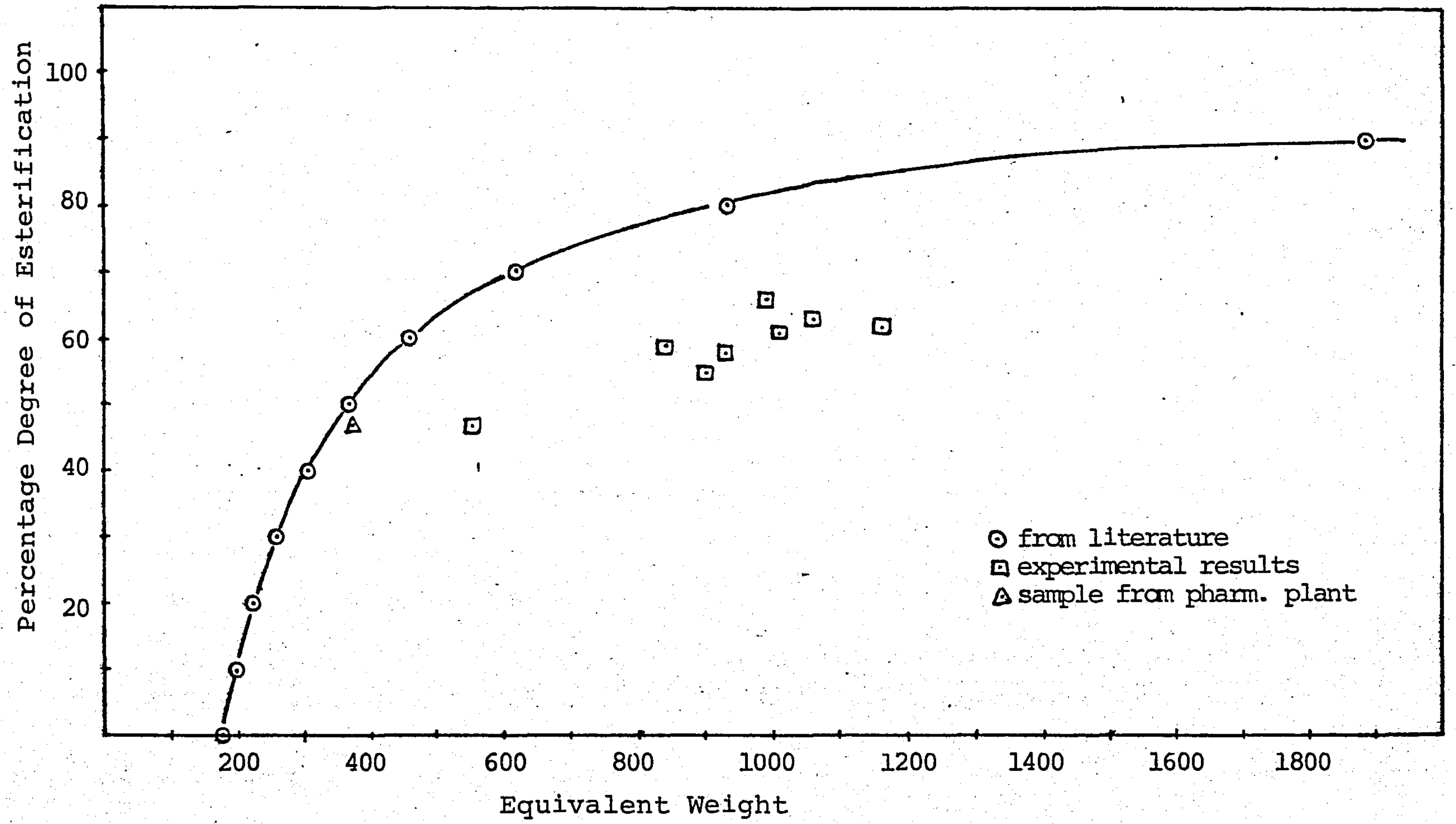

Fig. 22. Relation Between Degree of Esterification and Equivalent Weight of a Pure Pectinic Acid (%A.U.A.∽92.64),(Nagy,et.al.,1977)

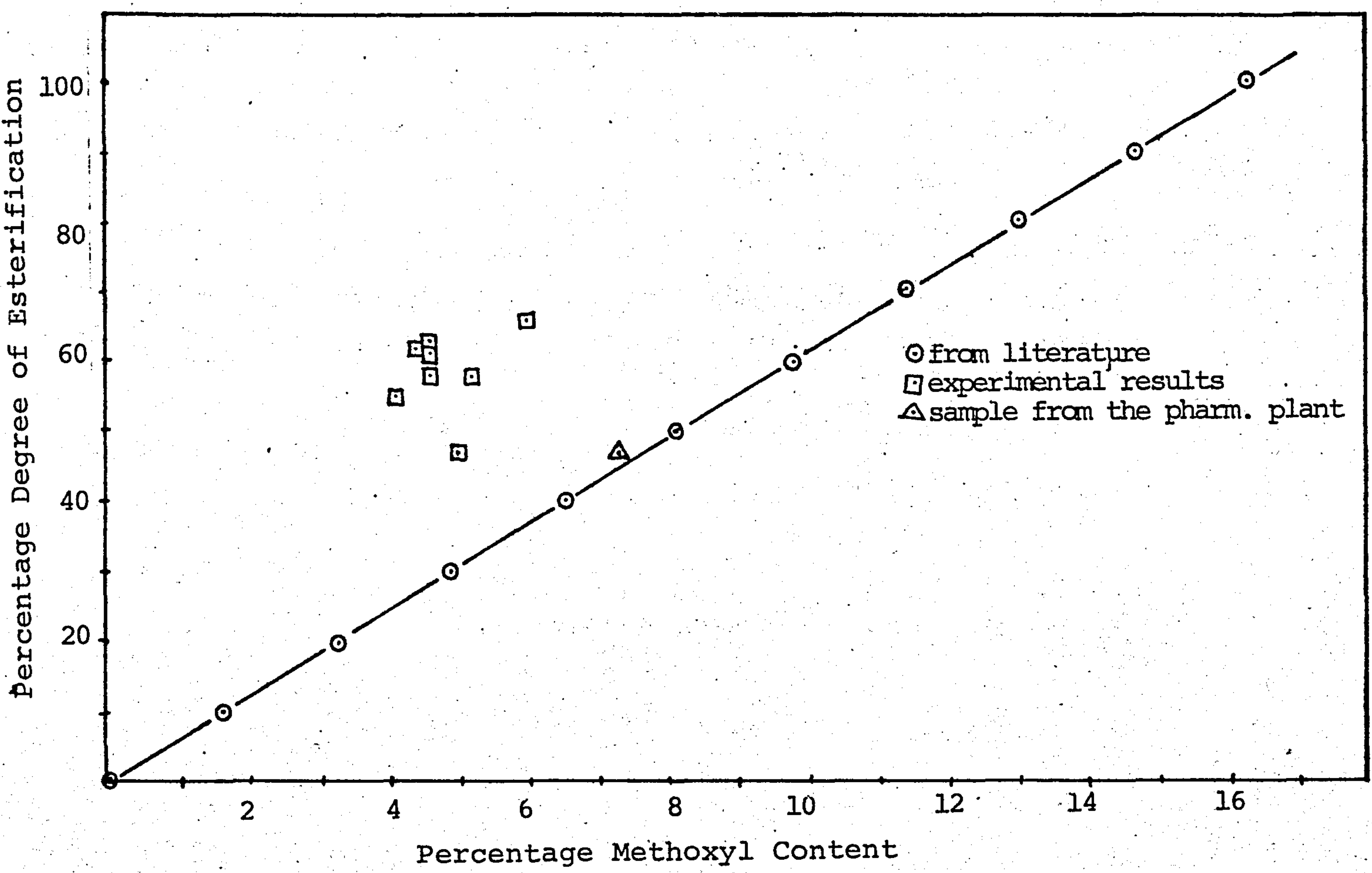

Fig. 23. Relation Between Degree of Esterification and Methoxyl Content of a Pure Pectinic Acid (% A.U.A.∽92.64), (Nagy, et. al., 1977)

liquor(Nagy,et.,al.,1977) was attempted in the earlier stages of this work. However, the products obtained from these purification stages gave rise to filtering problems of the liquor, so that pectins precipitated from this liquor were quite different, almost like cakes of silty soil. Thus, those filter aids were not used further in this work.

Pectins with the highest degree of esterification were obtained from extractions under mild conditions such as Run 12 (pH=2.5, T=60°C, t=30 min) and Run 18 (pH=3.0, T=75°C, t=45 min) which is very logical and as expected.

The errors in the determination of equivalent weight and methylester content may also lie in the possibility of missing the end point of the titration with the alkaline solution. As shown on Figure 24 in the Appendix there is a sudden change in pH around 7.5. Even one drop of excess alkali causes a large deviation from the end point.

V. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

From the experimental results obtained in this work it may be concluded that at mild conditions, that is at low extraction temperature and low acidity, low yields of pectin with high degree of esterification are obtained. Duration of the extraction process does not seem to strongly affect the results at these conditions. There seem to be no appreciable difference in extraction yields at levels of pH 2.0 and pH 2.5. However, at pH 2.5 higher quality pectin is obtained. The results show that at pH 2.5 60°C extraction temperature acceptable levels of pectin yield and quality are obtained.

As seen in Section IV.A and discussed in Section IV.C the results, that is the equivalent weight, the methylester and anhydrouronic acid contents of pectins obtained, show deviations from the literature. As stated in Chapter IV, filter aids could not be used to clarify the pectin liquor, and this may cause the deviations observed. Therefore, first of all the proper way of using the filteraids in clarifying the pectin extract should be investigated in order to see whether the employment of such clarification aids avoids the deviations in the results. Seperation of filteraids from the extract in Bücher funnel may be inefficient as very particular filteraids, such as diatameous earth, may pass through the sides of filter paper into the filtrate. The employment of other filtering equipment may improve the filtration process.

Dispersion of dry pectin in water is very difficult. When introduced into water particles hydrate on the surface forming a gummy coating, and absorbtion of water is slowed down. This difficulty may be avoided by wetting the powder pectin with alcohol. However, this treatment is useless when dissolving the pectins which are dried in sheet form. Dispersion of them in water takes a longer time even if the solution is agitated vigorously. For easier dispersion in liquids the pectins should be either ground after drying in sheet form or the concentrated pectin liquor should be spray dried.

An optimization of the amount of water used as the extraction medium is necessary, because its amount affects the pectin yield, the amount of alcohol used to precipitate the pectin and other processing costs, i.e. pumping, filtering and concentrating the extract.

After improving all the steps mentioned above the experimental design cube in Figure 11 should be completed to obtain more reliable conclusions on process variables. At least extractions should be done at 75°C extraction temperature for 30 minutes and 60 minutes to provide the behaviour of the other two constant temperature lines at constant pH. The repetition of constant temperature and time extractions at varying pH for at least one other extraction temperature and time will be good to compare the effect of varying extraction pH at different extraction temperatures and times.

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APPENDIX

Table 6. Data for Titration Method to Determine the Quality of Pectins Obtained (before Moisture Determination)

Experiment no.	12	15	14	16	17	19	18	13
Amount of Pectin Dissolved in 100 ml water (g)	0.5060	0.5000	0.5012	0.5004	0.5013	0.5016	0.5000	0.4965
Normality of NaOH Solution	0.09318	0.09318	0.09318	0.09318	0.09318	0.09318	0.09318	0.09318
Normality of H_2SO_4 Solution	0.11959	0.11959	0.11959	0.11959	0.11959	0.11372	0.11372	0.11959
pH before first titration	7.5	7.5	7.5	7.5	7.5	7.5	7.55	7.5
pH after first titration	2.8	2.85	2.8	2.8	2.8	2.6	2.9	3.1
Volume of NaOH consumed in the first titr. (ml)	4.95	4.4	5.75	6.0	5.4	8.3	5.0	4.9
Amount of NaOH used for saponification of pectin (ml)	67	67	67	67	67	67	67	67

Table 6. continued.

Amount of H_2SO_4 used to stop the saponification (ml)	52	52	52	52	52	55	55	52
pH before second Titration	3.9	3.85	4.1	3.9	3.72	4.1	3.5	3.9
pH after second titration	7.5	7.5	7.5	7.5	7.45	7.5	7.5	7.5
Volume of NaOH consumed in the second titration	7.83	7.3	6.9	8.4	7.45	7.35	9.6	7.65

Table 7. Data for Titration Curve of Pectin Solution (1.0 g Pectin / 100 ml water)(Fig.24)

Amount of NaOH (ml)	pH of pectin soln.	Amount of NaOH (ml)	pH of pectin soln.	Amount of NaOH (ml)	pH of pectin soln.
0	3.15 3.15	6.25	4.38	11.25	9.90
1.00	3.18	6.50	4.48	11.50	10.00
1.25	3.19	7.00	4.68	11.75	10.00
1.50	3.20	7.50	4.90	12.00	10.05
1.75	3.23	8.00	5.50	12.50	10.20
2.00	3.25	8.25	5.85	13.00	10.30
2.25	3.30	8.50	6.43	13.50	10.35
2.75	3.40	8.75	7.30	14.00	10.40
3.25	3.50	9.00	7.88	14.50	10.45
3.50	3.60	9.25	8.42	15.00	10.50
4.00	3.70	9.50	8.81	16.00	10.60
4.50	3.80	9.75	9.15	17.00	10.70
5.00	3.95	10.00	9.40	18.00	10.75
5.25	4.03	10.25	9.50	19.00	10.80
5.50	4.13	10.50	9.65	20.00	10.85
5.75	4.20	10.75	9.75	21.00	10.90
6.00	4.30	11.00	9.85	22.00	10.90

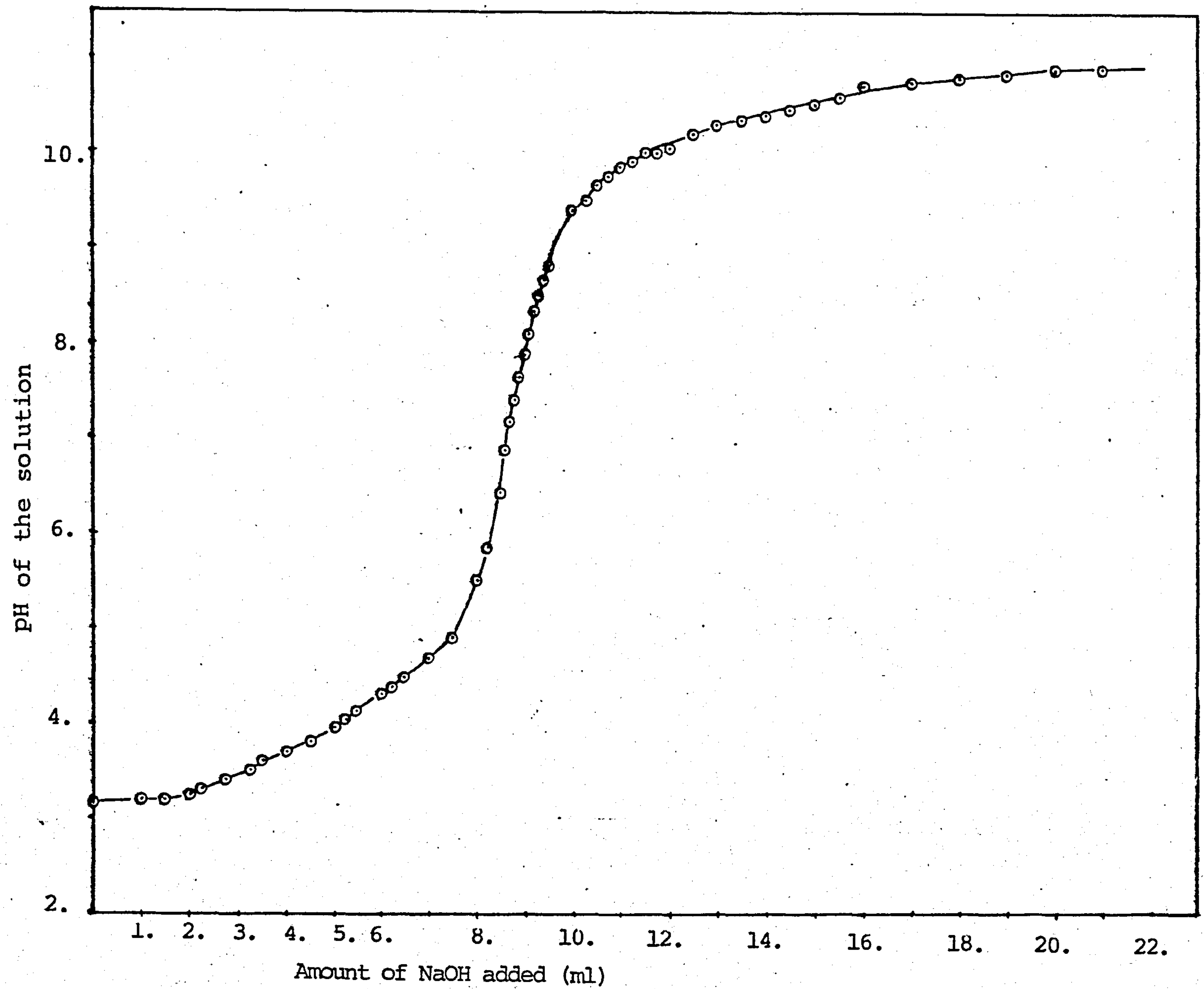

Fig. 24. Titration Curve of 0.5 Weight Per Cent Pectin Solution

Table 8. Data for Moisture Determination of Pectin Samples Obtained

Experimental Run	12	15	14	16	17	19	18	13
Weight of Empty Crucible (g)	19.0179	19.5113	18.3385	19.5800	18.7694	19.1450	18.4145	18.4100
Weight of Crucible and Pectin before Drying (g)	20.6646	20.5972	19.9411	21.0975	19.7543	20.2510	20.2248	20.5114
Weight of Crucible and Pectin after Drying (g)	20.6137	20.5487	19.8765	21.0045	19.6896	20.0908	20.0870	20.4524

Table 9. Data of Viscosity Measurements (1.0 g pectin / 100 ml water)

Experimental Run	12	15	14	16	17	19	18	13
Temperature of the Solution (C)	25.2	25.0	25.1	25.1	25.2	25.1	25.0	
pH of the solution	2.6	2.6	2.7	2.6	2.5	2.4	2.6	2.5
S (Scale Reading at U 1)	6	8	17	13	13.5	5	9	6.2
S (Scale Reading at U 4)	1.5	2	4.5	3	3.5	1	2.5	1.5

Table 10. Data for Experiments to Test the Precision of Analytical Methods

Experiment No.	16(1)	16(2)
Amount of Pectin Dissolved in 100 ml water (g)	0.5004	0.5000
Normality of NaOH Solution	0.09318	0.09318
Normality of H_2SO_4 Solution	0.11959	0.11959
pH before first titration	2.8	2.9
pH after first titration	7.5	7.5
Volume of NaOH consumed in the first titration (ml)	6.0	6.25
Amount of NaOH used for Saponification of Pectin (ml)	67	67
Amount of H_2SO_4 used to Stop the Saponification (ml)	52	52
pH before second titration	3.9	3.6
pH after second titration	7.5	7.5
Volume of NaOH consumed in the second titration (ml)	8.4	8.4