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PILOT SCALE PRODUCTION OF USP GRADE PECTIN FROM CALAMANSI FRUIT WASTES

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ABSTRACT

Commercial pectin is an indispensable product for both the pharmaceutical, cosmetic and food industries. Its extraction and conversion to a marketable product is described. The technological problems and economic feasibility of commercial pectin product are discussed in relation to its function and physical and chemical properties. Some constraints upon the production and marketing of the product are identified.

INTRODUCTION

Pectin, which comes from the Greek word "Pektos" meaning dense, thicken or coagulated, is a mucilaginous substance of higher plants. The pectic substance which gives the so-called "protopectin" is associated with cellulose and gives cell walls the ability to absorb water in large quantities. Cellulose has an important role in the structure as it gives rigidity to the cells while pectic substances contribute to their texture.

Pectin, with the structure shown below, is primarily composed of α-D-galactopyranosyluronic acid units, partially esterified with methanol. They contain in a lesser extent B-L-rhamnose residues and other neutral units (galactose, arabinose, xylose).

**NOTE TO STRIPPER!!!
PLS. SEE ORIGINAL COPY
FOR THE DRAWING**

α-D-galacturonic acid residues with the 4C conformation are joined by (1---->4) linkage to form the main chain.

Pectin production is limited largely to the highly industrialized countries of America and Europe and is typically produced on a large scale in one or two factories in each country. The most favored raw materials are wastes from fruit juicing industries.

Since pectin is used directly by food, pharmaceutical and cosmetic manufacturers as an emulsifier, extender or stabilizer in processed foods, in pharmaceuticals or cosmetic products; it is essential to establish at what level local processors incorporate pectin in their formulations and their views on the substitution of imported pectin by a local product. It is also essential to establish that a cheap and readily accessible raw material for pectin extraction is available for an indigenous fruit processing operation, and preferably this should be citrus (calamansi) wastes.

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This project, therefore, aims to undertake the pilot scale production of USP grade pectin from calamansi fruit wastes. To determine the optimum operational conditions of the pilot plant and to produce pectin that meets both USP and Philippine quality standards are the other objectives.

EXPERIMENTAL

A. RAW MATERIALS

Calamansi Fruit Wastes (Fresh/Dried)

Demineralized Water

Concentrated Hydrochloric Acid

95% Isopropyl Alcohol

B. PREPARATION OF RAW MATERIAL

Fresh calamansi fruit wastes (peel with pulp) were steam-distilled for 2 hours to remove the essential oil. The oil-free calamansi fruit wastes were then blanched in boiling water for 5 minutes. Approximately 2 parts water were needed for one part peel. Tap water was added to lower the temperature of the mass to below 60°C. The slurry was drained and rinsed. Most of the water-soluble components had been leached out and ready for the extraction of pectin or for drying for future use.

C. EXTRACTION OF PECTIN

The blanched, drained and rinsed calamansi fruit wastes were subjected to hydrolysis. It was carried out with 0.2% hydrochloric acid (pH of 1.6) at a temperature of 80°C for 30 minutes. Immediately after hydrolysis, the extract was cooled to 60°C and filtered to remove waste solids/insoluble matters from the liquid, then passed through a centrifugal separator to obtain a clear pectin liquor. The pectin liquor was then precipitated in isopropyl alcohol. One volume of concentrate or pectin liquor was added to 2 volumes of 95% isopropyl alcohol in fine streams and was mechanically stirred. The fibrous coagulum (pectin) was recovered from the liquor by passing this through a centrifugal basket. The pectin obtained was purified by 2 to 3 successive washings with the recovered isopropyl alcohol (75% IPA) and a final rinse was made with 95% isopropyl alcohol. The fibrous pectin was shredded, dried at a temperature not in excess of 60°C, ground to a fine powder, sieved and stored in clean, dry air-tight containers.

The schematic diagram for the extraction of pectin is shown in Fig. 1.

D. ASSAYS

Assay for methoxy groups, galacturonic acid and degree of esterification

Transfer 5.0 g of pectin to a suitable beaker, and stir for 10 minutes with a mixture of 5 mL of concentrated hydrochloric acid and 100 mL of 60 percent isopropyl alcohol. Transfer to a sintered-glass filter (30 to 60 mL crucible or Buchner type, coarse), and wash with six 15-mL portions of the hydrochloric acid - 60 percent isopropyl alcohol until the filtrate is free from chlorides. Finally wash with 20 mL of anhydrous isopropyl alcohol, dry for 2.5 hours at 105°C, cool and weigh. Transfer exactly one-tenth of the total net weight of the dried sample (representing 500 mg of the original unwashed sample) to a 250 mL conical flask, and moisten with 2 mL of alcohol. Add 100 mL of carbon dioxide-free water, insert the stopper, and swirl occasionally until the pectin is completely dissolved. Add 5 drops

of phenolphthalein TS, titrate with 0.1 N sodium hydroxide VS, and record the results as the **initial titer**. Add 20.0 mL of 0.5 N sodium hydroxide VS, insert the stopper, shake vigorously, and allow to stand for 15 minutes. Add 20.0 mL of 0.5 N hydrochloric acid VS, and shake until the pink color disappears. Add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS to a faint pink color that persists after vigorous shaking; record this value as the **saponification titer**.

Calculate the degree of esterification (DE), degree of carboxylation (DC), methoxyl (OCH_3) and galacturonic acid ($C_6H_{10}O_7$) contents by the following formulas:

$$DE = \frac{100 \times V_2}{V_t}$$

$$DC = 100 - DE$$

$$\text{Methoxyl units} = DE \times 190$$

$$\text{Carboxyl units} = DC \times 176$$

$$\text{Total Weight} = \text{Methoxyl units} + \text{carboxyl units}$$

$$\%\text{—}OCH_3 = \frac{DE \times 31}{\text{Total Weight}} \times 100$$

$$\%\ C_6H_{10}O_7 = \frac{MgC_6H_{10}O_7}{\text{Wt. of sample in mg.}} \times 100$$

$$Mg\ C_6H_{10}O_7 = 19.41\ V_t$$

Determination of Viscosity:

Weigh exactly 1 g. of pectin in an Erlenmeyer flask, moisten with 2 ml isopropyl alcohol and add 98 ml distilled water. Swirl occasionally until the pectin is completely dissolved. Determine viscosity of pectin solutions at 25°C using an Ostwald viscometer size #50.

Compute for viscosity in centipoise by the formula:

$$\text{Viscosity} = \frac{\text{Flow rate in secs.}_{\text{sample}} \times \text{Density}_{\text{sample}} \times \text{Viscosity}_{H_2O} \times 100}{\text{Flow rate in secs.}_{H_2O} \times \text{Density}_{H_2O}}$$

Determination of Setting Time and Temperature and Gel Strength

Sugar jellies were prepared by the following method: Mix sixty (60) grams sugar, 0.4 grams pectin and 39.5 ml water in a beaker. Heat the mixture to 106°C and 0.5 grams and citric acid was added. After mixing thoroughly the liquid was poured into aluminum molds. Identical jellies were prepared using the prepared pectin and 150 grade citrus pectin. The time elapsing between the finish of preparation of the sample mixture and the initial moment when the setting begins was observed. This is the setting time. Likewise, the temperature when the gel sets was noted and recorded as the setting temperature.

After 3 hours of setting at ambient temperature (30° to 35°C) the breaking strength or gel strength of the jellies was determined using the Precision Penetrometer.

Other Assays

Identification tests on the produced pectin, test for the presence of sugars and organic acids, loss on drying and determination of total ash and acid-insoluble ash were likewise carried out following the procedures specified in USP XXII.

RESULTS

The percentage breakdown of the physical composition of calamansi fruit is shown in Table I.

The physical properties of the pectin of ITDI produced from calamansi fruit wastes (fresh and dried) are shown in Table II.

Likewise, other parameters of ITDI pectin were analyzed and compared with the USP requirements. This is shown in Table III.

Results indicate that the produced pectin from both fresh/dried fruit wastes falls under high methoxy pectin with a degree of esterification higher than 50% as shown in Table III.

As shown in Table IV, the higher the setting temperature the shorter the setting time.

Another characteristic determined was the gel strength of the pectin obtained from the fresh and dried fruit wastes of calamansi and the results are shown in Table V.

DISCUSSION

The production of pectin is a straight forward process consisting of extraction, purification and precipitation. The process conditions are a compromise designed to give the largest yield of the highest quality in as short a time and as cheaply as possible. Pilot scale production of USP grade pectin is presented in Figures 2 to 13.

The raw material for extraction is either the fresh or dried calamansi fruit wastes. If the fresh material is used, it must be quickly extracted to prevent enzymatic destruction of the pectin. The advantages of using the dried-fruit wastes is that the pectin plant can operate all year and not just in the main harvest period. Also, dried feedstocks can be stored to a moisture content between 6 and 10%, and this can be sold to other pectin industries who may need a constant supply of the raw material.

The bleaching process prior to extraction of oil/drying removes soluble solids (mainly sugars) and yields a product which can be easily dried.

It was found that the fruit waste materials could be satisfactorily dried using forced convection oven drying at 60°C for 2 to 4 hours, see Fig. 10, and this did not significantly affect the quality of pectin extracted as shown in Table III.

Comparison of the specifications of a U.S.P. Grade Pectin and the pectin produced is shown in Table III. Examination of the given specifications reveal that the ITDI Pectin conformed with the U.S.P. requirements.

It was observed that the higher the degree of esterification, the higher the setting temperature and the shorter the setting time as shown in Table IV.

Pectin solutions exhibit low viscosities but their commercial importance is due to its ability to form gels.

Gelation is interdependent of the following factors (Harvey, 1950; Kertesz, 1951):

1. Soluble Solids: As the amount of sugar increases, the temperature and the speed of gelation increases, no gel can be obtained under 55% of soluble solids.

2. Lowering of the pH - value has the same effect; the optimum pH-value (bet. 3.3 and 2.6) is a function of the degree of methylation (DM).

3. Degree of esterification (DE).

Possibilities of pectin production in the Philippines - The process used for producing commercial pectin has been improved. The economic viability of the modern process, which employs an advanced technology, is based upon a readily available and cheap source of pectin, which is the calamansi fruit wastes. This is the by-product of a fruit processing factory. In cases where this material cannot

be used immediately, this can be dried under controlled conditions to prevent deterioration and microbial contamination which is of particular importance in the production of pectin.

The use of organic solvents as precipitants for producing solid pectin is costly unless efficient recovery can be assured.

In the final stages of solids - liquid separation in which the precipitated pectin is recovered, hand-operated hydraulic presses could provide the alternative to high-cost capital equipment such as centrifuge separators. They are comparatively trouble-free, easy to service, and ideal for small batch-production.

Constraints on the production of pectin are:

1. Extraction of pectin has to be a large scale to be economical, usually insufficient material is available or it is seasonal.

2. The raw material source has to contain sufficient pectin.

3. The activity of (pectin) enzymes, naturally present in fruits during storage, may degrade pectin during the preparation and initial extraction of the pectin from the waste product.

4. Problems of handling the large amounts of raw material needed.

5. The large volumes of pectin solution produced.

6. The methods of precipitating pectin.

7. The cost of equipment and chemicals.

The economic constraints on pectin production maybe due to the cost of erecting the plant, the competitive price of pectin on the market, and the astringent food/pharmaceutical commodity regulations.

Where the multi-stage process is not possible, part production maybe, for example, the dehydration of the fruit wastes, where only some form of drying is needed. This may attract capital from pectin producers overseas, provided the dried product could fulfill the requirements of an agreed specification. A benefit of such pre-treatment is the lower cost of transport as waste fruit products contain large quantities of water; less of this heavy constituent would reduce transport costs and facilitate easier movement.

SUMMARY AND CONCLUSION

In the first instance, samples of calamansi fruit wastes (fresh/dried) were evaluated by ITDI to establish the characteristics of the pectin obtained.

Until lately, the introduction of new food/pharmaceutical/cosmetic products containing a high proportion of pectin led to an unprecedented pressure on world processing capacity. This can be alleviated when a new local plant comes into operation. It is essential however, that any newly established pectin manufacturer offers his/her product at a competitive price, it must be of commercially acceptable quality and be regularly available.

The success of any new major use for pectin will depend on this improved technology, and we can look ahead to a great increase in demand.

ACKNOWLEDGMENT

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Table 1. The percentage breakdown of the physical composition of Calamansi Fruit

COMPONENT	PERCENT (%)
Juice	31
Pulp	20
Seed	19
Rind	30

Table 2. Physical Properties of ITDI Pectin

PARAMETERS	VALUE/DESCRIPTION	
	FRESH	DRIED
Solubility (25°), % by wt.	1 g/20 mL	1 g/20 mL
Color	Beige	Beige
Odor	Odorless	Odorless
Taste	Slightly Acidulous Taste	Slightly Acidulous Taste

Table 3. Conformance of ITDI Pectin with U.S.P. Requirements

PARAMETERS	CALAMANSI PECTIN		U.S.P. REQUIREMENTS
	FRESH	DRIED	
Loss on Drying	6.19%	4.94%	n.m.t. 10%
Ash (Total)	2.98%	3.66%	n.m.t. 10%
Methoxyl content	11.32%	12.64%	n.m.t. 6.7%
Galacturonic acid Content	87.74%	78.23%	n.l.t. 74%
Degree of esterification	73.20%	76.08%	n.l.t. 50%
Sugars and Organic Acids	20.65 mg.	21.0 mg.	n.m.t. 20.0 mg.
Acid-insoluble Ash	0.847%	0.106%	n.m.t. 1.0%
Viscosity of 1% Solution at 25C (Ostwald Viscometer)	4 cps.	50 cps.	not specified

*n.m.t. - not more than
*n.l.t. - not less than

Table 4. - Comparison of the setting temperature and time of ITDI Pectin and USP grade pectin

	DE	TEMP. (°C) started to gel	TEMP. (°C) complete setting	TIME (min.)
USP Grade pectin from UNILAB (United Laboratories)	74.61%	77°C	65°C	10
ITDI pectin				
Fresh	73.20%	75°C	60°C	15
Dried	76.08%	80°C	70°C	5

Table 5. Penetrometer readings and total soluble solids content of ITDI pectin and USP grade pectin

	% PECTIN	% TOTAL SOLUBLE SOLIDS	GEL STRENGTH
USP Grade pectin from UNILAB (United Laboratories)	0.55%	75	26.8 mm
ITDI Pectin			
Fresh	0.55%	73	35.0 mm
Dried	0.55%	79	28.3 mm

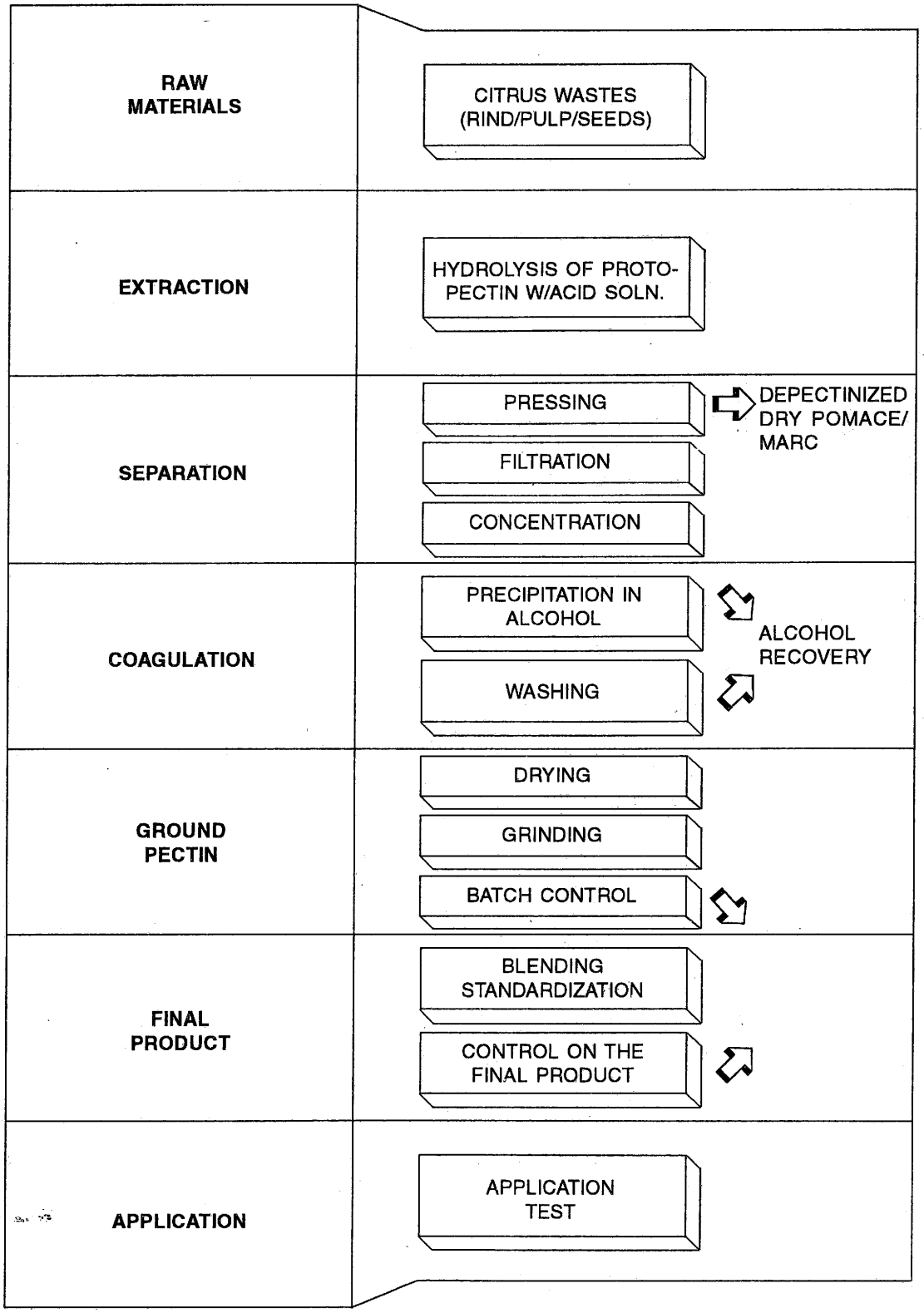

Figure 1. Procedure for the Extraction of Pectin

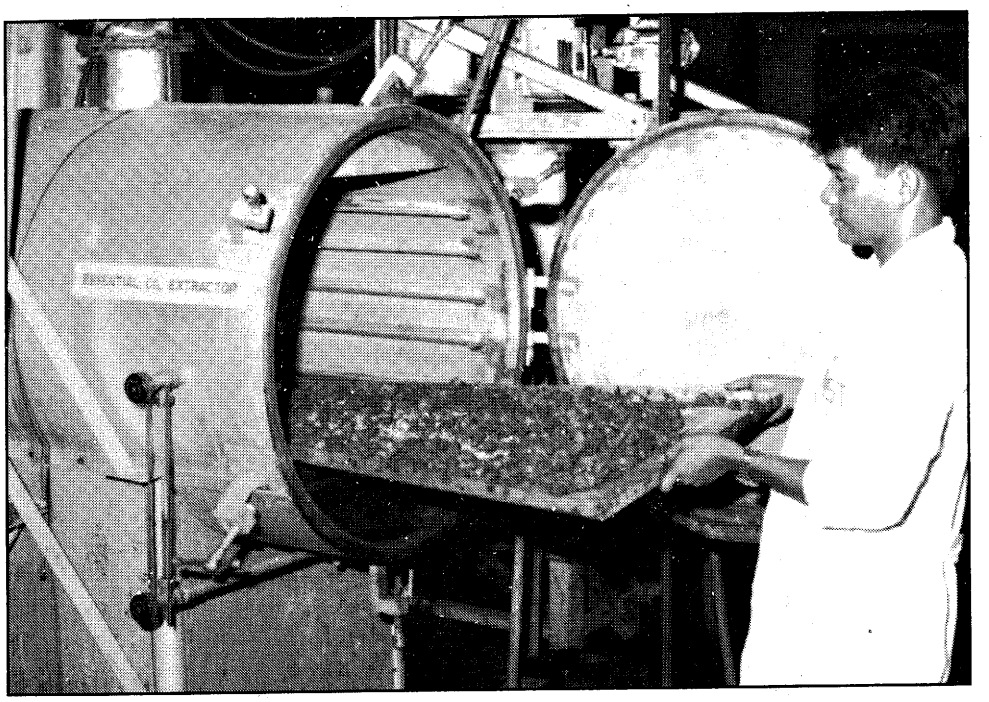

Figure 2. Loading Leached Calamansi Fruit Waste in Essential Oil Extractor.

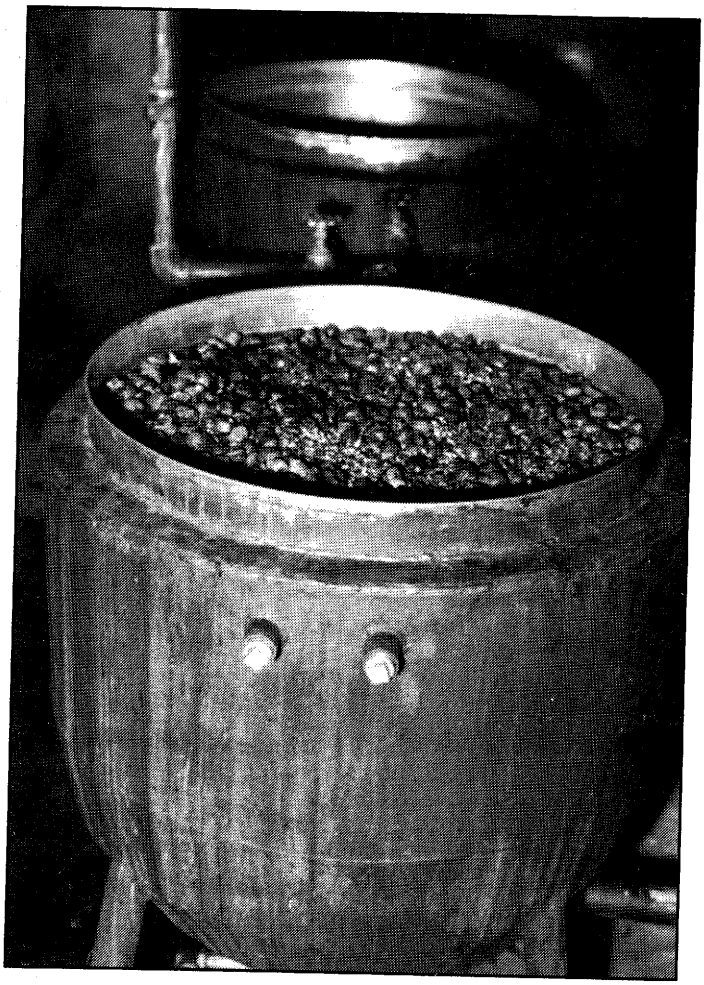

Figure 3. Fruit Wastes in the Acidification/Solubilization Tank.

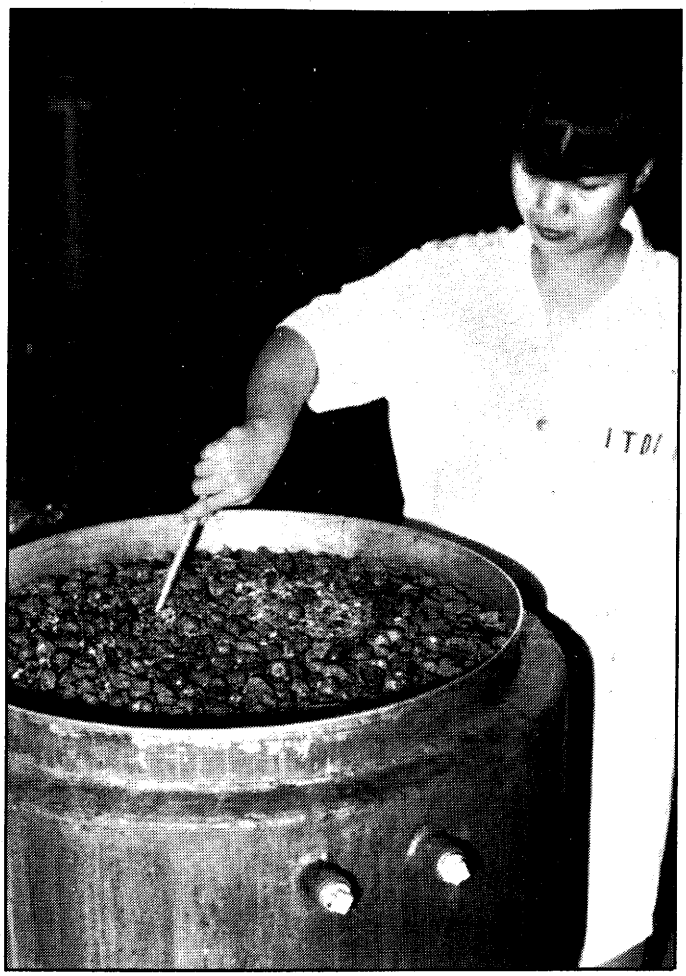

Figure 4. Stirring of Acidified Calamansi Fruit Wastes While Heating at 80°C for 30 minutes.

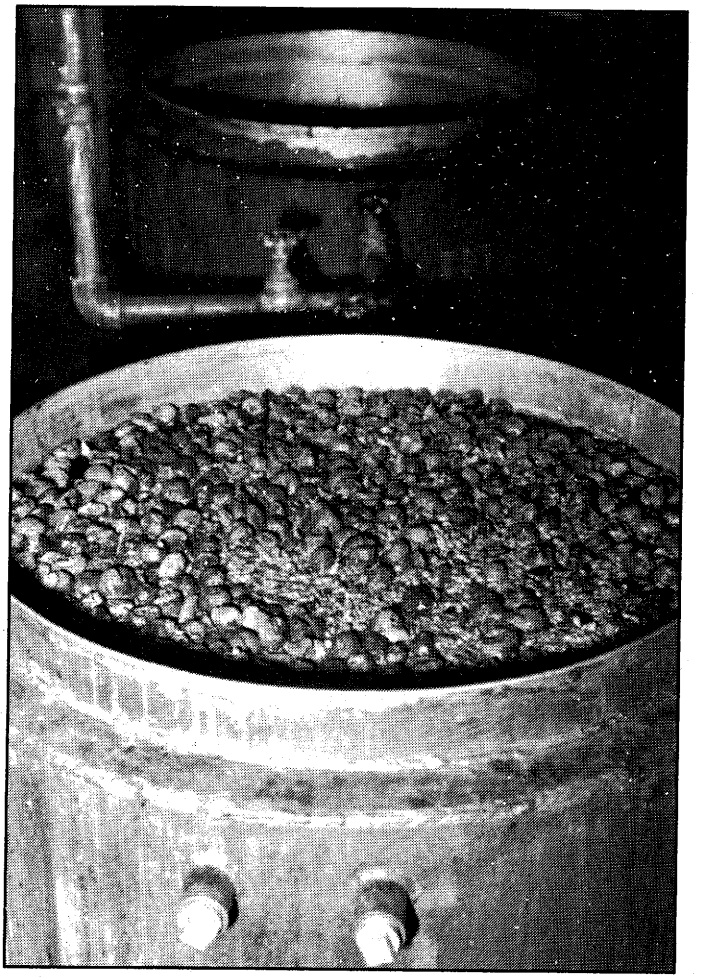

Figure 5. Solubilized Calamansi Fruit Wastes Prior to Filtration.

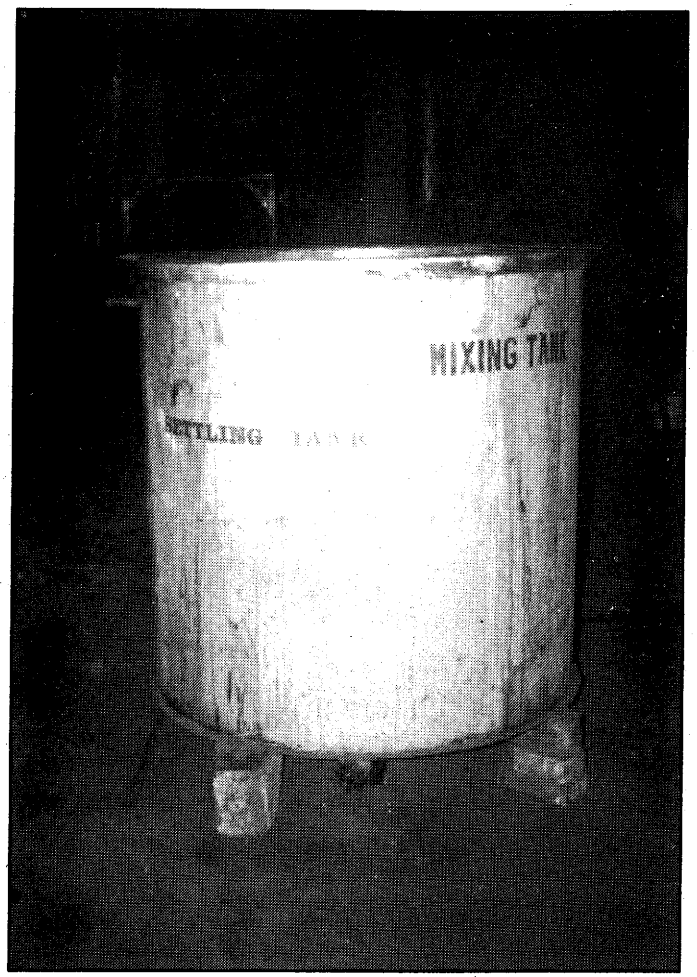

Figure 6. Precipitation of Pectin in 95% Isopropyl Alcohol (Precipitation tanks are made of stainless steel).

Figure 7. Precipitated Pectin Passed Through Centrifugal Separators.

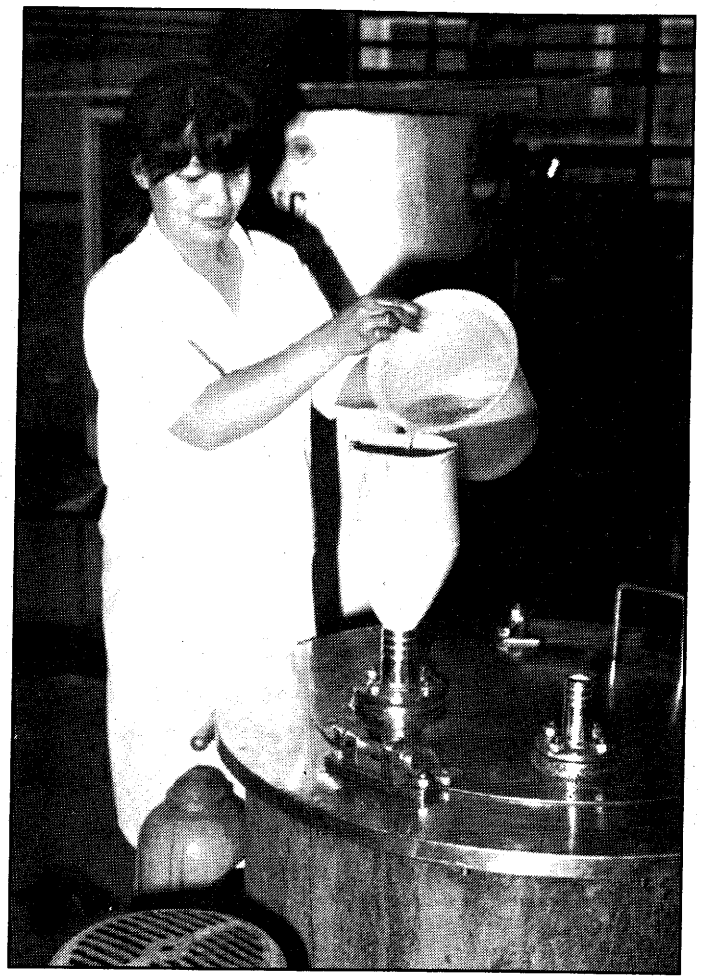

Figure 8. Pectin Washed 3x to Remove Impurities.

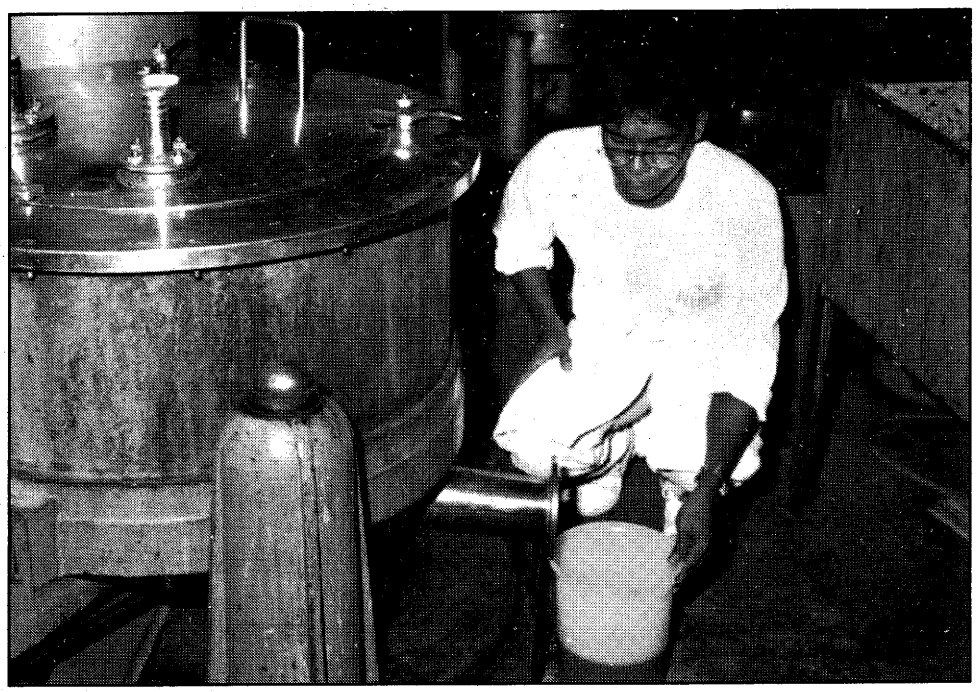

Figure 9. Used Isopropyl Alcohol Being Retrieved.

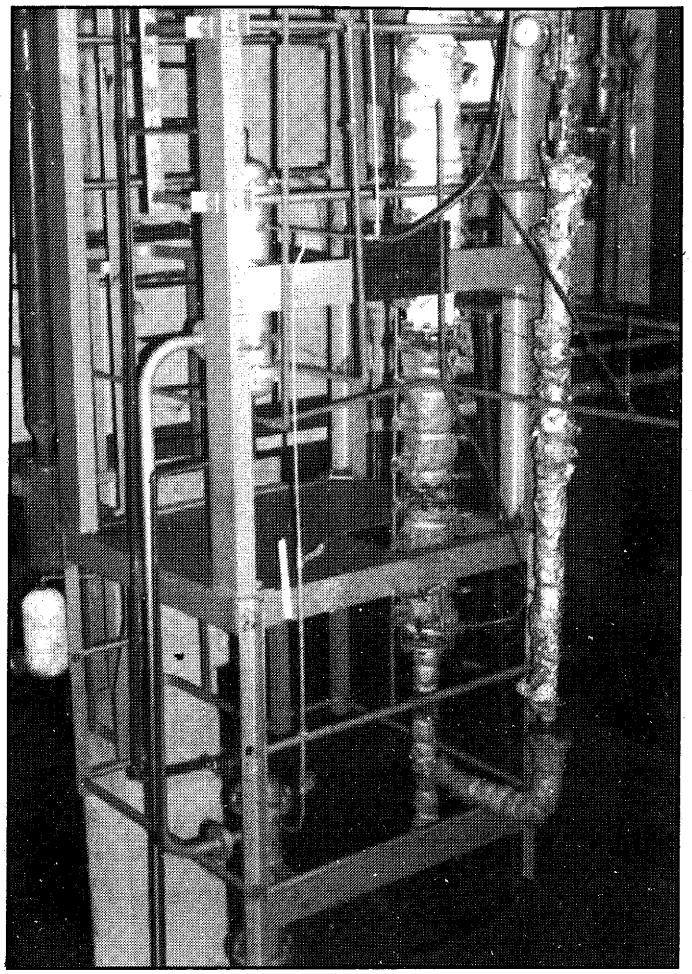

Figure 10. Distilling Retrieved Isopropyl Alcohol.

Figure 11. Drying of Extracted Pectin at 60°C in Fabricated Drying Oven.

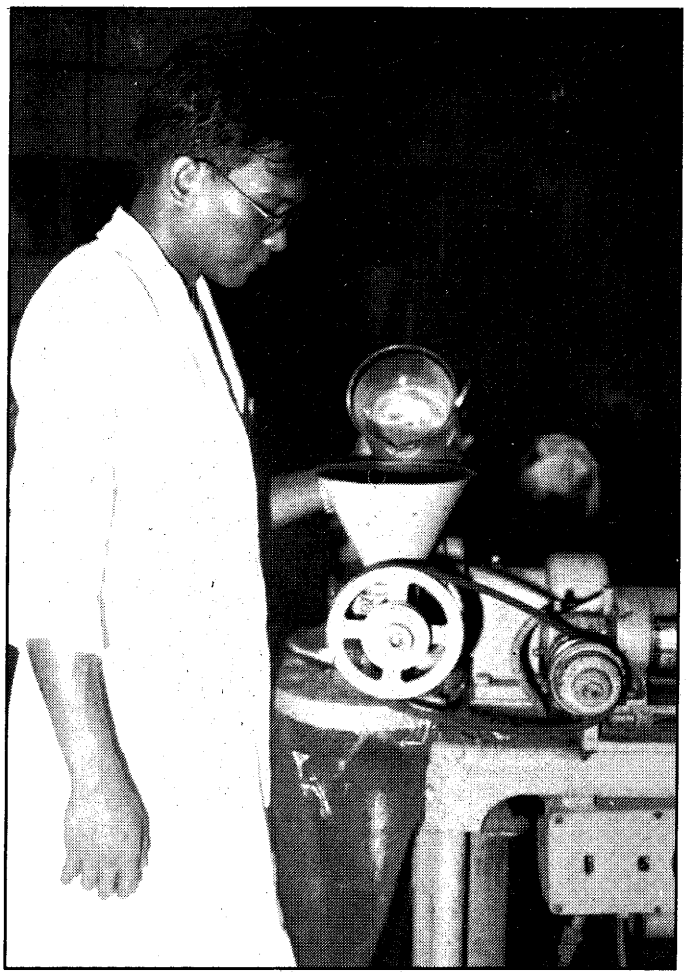

Figure 12. Grinding of Dried Pectin.

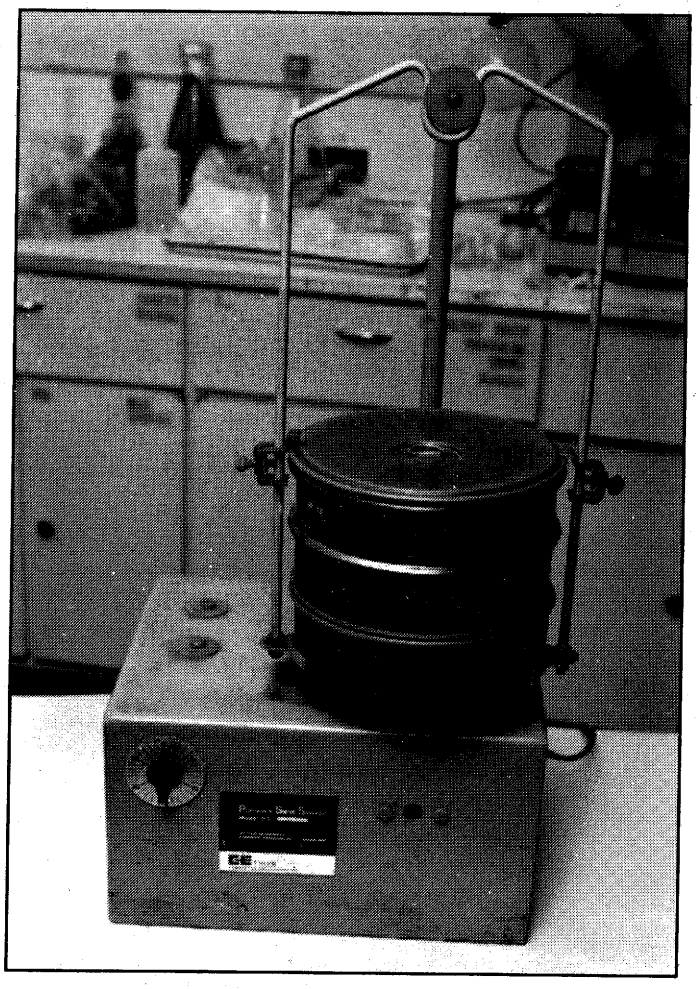

Figure 13. Ground Pectin Passed Through Sieves Using a Sieve Shaker.