

# Fractionation and Physicochemical Properties of Pectic Substances Extracted from Grapefruit Peels

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## Abstract

The aim of this work was to extract pectic fractions from grapefruit peels. The Alcohol Insoluble Solids (AIS) were prepared from peels and fractionated sequentially with hot distilled water, ammonium oxalate and hot 0.05 HCl. Water, oxalate and acid-extracted peels pectin were obtained and compared in terms of yield, physicochemical properties and neutral sugars. The results showed that the major part of grapefruit peels pectin was extracted in acid fraction. The water soluble fraction gave higher moisture and ash contents and exhibited higher methoxyl content compared to other fractions. The acid soluble fraction afforded higher degree of esterification, anhydrouronic acid, equivalent weight, intrinsic viscosity and molecular weight. Based on degree of esterification and methoxyl content all fractions can be categorized as high methoxyl pectin. The investigation of neutral sugars using TLC showed that all fractions contained similar sugars arabinose, galactose, rhaminose and xylose. Depending on the result obtained it could be concluded that besides material source, pretreatment procedure, temperature, concentration and extractant type the extraction sequence are critical for best extraction conditions and the yield of pectin.

**Keywords:** Grapefruit; Peel; Fractionation; Characterization; Pectin

## Introduction

Citrus peel and apple pomace are the major raw materials used for the production of commercially acceptable pectins [1]. Other sources such as mango peel [2-4] sugar beet residues [5], sunflower heads [6] and cactus (*Opuntia* spp.) [7] Have been considered for the extraction of commercial pectins. In Sudan grapefruit (*Citrus paradisi*) is one of the tropical fruits with an important production (total production of 65000 million metric tons) [8]. Sudanese grapefruit is well known for its large size, excellent quality and good coloration. A productivity as high as 40 tons per hectare is obtained [9]. The post-harvest loss which represents about 22% of the production is due to a short period of high production associated to a high perishability of the fruit. Processing of grapefruit into jam and upgrading of the byproducts could constitute a way to reduce this loss. Grapefruit peels represent about 16–19% of the total weight of the fruit [10]. These peels are most of the time thrown into nature or used for animal feeding. They have been reported to be a potential source of pectins [11], fibers [12] and polyphenols. Grapefruit waste contains about 37.5% pectin, 17.2% soluble sugars, and 14.3 holocellulose of its dry weight [13]. The pectin composition varies with the source from which it is isolated, as well as with conditions used during isolation and purification. The industrial demand for pectin with varying ability to gel or stabilize fruit and dairy products increases the need for accessing pectin of different types or pectin derivatives with tailored properties [14].

Pectin is a polysaccharide consisting mostly of two moieties. These are homogalacturonan, (1-4) linked,  $\alpha$ -Dgalacturonic acid and its methyl ester; and rhamnogalacturonan I, (1-2) repeating linked,  $\alpha$ -L-rhamnose-(1-4)  $\alpha$ -Dgalacturonic acid disaccharide. Rhamnogalacturonan II contains arabinan, galactan and arabinogalactan side chains. These monosaccharide units comprise most of sugar units found in pectin [15]. Pectin occurs as a white to light brown powder or granular, and odorless or has slightly characteristic odor. According to the FAO [8], pectin is considered to be safe additive that can be taken daily without limits. Pectin have been used in food industry as gelling, thickening and stabilizing agents [16]. The composition, structure and physiological properties of pectin might be influenced by conditions of extraction as well as sources, location and many other environmental

factors [17]. Therefore, sequential extraction by using various chemical agents such as EDTA, CDTA, ammonium oxalate, sodium carbonate, sodium hydroxide and hydrochloric acid has generally been used for the fractionation of pectins [18,19]. Enzymes and strong acids are also commercially used in the extraction of pectin [20-22].

The extraction of pectin basically involve the aqueous extraction of pectin from the raw material (plant), the isolation of the extracted pectin and purification, followed by drying process. The pectin extraction process should use a suitable method to obtain the maximum yield and quality of pectin. This research work was initiated to evaluate the impact of different extractant on the yield of pectic fractions of grapefruit peels and to investigate their physicochemical properties.

## Materials and Methods

### Materials

**Raw material:** Fresh grapefruits were purchased from local market three kilogram for each type white and red. The peels were cut into small pieces and dried at 550°C in oven for 48 hrs [23]. All the chemicals and reagents were of analytical grade.

### Methods

**Proximate chemical analysis:** Dry matter was determined by using the method of AOAC [24] by drying samples at 105 °C for 12h.

Ash content was determined by measuring the residue remaining after incinerating the sample overnight in a muffle furnace at 600°C [25].

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Protein content was determined by the kjeldahl method then crude protein was calculated by multiplying the N value with the factor 6.25.

Crude fat was determined by extracted the dried sample with petroleum ether (boiling range 40-60°C) in Soxhlet extraction apparatus for 6 hr. The ether extract was filtered in pre-weighed beakers, petroleum ether was evaporated completely from the beakers and the increase in weight of beaker represented the fat (AOAC, 1997).

Crude fiber content was determined by using the method of AOAC [26] samples were digested with 200 ml (0.225N) H<sub>2</sub>SO<sub>4</sub> acid and 200 ml (0.312N) of NaOH solutions and the residue calcined. The difference in weight after calcination indicates the quantity of fiber present.

Total carbohydrate (nitrogen free extract) was determined by difference.

All the results were expressed on a dry weight basis. All the experiments in this study were conducted in duplicate.

**Determination of Alcohol Insoluble Solids AIS:** Alcohol insoluble solids were determined by method described in AOAC (1980) [27]. Twenty grams from each type of grape fruit peels were weighed into a 600 ml beaker then 300 mls of 95% alcohol were added, stirred, brought to boiling, simmered for 30 min and then filtered through Buchner fitted with filter paper which was dried previously in a bottomed dish for 2 hours at 100°C, covered with fit cover and weighed. The residue then washed with 80% alcohol until washings are clear and colourless. The paper was then transferred to the previous dish and dried at 100°C for 2 hours. The final weight minus first weight was recorded as weight of alcohol insoluble solids and its percentage was then calculated as follows:

$$\text{AIS\%} = \frac{\text{final weight} - \text{first weight}}{\text{Weight of sample}} \times 100$$

**Determination of Calcium and Magnesium:** The calcium and magnesium were determined by the method of Elmer [28]. One gram of the peel is ignited in a muffle furnace at 500°C overnight. The contents were dissolved into 5 ml of 20% HCl, the solutions were warmed and filtered through acid washed filter paper into 50 ml volumetric flask and made to volume with distilled water. From one milliliter of this solution, the amount of calcium and magnesium were determined spectrophotometrically at wave length of 422.7 nm and 285.2 nm respectively using Atomic Absorption Spectrophotometer (3110)

**Determination of total pectin:** This is carried out according to the method of Luh et al. [29]. Duplicate samples (0.1 gm each) of AIS were mixed with 300 ml of 0.05 of the sodium salt of Ethylene Diamine Tetra Acetic Acid (EDTA), treated with 1N NaOH to reach pH of 11.5, allowed for 30 min at room temperature and the pH was adjusted to 5.0 with 1N acetic acid. To this mixture, 0.1 gm pectinase was added and stirred for about an hour, diluted to 500 ml with distilled water, filtered through whatman No.1 first few filtrates were discarded. Two milliliters of the filtrate were diluted to 50 ml from which two milliliters were taken for colorimetric determination in spectronic 20 the absorbance of total pectin was measured at wave length 520 nm with known amount of galacturonic acid ranged from 0.5-3.5 mg/2 ml.

**Preparation of Alcohol Insoluble Solids AIS:** Alcohol insoluble solids were prepared according to the method of Luh et al. [29] the grapefruit peels were separated from fruit, sliced into small cubes. The cubes were treated with 400 ml 95% ethanol preheated to 70°C. The mixtures were kept for 10 min at 70°C and were cooled in water bath

and mixed in a blender for a minute. Then they were filtered through filter paper No.1 under suction, after which the insoluble materials were washed twice with warm 70% ethanol to remove sugars and 95% ethanol at 70°C for removing of alcohol-soluble low molecular weight substances and for inactivation of endogenous enzyme system. The alcohol-insoluble solids (AIS) from grapefruit peels, thus obtained were dried at room temperature then ground to pass through 60-mesh and stored at room temperature until use.

**Sequential fractionating extraction:** Peels pectin fractions were extracted according to the method described by Gordon et al. [30]. One hundred grams sample of the peels AIS were treated with distilled water (dH<sub>2</sub>O) at a ratio of 1:3 at room temperature for 2 h with continuous stirring and then filtered. Each residue was treated in the same way with 300 ml dH<sub>2</sub>O for 10 min and then filtered. The crude filtrates with water-extracted pectin (WEP) were obtained. The insoluble residue from the second water extraction was added to 300 ml. 0.75% ammonium oxalate solution. Two hours extraction was made and the mixture was filtered. The extractions were repeated in the same manner for 10 min. The combined filtrate contained the oxalate extracted pectin (OEP). Both residues were separately further extracted with 300 ml 0.05% HCl for 2 h at 80°C and continuous stirred then filtered. Each residue was treated in the same way with 300 ml 0.05% HCl for 10 min at 80°C and then filtered. The crude filtrates with acid-extracted pectin (AEP) were obtained. The crude extracts of various fractions were precipitated with two volumes cold 95% ethanol and left for an hour. Water extract pectin (WEP) were coagulated with acidic ethanol (0.05% HCl) then left for 12 h. The precipitated crude pectin were separated by filtration, washed once with 100 ml of 80% ethanol, then with 70% ethanol to a neutral pH and finally with 100 ml of acetone. Pectin were dried at 60°C in a laboratory dryer ground to pass a60 mesh and kept in separate labeled bottles for further analysis.

### Analysis and characterization of pectic fractions

Analyses were performed at least in duplicates

**Determination of ash and moisture contents:** The ash content was determined by weighing 1 g of pectin in a tared crucible and then heated in a muffle furnace at 600°C for four hours. The residue was cooled in desiccators and weighed to constant weight. In determining the moisture content, 1 g of pectin was weighed and dried at 100°C for four hours to a constant weight.

**Determination of ash alkalinity:** The ash was dissolved in 25 ml of 0.1N HCl heated gently and then titrated with 0.1N NaOH using phenolphthalein indicator. Then ash alkalinity calculated as the number of milliliters of acid required neutralizing one gram ash.

**Determination of equivalent weight:** The determination of methoxyl and AUA contents and the equivalent weight were conducted following the method described by Owens et al. [31]. The values of equivalent weights were used for calculating the anhydrouronic acid (AUA) content and the degree of esterification.

Equivalent weights were determined by weighing 0.5 g pectin in a 250 ml conical flask and moistening it with 5 ml of ethanol. One gram of sodium chloride was added to sharpen the end point. Free carbon dioxide distilled water (100 ml) and six drops of phenol red indicator were added. The mixture was then stirred rapidly to ensure that all the pectic NaOH until the color of the indicator changed to pink (pH 7.5) and persisted for at least 30 seconds. The neutralized solution was used for the methoxyl determination. The following equation was used to calculate the equivalent weight:

Equivalent Weight =  $\frac{\text{Weight of sample (mg)}}{\text{meq. of sodium hydroxide}}$

where

meq. of sodium hydroxide = normality x titer value

This titer is known as initial titer (IR) or free acid titer.

**Methoxyl content:** The determination of the methoxyl (MeO) content was performed by adding 25 ml of 0.25 N NaOH to the neutralized solution which was shaken thoroughly and allowed to stand for 30 min at room temperature in a Stoppard flask. Twenty-five milliliters of 0.25 N HCl was then added and the mixture was adjusted to pH 7.5 by adding 0.1N of NaOH (titer figure). The following equation was used to calculate the methoxyl content:

$$\text{Methoxyl content} = \frac{\text{meq. of NaOH} \times 31 \times 100}{\text{Weight of sample}}$$

Where:

Meq of NaOH = normality of NaOH x titre figure

31 = formula weight of methoxyl group.

**Anhydrouronic acid (AUA) analysis:** By using the values of the equivalent weight and the methoxyl content, the anhydrouronic acid content was calculated from the expression given below:

$$(\text{AUA}) = \frac{176 \times 100}{Z}$$

Where Z = Weight of sample meq. of alkali for free acid + meq. of alkali for methoxyl

176 = is the molecular weight of AUA and

**Degree of esterification:** The degree of estrification was calculated as follows:

$$\text{Degree of esterification} = \frac{\text{ST} \times 100}{\text{ST} + \text{corrected IT}}$$

Where ST = is the saponification titre

IT = is initial titre.

**Acetyl content:** Acetyl value of pectin samples was determined according the method adopted by Pippen et al. [32]. Pectin samples (0.5 g) were dissolved in 0.1 N NaOH solutions with stirring and allowed to stand overnight. The contents were diluted to 50 ml with distilled water and an aliquot (20 ml) was placed into the distillation apparatus. Twenty milliliters magnesium sulphate– sulfuric acid solution (100 g magnesium sulphate and 1.5 g of sulphuric acid diluted to 180 ml) were also transferred to distillation apparatus, distilled, and about 100 ml of distillate was collected. The distillate was titrated with 0.5 N NaOH using phenol red indicators. A blank distillation using 20 ml of the magnesium sulphate–sulfuric acid solution was carried out and the distillate was titrated. The acetyl content was calculated according to the following equation:-

$$\text{Acetyl content \%} = \frac{\text{net ml of NaOH} \times \text{normality of NaOH} \times 4.3}{\text{Weight of sample (g) in the aliquot}}$$

**Determination of neutral sugars:** Thin Layer chromatography (TLC) was conducted on a plate (20x20) samples and reference sugars (0.1) were dissolved in 10% isopropanol and were spotted. The plates

were developed by the ascending method using solvent n-butanol-acetic acid-distilled water in ratio (5:4:1 v/v). The sugar was detected by spraying the plates with allocating reagent prepared from (4 gm diphenylamine, 4 ml aniline, 20 ml orthophosphoric acid in 200 ml acetone) as described by Baily and Bourne [31-33].

**Viscosity:** Viscosity of pectin solution was measured by viscometer (Ostwald-Cannon-Fenske No.1098). Pectin samples (0.1 gm) of each was prepared in 50 ml of distilled water and the pH was adjusted to 4.8 by 0.1N sodium hydroxide. The solution was stirred for 2 h, then 15 ml of calgon were added and stirred for further hour. The pH then was adjusted to 6.0 with 0.1N acetic acid the solution was completed to 100 mls with distilled water in volumetric flask the viscosity was determined within an hour in room temperature. The efflux time was determined in the same instrument for the solvent of calgon. The relative viscosity was calculated as the ratio of the time of efflux for solution to that of solvent. To determine the intrinsic viscosity another three concentrations (0.15, 0.1 and 0.05 g/100 ml) were prepared as mention before. The relative viscosity was calculated. To get the intrinsic viscosity the ratio (mr-1/c) was plotted against c and extrapolated to zero to obtain the intrinsic viscosity

C = concentration

$\mu r$  = relative viscosity

The average molecular weight was determined from intrinsic viscosity data according to the equation:-

$$N = 1.4 \times 10^{-6} M^{1.34}$$

Where

N = intrinsic viscosity

M = molecular weight

## Results and Discussion

The moisture content of grapefruit peels was (75.25%, 75.37%) for white and red samples respectively which was in the range of 66-96% generally for fruit and vegetable and is known to be variable even in the same variety depending upon locality and other environmental factors [34]. The grapefruit peels had ash (1.5, 1.6%), protein (1.05, 1.15%) and crude fiber content (1.73, 1.82%). Quantities of calcium for both types were amounted to (0.69, 0.71) and for magnesium 0.17 mg/100 gm for both type respectively (Table 1). The AIS obtained from red fresh peels was 9.5 g/100, while 10.5 g/100 g from white fresh peels. The AIS taken from grapefruit peels contained (7.01, 7.17%) moisture, (3.27, 3.37%) ash, (0.06, 0.08%) protein, (1.83, 1.04 mg/100 gm) calcium, (0.17, 0.19 mg/100 gm) magnesium with total pectin amounted to 25.00 and 25.26%, lignin was (0.08, 0.09%) for red and white samples respectively (data not shown). The AIS was fractionated by sequential extraction, resulting in obtaining 3 pectic substance fractions such as WEP, OEP and AEP. In our study we obtained (6.38, 8.20) water pectin, (5.23, 6.92) oxalate pectin and (7.80, 11.20) acid pectin for the red and white types (Table 2). This result showed that the major part of grapefruit peel pectin is extracted in the acid fraction. Yapo et al. [35] carried out sequential fractionating extraction from AIS of industrial citrus peels (1:30 AIS: extractant) with dH<sub>2</sub>O, oxalate, hot HCl 0.05M, and cold 0.05 M NaOH. They obtained 5.8 g/100 g AIS water extracted pectin and 27.3 g/100 g AIS acid extracted pectin. In Georiev et al. conducted sequential fractionating on orange and lemon peels they obtained 10.94 g and 16.3 g water extracted orange and lemon peels pectin per 100 g AIS, and 18.44 g and 18.00 g acid extracted orange and lemon pectin

| Parameters (%)                | Grapefruit Peels Type |                   |
|-------------------------------|-----------------------|-------------------|
|                               | Peels of White Type   | Peels of Red Type |
| Moisture                      | 75.25                 | 75.37             |
| Ash                           | 1.50                  | 1.60              |
| Protein                       | 1.05                  | 1.15              |
| Oil                           | 0.20                  | 0.40              |
| Fiber                         | 1.73                  | 1.82              |
| Carbohydrate (by difference)  | 20.27                 | 19.66             |
| Reducing sugars               | 10.4                  | 10.2              |
| Alcohol Insoluble Solids(AIS) | 10.5                  | 9.5               |
| Calcium mg/100g               | 0.69                  | 0.71              |
| Magnesium mg/100g             | 0.17                  | 0.17              |

Each value is a mean of duplicate determinations

**Table 1:** Components of Grapefruit Peels

| Parameters (%)                  | Grapefruit Peels Type |                   |
|---------------------------------|-----------------------|-------------------|
|                                 | Peels of White Type   | Peels of Red Type |
| Water Soluble Pectin            | 8.20                  | 6.38              |
| Ammonium Oxalate Soluble Pectin | 6.92                  | 5.23              |
| Acid Soluble Pectin             | 11.20                 | 7.80              |

**Table 2:** Pectin Content of Water, Oxalate and Acid Fractions

| Parameters (%)                    | Water Soluble Pectin |          |
|-----------------------------------|----------------------|----------|
|                                   | White Type           | Red Type |
| Moisture                          | 8.21                 | 8.35     |
| Ash                               | 11.05                | 7.05     |
| Ash Alkalinity                    | 2.26                 | 2.05     |
| Methoxyl Content                  | 7.50                 | 8.04     |
| Acetyl Content                    | 0.55                 | 0.52     |
| Degree of Esterification          | 52.03                | 53.23    |
| Equivalent Weight                 | 298.000              | 305.000  |
| Anhydrouronic acid(AUA)           | 31.8                 | 31.8     |
| Protein                           | 0.06                 | 0.07     |
| Intrinsic Viscosity               | 1.5                  | 1.5      |
| Molecular Weight x10 <sup>4</sup> | 3.126                | 3.126    |
| Calcium mg/100g                   | 0.39                 | 0.36     |
| Magnesium mg/100g                 | 0.005                | 0.005    |

Each value is a mean of duplicate determinations

**Table 3:** Properties of Water Soluble Pectic Fraction

per 100 g AIS. Comparing above mentioned results with those obtained in this study, it could be concluded that besides material source and pretreatment procedure the extractant type, temperature, concentration and extraction sequence are critical for best extraction conditions.

### Analysis and characterization of pectic fractions

The water soluble fraction gave higher moisture (8.21%,8.35%) and ash (11.05%,7.05%) contents compared to the oxalate fraction(8.20%,7.75%) moisture and (5.7%,4.4%) ash which ranked secondly and the acid fraction which had lower moisture (6.20%,5.22%) and ash contents for white and red respectively, the results presented in Tables 3-5). These results agree well with findings of El shafie [36] for pumpkin pectic fractions who reported (8.6%,7.3%,6.8%) for water soluble fraction,(3.3%,3.4%,3.5%) for oxalate soluble fraction and acid fraction which gave lower ash content(3.1%,3.2%,3.3%). Also results supported by the highest value of ash alkalinity-measure of mineral constituent combined with organic groups-for water soluble fraction with value up to 2.26 meq NaOH/gm for white type. The maximum limit of ash for good gel formation and good pectin quality is 10%. Pectin should have as low as moisture content as possible for safe storage and

to inhibit the growth of microorganism that can affect the pectin quality due to the production of pectinase enzyme. The difference in moisture content of different pectic fractions could be due to the difference in hygroscopic nature of pectin with different degree of esterification. The water soluble fraction had highest methoxyl content (7.50%, 8.04%) compared to other fractions. This compatible with findings of El shafie [36] who obtained (5.42%, 5.69%), (4.49%, 4.68%) and (4.09%, 4.25%) for water oxalate and acid fractions respectively. Generally the water soluble fraction consists of substances of high methoxyl content [37]. The results indicated the acid soluble fraction ranked first in the degree of esterification (57.54%, 56.56%) followed by oxalate soluble fraction (53.26%, 55.34%) and water soluble fraction had lower value (52.03%,53.23%). This result is in contrast with findings of El shafie [36] who reported high degree of esterification for water soluble fraction followed by oxalate and acid fractions respectively. The degree of esterification for all fractions was above 50% indicating that the fractions were characterized as high methoxyl pectin. The equivalent weights obtained were used in the calculations of the % AUA and %DE. The acid soluble fraction gave high value of equivalent weights (1041.00, 1428.00) followed by oxalate (819.000, 1250.000) and lower value (298.000, 305.000) for water fractions for both type respectively. The differences in equivalents weight between the fractions might be due to the variability in non uronide materials [38]. The content of AUA indicates the purity of the extract pectin and suggested to be not less than 65% [39]. However, the AUA obtained under all extraction conditions

| Parameters (%)                    | Oxalate Soluble Pectin |          |
|-----------------------------------|------------------------|----------|
|                                   | White Type             | Red Type |
| Moisture                          | 8.20                   | 7.75     |
| Ash                               | 5.70                   | 4.40     |
| Ash Alkalinity                    | 1.56                   | 1.42     |
| Methoxyl Content                  | 6.8                    | 5.84     |
| Acetyl Content                    | 0.20                   | 0.20     |
| Degree of Esterification          | 53.26                  | 55.34    |
| Equivalent Weight                 | 819,000                | 1250,000 |
| Anhydrouronic acid(AUA)           | 30.40                  | 27.70    |
| Protein                           | 0.08                   | 0.07     |
| Intrinsic Viscosity               | 2.50                   | 2.50     |
| Molecular Weight x10 <sup>4</sup> | 4.677                  | 4.677    |
| Calcium mg/100g                   | 0.08                   | 0.15     |
| Magnesium mg/100g                 | 0.01                   | 0.02     |

Each value is a mean of duplicate determinations

**Table 4:** Properties of Ammonium Oxalate Soluble Pectic Fraction

| Parameters (%)                    | Acid Soluble Pectin |          |
|-----------------------------------|---------------------|----------|
|                                   | White Type          | Red Type |
| Moisture                          | 6.20                | 5.22     |
| Ash                               | 4.20                | 4.40     |
| Ash Alkalinity                    | 1.14                | 0.62     |
| Methoxyl Content                  | 3.07                | 2.45     |
| Acetyl Content                    | 0.61                | 0.60     |
| Degree of Esterification          | 57.54               | 56.56    |
| Equivalent Weight                 | 1041,00             | 1428,00  |
| Anhydrouronic acid(AUA)           | 33.9                | 36.04    |
| Protein                           | 0.13                | 0.09     |
| Intrinsic Viscosity               | 2.0                 | 3.25     |
| Molecular Weight x10 <sup>4</sup> | 3.981               | 6.310    |
| Calcium mg/100g                   | 0.09                | 0.07     |
| Magnesium mg/100g                 | 0.02                | 0.01     |

Each value is a mean of duplicate determinations

**Table 5:** Properties of Acid Soluble Pectic Fraction.



| Samples                | AUA   | Arabinose | Galactose | Xylose | Rhamnose |
|------------------------|-------|-----------|-----------|--------|----------|
| Water soluble pectin   |       |           |           |        |          |
| Red type               | 31.80 | *         | *         | *      | *        |
| White type             | 31.80 | *         | *         | *      | *        |
| Oxalate soluble pectin |       |           |           |        |          |
| Red type               | 27.70 | *         | *         | *      | *        |
| White type             | 30.40 | *         | *         | *      | *        |
| Acid soluble pectin    |       |           |           |        |          |
| Red type               | 36.04 | *         | *         | *      | *        |
| White type             | 33.90 | *         | *         | *      | *        |

\*indicates presence of the sugar

**Table 6:** Sugars separated by partial acid hydrolysis of pectic fractions.

was less than 65% this indicates the extract may not be sufficiently pure due to the possible presence of proteins, starch and sugars in precipitated pectin. The acid soluble fraction showed high (36.4%, 33.90%) AUA followed by water soluble fraction (31.8%, 31.8%) and finally the oxalate soluble fraction (30.4%, 27.7%). It was evident from the data generated on the acid soluble fraction higher purity comparable to other fractions, as higher AUA and lower ash content are the two criteria governing the purity of pectin [40]. The same pattern was appearing in the results of protein content in all fractions the acid gave higher protein content (0.13%, 0.09%) followed by oxalate (0.08%, 0.07%) and finally the water fraction (0.06%, 0.07%), coinciding with findings of El shafie [36]. The acetyl content was found to give higher score (0.61%, 0.60%) for acid fractions followed by water fractions which recorded (0.55%, 0.52%) and the lower value obtained by oxalate fractions (0.20%, 0.20%) for red and white types respectively. Ranganna [41] reported that the gelling capacity of pectin decreased with increase in the degree of acetylation. If acetyl group is present in pectin, it inhibits jell formation. Schultz [42] reported that samples containing 3.5%-4.0% acetyl gives weak gels while gelling power restored at levels around 2.4% acetyl. The acid soluble fraction realized high intrinsic viscosity (2.5-3.25 dl/g) followed by oxalate (2.5 dl/g) and finally water (1.5 dl/g) for red and white types respectively. The results are in agreement with findings of El shafie [36] who reported high value of intrinsic viscosity for acid and lower value of intrinsic viscosity for water soluble fraction. It is possible that the acid fraction was composed of molecules of originally high molecular weight and that part of the abundant calcium in these fractions is in the adsorbed form [43]. The acid soluble fraction contained (0.07-0.09) mg/100 g, while oxalate (0.08-0.15) mg/100 g and water contained (0.36-0.39) mg/100 g calcium and this explains the high ash content and high ash alkalinity in this fraction. Since the intrinsic viscosity is a function of a molecular weight, the highest values for molecular weight were recorded for the acid soluble fraction as shown in Table 5.

### Sugar composition of pectic fractions

The investigation of neutral sugars using TLC showed that all fractions contained similar sugars arabinose, galactose, rhaminose and xylose together with galactouronic acid (Table 6). The presence of sugars also confirmed by McCready and Gee [44] El shafie [36] Koubala et al. [45,46] and Georgiev et al. [47].

### Conclusion

Pectin was successfully extracted from grapefruit peels. Three different fractions were obtained after sequential fractionating, extraction with hot water, ammonium oxalate and diluted HCl. The HCl appeared to be the most effective extractant in solubilizing and releasing pectin from grapefruit peels compared to other extractant. The pectin extracted by acid has higher purity as compared to the

water and ammonium oxalate pectin fractions. However, the data obtained showed that extractant have important effects on the features of extracted pectic fractions of two types of grapefruit peels. The ability of individual pectic fraction to form gel is dependent on level of present galacturonic acid in extract in addition to the composition and structure of extracted pectic fractions. The pectic fractions extracted by different extractants were highly methylated, (more than 50%) which indicates their suitability in the food industries as thickening agent and stabilizer. The exploitation of grapefruit peels as a source of functional compounds and their application in food is a promising field requires inter disciplinary research of food technologists, food chemists, nutritionists and toxicologists. In the near future, we are challenged to respond to this research results.

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