Extraction And Characterization of Pectin From Guava Fruit Peel.

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Abstract

The main objective of this research was to determine a practical follow-up to the extraction of pectin from Guava Fruit Peel and to characterize it in a laboratory, aiming at establishing the optimum conditions for acid extraction and to explore its potential for commercial production of pectin. Pectin was extracted from Guava peel powder using two different acids (HCl and Citric Acid) and at three different temperatures, time and pH viz (65, 75 & 85°C), (30, 45 & 60 min), (2.0, 2.5 & 3.0 pH) respectively. Pectin yield extracted by using Hydrochloric acid and Citric acid as reagent medium varied from 3.87% to 16.8% and 2.65% to 11.12% respectively. The best extraction condition by both the extraction reagents showed higher in yield by using Hydrochloric acid at 85°C, 60 min, 2.0 pH. The isolated pectin using Hydrochloric acid and Citric acid as reagents contained 685.3 and 345.4 equivalent weight, 4.25 and 3.50% methoxyl content, 67.4% and 82.1% anhydrouronic acid respectively. The degree of esterification of extracted pectin showed 52.85% and 44.3% for Hydrochloric Acid and Citric acid respectively indicating pectin obtained is low methoxyl pectin. The ash and moisture content of isolated pectin using hydrochloric acid and Citric acid were found to be (1.8%, 3.2%) and (8.2%, 6.0%) respectively. The sensory quality of jelly developed by using pectin obtained by Citric acid Extraction was found to be the best.

Keywords: Equivalent weight, %DE, %Methoxyl content, Pectin yield, pH, Reagents, Time, Temperature, Anhydrouronic Acid, HCl, Citric Acid.

1. Introduction

Pectin is a family of complex variable polysaccharides extracted from the primary cell wall of higher plants. Chemically, pectin consists of linear polymers of D-α-(1-4) anhydrogalacturonic acid. Part of the carboxyl groups of the anhydro-galacturonic acid is esterified with methanol (Wosiacki, 1977). Vauquelin stated its chemical nature in 1790 and Braconnot showed the characteristic of gellification and gave it the name pectin (Berk, 1976). Pectin is widely used as a functional ingredient in the food industry due to its ability to form aqueous gels and has been used in jams and jellies, fruit preparations, fruit drink concentrates, fruit juice, desserts and fermented dairy products. It has also wide application in the pharmaceutical industries. It has also been used as a fat substitute in spreads, ice cream and salad dressings. Liu et al. (2006) reported that in terms of nutrition, pectin has been shown to lower blood cholesterol levels and low density lipoprotein cholesterol fractions, which is beneficial for human health. It is also stated that pectin may help decrease cancer tumour formation. According to Memorial Sloan-Kettering Cancer Centre, the pectin acts as ligand for galectin-3, a protein involved in cell growth and cell cycles. Elevated galectin-3 is associated with inflammation of the heart and cancer tumours. Commercial pectin is currently classified according to the degree of esterification (DE). There are three classifications of pectin: HM (high ester); LMC (low ester conventional) and LMA (low ester amidated) according to CP Kelco (2002). Currently commercial pectin’s are almost utterly derived from citrus peels. According to the literature, fresh weight of plant material accomplishes 0.5%-4.0%. 
Guava (Psidium guajava L.), which belongs to the Myrtaceae family, is a native of tropical America and is widespread throughout the tropical and subtropical areas (Chopda and Barrett, 2001). Guava is consumed fresh or made into processed products such as juice, nectar, puree, jam and jelly (Kashyap et al., 2001). Guava fruit not only has exotic flavor but also is a rich source of relatively low methoxylated pectin (50%) amounting to more than 10% of the dry weight. Commercial guava juice processing normally involves the use of pectinase enzymes to increase juice yield from pressed guava peel and pulp (Alkorta et al., 1998). Guava cake (peel, pulp and seeds) a by-product from juice production, accounts for 30% of the guava fruit weight and is commonly used as animal feed or fertilizer. However, very recent investigations indicate that guava peel and pulp can also be used as a new source of dietary fiber (DF) and antioxidant phenolic compounds (Jimenez-Escrig et al., 2001). The DF includes plant substances that resist the DF includes plant substances that resist the action of human digestive enzymes. Total DF is divided into two major fractions: water-soluble (pectin, gum) and water-insoluble (cellulose, lignin, some of the hemicellulose). The objectives of this work were to extract pectin from Guava Fruit Peel and to characterize it in order to observe the influence of some factors on the yield of pectin.

2. Materials and methods.

2.1 Sample collection
Guava Fruit was obtained from the local market of Mahewa, Naini, Allahabad. The guava fruit was washed with warm water to remove any adherent dirt and dust and then peeled. Peels were then cut into small pieces for efficient drying and were blanched for 5 minutes to inactivate the enzymes which may cause undesirable changes latter on. The peels were removed from blanching pan and were then treated with warm absolute ethanol for 25 minutes to remove oily substances from the peel. The Ethanol treated peels were then dried in a tray drier at 55°C for overnight. The Dried peels were then grinded to prepare fine powder which was then used for extraction.

2.2 Extraction of pectin
Extraction of Pectin was carried out by using two different acids viz Hydrochloric Acid and Citric Acid. 5g of powder was weighed and placed in a beaker, 250ml of water was added to the powder followed by the adjustment of pH to a desired value. For maintaining 2.0, 2.5 and 3.0 pH of extraction medium, required 15g, 10g and 7g citric acid (99.9% conc.) respectively. Likewise for maintaining the above three pH of extraction medium, required 1.0ml, 0.8ml and 0.5ml citric acid (72% conc.) respectively. Extraction was done by hot water bath procedure. Thereafter, the mixture was heated for each different pH medium of extraction while stirred at 65.75 and 85°C for each different time 30.45 and 60min. The hot acid extract was filtered through muslin cloth. For each acid, three different pH medium of extraction at three different range of time and temperature, extraction was carried out and collected the extract separately for further experiments. The filtrate was cooled to 4°C of temperature. 96% ethanol was added on equal volume basis of extract to the extract and kept for one hour. The ethanol caused cogulation of the pectin which was recovered by filtration. The cogulated pectin was put in petridishes and kept in tray drier at 55°C for 5 hours. The pectin obtained was then grinded for analysis.

3.0 Analysis and Characterization of pectin

3.1 Yield
The pectin yield was calculated by using Equation 1

\[
\text{Pectin (g/100g)} = \frac{\text{Weight (g) of dried pectin}}{\text{Weight (g) dried pecan powder taken for extraction}} \times 100
\]

3.2 Moisture content (Ranganna 1995)
5gm of sample in previously dried and tared dish was weighed and placed in hot air oven for 2 hours at 130°C. The dish was removed and cooled in a decicator and then weighed.

\[
\text{Moisture content} = \frac{W_2 - W_1}{W_1} \times 100
\]

Where,
- \( W \) is Weight of petridish (g), \( W_1 \) is Weight of petridish with sample (g), \( W_2 \) is Weight of petridish with dried sample (g).

3.2 Ash content (Ranganna 1995)
Weighed 1.2g of pectic substance (sample). The sample was ignited slowly, then heat for 3-4 hr at 600°C. Then cooled the crucible to room temperature in a desiccators and weighted properly. The process will be weighted till constant weight come and final weight will be noticed.

\[
\text{Ash} = \frac{W_2 - W_1}{W} \times 100
\]

Where,
3.3 Equivalent Weight:
Equivalent weight is used for calculating the
anhdroxyuronic acid content and degree of esterification. It
is determined by titration with sodium hydroxide to pH 7.5
using either phenol red or Hinton’s red indicator.
Equivalent weight was determined by Ranganna’s method
(1995). 0.5 g sample was taken in a 250 ml conical flask
and 5 ml ethanol was added. 1 g of sodium chloride to
sharpen the end point and 100 ml of distilled water were
added. Finally 6 drops of phenol red or Hinton’s indicator
was added and titrated against 0.1 N
NaOH. Titration point was indicated by purple color. This
neutralized solution was stored for determination of
methoxyl content.

\[
\text{Equivalent weight} = \frac{\text{Weight of sample} \times 1000}{\text{ml of alkali} \times \text{Normality of alkali}}
\]

3.4 Methoxyl content
Determination of MeO content was done by using the
Ranganna’s method (1995). The neutral solution was
collected from determination of equivalent weight, and 25
ml of sodium hydroxide (0.25 N) was added. The mixed
solution was stirred thoroughly and kept at room
temperature for 30 min. After 30 min 25 ml of 0.25 N
hydrochloric acid was added and titrated against 0.1 N
NaOH to the same end point as before like in equivalent
weight titration.
The methoxyl content or degree of esterification is an
important factor in controlling the setting time of pectins,
the sensitivity to polyvalent cations, and their usefulness in
the preparation of low solid gels, fibres and film. It is
determined by saponification of the pectin and titration of
the liberated carboxyl groups.

\[
\text{Methoxyl content} \% = \frac{\text{ml of alkali} \times \text{Normality of alkali} \times 3.1}{\text{Weight of sample}}
\]

3.5 Anhydrouronic Acid Content.(Ranganna)
Pectin which is partly esterified polygalacturonide, contains
10% or more of organic material composed of
arabinose, galactose and perhaps sugars. Estimation of
anhydrouronic acid content is essential to determine the
purity and degree of esterification, and to evaluate the
physical properties. When the Equivalent Weight and
methoxyl content of pectin is known, its AUA was
calculated as follows.

\[
\text{AUA} = 176 \left( \text{M.Eq. of alkali free acid} + \text{M.Eq. alkali for saponification} + \text{M.Eq. for ash} \right)
\]

\[
\text{Weight of sample}
\]

3.7 Degree of Esterification
The degree of esterification (DE) of pectin was determined
using following equation.

\[
\% \text{DE} = \frac{176 \times \% \text{MeO}}{31 \times \% \text{AUA}} \times 100
\]

4.0 Results And Discussion
4.1 Effect of Different Acids on pectin yield
4.1.1. Effect on yield of pectin from Guava peel
using Hydrochloric Acid As Reagent For Extraction.
The % yield of pectin extracted by using Hydrochloric acid
from Guava peel powder (GPP) ranged from 3.87% to
16.8%. The percent yield was maximum (16.8%) for
Guava peel powder using Hydrochloric Acid acid at
treatment combination of 2.0 pH, 60min. and 85°C. The
yield was minimum (3.87%) at treatment combination of
3.0 pH, 30min. and 65°C.

The above results show that yield increases with increase
in the time and temperature as the protopectin naturally
present in cells takes time to solublise and come into the
solution.
4.2 Effect of various parameters on yield of pectin

4.2.1 The effect of extraction time, pH of solution and temperature on pectin yield extracted from GPP using HCl

The percent yield of pectin extracted from GPP using HCl at 2.0 pH for 30 min at temperature 65, 75 and 85°C are 7.6%, 8.8% and 10.0% respectively. Likewise at 2.0 pH for 45 min at temperature 65, 75 and 85°C are 14.8, 15.8 and 16.7% respectively. Likewise at 2.0 pH for 60 min at temperature 65, 75 and 85°C the % yield are 16.0, 16.5 and 16.8% respectively. These Results are shown graphically in fig 4.1.

![Fig.4.1](image)

**Fig.4.1 Effect of time and temp. on pectin yield at pH 2.0 HCl.**

The Yield (%) of pectin extracted from Guava Peel Powder at pH 2.5 using Hydrochloric Acid for 30 min at temperatures of 65, 75 and 85°C was found to be 5.8%, 6.37% and 6.70% respectively. Likewise at temperatures of 65, 75 and 85°C the % yield are 12.1%, 13.4% and 14.63% respectively. These results are shown graphically in fig 4.2.

![Fig.4.2](image)

**Fig.4.2 Effect of time and temp. on pectin yield at pH 2.5 HCl.**

The yield (%) of pectin extracted from Guava peel Powder at pH 3.0 using Hydrochloric acid, temperatures 65, 75 and 85°C for 30 min was found to be 3.87%, 4.85% and 5.37% respectively. At pH of 3.0, temperatures of 65, 75 and 85°C for 45 minutes of time pectin yield was found to be 5.67%, 6.37% and 6.70% respectively. Likewise at temperatures of 65, 75 and 85°C for time of 60 min, pectin yield was found to be 8.32%, 9.55% and 10.10% respectively as shown in fig 4.3.

![Fig.4.3](image)

**Fig.4.3 Effect of time and temp. on pectin yield at pH 3.0 HCl.**

4.2.2 The effect of extraction time, pH of solution and temperature on pectin yield extracted from GPP using citric acid.

The yield (%) of pectin from Guava peel powder (GPP) using citric acid at pH 2.0, temperatures of 65, 75 and 85°C for 30 min was found to be 6.2%, 7.0% and 7.2% respectively. At pH 2.0, temperatures 65, 75 and 85°C for 45 min, pectin yield (%) was found to be 7.5%, 7.8% and 8.6% respectively. Similarly for 60 min at temperatures of 65, 75 and 85°C yield (%) was found to be 10.15%, 10.70% and 11.12% respectively as shown in fig 4.4. There was less increase on the pectin yield for 30 and 45 min of extracted. This is shown in fig 4.4, as the temperature and time increases then more in the pectin yield. Lower pH values negatively affected the galacturonic acid content of pectin, but increased the pectin yield. Thomas et al., (2008)

![Fig.4.4](image)

**Fig.4.4 Effect of time and temp. on pectin yield at pH 2.0 Citric Acid.**

The yield (%) of pectin from Guava peel powder (GPP) using citric acid at pH 2.5, temperatures of 65, 75 and 85°C for 30 min was found to be 6.55%, 7.0% and 7.45% respectively. At pH 2.5, temperatures 65, 75 and 85°C for 45 min, pectin yield (%) was found to be 7.32%, 7.62% and
literature shows different ash content for different fruits like Kinnow (7.1-8.1%), orange peel (5.5%-6.5%). Soy hull (1.2-3.0%). The difference is due to different methodologies used by different researchers. The upper limit of ash content for good-quality pectin is considered to be 10% from the viewpoint gel formation. Therefore, with respect to this parameter, the pectin isolated in this study may be considered to be of satisfactorily good quality.

4.4 Moisture content.
The pectin extracted using Hydrochloric acid and citric acid has moisture content of 8% and 6% respectively. The above results are less than (12.44%) obtained by Neungnapa et al. The variation is due to difference in methodology used and acids used for extraction. Pectin should have low moisture content for safe storage and to inhibit the growth of microorganisms that can affect the quality due to the production of pectinase enzyme.

4.5 Equivalent Weight:
The Equivalent weight of pectin extracted from Guava peel powder ranged from 345.4 to 685.30 which is lower than 735-833 cited by Rangit Kumar Shaha et al. (2012). Pectin extracted using Hydrochloric Acid had higher equivalent weight than that of pectin extracted by using Citric acid. The lower equivalent weight could be higher partial degradation of pectin. The increased or decreased of the equivalent weight might be also dependent upon the amount of free acid (Ramli and Asmawati, 2011). The equivalent weight is used in the calculation of Anhydroarounic acid (AUA) content and Degree of esterification (DE).

4.6 Anhydroarounic acid content
The content of Anhydroarounic Acid (AUA) indicates the purity of the extracted pectin and is suggested to be not less than 65%. The AUA content of pectin extracted from guava peel powder using hydrochloric Acid and Citric Acid was found to be 67.4% and 82.1% respectively which shows that pectin extracted using citric acid is more pure than that of pectin extracted by using Hydrochloric Acid. The less AUA for Pectin extracted by using Hydrochloric Acid may be due to presence of sugars in the precipitated pectins. This problem can be decreased by using effective purification methods like metal-ion precipitation. Low value of AUA means that the extracted pectin might have a high amount of protein (Ismail et al., 2012).

4.7 Methoxyl content
The Methoxyl content of pectin extracted from Guava peel powder by using Hydrochloric Acid and Citric acid was...
found to be 4.25% and 3.50% respectively. The Methoxyl content reported in literature ranged from 1%-4% for pectin extracted from Kaffir Lime (Ranjit kumar Shaha et al.2013). The Methoxyl content is an important factor in controlling the setting time of pectins, their combining power with metallic ions and the ability of the pectin to form gels.

4.8 Degree of Esterification
The Degree of esterification of pectin extracted from pectin using Hydrochloric Acid and Citric Acid was found to be 67.8% and 53.4% respectively. From the above results pectin from Guava Peel powder can be categorized as high methoxyl pectin (HMP) because it has Degree of Esterification (%DE) that is higher than 50% . The Degree of Esterification reported in literature ranged from 62.55%-72.05% for passion fruit (Erika Kliemann et al. 2008 ), 76.30% for Citrus Maxima (Uthai Sotanaphun et al.2011), 31.05%-46.96% for Dragon Fruit (Norazelinia shah et al.2012 ). The types of pectin determine the mechanism for gel formation. Low Methoxyl Pectin (LMP) can form gels with the addition of low amount of sugar or without sugar or without addition of sugar in divalent cations. LMP produce gels independent of sugar content .They also are not as sensitive to pHs as HM-pectins are. The rate of gel formation is significantly affected by the Degree of esterification. A higher Degree of Esterification causes more rapid setting of gels.

5.0 Sensory Evaluation of organoleptic attributes
The pectin obtained from Guava fruit peel using HCl and Citric acid for extraction was used in the preparation of guava jelly . Three samples were prepared viz control with no added pectin , with pectin obtained using HCl and third with pectin obtained using citric acid. The pectin %age was fixed to 2% and was then presented to panel for judging, sensory attributes were evaluated using nine point hedonic scale. The jelly with pectin obtained using citric acid was the rated best as shown in the graph below.

Fig 4.7 Organoleptic score of three samples of jelly

6. Conclusions
In the present study, pectin was extracted successfully from Guava fruit using different Acids and extraction conditions. The results indicated that different extractants, pH, extracting temperature and time effect on the extraction yield of pectin. The best condition were, extracting temperature at 2.0 pH, time 60min and temperature 85°C using Hydrochloric Acid for Extraction giving yield of 16.8%. This study was intended to identify if guava fruit peel could be used as a potential source of pectin and if there is any, the optimum conditions could be determined. From the results obtained, guava peel gives a significant amount of pectin whereby it can be considered in commercial production of pectin along with other citrus sources.

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