PROCESS OPTIMIZATION OF PECTIN EXTRACTION FROM KAFFIR LIME (*CITRUS HYSTRIX*)

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ABSTRACT

Pectin was extracted from lemon peel under pH (1.2 and 2), temperatures (60°C and 100°C), time (60 min) and ethanol extract ratio (1:0.5, 1:1.0 and 1:1.5). Pectin extracted with distilled water was characterized in terms of yield, moisture content, ash content, equivalent weight, methoxyl content, pectin grade and degree of esterification. Preliminary results showed that the optimum condition for extraction of pectin was found at a temperature of 100°C, pH 2 and extraction ratio 1:1 on the basis of pectin yield extracted with distilled water. Data obtained were analyzed statistically using ANOVA (no blocking) at 5% level of significance, which showed that there was significant difference ($p<0.05$) between the yield of pectin extracted.

Keywords: Pectin, peel, ethanol extract, methoxyl content, esterification, *Kaffir lime*

INTRODUCTION

Pectin is defined as complex mixtures of polysaccharides that make up approximately one third of the cell-wall dry substance of most types of plants (Van Buren, 1991).Pectin is capable of forming gels with sugar and acid. Because of this gelling ability one of the well-known uses of pectin is in high sugar jams and confectionery jellies, dating back to at least the 18th century.Apart from that, it is a natural additive for foods andis now used as thickeners, water binders, and stabilizers. It is used in yogurts and pastry glazes and as a stabilizer in drinkable yogurts and blends of milk and fruit juices (Mayer, 1990).It is also being used as a texturizing fat replacer to mimic the mouth-feel of lipids in low-calorie foods and shorter chain galacturonic acids have been considered as clarification agents in fruit juices (Braddock, 1999).Pectin was first isolated and described in 1825 by Henri Braconnot, though the action of pectin to make jams was known long before this date. During industrialization, the makers of fruit preserves turned to producers of apple juice

to obtain dried apple pomace that was then cooked to extract pectin. In the 1920s and 1930s, factories were built that commercially extracted pectin from dried apple pomace and later citrus peel (Kalapathy and Proctor, 2001).Pectin is produced commercially from citrus peel and apple pomace.The extraction conditions vary from facility to facility and are dependent on the pectin source. Extraction most commonly occurs using a dilute mineral acid, usually hydrochloric, sulfuric, or nitric acids (Campbell, 2006).

The Kafir lime (*Citrus Hystrix*) is a small evergreen tree native to Asia. The tree's ellipsoidal yellow fruit is used for culinary and non-culinary purposes throughout the world, primarily for its juice, though the pulp and rind (zest) are also used in cooking and baking. Its juiceis about 5% to 6% citric acid, which gives lemons a sour taste(Kalapathy and Proctor, 2001).In the citrus fruits (like lime), pectin is mainly located in the albedo, the inner white layer of the peel that surround the juicy vesicles, and the lamellae. Hence, the peel and the albedo can be used to make the pectin (Braddock, 1999).

The significance of the study is to proper use of wastage of lime peel, to minimize the import of pectin and control the losses or damages of fruits and vegetables.This study was intended to identify if Kaffir lime peel could be a potential source for pectin source and if there is any, the optimum conditions could be determined. The overall goal of this project was to optimize the variables within extraction method in order to produce the highest yield of pectin from lemon peel.

MATERIALS AND METHODOLOGY

Material collection

Lemon was collected from local market of Dharan. Matured lemons with greenish yellow skin were taken.

Methods

Clean, non- rusted and sharp stainless-steel knives were used to cut the lemon.The collection, handling and storage of the dried peelwas done in clean glass containers. For the extraction of pectin, aluminum containers were used. A clean muslin cloth was used to filter the pectin.

Lemons were washed carefully with clean water and cut into slices (2-3 mm thickness) with a sharp knife. A juice extractor was used for juice extraction. After juice extraction the residue was dried at 60˚C for 24 hours in a temperature-controlledcabinet drier followed by grinding into powder by using a blender and sieved using sieve (20and 80 mesh size) to get fine powdered pectin. The obtained powder was then packed in low density polyethylene bag (thickness 75µm); sealed and stored at 6˚C for further study.

Extraction Method

Three hundred mL of deionized (DI) water was measured into a 1000 mL Erlenmeyer flask and maintained at the desired temperature (60℃ or 100℃) using the shaking water bath. Pressed peel (50 gm) was added to the water and then dilute acid was added to the peel-water mixture until the desired pH (1.2 or 2) was obtained. The mixture was agitated at a constant temperature until the desired extraction time (60 minutes) had elapsed. The pH (1.2 and 2) and temperature (60 and 100)˚C were recorded and the mixture was allowed to cool in an ice water bath until it reached 55˚C. The mixture was centrifuged at 1180×g (5050 rpm) for 10 min. The filtrate was vacuum filtered using Whatman filter paper and the solids were re-suspended in 400 mL of 60℃ DI water for 5 min.

The centrifugation and filtration steps were repeated. The filtered solutions were combined and approximate volume of ethanolas per ethanol extraction ratio (1:05, 1:1 and 1:1.5) was added for overnight precipitation. The pectin was separated from the alcohol solution by filtering through double layer of muslin cloth and the samples were washed three times with 70% alcohol and once with un-diluted alcohol to remove any impurities. The extracted pectin was dried under vacuum at temperature about 50˚C in aluminum sample dishes until all moisture was removed. Samples were cooled, weighed and ground using a mortar and pestle or a blender and then sieved (20and 80 mesh size) to get fine powders of pectin. The powdered pectin samples were stored in small plastic sample bags at 6℃ for further study (Campbell, 2006).

Characterization of extracted pectin

The dried pectin obtained was subjected to the following tests for characterization.

a) **Color:** Color of the extracted pectin was determined from the process given by Karki (1990).

b) **Equivalent weight:** Equivalent weight was determined by process given in Ranganna (1995). Equivalent wt. is used for calculating the anhydrouronic acid content and the degree of esterification.

c) **Methoxyl content (MeO)**: It was determined by method given in Rangana (1995).

d) **Moisture content:** Moisture content of pectin was determined by hot air oven method given by Ranganna (1995).

e) **Total Anhydrouronic Acid Content (AUA):** Estimation of anhydrouronic acid content is essential to determine the purity and degree of esterification, and to evaluate the physical properties. Total AUA of pectin was

obtained by the following formula (Mohamed and Hasan, 1995).

$$\%\, ofAUA = \frac{176 \times 0.1z \times 100}{W \times 1000}$$

$$W + \frac{176 \times 0.1y \times 100}{\times 1000}$$

where, z = ml (titer) of NaOH from equivalent weight determination, y = ml (titer) of NaOH from methoxyl content determination, W = weight of sample.

f) Degree of Esterification (DE): The DE of pectin was measured on the basis methoxyl and AUA content (Owens *et al.,*1952) and calculated by following formula.

$$\%D = \frac{176 \times \%MeO}{31 \times \%\text{AUA}} \times 100$$

g) Ash content: Ash content was determined by dry ashing method given by Ranganna (1995).

h) Pectin grades: The grading method given byRanganna (1995) was used for determining pectin grades.

i) Pectin yield: The pectin yield was determined according to process given in Ranganna (1995).

Two-way ANOVA (no blocking) was done for the data obtained from the yield of pectin in different conditions at 5% level of significance. For this LSD method (GenStat 5 Release 7.1 software program developed by Lawes Agricultural Trust, Rothamsted Experimental Station, 1985) was used and means of the data were subjected to test of significance by using Fisher's LSD (least significant difference) method at 5% level of significance.

RESULTS AND DISCUSSION

Extraction yield at different sets of temperature

Extraction yield at 60°C

At 60°C, pectin extraction was done for 6 samples namely A_1, B_1, C_1, D_1, E_1 and F_1. The yield is shown in Table 1.

Table 1 Extraction yield at 60°C

Sample	pH	ER	Product (gm)	(%) Yield
A_1	1.2	1:0.5	3.9	7.8
B_1	1.2	1:1	4.4	8.86
C_1	1.2	1:1.5	5.0	10.18
D_1	2	1:0.5	4.1	8.44
E_1	2	1:1	5.2	10.48
F_1	2	1:1.5	4.3	8.69

Extraction yield at 100°C

Also, at 100°C, pectin extraction was done for 6 samples namely A_2, B_2, C_2, D_2, E_2 and F_2. The yield is shown in Table 2.

Table 2 : Yield at 100°C

Sample	pH	ER	Product (gm)	(%) Yield
A_2	1.2	1:0.5	2.6	5.2
B_2	1.2	1:1	4.9	9.8
C_2	1.2	1:1.5	4.5	9.0
D_2	2	1:0.5	3.6	7.3
E_2	2	1:1	8.3	16.71
F_2	2	1:1.5	7.7	15.50

From the above tables (1 and 2), highest pectin yield was obtained at sample E_2 (pH 2, ER 1:1 at 100°C). The yield was 16.71% which was similar with amarelle peel pectin (13 to 17%) and mango peel pectin (4.6 to 18.5%) (Koubala et al., 2008). However, the yield of lemon peel pectin was higher than that as reported by Yapo, 2007 for passion fruit (7.5%) and lower than golden apple (22%) (Rha et al., 2011).

Physical and Chemical analysis.

The physical and chemical analysis was done for the sample with highest pectin yield i.e., Sample E_2 and is shown in Table 3.

Table 3 Physical and Chemical analysis of sample E_2.

Parameters	Results
Yield	
Wet basis	16.71%
Dry basis	20.21%
Moisture content	16.67%
Equivalent wt.	694.40
Methoxyl content	4.46%
Ash content	1.24%
Anhydrous acid content	65.15%
Degree of esterification	22.15%
Pectin grade	150
Color	Whitish grey
Odour	Disagreeable

The moisture content of extracted pectin was found to be 16.66%. This observation was comparable to that found by Ismail *et al.* (2012) who reported moisture content of dragon fruit pectin varied from 11.13 to 17.33%.The ash content was found to be 1.24%. It increases as the pectin yield decreases, indicating that the sugar content and others constituent increases significantly due to ripening of the fruit. Low ash content (below 10%) and maximum limit of ash content 10% are one of the good criteria for gel formation (Ismail *et al*, 2012). The Kaffir lime pectin produced in this study can be categorized as low methoxyl pectin (LMP) because it has a % Degree of Esterification that is lower than 50%.The AUA was found to be 65.15 which shows that the pectin was pure.

Variation in ER at temperature 60°C and pH 1.2

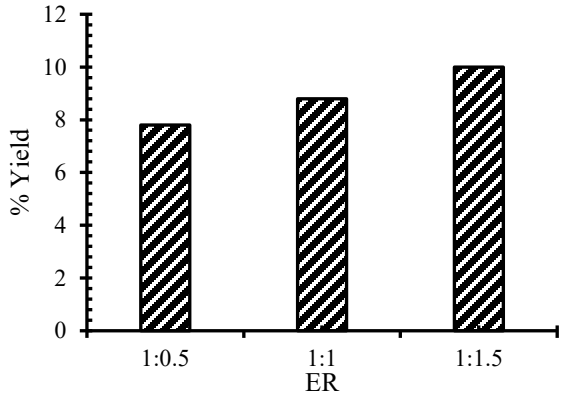

Figure1. Effect on extraction yield at temperature 60°C and pH 1.2

Figure 1 shows that mean score of samples A_1, B_1 and C_1 are 7.8%, 8.8% and 10% respectively. The yield % was significantly different between the samples extracted at 60°C, pH 1.2. So, sample C_1 was found to be best in terms of % yield since % yield was taken as the basis for best sample selection.

Variation in ER at temperature 60°C and pH 2.

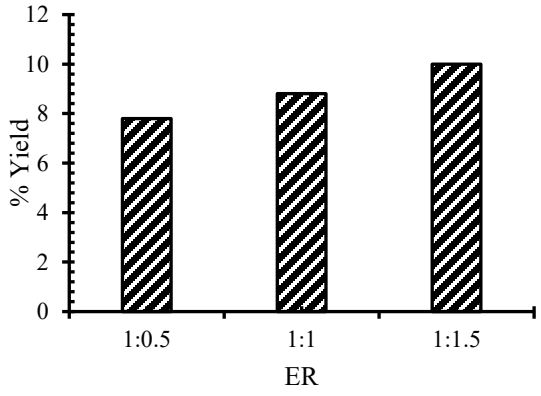

Figure2 Effect on extraction yield at temperature 60°C and pH 2

Figure 2 shows that mean score of samples D_1, E_1 and F_1 are 8.44%, 10.48% and 8.69% respectively. So, sample E_1 has the highest % yield than sample D_1 and F_1.There was significance difference between the samples. Thus, % yield was taken as the basis for best sample selection. So, sample E_1 was taken as best sample.

Variation in ER at temperature 100°C and pH 1.2

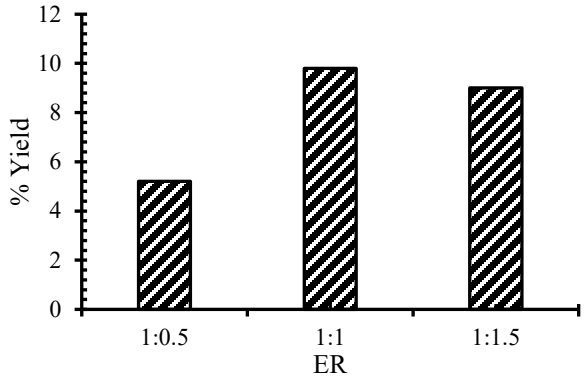

Figure3. Effect on extraction yield at temperature 100°C and pH 1.2

Figure 3shows that mean score of samples A_2, B_2 and C_2 was 5.2%, 9.8% and 9.0% respectively. The yield % was significantly different between the samples extracted at 100°C, pH 1.2. So, sample B_2 was found to be best in terms of % yield since % yield was taken as the basis for best sample selection.

Variation in ER at temperature 100°C and pH 2.

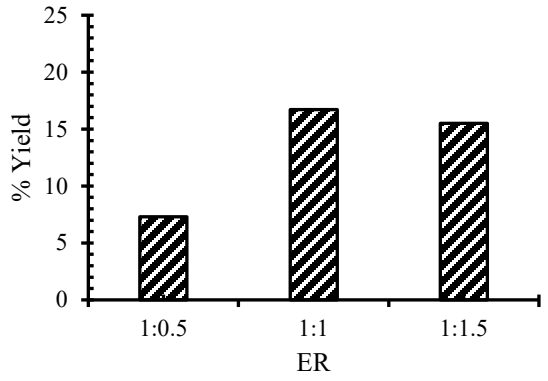

Figure 4 Effect on extraction yield at temperature 100°C and pH 2

Figure 4shows that the mean score of samples D_2, E_2 and F_2 was 7.3%, 16.71% and 15.50% respectively.The yield % was significantly different between the samples extracted at 100°C, pH 2. So, sample E_2 was found to be best in terms of % yield since % yield was taken as the basis for best sample selection.

Comparison between the samples

Comparison between the sample C_1 (extracted at pH 1.2) and E_1 (extracted at pH 2) was done statistically. The yield was found to be different. It showed Sample E_1 was best among the other extracted at 60℃. Between sample B_2 and E_2 yield was found to be different. It showed thatsample E_2 was best, at 100℃, among others. Finally, comparison between sample E_1 and sample $E_2$was statistically done. Here, % yield was taken as the basis for best sample selection. So, sample E_2 was found to be best among all the other samples.

CONCLUSIONS

The best condition for pectin extraction was at pH 2, ER 1:1 and temperature 100°C.The yield was found to be 16.71%. The AUA % was mostly above 65% which indicates that the pectin was pure. Also, the pectin isolated fromKaffir lime peels can be classified as low methoxyl pectin as it demonstrated low degree of esterification and methoxyl content.The overall results demonstrated that the lemon peel may be as a rich source ofpectin as well as extracted pectin might be used as thefunctional food ingredients domestically and industrially.

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