A COMPARATIVE STUDY OF EXTRACTION OF PECTIN FROM WET AND DRIED PEELS USING WATER BASED AND MICROWAVE METHODS

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ABSTRACT: This research effort aim at determining the effect of drying on the extraction of pectin from lemon peels. Two methods were used to extract pectin from lemon peels namely: the water base extraction method and the microwave extraction method. These methods were carefully carried out on both the fresh and the dry peels samples. First, the samples were washed, partially dried for the fresh peel and completely dried for the dry peel. Sulphuric acid was used in setting the pH of the solutions to 1, 2 and 3 in the water based extraction method while the use of Ethylene DiamineTetraacetic Acid (EDTA) and Sodium Hydroxide (NaOH) were used to set the pH to 1.5 and 10 respectively for the microwave extraction method. The solvent systems were then treated with the pectin samples at different times and temperatures to obtain varying results. The effect of time, temperature, pH and solvent systems were then studied and the yields obtained were recorded and compared. The results obtained shows that when a peel is completely dried using sun, it losses some amount of pectin probably due to thermal degradation of the pectin in the albedo resulting in pectin yield becoming small. The result also revealed that increase in acidity, extraction time and temperature increases the yield by a range of 0.01 to 0.50.

KEYWORDS: Albedo, Pectin, microwave method, water based extraction, solvent systems, fresh and dry peels.

INTRODUCTION

Pectin is a naturally-occurring thickening agent that is most often used by adding it to jams, jellies and similar products to help them gel and thicken. Pectin creates a thick, clear set when it gels. It is a carbohydrate (a polysaccharide) found in and around the cell walls of plants, and helps to bind those cells together. All fruit has pectin in it, but the amount varies widely. Apples and oranges contain the most pectin, and the pectin from both fruits is used commercially to thicken many different types of products. Pectin generally needs high sugar content and some acid, such as citric acid, to activate, and some commercially available pectins include citric acid as an ingredient to help ensure that consumers get their desired result when working with their products. Pectin can be bought at the grocery store in both powder and liquid forms, and it can also be introduced to a recipe by adding fruit that has high natural pectin content, such as apples or plums.

Gelatin and pectin both produce clear gels with a high sheen, but the products are not the same. Pectin is a water-soluble fiber, while gelatin is a protein derived from animals. Pectin is used almost exclusively in high-sugar products, like jams. Gelatin, on the other hand, is used in a much wider variety of foods, including mousses, marshmallows and frostings because gelatin sets in a cool environment and does not require that specific ingredients be included to activate it. Pectin is a complex mixture of polysaccharides that makes up about one third of the cell wall dry substance of higher plants. The highest concentrations of pectin are found in the middle lamella of cell wall, with a gradual decrease as one passes through the primary wall toward the plasma membrane (Kertesz, 1951). Because the ability of pectin to form gel depends on the molecular size and degree of esterification (DE), the pectin from different sources does not have the same gelling ability due to variations in these parameters. Therefore, detection of a large quantity of pectin in a fruit alone is not in itself enough to qualify that fruit as a source of commercial pectin. At present, commercial pectin are almost exclusively derived from citrus peel or apple pomace, both by-products from juice (or cider) manufacturing. Apple pomace contains 10-15% of pectin on a dry matter basis. Citrus peel contains of 20-30%. From an application point of view, citrus and apple pectins are largely equivalent. Citrus pectins are light cream or light tan in colour; apple pectins are often darker. Alternative sources include sugar beet waste from sugar manufacturing, sunflower heads (seeds used for edible oil), and mango waste

METHODOLOGY

The methodology consists basically of extracting pectin from dry and wet lemon peels using two methods of extractions and comparing the results in order to determine the material containing the highest yield.

Materials needed

Laboratory Equipment: Microwave Oven (0.75kW, Volts 240 A.C 1PH 50HZ), Waterbath Heater, pH Meter, Centrifuge, filter paper

Reagents: Sulphuric Acid (H_2SO_4), Ethanol, Acetone, EDTA (Ethylene Diamine Tetra-acetic acid), Distilled Water, NaOH Sample Preparation, ruthenium red (ammoniated ruthenium oxychloride), NH_2OH-$FeCl_3$, Strong mineral acid

For the fresh peel sample

Fresh unripe lemons were peeled. The albedo portion was minced and washed with cold water to prevent any adhering juice and to remove the Glycosides, the bitter taste of the peels. The albedo was then air dried and grounded to fine powder before the extraction.

For the dry peel sample

After the peels were washed with cold water, they were further sun-dried until there was constant weight of peel samples.

Waterbased Extraction

Water based Extraction is extraction using acidified water. A buffer solution of pH 4 at 25^{o}C was used to calibrate the pH meter. pH of 1, 2 and 3 were produced by adding concentrated Sulphuric acid into three different beakers that contained 900 ml of potable water. 10 grams of the grinded sample was measured in 27 places and poured into 27 beakers. After this 100 ml each of pH 1, 2 and 3 were poured into the 27 beakers containing the measured peel sample and taken into a water bath and set at temperatures 80^{o}C, 90^{o}C and 95^{o}C for 45 mins, 60 mins and 90 mins. After the duration, the samples were brought out of the water bath for further analysis. This procedure was done for both the fresh grounded peel sample and the dried grounded peel sample.

After cooling the solution, it was filtered using filter paper. 25mls of Ethanol was added to all the filtered solutions to facilitate filtration of pectin. The solution was then centrifuged at 4000 rpm for 15 mins to separate jelly pectin which was dried under vacuum at 50^0C. The centrifugation and filtration steps were repeated. Twice the volume of alcohol was added for overnight precipitation. Samples were cooled, dried, weighed and ground using a mortar and pestle.

The same procedure was carried out for both the wet and dried peel samples.

Microwave extraction method

0.05M ethylenediamine tetra acetic acid and 1 M sodium hydroxide were added into 100mls of distilled water separately and used to maintain pH up to 1.5. Extraction periods of 5min, 10min and 15mins were used to extract the pectin. After these periods the samples were removed from the microwave, filtered and allowed to cool. 25mls of Alcohol was also added to the samples to enable precipitation of pectin.

Further the samples were centrifuged at 4000rpm for 15mins and 50mls of ethanol was then added for overnight precipitation just as in the case of water based extraction method.

Y_{pec} (%) = Y= 100 x P/Bi

Where, Y_{pec} = the extracted pectin yield in percent (%),

P = the amount of extracted pectin in g and

Bi = the initial amount of ground lemon peel.

Detection of pectin

The detection of pectin is based primarily on the chemistry of galacturonic acid. Pectin is usually first isolated from most of the congeneric substances occurring in crude plant extracts and formulated foods, then purified, before being subjected

to confirmative tests. Some tests are based on the incompatibility of pectin with organic solvents.

Qualitative tests:

• Colour: This was done by visual observation

• Solubility of dry pectin in cold and hot water: of the pectin samples were separately placed in a conical flask with 9.5mls ethanol added followed by 50 mL distilled water. The mixture was shaken vigorously to form a suspension which was then heated at 85-95°C for 15 min (Fishman et al., 1984).

• Solubility of pectin solution in cold and hot alkali (NaOH): To 1 mL of NaOH was added 5ml pectin solution and then heated at 85-90°C for 15 min, (Joslyn, 1980).

• Precipitation: The alcohol insolubility of pectin has been developed into a test for traces of pectin in fruit Juices. A positive reaction is indicated by the development of a stringy, gelatinous deposit.

• Chromophore Formation:In histological testing, pectin may be distinguished from surrounding non pectin material by staining with ruthenium red (ammoniated ruthenium oxychloride). A positive test is evidenced by the typical pink color (pectin) on a gray background (lignin and cellulose).

• Differential staining (in leaf sections) was accomplished with alkaline NH_2OH-$FeCl_3$. The reaction converts pectin carboxyl groups to hydroxamic acids, resulting in water-insoluble, red complexes in excess Fe(UI). The test is specific for pectin.

• Decarboxylation: A vigorous evolution of CO_2 and appearance of a pentose derivative, notably furfural, resulting from the action of strong mineral acids and heat on pectin, is claimed to be a good indication of the presence of pectin.

RESULTS

Water based extraction using fresh peel

The maximum yield of pectin was obtained at pH of 1, extraction temperature of 95^0C for 90mins with a value of 4.6 . Also substantial yield was observed at pH of 2, extraction temperature of 95^0C for 90mins. However, poor and negligible yield were obtained at pH of 3 for 45mins,60mins and 90mins at temperature of 45^0C with values of 0.0,0.1 and 0.3 .

For the rest of the result the pH of 1 produced the highest yield probably due to the strong concentration of the acid breaking and penetrating into the walls of the albedo causing it to degrade to its maximum peak. The length of the extraction period greatly and positively influenced the yield of the pectin.

Water based extraction using dry peel

Similar to the case of results obtained from using the wet peel, the maximum yield of pectin was also observed at pH of 1, extraction temperature of95^0C for 90mins with a value of 1.6. Very Negligible yield was found in pH 3 at extraction temperature of 45^0C and extraction periods of 45mins,60 mins,90min with values of 0.00 .

Effect of pH on the dry and fresh peel using water based extraction method

The effect of pH on pectin yield was determined using increasing amounts of 1M of Sulphuric acid to lower the pH to the desired values of approximately 1.0, 2.0, 3.0

Table : Effect of Ph on pectin yield from wet and dry peels

pH	% yield (dry)	% yield (wet)
1	16	46
2	6	45
3	1	10

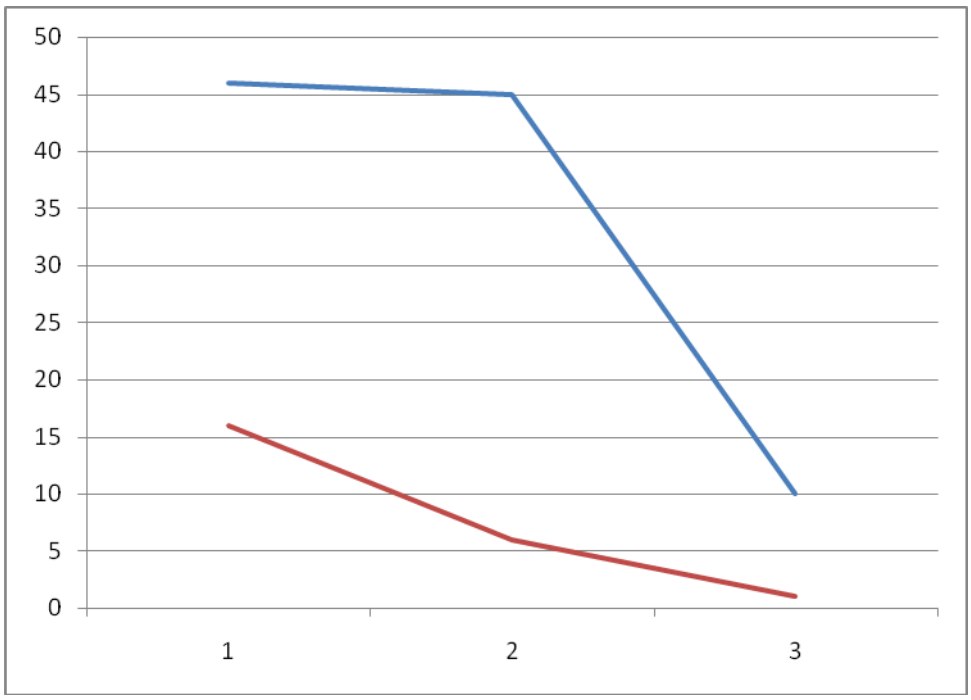

Fig. 1: Effect of pH on yield of pectin from wet and dried peels respectively.

Figure 1 shows the graph of % yield against pH for the fresh and dry peels. It shows that the yield reduces as the pH increases in value and acid concentration.

Also, it was observed that the three parameters that were varied immensely contributed positively to the result of pectin obtained. The more acidic the solvent was the more the yield obtained. Kertesz reported that high concentration of hydrogen ions present in the solvent (at low pH) stimulates the hydrolysis of protopectin. GreveMcArdle,Gohlke&Labavitch(1994) reported that at lower pH, the highly hydrated carboxylate groups are repressed in the larger hydrogen ion concentrations and therefore, converted into slightly hydrated carboxylic acidgroups. Pectin yield being lesser in higher pH might be due to some pectin still attached to the cell wall components, although pectin molecules can be partially solubilized from plant tissues without degradation by weakly acidic aqueous solvents.In order to improve the yield, this type of pectin constituent(protopectin) is suggested to be hydrolysed by acid. Alkaline conditions were found by Knee and Jarvis et al to break the bonds between the

pectin molecule and the cell wall in a similar manner to acidic solvents. Knee38 and Jarvis et al found that substantial amounts of pectin were extracted under alkaline conditions as compared with neutral conditions. Nevertheless, alkaline conditions cause instability in the backbone of pectin molecule (galacturonic acid) and consequently, the pectin molecule tends to decompose. Due to the decomposition of pectin molecules, the extracted pectin cannot be precipitated with alcohol. Therefore, the recovery of the extracted pectin tends to be reduced under alkaline conditions. Thus low pH is essential for higher yield that is not achievable at higher pH condition.

The longer the extraction period was the greater the yield of the pectin probably due to increase hydrolysis of neutral sugar side chains and the decrease in pectin yield by the increase in extraction period may be due to the thermal degradation of the extracted pectin.The higher the temperature of the pectin became the more the yield obtained (Vriesmann et al (2012), Aravantinos-Zafiris and Oreopoulou (1992).

Effect of time on the fresh and dry peel using waterbased

The effect of time on pectin yield was examined to determine if increasing extraction time would increase pectin yield. There was increase in the yield as the extraction time increased.

Time (mins)	% yield (wet peels)	% yield (dry peels)
45	40	6
60	42	10
90	46	16

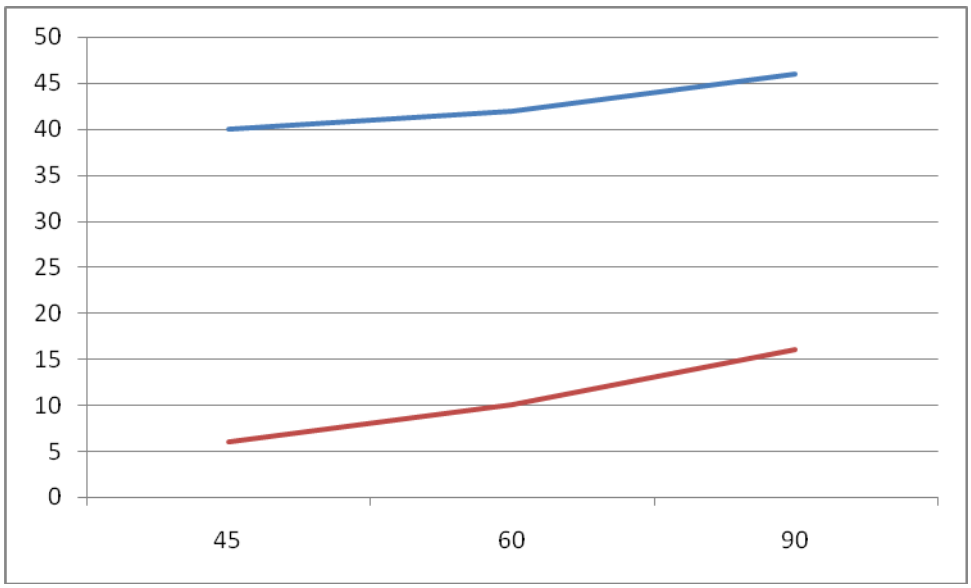

FIG 2: Yield of pectin(%) with time

Effect of temperature using waterbased method on the fresh and dry peel .

There was noticeable increase in the yield as the extraction temperature increased.

% yield is plotted against the highest values of temperature 80^0C,90^0C and 95^0C

Temperature (^{o}C)	% yield(wet peels)	% yield(dry peels)
80	49.5	12
90	50.0	15
95	50.0	16

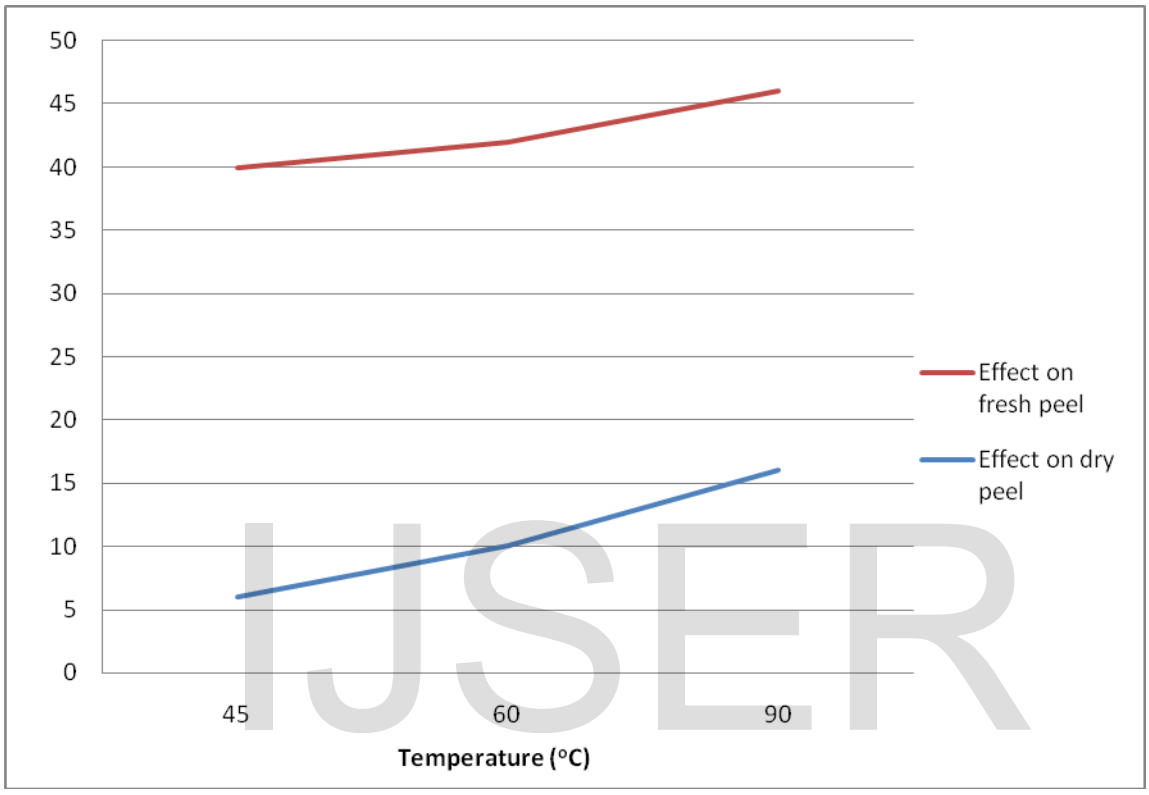

Fig. 3: Effect of extraction temperature on yield of pectin

Fig. 3 shows that the % yield increased from 80^0C to 90^0C for both wet and dry peels and stayed constant at 95^0C which could be due to the decrease in pectin yield by the increase in extraction period may be due to the thermal degradation of the extracted pectin.

. After comparing the results obtained in the extraction from wet and dry peels using the two methods it can be deduced that drying of the peels completely using sun as a dryer results in an apparent reduction in the yield of pectin recovered from the experiment. Reason for that can be due to the method of drying which is by sun. When Ranajit Kumar Shaha (2012) carried out extraction

of pectin on citrus peels varying sun and microwave oven as dryers,the result obtained showed that the yield from microwave dried peel gave a higher result (61.80%) compared to the yield of the sun dried peel (41.20%).

For microwave oven using EDTA and NaOH on freshpeel

The results observed in table 4.6 and 4.7 showed that the maximum amount of yield was obtained using EDTA at extraction time of 15mins with value of 4.0 and also at extraction time of 15mins with value of 3.5 using sodium hydroxide.

For microwave oven using EDTA and NaOH on dry peel

The results obtained and shown in table 4.8 and 4.9 reveals that the maximum amount of yield was obtained using EDTA at extraction time of 15mins with value of 2.5 and also at time of 15mins with value of 2.0 using sodium hydroxide.

Effect of solvent used (EDTA and NaOH) using microwave method on fresh and dry peel

It can be observed from the 12 results of the yield obtained that the use of EDTA produced more yield compared to NaOH.

Effect on fresh peel

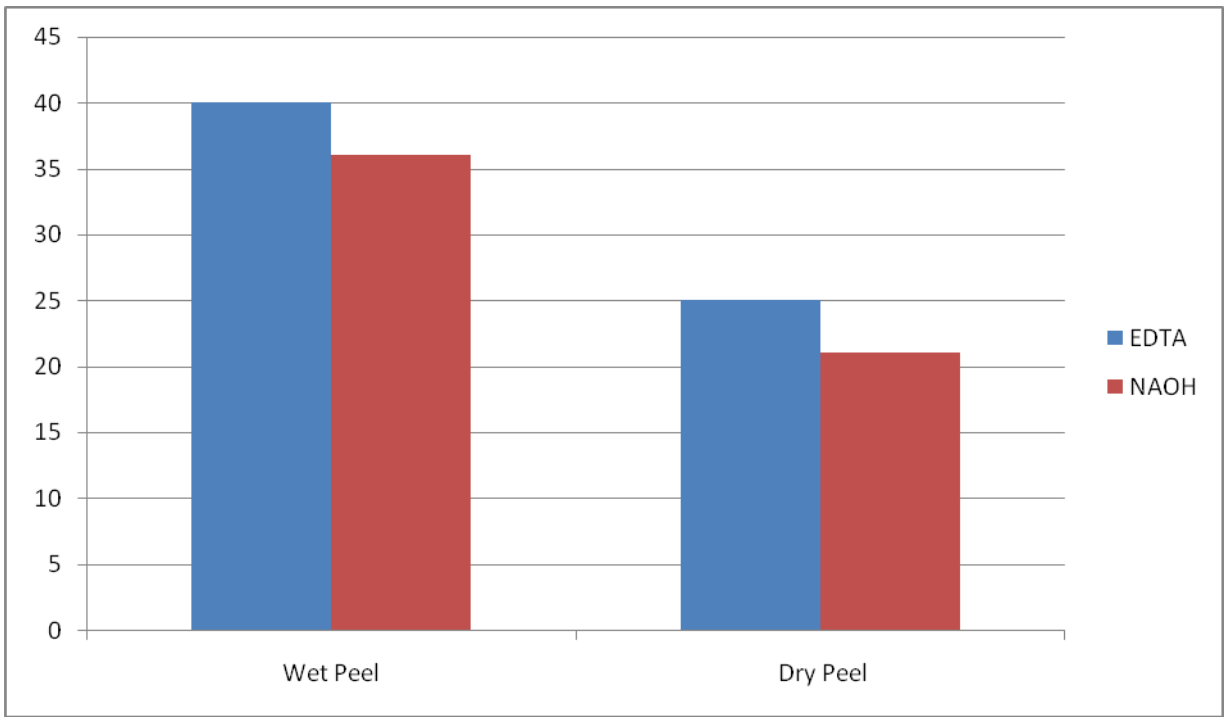

Fig.4: Effect of solvent(EDTA and NaOH) on yield of pectin for dry and wet peels

It shows that EDTA is a better solvent to extract using microwave oven method compared to NaOH because an optimum yield is produced when treated on fresh peel.

.Even such a mild concentration of EDTA was reported to cause degradation of some pectin molecules(S. Yeoh, J. Shi, T.A.G. Langrish 2008) . Degraded pectin molecules cannot be easily recovered from the solvent, and thus the yield of pectin is affected. Therefore, high concentrations of EDTA are not suitable for extraction. Also extraction period played a vital role in the extraction method in the sense that the increase in time increased the yield obtained.

Effect of extraction period using microwave method on fresh and dry peel

It can be observed from that as the time of extraction increased by 5mins the pectin yield also increased within the range of 0.01-0.5 .

Effect on fresh peel

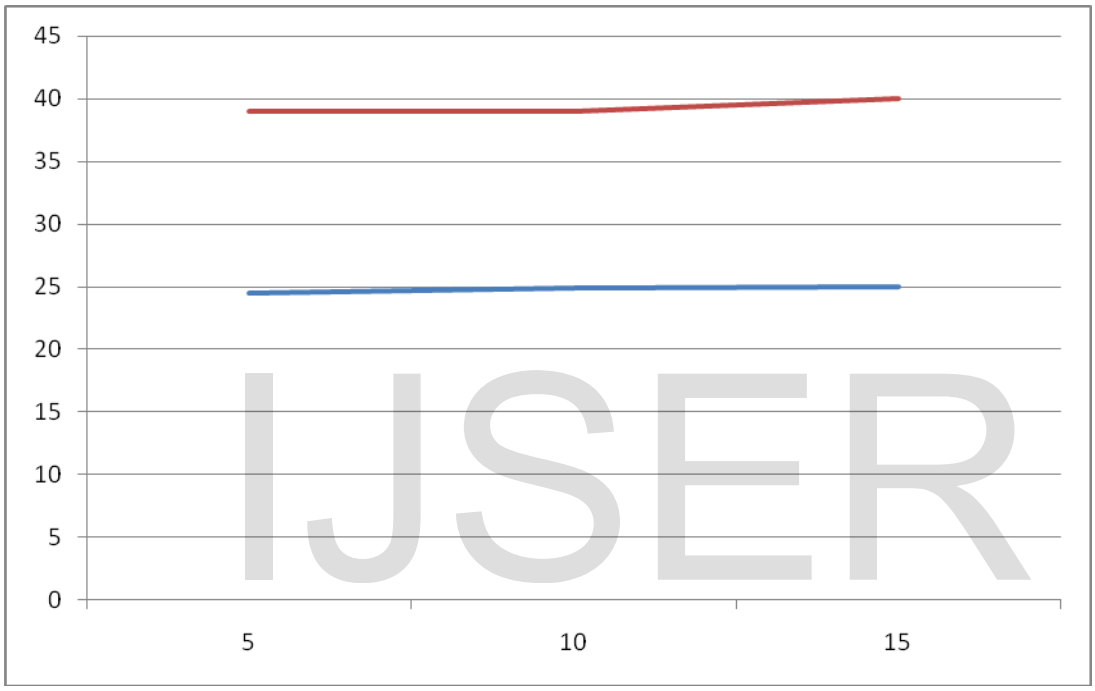

FIG.5: **Effect of extraction period using microwave method on fresh and dry peel**

It shows that increase in temperature increase the % yield of pectin for fresh peel extract just as in the case of water based method although it was constant from temperature 80^0C to 90^0C .

For dry peel extract the temperature varied directly proportional to the % yield just as in water based extraction method.

Effect of pH using microwave method on fresh and dry peel

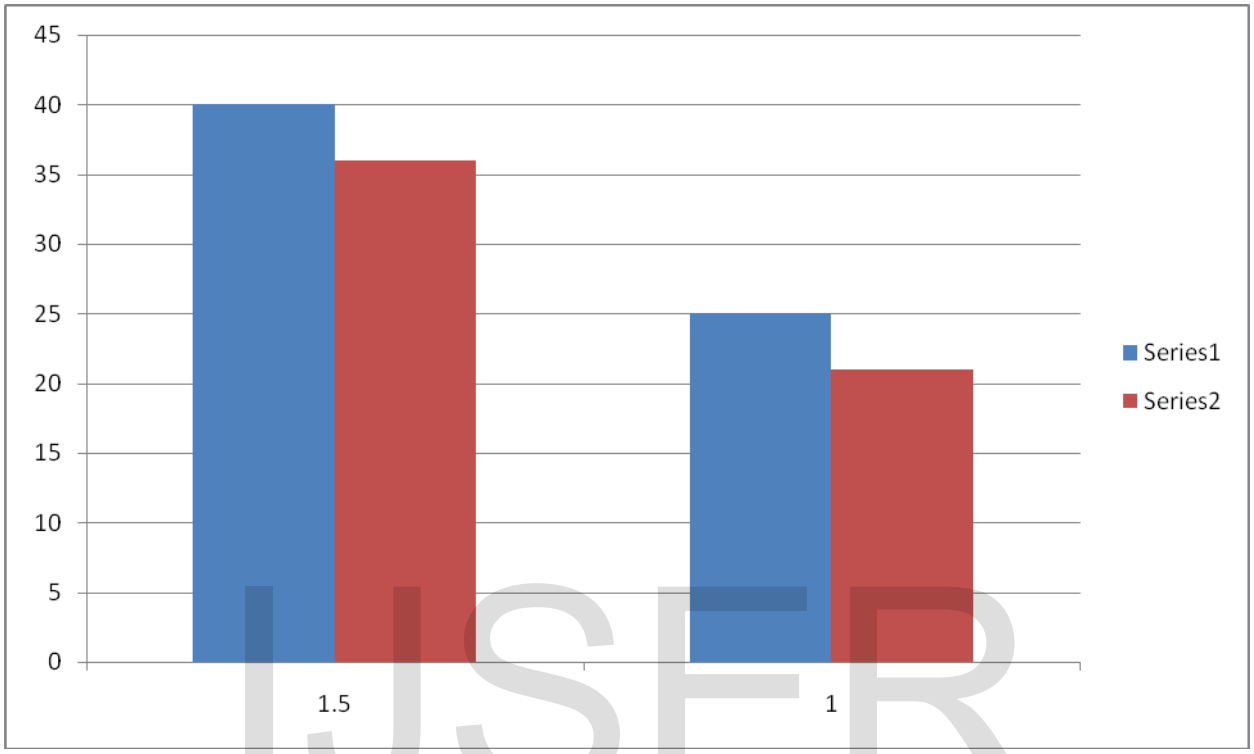

FIG.6: **Effect of pH on yield of pectin using microwave extraction.**

It shows that the % yield increased as the pH value increased in the fresh peel extract.In other words, the more acidic the solvent is, the more the yield of the pectin.

In fig.6 the declination of the slope showed that solvents with low pH produces low yield of pectin and so therefore for optimum yield concentrated acidic solvents should be used to extract pectin. The same result was observed in the extraction of pectin from fresh/dry peels using water based extraction and also that obtained from using fresh peel to extract pectin in microwave extraction method.

Qualitative tests

In cold alkali, (NaOH), the pectin suspension obtained from the fruits gave a yellow gelatinous color which turned white when heated at 85-90°C for 15 min, however, (Fishman, 1993) stated that pectin is unstable under alkaline solution which corresponded with what was obtained from this research.

Parameter	Wet lemon Peel	Dry lemon peel
Colour	Brown	Dark brown
Solubility in cold water	Dissolved slightly and form suspension after vigorous shaking	Dissolved slightly and form suspension after vigorous shaking
Solubility at 85-90°C for 15 min	The mixture dissolved	The mixture dissolved
Solubility of pectin suspension in cold alkali	The pectin suspension forms yellow precipitate	The pectin suspension forms yellow precipitate
Solubility of pectin suspension in hot alkali	The pectin suspension dissolved and turned milky	The pectin suspension dissolved and turned milky
Staining with ruthenium red	Turned pink color on a gray background	Turned pink color on a gray background
Addition of 2mls of alkaline NH_2OH-$FeCl_3$	Became water-insoluble, red Complexes in excess Fe	Became water-insoluble, red Complexes in excess Fe
Precipitation	Development of a stringy gelatinous deposit.	Development of a stringy gelatinous deposit.
Addition of strong mineral acid and heat	A vigorous evolution of CO_2	A vigorous evolution of CO_2

CONCLUSION AND RECOMMENDATION

From the yield obtained it can be concluded that water based method is the best method for the extraction of pectin from both fresh and dry lemon peel compared to microwave method. Although the yield difference for the two method is about 0.5,, microwave method is preferred to water based due to the time constraints between them. According to the result it would take 90mins to produce 24g of pectin from 60g of lemon peel in Microwave method while in water based method it would take the same 90mins to produce 30g of pectin from 60g of lemon peel. The use of EDTA led to the production of more pectin than Sodium hydroxide by 0.4 .This can be concluded based on the result obtained from the experiment. After comparing the results obtained in the extraction from wet and dry peels using the two methods it can be concluded that drying of the peels completely using sun as a dryer resulted into an apparent declination on the yield of pectin recovered from the experiment.

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