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RESEARCH ARTICLE

EXTRACTION AND OPTIMIZATION OF PECTIN FROM COFFEE ROBUSTA PULP AND IT'S APPLICATION IN THE FOOD PRODUCT

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Abstract

Coffee is one of the paramount agricultural commodities and the most consumed beverages in the world. A serious problem with the coffee industry now-a-days is huge amount of coffee pulp gets wasted once the coffee beans were separated from its cherries. It is found essentially rich with carbohydrates, proteins, minerals, fat and contains a sufficient level of minor compounds such as pectin, caffeine, tannins, polyphenols. Pectin was extracted by two different methods such as acid hydrolysis and enzymatic extraction at different pH and temperature. Pectin was evaluated for its proximate analysis and physio-chemical analysis such as ash content, moisture content, pectin yield, methoxyl content, equivalent weight, anhydrouronic acid content, degree of esterification, gel forming ability, viscosity & galacturonic acid content. The project was carried out with extraction and optimization of pectin and to incorporate in to the food product. The pectin act as thickening agent and gelling agent and it is incorporated with jam to study its characteristics with commercial food grade.

Introduction:-

Coffee is one of the important agricultural commodities and the most consumed beverages in the world. Coffee berries belongs to the family Rubiaceae and genus Coffea. The two main species of coffee are arabica and robusta. Traditionally, India has been a noted producer of arabica coffee but in the last decade robusta beans replaces the arabica coffee with over 60% of production in India. Pectin is a soluble gelatinous polysaccharide which is found in the cell walls of higher plants, where they function as a hydrating agent and cementing material for the cellulosic network. They are commonly produced during the initial stages of primary cell wall growth and make about one third of the cell wall of dry substances of dicotyledonous and some monocotyledonous plants. A serious problem with the coffee industry now-a-days is huge amount of coffee pulp being wasted once the coffee beans were separated from the coffee cherries. Coffee pulp is a fibrous mucilaginous material is essentially rich in carbohydrates, proteins, minerals, fat and contains a sufficient level of minor compounds such as pectin, caffeine, tannins, polyphenols. The drying of industrial coffee pulp waste was carried out by two methods such as sun drying method and oven drying method. Comparing both the methods, sun drying method is more cost effective. The chemical agents used for pectin extraction are water and buffers, calcium-ion chelators, acids and bases. Acids are the strongest extracting agents of pectin as they facilitate extraction of insoluble pectin that is tightly bound to the

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cell matrix of the plant material and result in higher yields. The characterization of the extracted pectin was done by calculating the ash content, moisture content, equivalent weight, pectin yield, methoxyl content, anhydrouronic acid content and degree of esterification. Pectin can be used as gelling agent in jams, jellies and desserts. It has the ability to form gel and can also be used as a stabilizing agent which thickens soups and sauces without causing them to curdle. In this study, pectin is extracted by using acid hydrolysis and enzymatic extraction method from coffee pulp waste and optimized at different pH, time and temperature. Physio-chemical properties of extracted pectin was investigated and its application in the food product like jam.

Specific objectives of this study

i. To extract the pectin from coffee pulp waste (acid hydrolysis, enzymatic extraction).

ii. To investigate the physiochemical properties (pH, temperature, moisture content, ash content, viscosity etc.)

iii. To investigate the application of pectin in food products like jams and jellies.

Materials and Methods:-

Collection of raw materials and dry powder preparation

Coffee pulp was collected from Sree Ram coffee pulping unit,Wayanad. Cellulase enzyme was bought from Sastha Biozyme India, Rajapalayam. Hydrochloric acid and ethanol were bought from Sri Sakthi Agro Chemicals, Coimbatore, and the equipment used arewater bath, hot air oven, refractometer, pH meter. It is kept for sun drying for 3-4 days and oven drying at 80°C for 1hour and then crushed using mixer grinder. The sun dried and oven dried coffee pulp powders were labelled and stored properly in separate air tight containers.

Fig 1:- Dried coffee pulp.

Fig 2:- Coffee pulp powder.

Extraction Methods

The extraction of pectin from coffee pulp waste was carried out by using acid hydrolysis and enzymatic extraction.

Acid Hydrolysis

Pectin was extracted by acid hydrolysis method using Hcl by stirring and heating. It is filtered to remove the fiber material then precipitated and washed with equal amount of ethanol. Filtration is done again to remove the solvent and dried using hot air oven for 1 to 2 hours. The obtained pectin extract was labelled and stored properly in separate containers for the analysis of pectin.

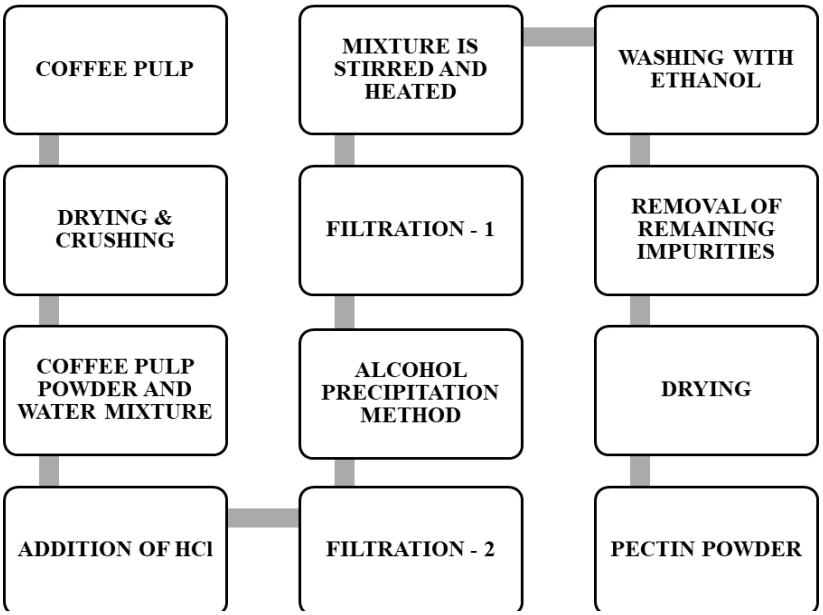

Fig 3:- Pectin extraction using acid hydrolysis.

Enzymatic extraction

Pectin was extracted by enzymatic method using cellulase and Hcl. It is stirred while boiling and filtered to remove the fiber material later allowed to precipitate. The precipitate is washed with equal amount of ethanol and filtered to remove the solvent. It is then dried using hot air oven for 1 to 2 hours. Then, the obtained pectin was labeled and stored properly in separate containers for the analysis of pectin.

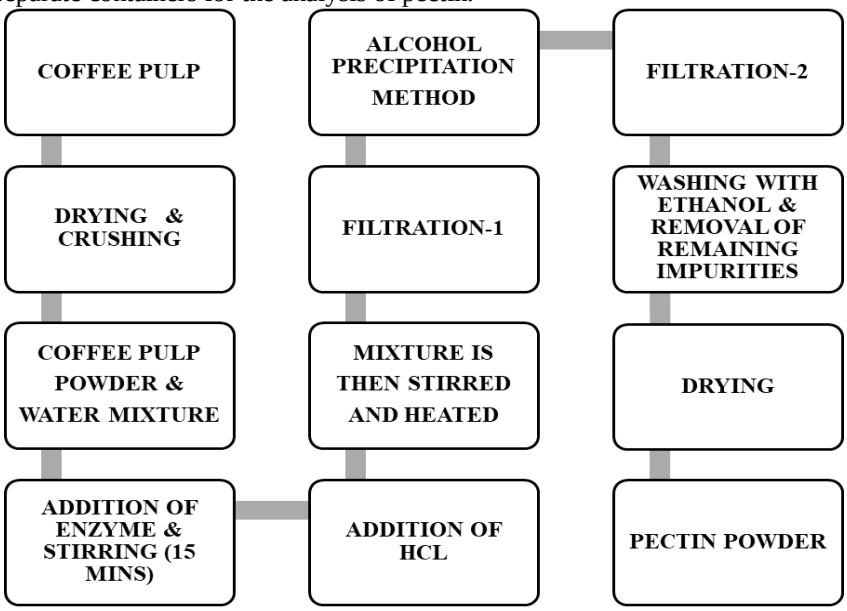

Fig 4:- Pectin extraction using enzymatic extraction.

Application of pectin in pineapple jam

The pectin which is extracted by using the extraction methods such as acid hydrolysis and enzymatic extraction. It is used as a gelling agent in the preparation of Jam.

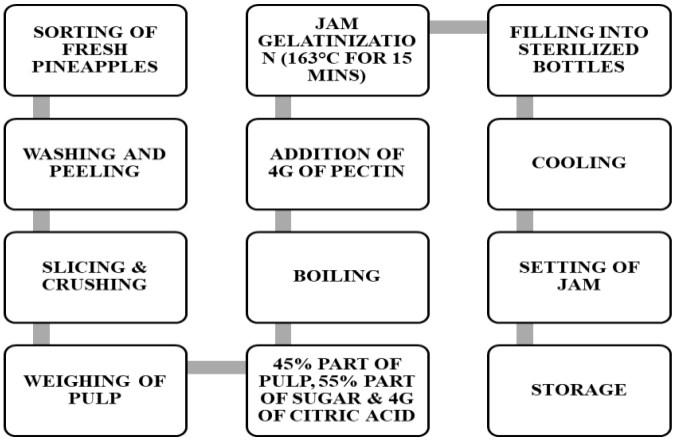

Fig 5:- Preparation of jam.

Physiochemical properties of pectin

The physiochemical properties of pectin such as pectin yield, moisture content, ash content, equivalent weight, methoxyl content, anhydrouronic acid content, degree of esterification, gel forming ability, viscosity, galacturonic acid content.

Pectin yield

Yield of pectin obtained by the process of extraction and the percentage yield of pectin was calculated by the weight of dried pectin to the weight of dried pulp powder taken for extraction.

$$Pectin\ (g/100g) = \frac{Weight\ (g) of\ dried\ pecin}{Weight\ (g)\ of\ dried\ pulp\ powder\ taken\ for\ extraction} \times 100 \quad (1)$$

Moisture content

Water content or moisture content is the quantity of water contained in a material, such as soil(called soil moisture), rock, ceramics, crops or wood.1.0 g of pectin was transferred into crucible and dried at 130^0C in hot air oven for 1 hour to determine the moisture content using the f following formula

$$Moisture\ \% = \frac{100(w_1 - w_{2)}}{(w_1 - w)}, \quad (2)$$

w1= weight in g before drying

a) w2=weight in g after drying

b) w=weight in g of the empty dish

Fig 6:- Moisture Balance.

Ash content

The ash content is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of specific inorganic components present within a food, such as Ca, Na, K and Cl.

The ash content was determined by weighing 1.0 g of pectin sample in a crucible and heated in a muffle furnace at 600^0C for four hours. Then the residue was cooled in desiccator and weighed to calculate the ash content by the following formula,

$$Ash\,\% = \frac{Weight\ of\ ash\ (g)}{Weight\ of\ pectin(g)} \times 100 \quad (3)$$

Fig 7:- Muffle Furnace.

Determination of equivalent weight

Equivalent weight of pectin is the total content of free galacturonic acid (not esterified) in the molecular chains of pectin. Pectin produced at low pH has higher equivalent weight, because low pH can cause polymerization of pectin into a longer chain.

The values of equivalent weights were used for calculating the anhydrouronic acid (AUA) content and the degree of esterification. 0.5 g pectin was weighed in a 250 ml conical flask and moistening it with 5 ml of ethanol. 1.0 g of sodium chloride was added to sharpen the end point and 100 ml of carbon dioxide free distilled water was added and the mixture was dissolved completely. Six drops of cresol red indicator was added and titrated against 0.1 N NaOH until the color of the indicator changed to violet (pH 7.5) and persisted for at least 30 seconds. The neutralized solution was used for the methoxyl content determination. (UzmaAltafet al., 2015) The following formula was used to calculate the equivalent weight,

c) $$Equivalent\,weight = \frac{weight\ of\ sample(g)}{ml\ of\ NaOH \times Normality\ of\ NaOH} \times 1000 \quad (4)$$

Determination of methoxyl content

Methoxyl content is defined as the number of moles of methyl alcohol in 100 molgalacturonic acid. Methoxyl content of pectin is important to control the gel strength, setting time, sensitivity to metal ions and to determine the functional properties of pectin solutions and pectin gel texture. Methoxyl content was determined by using the neutralized solution obtained during the equivalent weight determination by the saponification of the pectin followed by titration of the liberated acid. 25 ml of 0.25 M NaOH was added to the neutralized solution used in the equivalent weight determination. The mixture was mixed thoroughly and allowed to stand for 30 minutes at room temperature. (UzmaAltaf et al., 2015) The methoxyl content (MeO) % was calculated using the following formula,

d) $$MeO\% = \frac{Volume\ of\ NaOH \times Normality\ of\ NaOH \times 31}{Weight\ of\ pectin\ sample(mg)} \times 100 \quad (5)$$

e) Where31 is the molecular weight of carboxyl group.

Determination of anhydrouronic acid

The anhydrouronic acid content will be higher by increasing time of extraction. A minimum value of 65% AUA for commercial pectin has been specified by FAO. (UzmaAltaf et al., 2015)

From the values of the equivalent weight and the methoxyl content, the anhydrouronic acid content was calculated by using the following formula,

$$Z = \frac{weight\ of\ pectin\ (mg)}{ml\ of\ NaOH\ for\ eq.wt\ +\ ml\ of\ NaOH\ for\ MeO \times Normality\ of\ NaOH} \quad (6)$$

$$AUA\% = \frac{176 \times 100\%}{Z}$$

f) Where 176 is the molecular weight of AUA

Determination of degree of esterification

The degree of esterification (DE) is the ratio of the esterified galactouronic acid groups to the total galactouronic acid groups present. It is an important property which determines the gelling nature of pectin. (UzmaAltafet al., 2015)

Degree of esterification was calculated by using the AUA% and MeO% from the following formula,

$$\%DE = \frac{176 \times MeO\% \times 100\%}{31 \times AUA\%} \quad (7)$$

Food Chemical Codex (FCC, 1981) and USP 26 NF 21 (2003) with slight modification. 0.5 g dried pectin is transferred to 250 ml conical flask. To this add 2 ml absolute ethanol then dissolve it in 100ml carbon-di-oxide free water. 5 drops of phenolphthalein was added and titrated with 0.5M NaOH to the appearance of pink color and the reading was noted as (a). Then 10 ml of 0.5 M NaOH was added and shaken vigorously and kept for 15 minutes. Then 10 ml of 0.5M HCl was added to this and it was shaken until the disappearance of the pink color.

Five drops of phenolphthalein was added to this and again titrated with 0.5 M NaOH to get the end point (appearance of pink colour) and the titer value was noted as (b). Degree of esterification was calculated using the following formula.

$$\%DE = \frac{b \times 100\%}{(a + b)} \quad (8)$$

Testing of gel forming ability

Jelly grade is the quality of the pectin, found by the testing gel forming ability of a pectin. The pectin should be able to form satisfactory jelly with 65% sugar content at pH 3.0±0.5 by boiling in microwave oven. The strength of that gel was tested by finger pressure test and the jelly grade is mentioned as ^{0}SAG.

Viscosity

Viscosity is the state of being thick, sticky and semifluid in consistency, due to internal friction. The viscosity of pectin solution was measured by viscometer (Brookfield DV-III Rheometer)

Fig 8:- Viscometer.

Determination of galacturonic acid

Pectin estimation bygalacturonic acidis treated with Carbazole in the presence of H_2SO_2 and the color developed is measured at 520nm. 100 mg pectin was weighed and dissolved in 100 ml of 0.05N NaOH. It is allowed to stand for 30 min to de-esterify the pectin. Take 2 ml of this solution and make up to 100 ml with water. Then pipette out 2 ml of de-esterified pectin solution and add 1 ml carbazole reagent. A white precipitate will be formed. Following that, add 12 ml conc. H_2SO_2 with constant stirring. Close the tubes with rubber stopper and allow to stand for 10 min to develop the color. To set a blank, add 1 ml of purified ethyl alcohol in the place of carbazole reagent. Then read the color at 525nm against blank, exactly 15 min after the addition of acid.

Phytochemical assay

Phytochemical assay refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants such as total polyphenol content and tannin content were determined

Determination of total Polyphenol content

The concentration of phenolics in plant extracts was determined using spectrophotometric method. Folin-ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1ml of extract and 9ml of distilled water was taken in a volumetric flask (25 ml). One milliliter of folin-ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes,10 ml of 7% sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25ml. A set of standard solutions of gallic acid (20,40,60,80 and 100 mg/ml) were prepared in the same manner as described earlier. Incubated for 90 minutes at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an ultraviolet (UV)/ visible spectrophotometer. Total polyphenol content was expressed as mg of GAE/gm of extract. (Taha m. Rababahet al., 2012)

Determination of Tannin Content

The tannins were determined by Folin- Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water, 0.5 ml of Folin - Ciocalteuphenol reagent, 1 ml of 35% Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20,40,60,80 and 100 mg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm within UV/ visible spectrophotometer. The tannin content was expressed in terms of mg of GAE/g of extract. (Taha m. Rababahet al., 2012)

Results and Discussions:-

Extraction methods

The extraction of pectin is carried out by different extraction techniques like acid hydrolysis method andenzymatic extraction using Hydrochloric acid and enzyme Cellulase.

Acid hydrolysis: sun driedand oven dried coffee pulp

Table 1:- Acid hydrolysis: sun dried and oven dried coffee pulp.

SUN DRIED COFFEEPULP			OVEN DRIED COFFEE PULP	
TEMPERATURE (°C)	pH	YIELD (g)	pH	YIELD (g)
65°C	1.0	0.03	1.0	0.10
	2.0	0.05	2.0	0.11
	2.5	0.06	2.5	0.10
	3.0	0.08	3.0	0.12
75°C	1.0	0.13	1.0	0.10
	2.0	0.15	2.0	0.12
	2.5	0.18	2.5	0.14
	3.0	0.17	3.0	0.15
85°C	1.0	0.16	1.0	0.13
	2.0	0.20	2.0	0.13
	2.5	0.22	2.5	0.18

	3.0	0.18	3.0	0.10

The extraction of pectin was carried out for various temperatures (65°C, 75°C and 85°C) and pH (1.0, 2.0, 2.5 and 3.0). Thus, compared to all the temperatures and pH combinations of extraction, the higher yield of pectin was obtained for the pH 2.5 at temperature 85°C in 45 minutes. It was observed that, for sun dried coffee pulp powder the pectin yield was higher as compared to oven dried coffee pulp powder.

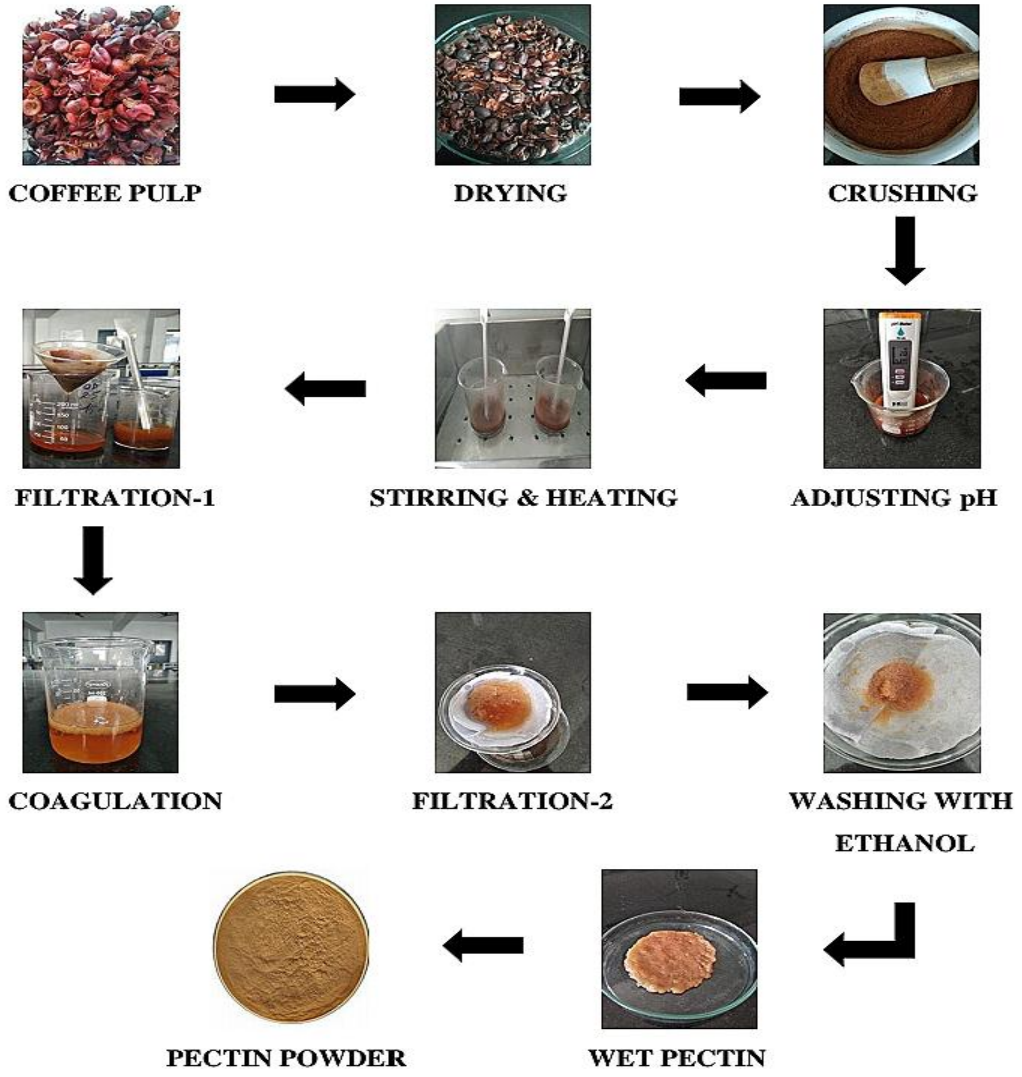

Fig 9:- Representation of acid hydrolysis.

Enzymatic extraction

The enzymatic extraction is carried out by using 0.5 g, 1.0 g and 2.0 g of cellulase enzyme.

ENZYME(g)	TEMPERATURE	PH	YIELD(g)
0.5	65°C	1.0	0.10
		2.0	0.13
		2.5	0.14
		3.0	0.12
	75°C	1.0	0.11
		2.0	0.14
		2.5	0.16
		3.0	0.14
	85°C	1.0	0.17
		2.0	0.14
		2.5	0.18
		3.0	0.16
1.0	65°C	1.0	0.11
		2.0	0.12
		2.5	0.10
		3.0	0.09
	75°C	1.0	0.21
		2.0	0.34
		2.5	0.91
		3.0	0.72
	85°C	1.0	0.94
		2.0	0.98
		2.5	1.12
		3.0	0.89
2.0	65°C	1.0	0.21
		2.0	0.17
		2.5	0.19
		3.0	0.20
	75°C	1.0	0.16
		2.0	0.19
		2.5	0.21
		3.0	0.14
	85°C	1.0	0.17
		2.0	0.12
		2.5	0.17
		3.0	0.15

Table 2:- Enzymatic Extraction using enzymes.

The Enzymatic Extraction was done by using 0.5g, 1.0g and 2.0g of enzymes and itwas treated with different temperatures and different pH of65°C,75°C,85°C and 1.0,2.0,2.5,3.0 respectively. As compared to all pH and temperatures, the best yield of pectin was obtained at pH 2.5 and temperature 85°C.

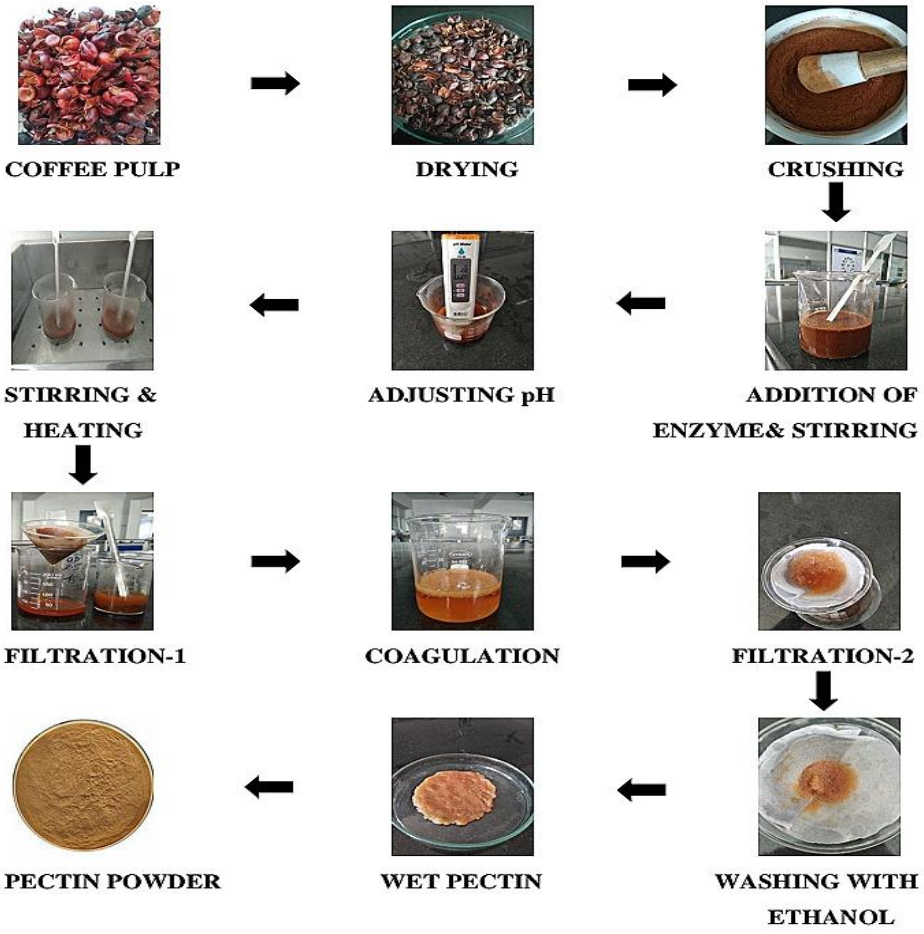

Fig 10:- Representation of enzymatic extraction.

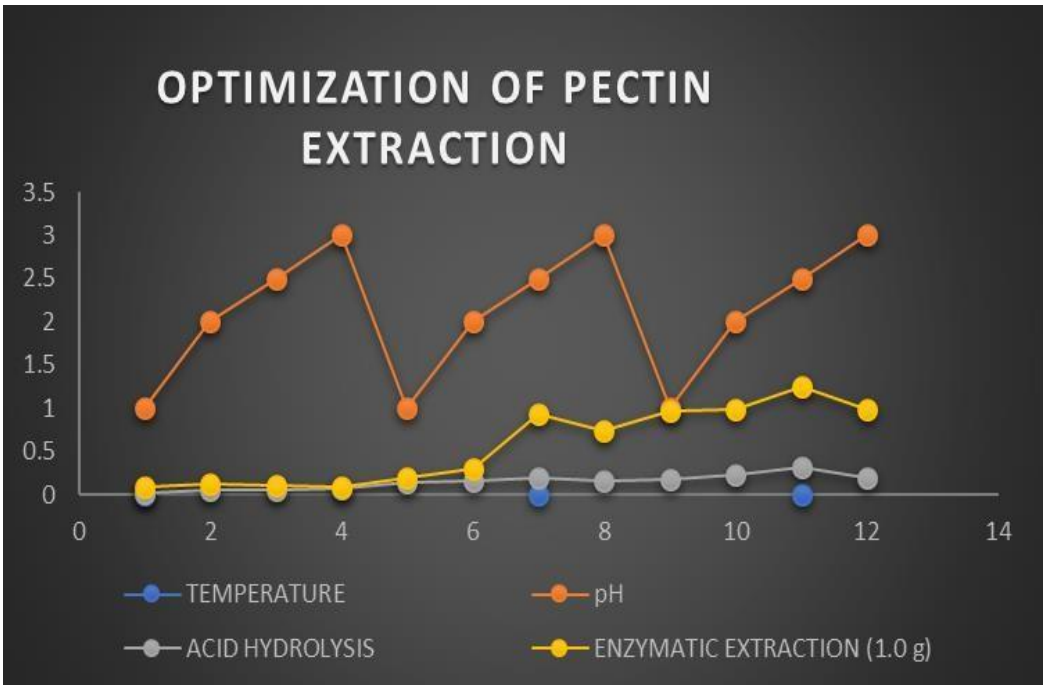

Fig11: Graphical representation of optimization of pectin extraction

The above graph describes about the Optimization of pectin extraction at different pH (1.0,2.0,2.5,3.0) and temperatures(65°C, 75°C, 85°C) for acid hydrolysis and enzymatic extraction methods. From the graph, it is observed that enzymatic extraction gives the highest yield of pectin at pH 2.5 and temperature 85°C. Four trails (Trail 1, Trail 2, Trail 3 and Trail 4) were carried out at 85°C for enzymatic extraction and the average of the trail was taken as the result.

Average yields of pectin at different pH

Average yield ofpectin at different pH was taken as a result.

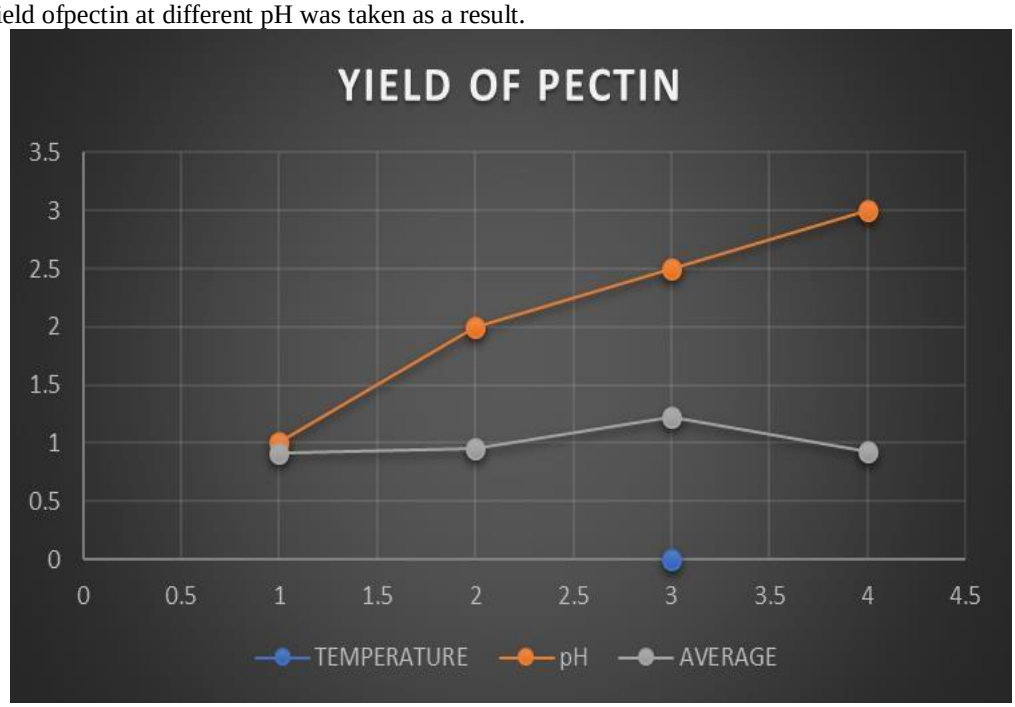

Fig 12:- Graphical representation of average yields of pectin at different pH.

The graph shows that the highest yield of pectin was obtained at pH 2.5 and temperature 85°C. Finally, it concludes that the best yield of pectin was obtained at pH 2.5 and temperature 85°C in enzymatic extraction method.

Application of pectin in food product

A valuable by-product that can be obtained from coffee pulp waste is pectin. Pectin's are commercially used as thickening agent and gelling agent in jams and jellies.

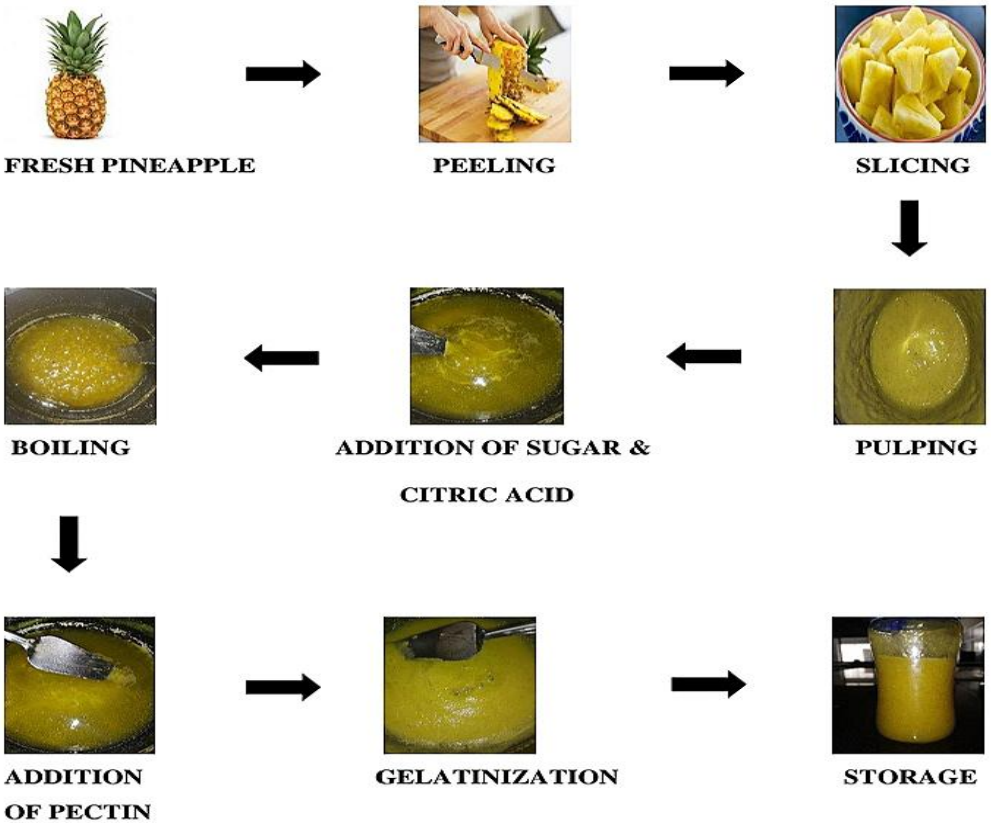

Fig 13:- Representation of preparation of jam.

Proximate analysis of pectin

The Moisture content of given pectin sample was analyzed by AOAC method - HOT AIR OVEN. It is estimated to have 86% in 10 gm of sample. The Ash content of given sample was analyzed by AOAC method – MUFFLE FURNACE. It is estimated to have 0.7% in 10 gm of sample.

Chemical analysis of pectin

The equivalent weight of given pectin sample was analyzed by AOAC method and is estimated to have 45.35gm in 50 gm of sample. Methoxyl content of coffee pulp pectin derived using Hcl was analyzed by AOAC method and it is estimated to have 2.21% in 50 gm of sample. The result shows that the anhydrouronic acid content of pectin extracted using hydrochloric acid was analyzed by AOAC method and it is determined to have 68.5% in 50 gm of sample. The galacturonic acid content of given pectin sample was analyzed by AOAC method and it is evaluated to have 78% in 50 gm of sample. The result shows that pectin extracted using hydrochloric acid was analyzed by AOAC method and it is estimated to have 18.32% in 50 gm of sample. The viscosity of given pectin sample was analyzed by AOAC method and it is evaluated to have 750cpu in 50 gm of sample. The phytochemical assay such as tannins and polyphenols were analyzed by AOAC method and it is determined to have 5.8% and 11% respectively.

Conclusion:-

The Utilization of coffee pulp for production of bioactive compound like pectin helps in overcoming the environmental issue associated with its disposal. Pectin has various functional properties and was extracted using acids and enzymes.

The pectin was extracted from dried coffee pulp powder by various extraction techniques such as acid hydrolysis and enzymatic extraction. Pectin was extracted at different time, temperature and pH combinations. The extraction conditions had major impact on the extraction yields of pectin. Extraction was done at temperatures 65°C, 75°C and 85°C and for time 30 minutes, 45 minutes and 60 minutes, and different pH 1.0, 2.0, 2.5 and 3.0 respectively. The yield of pectin was higher at enzymatic extraction method. The best yield of pectin was obtained at pH 2.5,

temperature 85°C and time duration of 45 minutes. It was also concluded that with increase in extraction time and temperature, the pectin yield increases and also investigated that the pH is inversely proportional to the pectin yield.

The Physio chemical properties of pectin were investigated by determining the values of pectin yield, ash content, moisture content, equivalent weight, methoxyl content, anhydrouronic acid content, degree of esterification, viscosity were investigated for the extracted pectin, and analyzed the phytochemicals assay of tannins and polyphenols. The extracted pectin was incorporated in jam, there was no significance difference between the control sample and developed sample. The properties were analyzed and was reported that the quality of this pectin almost meets out the specifications of commercial food grade pectin.

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