INTENSIFICATION OF EXTRACTION OF LIGNOCELLULIC PRODUCTS FROM SUSTAINABLE FEEDSTOCK

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ABSTRACT

The development of sustainable technology for extraction of value added products from the sustainable feedstock is gaining importance. Ferulic acidis a phenolic acid of low toxicity and one of the most important medical components possessing anti-oxidant properties. p-Coumaric acid is a hydroxycinnamic acid, an organic compound is a hydroxy derivative of cinnamic acid. It has an antioxidant property and is reduce the risk of stomach cancer. The present work is to study the extraction of different phenolic acids from agricultural wastes and to study the optimum extraction parameters. In this paper, the phenolic compounds (Ferulic acid and p-Coumaric acid) from agricultural wastes (Maize bran and bagasse peel) are extracted by using conventional method and sonochemical reactor extraction processes. The experiments is carried out in different levels starting from making different normalities solutions by using different alkaline, NaOH and KOH up to chromatography study of different phenolic compounds by using High-Performance Liquid Chromatography. Effects of extraction time, effect of extraction temperature, effect of solid/liquid ratio were optimized for each method. The optimum extraction conditions are - Extraction temperature 250C, extraction time 240 min, 3N, solid to liquid ratio 0.084 g of raw material/g of NaOH/KOH solution.

Index Terms— *Sonochemical reactor, Ferulic acid, p-Coumaricacid.*

INTRODUCTION

With better understanding of natural products, an increasing number of people are becoming interested in studying the active natural products as medicines or food additives. Phenolic acids are rather a small but important food component from the aspect of nutrition which is characterised by antioxidative activity. This basic property, which is very important for life, is associated with a number of biological functions such as antimutagenicity, anticarcinogenicity, deceleration of the organism ageing and many others. The phenolic compounds belonging to cinnamic acid derivatives (e.g. Ferulic and p-Coumaric acids studied by us) differ by a stronger antioxidative activity from the phenolic compounds of the group of benzoic acid derivatives. Phenolic acids occur in a large number of plants and as secondary metabolites they are classified according to their importance into the group of substances following flavonoids. They differ from other phenols by an acid character. In plants, they can be esterified by other small molecules of aliphatic alcohols, phenols, phenolic acids and alkaloids. Using the carboxylic and hydroxylic groups, they can form bonds with starch and other polysaccharides via hydrogen or covalent bonds, and can create bridges and transverse linkages.Hydroxycinnamic acids are antioxidant polyphenols common in the human diet although their potentialhealth benefits depend on their bioavailability. Dietary antioxidants that protect low-density lipoprotein (LDL) from oxidation may help to protect atherosclerosis and coronary heart disease. The antioxidant activity of the monomeric hydroxycinnamates decreased in the following order: Caffeic acid >Sinapic acid >Ferulic acid > p-Coumaric acid.

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a derivative of cinnamic acid. It is one of the most important medical components which possess antioxidative property by virtue of the phenolic hydroxyl group in its structure. Ferulic acid is an antioxidant found naturally in plant cell walls, leaves and seeds. A good amount of Ferulic acid is found in oats, brown rice, whole wheat, bagasse, maize bran, orange peel, peanuts, apples and pineapples. Molecular Formula:C10H10O4, Molecular weight: 194.184 g mol-1, Melting point: 168-1750C.

p-Coumaric Acid is the hydroxy derivative of Cinnamic Acid with antioxidant properties. p-Coumaric acid is a major component of lignocellulose. Studies suggest that p-Coumaric Acid may reduce the risk of cancer by reducing the formation of carcinogenic nitrosamines.p-Coumaric acid can be found in a wide variety of edible plants such as peanuts, navy beans, tomatoes, carrots, and garlic. It is also found in bagasse, maize bran, orange peel, barley grain. Molecular Formula: C9H8O3, Molecular weight: 164.16 g mol-1, Melting point: 210-2130C.

Maize (Zea mays, L) bran is loaded with insoluble fiber, which is important for digestive health, and soluble fiber, which helps to lower the cholesterol level in the blood. It contains many active components,p-hydroxybenzoic, vannilic, syringic and gallic acids, p-Coumaric, caffeic, ferulic and sinapic acids.

Bagasse peel is the dry dusty pulp (a mixture of cellulose fibers) that remains after juice is extracted from sugar cane or similar plants.It contains many active components.

The conventional technique for extracting phenolic compounds is by Orbital Shaker for 6 hrs. and at room temperature.

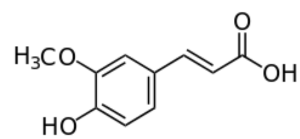
Sonochemical reactor used is high – intensity Ultrasound for 6 hrs. and at room temperature. Ultrasound, the term used to describe sounds ranging from 16 kHz to 1 GHz, usually generated by a transducer which converts mechanical or electrical energy into high frequency vibrations. The improvement in extraction efficiency due to ultrasound appears at certain values of so-called acoustic pressure. Among the most important phenomenon taking place in the acoustic field are: cavitation, friction at the boundary and interfacial surfaces, and increase in the diffusion rate.

MATERIAL AND METHODS

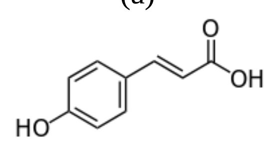
2.1Materials and Chemicals:

Ferulic acid (standard material), molecular weight - 194.19, melting point – 170 – 1720C, is purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai. p- Coumaric acid (standard material), molecular weight – 164.16, melting point – 2140C, is purchased from Sigma Life Science. Acetonitrile (HPLC Grade) and HPLC Grade Water (pH – 5-8) used as mobile phases are purchased from Merck, Mumbai. All Chemicals are used without further purification and are of analytical grade. The Chemical structures are shown in Fig.1.1

Maize bran, Bagasse peel is obtained from local vendors.



(a)



(b)

Fig.1.1 Chemical Structures of Ferulic acid (a) and p-Coumaric acid (b)

EXPERIMENTAL SETUP AND METHODS

2.2 Conventional-assisted Extraction

Conventional extraction experiments are performed in an Orbital Shaker. Orbital shaker is an ideal instrument for intensive mixing of samples in the regulated temperature. Mixing and heating modes can be used simultaneously and independently. The device can work as a shaker and as a thermostat. It has platform with clamps for flasks 100-250 ml capacity and having 8 places, having speed range – 50 – 450 rpm and temperature range – 250C – 1000C.The extraction of Ferulic acid and p-Coumaric acid is performed by adding calculated amount of sustainable feedstock (Maize bran, bagasse peel) in different normality solutions. i.e. 1N, 2N, 3N and 4N for 6 hours in a 250 mL flask.

The flask is then fixed into the orbital shaker. The sample is collected hourly i.e. at 1hr, 2hr, 3hr, 4hr, 5hr and 6hr. After extraction the solvents are centrifuge by using Laboratory Centrifuge at 4000 rpm for 20 min. After extraction, the extracts are filtered through Whatsman filter paper and then the pH of the solvent is reduced upto 3 by using 0.1 N HCl. And then solvent is analyzed using High Performance LiquidChromatography (HPLC).

2.3 Ultrasonic-assisted Extraction

Sound, including ultrasound, is transmitted through any physical medium by waves that compress & stretch the molecules spacing of the medium through which it passes Ultrasound enhances chemical reactions in a solution through the generation of Cavitation micro bubbles. The swift formulation and disintegration of thousands of minute bubbles in a liquid is known as 'Cavitation'. To develop a cavitation, a high intensity pressure wave from the transducer into a liquid is transmitted. Cavitation development and amount of energy released relies on a number of factors such as Ultrasonic Power Density, Vapor Pressure, Temperature, and Properties of the liquid.

Ultrasonic extraction experiments are performed in an ultrasonic bath is showing an ultrasonic bath, the bathis basically a rectangular container (23.5 cm x 13.3 cm x 10.2 cm), having capacity of 3L, to which 22 kHz ultrasonic transducers are annealed at the bottom. The bath power rating is 400 W on the scale of 0-10. The experiment temperature is 250C. The extraction of Ferulic acid and p-Coumaric acid is performed by adding calculated amount of sustainable feedstock (Maize bran, bagasse peel) indifferent normality solutions. i.e. 1N, 2N, 3N and 4N for 6 hours in a 250 mL flask. The flask is then partiallyimmersed into the ultrasonic bath. The sample is collected hourly i.e. at1hr, 2hr, 3hr, 4hr, 5hr and 6hr.

After extraction the solvents arecentrifuge by using Laboratory Centrifuge at 4000 rpm for 20 min. After extraction, the extracts are filtered through Whatsman filter paper and then the pH of the solvent is reduced upto 3 by using 0.1 N HCl. And then solvent is analyzed using High Performance Liquid Chromatography (HPLC).

2.4 HPLC analysis

HPLC (ELITE La Chrom) is using for analyzing Ferulic acid and p-Coumaric acid (Figure 5), Pump – L – 2130, Pressure – 45-50 bar (max. 392 bar), Lamp – UV Detector (L-2400), WL – 320 nm, Mobile phases – Solvent A - Acetonitrile (20%), Solvent B - Water (HPLC grade) (80%). The flow rate is 1.0 mL/min. The injection volume is 10 μl. The detector is set as 320 nm. All quantitativeanalyses are made by the external standard method, using Ferulic acid and p-Coumaric acid as an analytical standard.

%Yield = Area of Sample peak / Area of Standard peak x 100

RESULTS AND DISCUSSION

3.1 Calibration Curve

The progress of the yield of extraction of was monitored by measuring the concentration of ferulic acid and p-Coumaric acid using Hitachi High Pressure Liquid Chromatography (HPLC) at 320 nm wavelength. Initially the calibration curve was obtained by preparing the solutions with different concentration over the range of 10 to 300 ppm followed by analysis using HPLC.

3.2 Extraction of Ferulic acid

Conventional Method

Maize Bran Effects of NaOH and KOH on the extraction of Ferulic acid from Maize bran have shown in Figs 3.1 and 3.2 respectively. Both NaOH and KOH are strong alkalis the difference is in their solubilities in organic solvents and organic reactions. The results indicated that the yield increased rapidly with the extraction time for the first 300 min. However, the extraction yield had no noticeable enhancement when theshaking time increasing from 300 to 360 min; this may be because most of the extracts had already been extracted during the first 240 min. It has been observed that the yield of extraction is more using KOH (90.2%) compared to NaOH (88.5%) atoptimum concentration of 3 N.

These results can be justified based on the pKb value of NaOH and KOH. The pKb value of potassium hydroxide is 0.5, while the value for sodium hydroxide is 0.2 (the smaller the value of pKb, the stronger thebase). Therefore, NaOH is stronger than KOH. In addition,sodium is less electronegative than potassium, so NaOH is more willing to release the hydroxy group and it is stronger base (J. Phys. Chem. B, 2010, 114 (19), pp 6542–6548: April 22, 2010).

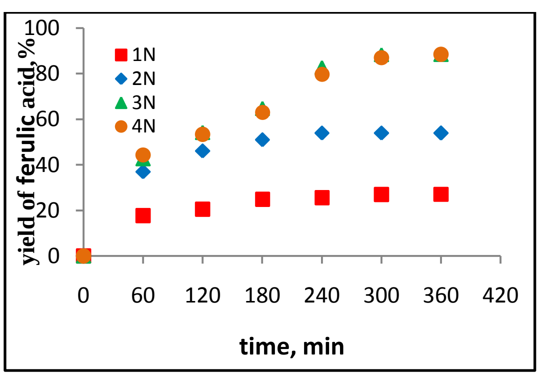


Fig. 3.1 Effect of NaOH on extraction of ferulicacid from Maize Bran using conventional method.

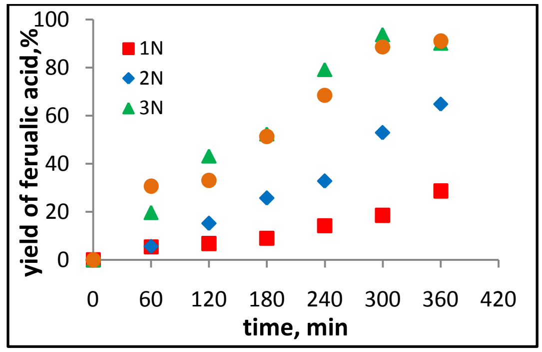


Fig. 3.2 Effect of KOH on extraction of ferulic acid from Maize Bran using conventional method.

Bagasse Peel The results of effects of NaOH and KOH on the extraction of Ferulic acid from Bagasse Peel using conventional method have shown in Figs. 3.3 and 3.4 respectively. As discussed earlier both NaOH and KOH are strong alkalis the difference is in their solubilities in organic solvents and organicreactions and it plays the significant effect on the extraction of phenolic compounds. The results indicate that the extraction yield increases with increase in concentration of alkali and time.
Ithas been observed that the maximum yield of ferulic acid observed for 4N and 3N alkali concentration for the NaOH and KOH respectively. Thoughextraction time requirement using both alkali same but there is increase in the yield of extraction incase of the KOH (86.4%) compared toNaOH concentration (82.1%). This fact can be explained based on the pKb values of NaOH and KOH.

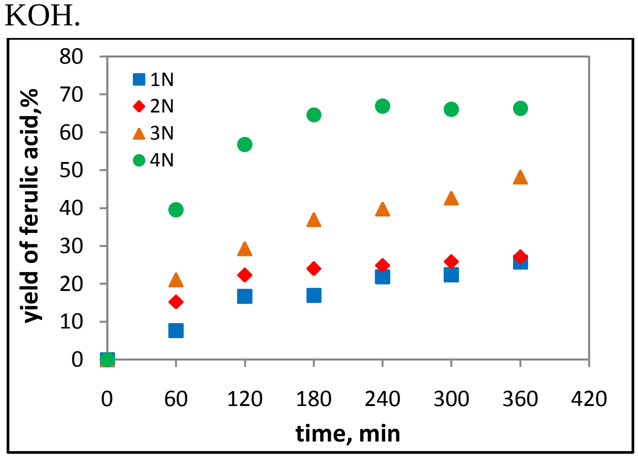


Fig. 3.3 Effect of NaOH on extraction of ferulic acid from Bagasse Peel using conventional method.

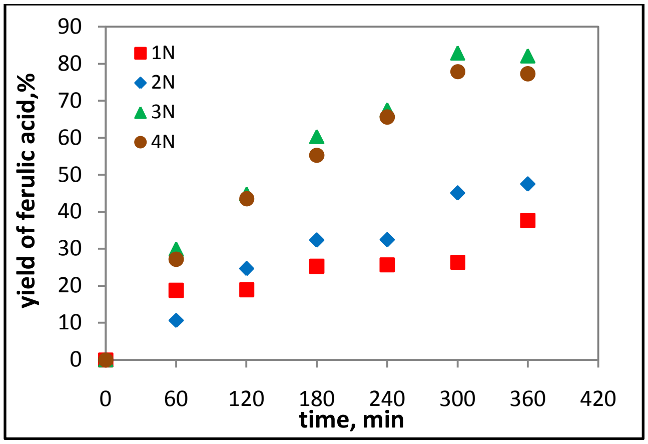


Fig. 3.4 Effect of KOH on extraction of ferulic acid from Bagasse Peel using conventional method.

The pKb value of potassium hydroxide is 0.5, while the value for sodium hydroxide is 0.2 (the smaller the value of pKb, the stronger the base). Therefore, NaOH is stronger than KOH. In addition, sodium is less electronegative than potassium, so NaOH is more willing torelease the hydroxy group and it is stronger base.

3.3 Ultrasound Method

Maize Bran The influence of extraction time on the extraction of Ferulic acid from Bagasse peel using ultrasound have been shown in Figs 3.5 and 3.6 forNaOH and KOH respectively. The extraction was investigated at constant temperatureof 250C and varying alkaliconcentration from 1N, 2N, 3N and4N. The results indicatedthat the yield increased rapidly with the extraction time and alkali concentration up to 3N and thereafter there will be marginal increment in the yield of ferulic acid yield. Though the effect of concentration of alkali same for the using NaOH and KOH but there was significant increase in the yield of Ferulic acid extraction for KOH (93.7%) compared to NaOH (88.7%). The time required for exaction for both alkalis was same (300 min). Increase in yield using KOH can be justified based on the pKb value difference. pKb value of NaOH is more compared with KOH and release the more hydroxyl ions and affects the yield of extraction. Another interesting observation the yield of extraction using the ultrasound method is more compared with conventional method. This fact can be explained based on the physical and chemical effects of the ultrasound. When the ultrasound passed through the liquid medium cavitation is occurred.

Cavitation is generation of micro-bubbles in reactor, which results in high temperature and pressure shock waves. There are two effects of cavitation created physical effects (micro-emulsification and acoustic streaming) and chemical effect like formation hydroxyl radicals. During the extraction process the chemical and physical effects of cavitation increase the yield of extraction.

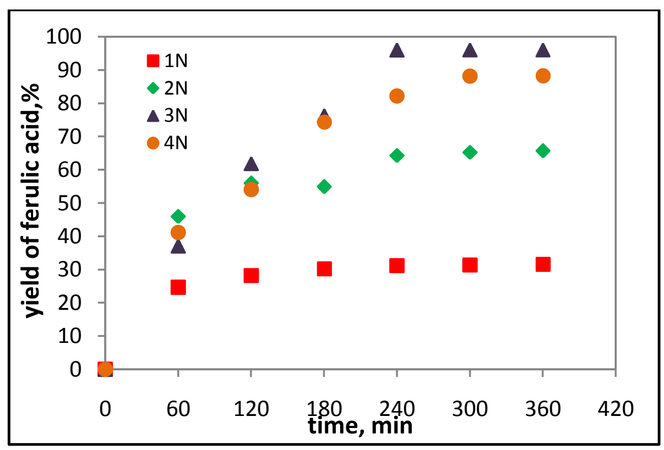


Fig. 3.5 Effect of NaOH on extraction of ferulic acid from Maize Bran using ultrasound method.

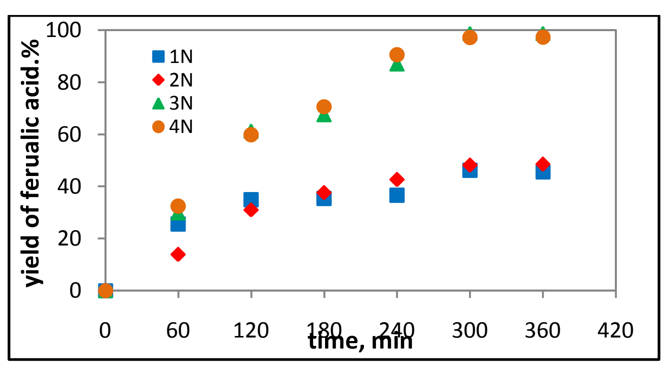


Fig. 3.6 Effect of KOH on extraction of ferulic acid from Maize Bran using ultrasound method.

Bagasse Peel The effect of concentration of NaOH and KOH on the extraction of Ferulic acid from Bagasse peel is shown in Figs 3.7 and 3.8. It has been observed that the yield of extraction of ferulic acid increase with increase in concentration from 1 N (31.3%) to 3 N (96%) and 1N (46.2%) to 3N (98.8%) for NaOH and KOH respectively. Further increase in the concentration has marginal effect on the increase in yield. Extraction time for the both alkalis concentration is same (300 min) but the yield of extraction is more in case of KOH. This effect can explain based on the pKb value of two values of two alkalis.

As pKb value of NaOH more compared with KOH which results in the higher concentration of OH radicals. These radicals may recombine to form the hydroperxyl radicals, which results in lower hydroxyl concentration. Another increasing fact has been observed in case of the ultrasound, the higher yield of ferulic acid compared to the conventional approach. This significant increase in yield can be explained based on the cavitation effect of the ultrasound, which results in, chemical and physical affects which useful in enhancing the yield of extraction. In ultrasound extraction, the phenomenon of cavitation in the solventmixture is affected by surface tension, viscosity and medium vapor pressure. In the presence of water, the intensity of ultrasonic cavitation in the solvent mixture increases as the surface tension increases while the viscosity and vapor pressure decreases. Water has a higher surface tension than ethanol, which need higher energy to produce cavitation bubbles that collapsed at a high intensity produces a shock wave that passes through the solvent enhancing mass transfer within the plant material (Ou et al., 1997; Mason et al., 1991; Roldan-Gutierrez et al., 2008; Amirah et al., 2012) which might have resulted in higher yield of aqueous extract compared to other solvent extracts.

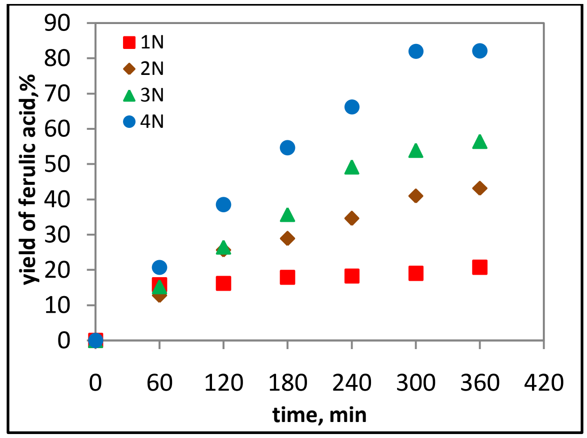


Fig. 3.7 Effect of NaOH on extraction of ferulic acid from Bagasse Peel using ultrasound method.

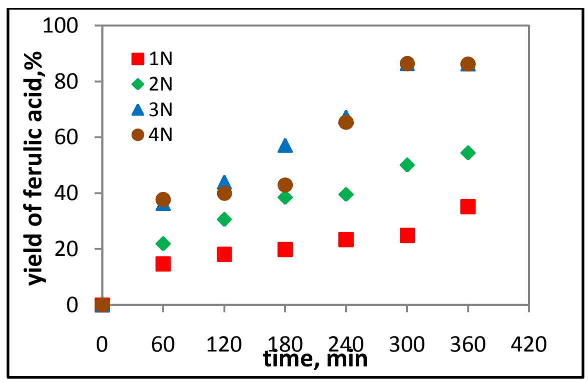


Fig. 3.8 Effect of KOH on extraction of ferulic acid from Bagasse Peel using ultrasound method.

3.4 Extraction of p-Coumaric acid Conventional Method

The lignocellulose biomass is mainly cellulose (a high molecular weight linear homopolymer of repeating d-glucose units), hemicellulose (a heterogeneous polymer composed mainly of low molecular weight polysaccharides), and lignin (a complex high molecular weight biopolymer composed mainly of phenolic compounds). The hemicellulose removal also increases the material porosity facilitating the diffusion and impregnation of the sodium hydroxide into the material in the subsequent steps. p-Coumaric acid is phenolic acid of great interest due to its chemo protectant and anti-oxidant properties. In addition, both acids are potential precursors in the bio catalytic production of value-added aromatic natural products. Development of cost effective extractive technique will be significant.

Maize Bran Effect of concentration of NaOH and KOH on extraction of p-Coumaric acid from maize bran using conventional approach have been shown in Figs 3.9 and 3.10 respectively. It has been observed the rate extraction increase with increase in alkali concentration and time. Yield of extraction was increase from 56.2 to 72.4% and 41.4 to 83.7 % for increase in concentration from 1N to 4 N forNaOH and KOH respectively.

Though the time required for extraction same for both alkalis but there was significant increase in yield of p-Coumaric acid using KOH as alkali. This effect is justified based on the pKb value or oxidizing potential of KOH and NaOH. The oxidizing potential of NaOH is more compared KOH, which may result into higher rate of formation of hydroxyl radicals, these higher radicals mayrecombine to form hydroperxyl radicals, which may reduce net oxidizing potential of NaOH. Lignocellulose material may be loose activity in presence of strong oxidizing agent, which result in lower yield of extraction of p-Coumaric acid.

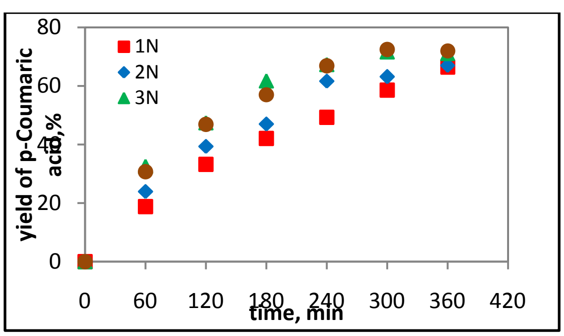


Fig.3.9 Effect of NaOH on extraction of p-Coumaric acid from Maize Bran using conventional method.

Maize Bran Results of alkaline hydrolysis of Maize Bran using ultrasound have been shown in Figs. 3.13 and 3.14 for NaOH and KOH respectively. It has been observed the yield of extraction increase with increase alkali concentration from 1N to 3 N and further increase in concentration has the marginal effect on increase in yield of extraction. Maximum is found tobe 95.1 and 98.3 % (240 min) forNaOH and KOH respectively.

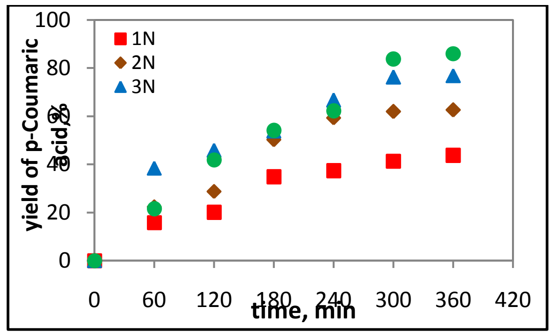


Fig.3.10 Effect of KOH on extraction of p-Coumaric acid from Maize Bran using conventional method.

Bagasse Peel

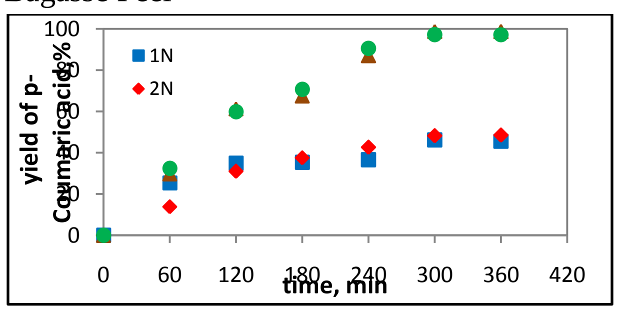


Fig.3.11 Effect of NaOH on extraction of p-Coumaric acid from Bagasse Peel using conventional method.

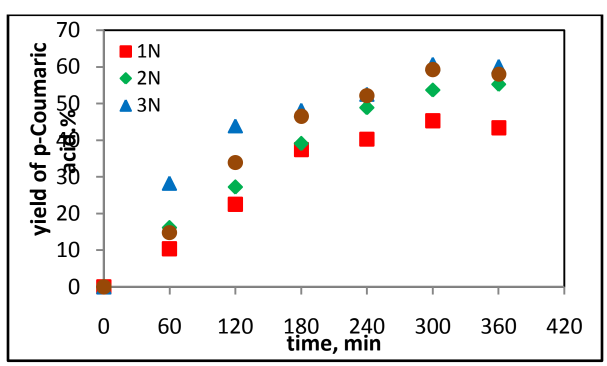


Fig.3.12 Effect of KOH on extraction of p-

Coumaric acid from Bagasse Peel using conventional method.

Similar trend were observed of extraction of p-Coumaric acid from Bagasse peel using conventional method and results are depicted in Figs 3.11 and 3.12 for concentration of NaOH and KOH respectively. Extraction yield was increase from 45.3 % (1N) to 60.8% (3N) for NaOH and 58.6 % (1N) to 72.4% (3N) for KOH. With further increase in concentration of alkalis dose not yield significant effect on the increase yield. Increase in yield of extraction using KOH can be justified based on difference in pKb values of NaOH and KOH.

3.5 Ultrasound method

The lignocellulose materials are composed by cellulose, hemicellulose and lignin, the last one being a polyphenol macromolecule from which phenolic acids can be isolated. Due to the low cost and large availability and is an underexploited raw material from which phenolic acids may be isolated, with only enzymatic extraction commonly practiced for isolation of these compounds from agricultural residues and alkaline hydrolysis also used. These processes are very slow and resulted into poor extraction yield. Physical effects associated with cavitation highly useful for enhancing the yield and considerable reduction in extraction time requirement.

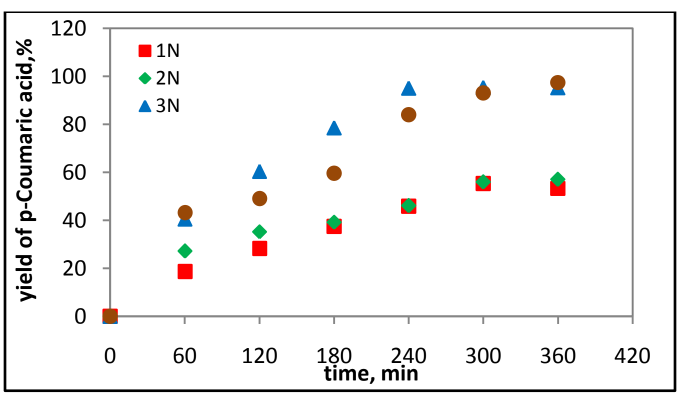


Fig.3.13 Effect of NaOH on extraction of p-Coumaric acid from Maize Bran using ultrasound method

Maize Bran Results of alkaline hydrolysis of Maize Bran using ultrasound have been shown in Figs. 4.13 and 4.14 for NaOH and KOH respectively. It has been observed the yield of extraction increase with increase alkali concentration from 1N to 3 N and further increase in concentration has the marginal effect on increase in yield of extraction. Maximum is found to be 95.1 and 98.3 % (240 min) forNaOH and KOH respectively.

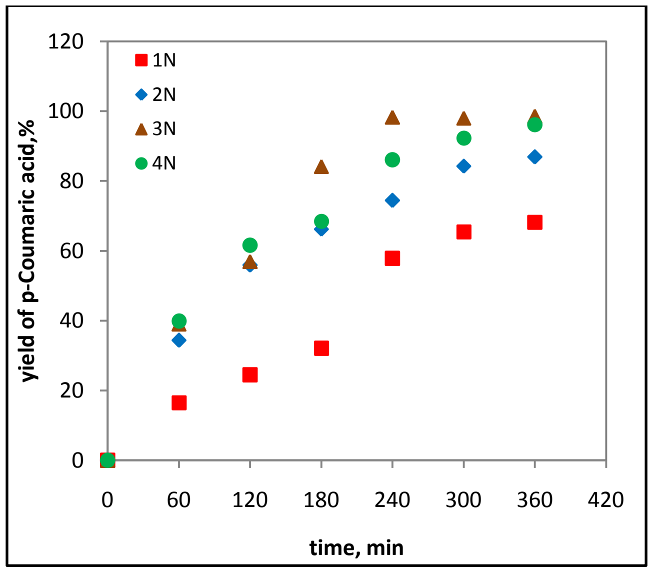


Fig.3.14 Effect of KOH on extraction of p-Coumaric acid from Maize Bran using ultrasound method.

Though there is marginal decrease in extraction time requirement compared to the conventional approach but there is considerable increase in yield of extraction. Increase in yield in ultrasound process due the physical effect of ultrasound like micro-emulsification and streaming in process. Physical effects increase the contact between the bulk and interface. Also the shocks waves created due the high temperature and pressure conditions are useful for rapture of cell wall of maize bran and release the phenolic compounds in bulkliquid.

Bagasse Peel Results of effect of NaOH and KOH on yield of p-Coumaric acid from bagasse Peel using ultrasound have been shown in Figs 3.15 and 3.16 respectively.

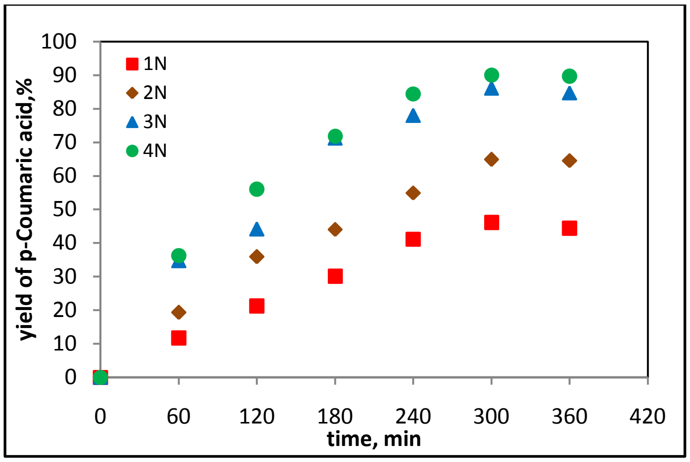


Fig.3.15 Effect of NaOH on extraction of p-Coumaric acid from Bagasse Peel using ultrasound method.

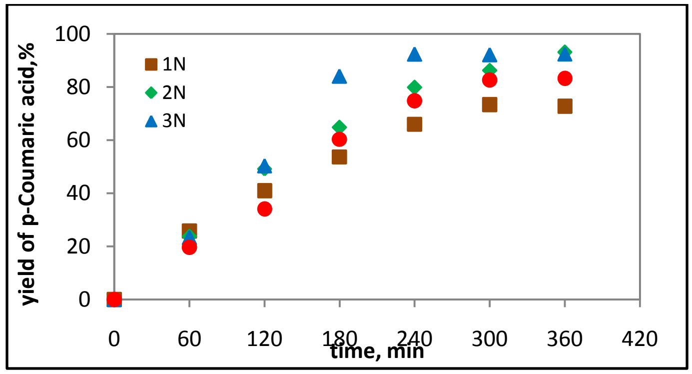


Fig.3.16 Effect of KOH on extraction of p-Coumaric acid fromBagasse Peel using ultrasound method.

It has been observed that the yield of p-Coumaric acid increase with increase in concentrationof alkali and maximum yield was obtained 90.1 and 92.4 % for 4N NaOH and 3 N KOH concentrations respectively. Increase yield using ultrasound method is higher than conventional approach though the time required for both process is same. The shock waves and associated physical effects of cavitation are useful for enhancing the yield of the extraction.

3.6 Optimized Results.

Optimized results for the extraction of Feruli acid and p-Coumaric acid have been shown in tables 4.1 and 4.2.

Parameters	Bagasse Peel		Maize bran	
	Conventional	Ultrasonic	Conventional	Ultrasonic
pH	3	3	3	3
NaOH Conc., N	4	4	3	3
KOH Conc., N	3	3	3	3
Temperature,0C	25	25	25	25
% Yield	NaOH – 60.8	NaOH – 90.1	NaOH – 72.4	NaOH – 95.1
	KOH – 72.4	KOH – 92.4	KOH – 83.7	KOH – 98.3
Extraction Time, min	NaOH - 300	NaOH - 300	NaOH – 300	NaOH - 240
	KOH - 300	KOH - 240	KOH - 300	KOH - 240

Table 3.1 Optimized parameters of extraction of Ferulic acid.

Parameters	Bagasse Peel	Maize bran		
	Conventional	Ultrasonic	Conventional	Ultrasonic
pH	3	3	3	3
NaOH Conc., N	4	4	3	3
KOH Conc., N	3	3	3	3
Temperature,0C	25	25	25	25
% Yield	NaOH – 60.8	NaOH – 90.1	NaOH – 72.4	NaOH – 95.1

	KOH – 72.4	KOH – 92.4	KOH – 83.7	KOH – 98.3
Extraction Time, min	NaOH - 300	NaOH - 300	NaOH – 300	NaOH - 240
	KOH - 300	KOH - 240	KOH - 300	KOH - 240

Table 3.2 Optimized parameters of extraction of p-Coumaric acid

The optimized result shows that the yield of ferulic acid and p-Coumaric higher in case of ultrasound compared to the conventional approach. Time required for extraction compound is marginal decrease in case of ultrasound compared to the conventional approach. As discussedearlier the results using ultrasound is more due the cavitation effect of ultrasound. The shocks wave created due cavitation (higher and temperature conditions for pico-sec) are highly useful for rupture of cell wall of agricultural residue and physical effects associated with cavitation can overcome the mass transfer barrier in the extraction process. Process develop in present work is highly useful for enhancing yield of extraction and decrease the energy requirement per unit of product extracted.

4. CONCLUSION

Considerable differences between the two techniques can be partly attributed to the different modes of action of the machines used in the extractions. We propose that the efficiency and accuracy of phenolic extraction can be enhanced by the utilization of the sonication method. Conventional Extraction method for extracting Ferulic acid and p-Coumaric acid has a number of shortcomings, including long extraction time, great consumption of solvents and lower extraction efficiency. The extraction yield by Ultrasonic extraction is compared to conventional approach. The optimum parameter for ultrasonic assisted extraction is for 3N at room temperature and extraction time of 240 min. Ultrasonic assisted method has been shown to be an effective method for extraction of phenolic compounds extracted from Maize bran and Bagasse peel compared to conventional method.

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