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PRODUCTION OF CITRIC ACID FROM LEMON AND CASSAVA WASTE PEELS

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ABSTRACT

The high demand for citric acid by many industries cannot be over emphasized. Presently, 70 % of the total citric acid produced globally is consumed by food industries, 12 % by pharmaceutical industries and the remaining 18 % by other industries. Moreover, rapid increase in food/beverage industries in Nigeria with no known company producing citric acid in Nigeria makes its scarcity severe. Cassava peels from cassava processing industries and lemon peels are abundant. Surface fermentation method was employed in production of citric acid at varying pH from 3.0 to 4.5. Physico-chemical properties and Fourier Transform Infra Red spectroscopic analysis were conducted using standard methods. The kinetics of the production process was also determined. The yields and physico-chemical properties of the citric acid obtained from both substrates were comparable to that of commercial citric acid. The yields obtained from cassava peels are lower when compared to those from lemon peels when the same quantities were used. The optimum yield obtained from lemon and cassava peels after 192 h of fermentation at optimum pH 4 were found to be 34.4 g/kg and 32.7 g/kg respectively. The infra red spectra of citric acid produced from cassava and lemon peels showed bands at 3497, 3290 and 3288 cm^{-1} depicting hydroxyl group bonded to carboxyl. The 1750 cm^{-1} carbonyl band was observed for both peels. There was increase in percentage yields of citric acid over 48 – 192 h fermentation periods for pH range of 3.0 – 4.5. The kinetics of the production process was observed to follow a pseudo-second order as it gave best fit compared to other models used. The range of pseudo – second rate constant fell within 1.045 x 10^{-3} – 2.411 x 10^{-3} kg/g.hr for pH range of pH 3.0 – 4.5 for lemon peels while that of cassava peel ranged from 1.071 x 10^{-3} to 98.91 x 10^{-3} kg/g.hr for pH 3.0 – 4.5 range. The possibility of production of citric acid in Nigeria from lemon and cassava waste peels could reduce importation of this product by industries and thus save enough revenue for the country.

Key Words: Citric acid, fermentation, kinetics, yield, physico-chemical properties

INTRODUCTION

Citric acid (2-hydroxylpropane-1, 2, 3-tricarboxylic acid), a white crystalline powder at room temperature is a weak organic acid found mostly in citrus fruits but can be produced from any substance containing sugar. It exists in either anhydrous or monohydrate form with a molecular weight of 192 and 210 g/mol respectively. It is a nearly universal intermediate product of metabolism and its traces are found in virtually all plants and animals [Papagianni, 2007]. It is solid at room temperature which melts at 153 °C and decomposes at higher temperature above 175 °C [Kubicek, 1998).].

The most notable sources of citric acid are citrus fruits such as lime, lemon and oranges, which contain the highest amount of natural citric acid. It is a natural preservative and conservative substance that can also be used to add acidic sour taste to food, soft drink, and other food products. Citric acid is used in food, beverage, pharmaceutical, chemical, cosmetic and other industries for applications such as acidulation, anti-oxidation, flavour enhancement, preservation and as a synergistic agent [Sarangbin, 1993, Archer, 2000]. The food industry consumes about 70 % of total citric acid produced while pharmaceutical industries consume about 12 %, and the remaining 18 % are consumed by other industries [Pandey *et al*., 2001; Soccol and Vandenberghe 2003]. Global production is now about 1.7 million tons and there is an estimated annual growth of 3.7–4.5 % in demand of citric acid [Soccol and Vandenberghe 2003; Soccol *et al.* 2006].

The production of citric acid can be done in three major ways namely extraction from citrus fruits, chemical synthesis and fermentation of substrates [Sarangbin, 1993]. Fermentation is the predominant way of producing citric acid and accounts for over 90% of the world production. Citric acid production could be carried out by different groups of microorganisms such as yeast, bacteria and other *Aspergillus species. Aspergillus niger* remained the organism of choice for commercial production, because it produces more citric acid per time unit [Soccol *et al.* 2006].

Although methods were well developed to synthesize citric acid using chemical reactions, better successes were achieved using microbial fermentations over the period of time [Mattey, 1992].

Citric acid production from whey with various controls such as addition of sugars (fructose, glucose, galactose and sucrose) plus other additives like riboflavin, methanol and so on have also been reported [Murad and Khalaf, 2003]. It has been reported that the nature of sugar source has a marked effect on the citric acid production by *Aspergillus niger* [Hossain, 1984]. Sucrose is the traditional commercial substrate for citric acid production while glucose, fructose and maltose have also been explored as substrates for citric acid. It was observed that citric acid production increases at different periods of fermentation in various substrates; and also differ with various fermentation media by *Aspergillus niger* 14/20 and 79/20 strains. The effects of initial pH (4.5, 5.5 and 6.5), methanol (volume fraction 1.0, 2.0 and 3.0 %) and ammonium nitrate (mass per volume ratio 0.01 %) on the production of citric acid has been examined and found that methanol and ammonium nitrate affected the yield obtained [Emine and Osman, 2004].

The increase in worldwide demand of citric acid is about 6.0×10^5 tonnes per year and it is bound to rise day by day [Hossain, 1984]. More importantly, there is a rapid increase in the number of food/beverage industries in Nigeria with little or no known company producing citric acid in the country.

Due to extensive farming and availability of cassava and lemon crops in Kwara State and Nigeria in general as well as the large application of citric acid; this research work exploits the potential of these agricultural products in production of citric acid. It is aimed at comparison of yields of citric acid from dried citrus lemon peels with the amount produced using cassava peels as substrate. It will also ascertain the optimum condition and kinetics of production process.

MATERIALS AND METHOD

Collection of Materials

Fresh lemon fruits were obtained from Owode Market in Offa, Offa Local Government Area of Kwara State. They were peeled using sharp knives and sundried for 2 weeks to remove moisture and were reduced in size using pestle and mortar. The pounded lemon peels were further pulverized using grinding machine into powdery form.

The cassava peels used were obtained directly from Tanke Market, Tipper garage in Tanke-Ilorin in Ilorin South Local Government Area of Kwara State. They were also sun-dried for two weeks followed by size reduction using pestle and mortar. Further size reduction was done using grinding machine to turn them to powdery form. The *Aspergillus niger* cultures used were supplied by Department of Microbiology, University of Ilorin, Ilorin, Nigeria.

Production of Citric Acid

Required quantities (10g) of the dried powdered lemon and cassava peels substrates were measured separately and placed into a 200 ml separate Erlenmeyer flasks. A quantity of 100 ml of distilled water was then introduced into each Erlenmeyer flask. The flasks were initially autoclaved for about 20 minutes at about 121^{o}C and pH of the pulverized lemon and cassava peels in each of the flasks was measured.

The initial pH was then adjusted to the desired range by addition of 0.1M HCl or 0.1M NaOH to lower or increase the value depending on the determined pH after the initial autoclaving. The samples were then autoclaved again before the introduction of *A. niger*. The autoclaved samples were then allowed to cool to room temperature, and inoculated with the *Aspergillus niger*.

The essence of the autoclaving is to sterilize the samples so that the microorganisms will not be contaminated. The various yields of citric acid were studied at different pH of 3.0, 3.5, 4.0 and 4.5. After separate inoculation of the substrates with *Aspergillus niger*, they were kept at 30^{o}C for fermentation periods of 2 days interval to a maximum of 8 days. The mash containing the fermented substrate and the mycelium were then filtered with absorbent cotton followed by microfiltration using vacuum pump [Laboni *et al*., 2010; Emine and Osman, 2004].

Recovery of Citric acid from Liquor

The two stages involved in the recovery of precipitates of citric acid from the cultured filtrates are:

Conversion of citric acid in the filtrate to calcium citrate using calcium hydroxide: The filtrate obtained which contained the citric acid was heated and continuously stirred while 2.5 g calcium hydroxide granules were added slowly to the filtrate until formation of precipitate was noticed. The solution was then stirred further for 40 minutes. The citric acid in the filtrate was converted to calcium citrate:

$$2C_6H_8O_7\,(aq) + 3Ca\,(OH)_2(s) \rightarrow Ca_3(C_6H_5O_7)_2.4H_2O + 2H_2O\,(l)$$

The calcium citrate was recovered by manual centrifugation for another 12 minutes until a sediment and clear solution were obtained [Emine and Osman, 2004].

For conversion of calcium citrate to citric acid, a 5 ml distilled water was then added to the recovered calcium citrate followed by 3 drops of concentrated sulphuric acid while stirring until the precipitate disappears. This led to formation of calcium sulphate precipitate and citric acid solution.

$$Ca_3.(C_6H_5O_7)_2.4H_2O(aq) + 3H_2SO_4 \rightarrow 2C_6H_8O_{7\,(aq)} + 3CaSO_4.2H_2O(s) + 2H_2O(l)$$

The solution was then stirred using glass rod for another 40 minutes and the solution containing the calcium sulphate precipitate was filtered using vacuum pump to separate the citric acid solution from the calcium sulphate. The filtrate (citric acid) was kept in freezer for 24 hours until crystal growth was observed. The liquid at the top was decanted and crystal formed was allowed to dry at room temperature. The yields of citric acid from the two substrates obtained were purified by crystallization and re-crystallization and dried. The yields of citric acid were determined in g/kg.

Physico-chemical properties of prepared and commercial citric acid

Physicochemical properties such as bulk density, ash content, moisture content, melting point, pH, yield and so on of the prepared citric acid from lemon and cassava waste peels as well as commercial citric acid (BDH) were determined using standard methods [AOAC 1990].

Furthermore, chemical confirmatory tests on the prepared citric acids and the commercial one were carried out by addition of 2 mL of aqueous $CaCl_2$ solution to about 2 ml of separate neutral solution of prepared citric acid from both substrates as well as the commercial one. No precipitate was obtained in all the samples while on heating; formation of white shinning crystals of calcium citrate confirmed the presence of Citric acid. Another confirmatory test was conducted by addition of 4-5 drops of concentrated sulphric acid to a mixture of small amount of prepared citric samples and β – naphtol, a blue colouration confirmed the presence of citric acid [Vishnoi, 1979].

FT-IR Analysis of prepared and commercial citric acid

Fourier transform infrared spectroscopic (FTIR) analysis was also conducted on prepared citric acid from both substrates as well as on commercial citric acid (BDH) using a Shimadzu 8400 FTIR machine. The samples were prepared by crushing the sample with KBr in the ratio of 1: 5 to form pellet in a IR disc. A beam of infrared light was then passed through the sample and measurement over the whole wavelength range generated absorption spectrum [Infra-Red *Spectroscopy,* 2011].

Kinetics of production of Citric Acid

The kinetics of production of citric acid was studied by measuring the quantity of yields of the acid produced at the end of the incubation periods (48, 96, 144 and 192 h) for each substrate per unit time and at various pH values.

RESULTS AND DISCUSSION

Physicochemical properties of citric acid: The physicochemical properties of produced and commercial citric acids are presented in Table 1. The bulk density of citric acid produced from lemon (0.893 kg/cm^3) and cassava (0.816 kg/cm^3) peels were found to be slightly lower than that of commercial citric acid (0.914 kg/cm^3). The melting points of citric acid prepared from both substrates were found to fall within that of commercial citric acid (Table 1). The conductivity values of citric acid derived from lemon and cassava peels is found to be very close to that of commercial citric acid (Table 1). The physical natures of the citric acids derived from both substrates as well as those from commercial source are similar while their colour and odour are also very close. The yields from the two substrates were greater than 30g/kg.

FTIR analysis

The spectra of the citric acid prepared from waste cassava peel, lemon peel and commercial (BDH) citric acid is presented in Figs. 1. The assignment of bands of infra red spectra of citric acid produced from cassava, lemon peels and commercial (BDH) are also shown in Table 2. The spectra of all the samples were characterized with their assignments as indicated in Table 2. These band values listed on Table 2 are in agreement with values reported in literature as 3424 – 3428 cm^{-1}(OH) stretching, 2925 cm^{-1} (CH) stretching asymmetric, 1743 – 1745 cm^{-1} (stretching C=O), 1640 – 1647 cm^{-1}(vibrational CO bond), 1457 – 1449 cm^{-1} (stretching CH) and 1155 – 1162 cm^{-1} (C – C stretch) [Bureau *et al.*, 2009; Mohamed *et al.* 2011]. The citric acid produced from waste cassava peels matched the commercial citric acid (BDH) better than that prepared from waste lemon peel (Table 2, Figs. 1a – 1c)).

Effect of time and pH on production of citric acid

The effect of time and pH on production of citric acid from cassava is shown in Table 3 while that of lemon is represented on Table 4.

From Table 3, it was observed that the yields obtained increased with increase in time interval with highest yield (32.7 g/kg) at 192 h after fermentation period and at pH 4.0 while at pH 3.0, the least yield (22.6 g/kg) at the same time interval was obtained. There is a decrease in yield of citric acid (27.2 g/kg) obtained at pH 4.5 after 192 h when compared to those obtained at pH 3.5 and 4.0 whose yields are 31.8g/kg and 32.7g/kg respectively. However, these amounts were observed to be more than that obtained at pH 3.0 (22.6g/kg). The amount of yield after 48 h was observed to be lowest for all pH values investigated. The optimum pH for production of citric acid via submerged fermentation using *A. niger* was found to be at pH 4.0 for any period of fermentation but highest yield was obtained after 192 h.

Similarly, Table 4 showed that the lemon yields obtained increased with increase in time interval with highest yield at 192 h for every pH value investigated. The pH 4.0 also gave the highest yield of 34.4 g/kg while pH 3.0 gave the least yield (24.3g/kg) at the same time interval when compared to yields obtained at other pH values. A decrease in yield (30.1g/kg) was also observed for fermentation of lemon peels at pH 4.5 and after 192 h of fermentation period when compared to those of pH 3.5 and 4.0 having 33.3g/kg and 34.4g/kg respectively at the same time interval. However, the yield was more than that obtained at pH 3.0 (24.3g/kg). The amount of yield after 48 h was observed to be lowest for all pH values investigated. The production of citric acid from lemon peels via submerged fermentation using *A. niger* was also optimum at pH 4.0. These observations are summarized in Figs. 2 – 5 for both cassava and lemon peels at various pH values for different periods of fermentation process.

Figs. 2 – 5 gave the yields obtained for both cassava and lemon peels at different pH values and time intervals (hours). It was also observed that production increased with increase in time, and pH values of 3.5 and 4.0 have little difference in the yields obtained with pH 4.0 having the highest yields for the two substrates. There is a decrease in yields obtained from both substrates at pH 4.5 as shown above. These findings in terms of trend are in agreement with literature report [Emine and Osman, 2004] as the values cited in literature showed trend of decrease in value from pH 4.5 (80.0 %) to pH 5.5 (76 %) which however increased to 81.0 % at pH 6.5 for under-sized semolina using *Aspergillus niger* by submerged fermentation method.

Table 1: Physicochemical properties of produced and commercial citric acid

Properties	Lemon Peels	Cassava Peels	Commercial (Citric Acid)	Literature values
Bulk Density(kg/cm^3)	0.893	0.816	0.914	0.9- 0.98
Melting Point (^OC)	154-160 ± 1.4	156-165 ± 0.7	152-157.7 ± 0.4	153
Nature of substance	Crystalline	Crystalline	Crystalline	Crystalline
Colour	White	Creamy	White	White
Odour	Odourless	Odourless	Odourless	Odourless
pH (1% solution)	2.46	3.02	2.36	2.3 – 3.0
*Conductivity (mS/cm)	1.73	1.78	1.71	N/A
**Yield (g/kg)	34.4	32.7	N/A	N/A

**Yield is measured after 192 h and pH of 4.5; *Conductivity is on 1% solution

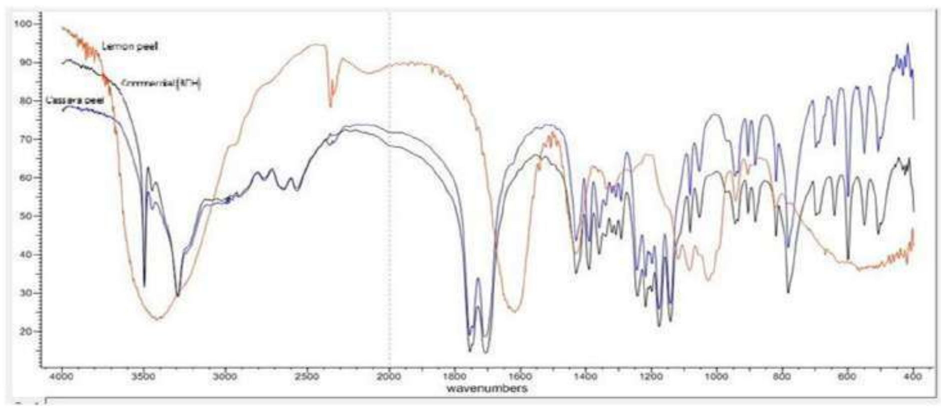

Fig. 1: Comparison of FTIR spectra of citric acid prepared from waste cassava and lemon peels with commercial (BDH) citric acid

Table 2: Assignments of Infra Red bands of citric acid derived from cassava and lemon peels

Wave numbers, cm^{-1}			Assignment of band
Cassava-based citric acid	Lemon-based citric acid	Commercial citric acid (BDH)	
3497 s	3491 s	3497 s	-OH stretch
1745 s	-	1745 s	-C = O stretch of acid
1708 w	1684 s	1705 w	Vibrational CO bond
1429 s	1449 w	1429 s	CH stretch
1141 – 1176 s	1116 – 1142 s	1142- 1177 s	C – C stretch

S means strong while w indicates weak vibration or stretch. Where – indicates no vibration or stretch was observed.

Table 3: Yields Obtained from Dried Cassava Peels at Different pH and Time Intervals

Time (h)	**pH**			
	3.0	**3.5**	**4.0**	**4.5**
48	18.3 g/kg	24.4 g/kg	24.9 g/kg	21.9 g/kg
96	20.1 g/kg	26.9 g/kg	27.5 g/kg	23.9 g/kg
144	21.0 g/kg	28.1 g/kg	28.9 g/kg	25.3 g/kg
192	22.6 g/kg	31.8 g/kg	32.7 g/kg	27.2 g/kg

Table 4: Yields obtained from dried lemon peels at different pH and time intervals

	pH			
Time (h)	**3.0**	**3.5**	**4.0**	**4.5**
48	20.1 g/kg	26.7 g/kg	27.1 g/kg	24.8 g/kg
96	22.2 g/kg	28.1 g/kg	28.8 g/kg	26.3 g/kg
144	23.6 g/kg	29.2 g/kg	29.9g/kg	28.4 g/kg
192	24.3 g/kg	33.3 g/kg	34.4 g/kg	30.1 g/kg

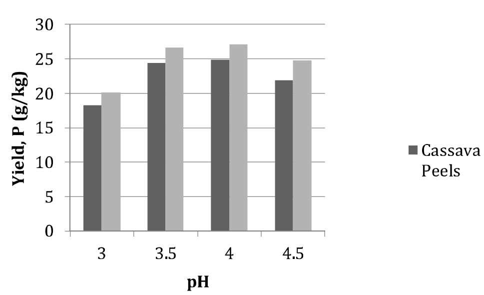

Fig. 2: Yields obtained at different pH values after 48 h for dried cassava and lemon peels.

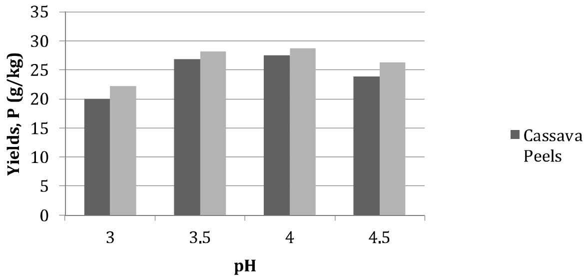

Fig. 3: Yields obtained at different pH values after 96 h for dried cassava and lemon peels.

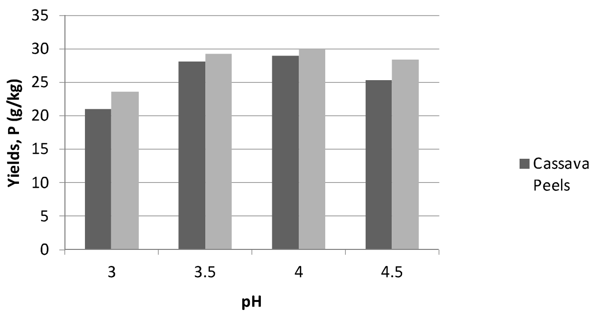

Fig. 4: Yields obtained at different pH values after 144 h for dried cassava and lemon peels.

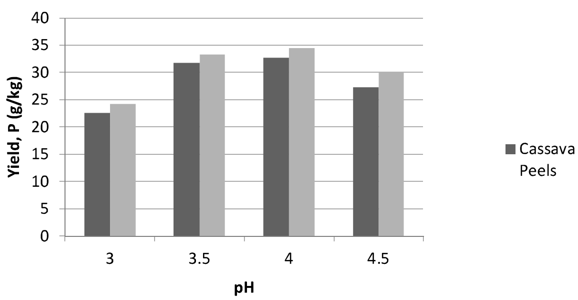

Fig. 5: Yields obtained at different pH values after 192 h for dried cassava and lemon peels.

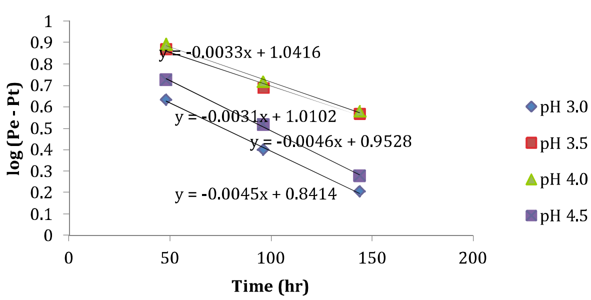

Fig. 7: Pseudo-First order plots for Citric Acid production from Cassava Peels at different pH values

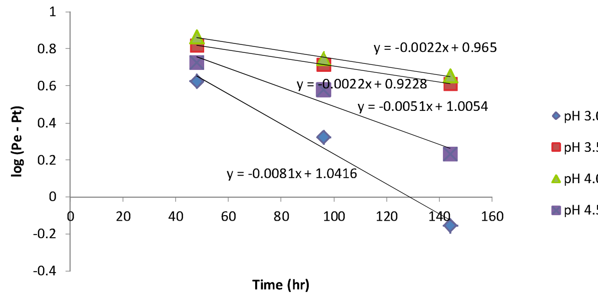

Fig. 8: Pseudo-First Order plots for Citric Acid production from lemon peels at different pH values

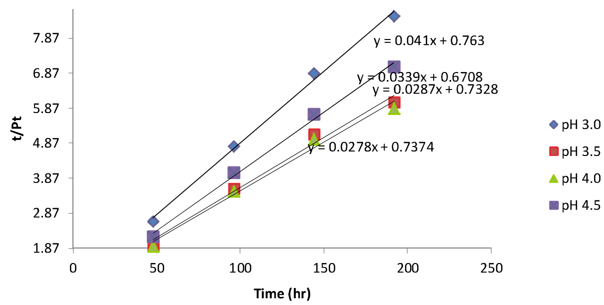

Fig. 9: Pseudo-second order plots for citric acid production from cassava peels at different pH values

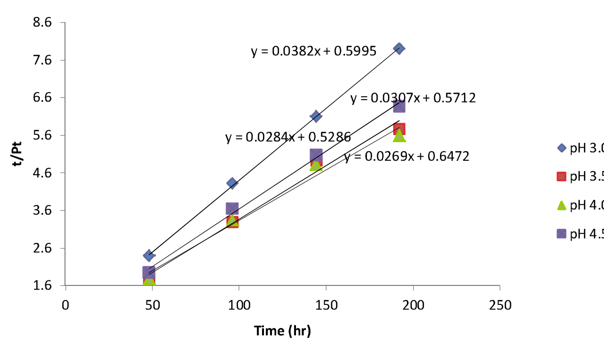

Fig. 10: Pseudo – second order plots for citric acid production from lemon peels at different pH values

Kinetics of Production of Citric acid

The kinetics of production of citric acid from cassava and lemon peels was investigated by fitting experimental data into each of the following equations (Ho and McKay, 2000):

i. First order production of citric acid kinetic model

$$\log P_t = \log P_o - \frac{k_1}{2.303}t \qquad 1$$

ii. Second order production of citric acid kinetic model

$$\frac{1}{P_t} = \frac{1}{P_o} + kt \qquad 2$$

iii. Pseudo - first order production of citric acid kinetic model

$$\log\left(P_e - P_t\right) = \log P_e - \frac{k_{s1}}{2.303}\, t \qquad 3$$

iv. Pseudo-second order production of citric acid kinetic model

$$\frac{t}{P_t} = \frac{1}{ks_2 P_e^{\,2}} + \frac{t}{P_e} \qquad 4$$

where P_t is the amount of citric acid obtained after time, t while P_e is the amount of citric acid obtained at equilibrium. The symbols k_1, k_2, ks_1 and ks_2 represent the rate constants of first, second, pseudo- first order and pseudo second order for citric acid production kinetics respectively.

It was observed that the separate production of citric acid from cassava and lemon peels at different pH does not follow first order model as plot of log P_t versus time, t did not agree with graphical representation of first order model (graphs not shown). Moreover, their slopes are positive instead of being negative which implies that the rate constant for first order, k_1, will be negative, confirming that the production process does not follow first order model. Similarly, the experimental data obtained for the production of citric acid from cassava and lemon peels did not fit into graphical representation of pseudo-second order model (graphs not shown). Thus, their graphs exhibited negative slopes depicting that second order rate constant values to be negative. This confirms that the production process cannot be explained to follow second order model.

However, the plot of log (Pe – Pt) against time, t for citric acid prepared from either waste cassava peel or lemon peels at various pH values gave linear graphs that fitted well into pseudo – first order kinetics (Figs. 7 and 8). This indicates that negative slopes are obtained and as such positive rate constants. Fig. 7 depicts plot of pseudo – first order kinetics. The experimental data obtained fitted well into pseudo – first order kinetics for both substrates. Their correlation coefficient was found to be high and fell within the range of 0.986 – 0.998 for all lemon pH values while those of cassava peels fell within range of 0.945 – 1.000. The graphical representation of pseudo – first order kinetic model confirmed that the production process might have followed pseudo first order.

Fig. 9 showed that the production process kinetics of citric acid from cassava peels at different pH values followed pseudo – second order since the graphical representation of this model exhibited a positive slope in accordance with pseudo – second order kinetics equation. Similarly, Fig.10 also depicted that the production process of citric acid from lemon also followed pseudo – second order kinetic process. Its positive slope shows graphical representation of pseudo – second order equation which confirmed that the production process followed pseudo – second order. From the pseudo –first order and pseudo – second order production process kinetics, the various rate constants were calculated and presented in Table 5. The pseudo-first order rate constants obtained for lemon peels were found to range from 4.606 x 10^{-3} h^{-1} to 18.424 x 10^{-3} h^{-1} while that of cassava peel ranged from 6.909 x 10^{-3} h^{-1} to 9.212 x 10^{-1} h^{-1} for pH range of pH 3.0 – 4.5. The range of pseudo – second rate constant fell within 1.045 x 10^{-3} – 2.411 x 10^{-3} kg/g.hr for pH range of pH 3.0 – 4.5 for lemon peels while that of cassava peel ranged from 1.071 x 10^{-3} to 98.91 x 10^{-3} kg/g.hr. The trend observed for the pseudo-first order rate constants obtained for lemon peels is that as the pH increased, the rate constant decreased until an optimum pH was passed when the rate constant increased again.

This same trend was followed by cassava peel and the decrease in pseudo first order rate constant as pH increased from pH 3 to pH 4 and then to pH 4.5 whereby the pseudo-first order rate constant increased again. For the pseudo-second order kinetic model, as pH value increased, its rate constant decreased until it increased again at pH 5 for preparation of citric acid from lemon substrate. The highest rate constant (98 x10^{-3} kg/g.h) was obtained during preparation of citric acid from cassava peel whose experimental data were fixed into pseudo – second order kinetics. It also occurred at the optimum pH of 4.

Table 5: Summary of rate constants obtained for citric acid production process kinetics

Substrate types	pH values	Pseudo- first order rate constants, h^{-1}		Pseudo-second rate constant, kg/g.h	
		$ks_{1x\,10}^{-3}$	R^2	ks_2 x10^{-3}	R^2
Cassava peel	3.0	9.212	0.998	2.203	0.996
	3.5	6.909	0.986	1.071	0.985
	4.0	6.909	0.996	98.910	0.985
	4.5	9.212	0.997	1.625	0.996
Lemon peel	3.0	18.424	0.973	2.411	0.999
	3.5	4.606	1.000	1.485	0.983
	4.0	4.606	0.997	1.045	0.979
	4.5	11.515	0.945	1.576	0.996

Ks_1 and ks_2 are pseudo – first and pseudo – second order rate constants; R^2 indicates correlation coefficient

CONCLUSION

Citric acid had been successfully produced from both lemon and cassava peels using surface fermentation and *Aspergillus niger* as the fermenting microorganism. It was found out that the yields obtained from lemon peels are a little greater than those from cassava peels when the same mass of raw materials were used. Among kinetic models investigated, for the production process for rate of formation from lemon and cassava peels, pseudo second order kinetics gave the best fit. Moreover, the highest rate constant was obtained at pH 4 for pseudo-second order kinetics using cassava peel as substrate in production of citric acid. The FTIR spectra of citric acid obtained using cassava peel is found to be comparable to that of commercial citric acid (BDH) (Table 2). Therefore, it was found that cassava peels can be used as a cheaper source of producing citric acid because of its abundance in our country, Nigeria being a major producer of cassava globally.

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