RESEARCH NOTE

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Studies on the Production of Protein Hydrolysates from Palm Kernel Meal and *Jatropha curcas* Seed Meal

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Three trials were conducted to study the optimum conditions required for production of protein hydrolysates from palm kernel meal and *Jatropha curcas* seed meal. Hydrochloric (HCl) and sulphuric (H_2SO_4) acids of varying concentrations (2, 4, 6, 8, 10 moles dm^{-3}) were tested for hydrolysis of each of the two plant sources at different temperatures (65, 80, 95, 110, 125°C) and for different durations (6, 12, 18, 24, 30 h). Results indicated that with both acids, protein hydrolysates yield and amino acid decomposition were positively and significantly correlated with acid concentration, temperature and duration of hydrolysis. However, HCl produced higher yield of hydrolysates with lower decomposition of amino acids from both protein sources. High yield of hydrolysates coupled with higher degree of amino acid decomposition were observed in *J. curcas* seed meal than in palm kernel meal. Optimum yield of hydrolysates was obtained from *J. curcas* seed meal when hydrolysed with either 6 moles dm^{-3} HCl or 8 moles dm^{-3} H_2SO_4 at 95°C for 18 h. In case of palm kernel seed meal, optimum yield was achieved at 110°C with either 8 moles dm^{-3} HCl or 10 moles dm^{-3} H_2SO_4 for 24 h and 18 h, respectively.

Keywords : Palm kernel meal, *Jatropha curcas* seed meal, Protein hydrolysates.

Protein hydrolysates obtained from both animal and vegetable protein sources find useful applications in food industries where they are utilised as condiments and amino acid sources in diets (Meister 1965). High production cost of hydrolysates associated with animal protein sources particularly in developing countries including Nigeria where animal production is low has made popular the use of vegetable sources (Pham and del Rosario 1983). The study reported here concerned determination of optimum conditions of acid hydrolysis of palm kernel meal and *J. curcas* seed meal proteins. The vegetable protein sources reported were chosen in view of their relatively high availability in the country and the low cost protein advantage that they offer.

J. curcas, physic nut tree, is an oleaginous shrub that grows spontaneously and under cultivation in dry tropical countries and in humid equatorial regions. Although it prefers cool soils, it grows vigorously with little or no care on arid escarpments and can adapt to long periods without rain. The seed is a good source of curcas oil used as fuel oil and for manufacture of soap, illumination and lubricating in wood industry. In Nigeria, the tree is planted only as hedging plant for demarcating boundaries in the households and as a windbreak and barrier against erosion on farm lands (Nir 1988).

Kernels and seeds of freshly harvested palm fruits and *J. curcas* fruits were oven dried at 80°C for 24 h, and ground in a Waring mill to produce powder, which passed through an 80 mesh (180 µm). Milled samples were de-fatted by extracting their lipids with cold acetone 1:3 w/v in three successive times prior to total N determination by using the Micro Kjeldahl method (AOAC 1990). Protein contents were estimated by multiplying % N with 6.25. The bulk of each of the milled samples was divided into three batches and then used in three hydrolysis trials conducted to determine optimum conditions for production of protein hydrolysates.

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Determination of optimum acid concentration for hydrolysis - Trial 1: In the first batch each of the two milled vegetable samples was subdivided into twenty equal portions (i.e ten duplicate groups) and then randomly allotted to a 2 x 5 factorial treatment combination. The factors were acid type (HCl and H_2SO_4) and acid concentration (2, 4, 6, 8, 10 moles dm^{-3}). Hydrolysis of proteins in samples was done with the respective acid types and concentrations at a fixed temperature of 110°C for 24 h. Yield of protein hydrolysates and the extent of decomposition of amino acid produced were measured as quantities of amino acid - N and ammonium - nitrogen (NH^{4+} - N) in hydrolysates, respectively (Pham and del Rosario 1983).

Determination of optimum temperature for hydrolysis - Trial 2 : In the second batch each of the milled vegetable samples was subdivided into twenty equal portions as in the first trial and then randomly allotted to a 2 x 5 factorial treatment combination. The factors in this case were acid type (HCl and H_2SO_4) and temperature (65, 80, 95, 110, 125°C). Acid concentration used for hydrolysis in each of the respective treatment group was based on the optimum concentration for each acid type and vegetable protein source resulting from the observations in the first trial. Duration of hydrolysis was maintained at 24 h. Yield of hydrolysates and decomposition of amino acid were measured as in the previous trial.

Determination of optimum duration of hydrolysis - Trial 3 : Ten duplicate groups (twenty equal subdivided portions) of the third batches of each of the milled samples were randomly assigned to a 2 x 5 factorial treatment combination in the third hydrolysis trial. The factors being acid type (HCl and H_2SO_4) and duration of hydrolysis (6, 12, 18, 24, 30 h). For each vegetable protein source, optimum temperature and acid concentration conditions resulting from the observations in the first and second trials were maintained for hydrolysis. Yield of hydrolysates and decomposition of amino acid were measured as in the two previous trials.

TABLE 1. EFFECT OF ACID CONCENTRATION AND TYPE ON YIELD OF PROTEIN HYDROLYSATES AND AMINO ACID DECOMPOSITION AFTER 24 H OF HYDROLYSIS OF PALM KERNEL MEAL (PKM) AND *JATROPHA CURCAS* (JC) AT 110°C.

Acid	Concentration, moles dm^{-3}	Yield of hydrolysates, g amino acid-N $100g^{-1}$		Decomposition of amino acid, mg NH_4^+ - N $100g^{-1}$	
		PKM	JC	PKM	JC
HCl	2	71.15^{d}	77.04^{d}	3.00^{c}	6.00^{c}
	4	88.94^{c}	91.34^{c}	3.50^{c}	6.50^{bc}
	6	93.18^{b}	97.70^{b}	4.00^{b}	7.50^{ab}
	8	96.56^{ab}	100.00^{a}	5.00^{b}	8.50^{a}
	10	100.00^{a}	100.00^{a}	6.50^{a}	9.00^{a}
	Mean1+	89.996	93.216	4.40	7.50
H_2SO_4	2	38.29^{t}	50.92^{s}	7.00^{r}	12.00^{s}
	4	59.64^{s}	79.72^{r}	9.50^{r}	17.00^{s}
	6	74.74^{r}	90.35^{q}	12.50^{q}	41.00^{r}
	8	85.70^{q}	97.05^{p}	14.50^{q}	71.00^{q}
	10	98.25^{p}	100.00^{p}	17.50^{p}	97.00^{p}
	Mean2+	71.324	83.608	12.20	47.60

Mean values in a column within an acid type followed by different superscripts (a-d for HCl group; p-t for H_2SO_4 group) differ significantly at $p<0.05$
1+mean values for the HCl group; 2+ mean values for the H_2SO_4 group

Statistical analysis : Results obtained were subjected to analysis of variance and regression and correlation analyses in accordance with the procedures of Steel and Torrie (1980). Significantly different treatment means were separated by multiple range test of Duncan (1955).

Effect of acid type and concentration - Trial 1 : Results of nitrogen determination showed that the protein contents of palm kernel meal and *J. curcas* seed meal were 19.67 and 38.98%, respectively. The yield of protein hydrolysates from both vegetable protein sources were remarkably influenced by the type and concentration of acid used for the hydrolysis at 110°C (Table 1). Irrespective of the protein source and type of acid used, yield was positively and significantly correlated with acid concentration (Table 2). However, the rates of release

TABLE 2. REGRESSION EQUATIONS (LINES) SHOWING RELATIONSHIP BETWEEN YIELD OF HYDROLYSATES (Y) OR DECOMPOSITION OF AMINO ACIDS (Y_o) AND CONDITIONS OF HYDROLYSIS-ACID CONCENTRATION, TEMPERATURE (θ) AND DURATION OF HYDROLYSIS (T).

Parameters	JC			PKM		
	Regression line	R^{2*}	r^*	Regression line	R^{2*}	I^*
Yield and acid concentration	$Y = 14.825\ Ln\ [HCl] + 68.746$	0.9409	0.8885	$Y = 17.4[HCl] + 61.244$	0.9598	0.9150
	$Y = 30.782[H_2SO_4] + 32.747$	0.9698	0.9192	$Y = 36.471Ln\ [H_2SO_4] + 11.123$	0.9898	0.9907
Yield and temperature (θ)	$Y = -0.0344\theta^2 + 7.8158\theta - 339.66$ (in HCl hydrolysis)	0.9806	0.8929	$Y = -0.0193\theta^2 + 5.144\theta - 237.95$ (in HCl hydrolysis)	0.9166	0.9323
	$Y = -0.0332\theta^2 + 7.64\theta - 335.67$ (in H_2SO_4 hydrolysis)	0.9764	0.9025	$Y = -0.0026\theta^2 + 2.0053\theta - 105.51$ (in H_2SO_4 hydrolysis)	0.9374	0.9677
Yield and time (t)	$Y = 0.1007t^2 + 4.7684t + 46.188$ (in HCl hydrolysis)	0.9659	0.8332	$Y = -0.1218t^2 + 6.1019t + 24.382$ (in HCl hydrolysis)	0.9234	0.8580
	$Y = -0.1006t^2 + 4.764t + 45.918$ (in H_2SO_4 hydrolysis)	0.9512	0.8268	$Y = -0.1054t^2 + 5.4897t + 24.722$ (in H_2SO_4 hydrolysis)	0.9890	0.9100
Decomposition and acid concentration	$Y_o = 1.923Ln\ [HCl] + 4.3251$	0.9195	0.9923	$Y_o = 2.3938e^{0.0952[HCl]}$	0.9795	0.9687
	$Y_o = 6.6266e^{0.2805[H2SO4]}$	0.974	0.9790	$Yo = 4.5782[H_2SO_4]^{0.5637}$	0.9884	0.9985
Decomposition and temperature (θ)	$Y_o = 0.0036\theta^2 - 0.5329\theta + 23.138$ (in HCl hydrolysis)	0.9600	0.9007	$Yo = 0.0038\theta^2 - 0.5931\theta + 26.091$ (in HCl hydrolysis)	0.9501	0.8584
	$Y_o = 0.8362e^{0.026[\theta]}$ (in H_2SO_4 hydrolysis)	0.9641	0.9194	$Yo = 0.8313e^{0.0251[\theta]}$ (in H_2SO_4 hydrolysis)	0.9618	0.9280
Decomposition and time (t)	$Y_o = 0.4444t^2 - 11.6t + 68.4$ (in HCl hydrolysis)	0.8996	0.7708	$Y_o = 2.151e^{0.0548t}$ (in HCl hydrolysis)	0.9354	0.7862
	$Y_o = 0.4609t^2 - 11.023t + 67.048$ (in H_2SO_4 hydrolysis)	0.9820	0.8544	$Y_o = 0.3081t^2 - 4.7768t + 22.16$ (in H_2SO_4 hydrolysis)	0.9107	0.9018

*Significant at $p < 0.05$

of amino acids from the hydrolysis of test protein sources differ with type and concentration of acid. With both test protein sources, mean yields of protein hydrolysates were more in case of HCl than H_2SO_4. Whereas 6 moles dm^{-3} HCl was optimum for hydrolysis of *J. curcas* seed meal protein, 8 moles dm^{-3} HCl was optimum for hydrolysis of palm kernel meal. Complete hydrolysis of *J. curcas* seed meal and palm kernel meal proteins was achieved with 8 and 10 moles dm^{-3} HCl, respectively. While complete hydrolysis of *J. curcas* was achieved with 10 moles dm^{-3} H_2SO_4, the highest acid concentration tested was inadequate for complete release of amino acids from palm kernel meal. However, 8 and 10 moles dm^{-3} of the acid appeared optimum for the hydrolysis of the proteins of the test sources. It appeared therefore that *J. curcas* was more susceptible to acid hydrolysis than palm kernel meal. Lawrence and Moore (1951) reported that the higher the protein concentration in the source the higher the rate of acid hydrolysis of protein. Furthermore, the authors noted that impurities such as fat and carbohydrate in the source inhibited the hydrolytic reaction of the acid and the peptide bonds of protein. Pham and del Rosario (1983) observed higher resistance to acid hydrolysis of protein from coconut compared with that of soybean due to higher fat and carbohydrate contents of the former. Lower protein content of palm kernel meal coupled with its higher carbohydrate content (Oyenuga 1968) might have accounted for the higher resistance of its protein to acid hydrolysis The higher mean yield of hydrolysates recorded with HCl as the medium over H_2SO_4 at the same concentration and for the same duration of hydrolysis is indicative of achievement of higher rate of hydrolysis with the former; and this finding confirmed earlier finding of Light and Smith (1963).

A serious problem associated with acid hydrolysis of protein is the destruction of some amino acids. Harfenist (1953) and Hirs et al (1954) showed that the degree of destruction is a function of the composition of the protein, the temperature, the time, and the concentration of protein used for hydrolysis. In the present study, the degree of decomposition of amino acids of the hydrolysates of both vegetable protein sources was significantly and positively correlated with the acid concentration, temperature and time used for the hydrolysis irrespective of the type of acid used. The regression equations describing the relationships, coefficients of estimation, and correlation coefficients are given in Table 2. Heating with H_2SO_4 caused more destruction of amino acids in the hydrolysates of both protein sources than when HCl was used for the hydrolysis. Also, greater losses of amino acids were noted in *J. curcas* seed meal protein hydrolysates than in the palm kernel meal protein hydrolysates when either of the acid types was used (Table 1). However, for both vegetable protein sources while 6 moles dm^{-3} HCl kept amino acid decomposition at marginal level, 4 moles dm^{-3} of the acid maintained the destruction at a low level. With H_2SO_4, 8 moles dm^{-3} caused marginal loss of amino acid in palm kernel meal protein hydrolysates, whereas 6 moles dm^{-3} of the acid caused marginal destruction in *J. curcas*. Low level of destruction in both protein sources was observed at 4 moles dm^{-3} of H_2SO_4.

Effect of temperature - Trial 2 : In order to optimise the energy utilisation for production of hydrolysates from the two

TABLE 3. EFFECT OF TEMPERATURE ON YIELD OF PROTEIN HYDROLYSATES AND AMINO ACID DECOMPOSITION AFTER 24 H OF HYDROLYSIS OF PALM KERNEL MEAL (PKM) AND *JATROPHA CURCAS* (JC) USING OPTIMUM CONCENTRATIONS OF HCl AND H_2SO_4.

Acid type	Temperature, °C	Yield of hydrolysates, g amino acid - N $100g^{-1}$		Decomposition of amino acid, mg NH_4^+ - N $100g^{-1}$	
		PKM	JC	PKM	JC
HCl	65	21.25d	24.50s	3.10b	3.30b
	80	32.90c	59.85b	3.50b	4.00b
	95	87.40b	98.80a	4.20b	5.40b
	110	95.73a	100.00a	5.40b	6.70b
	125	100.00a	100.00a	11.50a	13.10a
Mean[1+]		67.456	76.63	5.54	6.50
H_2SO_4	65	19.02s	22.85r	4.00r	4.60r
	80	28.50r	55.70q	7.45r	7.70r
	95	58.60q	97.36p	8.35r	9.25r
	110	96.60p	100.00p	11.50q	13.10q
	125	98.00p	100.00p	21.20p	25.35p
Mean[2+]		60.144	75.182	10.50	12.00

* Mean values in a column within an acid group followed by different letters (i.e. a - d for HCl group; p - t for H_2SO_4 group) differ significantly at $P>0.05$

[1+] Mean values for the HCl group;[2+] mean values for the H_2SO_4 group, optimum concentrations of HCl is 8 and 6 moles dm^{-3} and H_2SO_4 is 10 and 8 moles dm^{-3} for PKM and JC, respectively under both yield of hydrolysates and decomposition of amino acid.

protein sources, hydrolysis were carried out with each of the sources at the optimum acid concentration that resulted from the trial 1, that is, *J. curcas* (6 mole dm^{-3} HCl; 8 moles dm^{-3} H_2SO_4) and palm kernel meal (8 moles dm^{-3} HCl; 10 moles dm^{-3} H_2SO_4). Duration of hydrolysis was maintained at 24 h in all cases. Regardless of the acid type, the temperature of hydrolysis significantly affected rate of release of amino acids from both protein sources (Table 3) and the yield of hydrolysates and the temperature of hydrolysis were positively and significantly correlated (Table 2). Whereas complete hydrolysis of palm kernel meal protein was achieved at 125°C with 8 moles dm^{-3} HCl, lower acid concentration (6 moles dm^{-3}) at 110°C brought about complete hydrolysis of *J. curcas* protein in 24 h (Table 3). However, optimal yields of the hydrolysates were obtained from hydrolysis carried out at 110°C and 95°C with the respective acid strengths for palm kernel meal and *J. curcas,* respectively. In case of H_2SO_4, while 10 moles dm^{-3} was unable to bring about complete hydrolysis of palm kernel meal protein at the highest temperature tested (125°C) in 24 h, a lower strength of the acid (8 moles dm^{-3}) caused complete release of amino acids from *J. curcas* protein at 110°C within the same duration of hydrolysis. However, hydrolysis of palm kernel meal protein with 10 moles dm^{-3} H_2SO_4 at 110°C appeared adequate. For *J. curcas,* 8 moles dm^{-3} H_2SO_4 at 95°C seemed adequate.

Results of decomposition of the amino acids (Table 3) showed that hydrolysing protein from the two sources with HCl at the optimum concentrations as determined in trial 1 and heating at temperature up 110°C kept amino acid decomposition at a low level. On the other hand, hydrolysing proteins from both sources with H_2SO_4 at temperature up to 95°C using the respective optimum acid concentrations maintained low-level destruction of amino acids. Heating the hydrolysates at 110°C with H_2SO_4 resulted in marginal destruction of amino acids.

Effect of time - Trial 3 : In this experiment, hydrolysis trials were carried out for duration ranging from 6 to 30 h using the optimal acid concentrations and temperatures of hydrolysis observed for each protein source in the previous two trials. Irrespective of the protein source, acid type and concentration and temperature, yield of hydrolysates was significantly affected by the time (Table 4). Both variables were positively and significantly correlated (Table 2). The result seemed to suggest that yield of amino acid in acidic hydrolysis of protein is a function of time, and it is a view supported by earlier finding (Light and Smith 1963). With HCl as the medium, complete hydrolysis of *J. curcas* seed meal and palm kernel meal proteins were achieved in 24 and 30 h, respectively. However, optimum yields of hydrolysates were achieved in 18 h (*J. curcas*) and in 24 h (palm kernel meal). With this acid (i.e. HCl) at the optimum concentration and temperature, destruction was maintained at remarkably low level when continued for up to 24 h. In case of H_2SO_4, complete hydrolysis was achieved in 30 h in both test protein sources. Optimum yields were obtained in 18 h in both cases. Results of decomposition of the amino acids also indicated that least destruction was maintained when heating lasted 18 h.

From the foregoing, it may be concluded that hydrolysis of *J. curcas* seed protein was achieved with minimal amino

TABLE 4. EFFECT OF DURATION OF HYDROLYSIS ON YIELD OF PROTEIN HYDROLYSATES AND DECOMPOSITION OF AMINO ACID FROM PALM KERNEL MEAL (PKM) AND *JATROPHA CURCAS* (JC) USING OPTIMUM CONCENTRATION OF HCl AND H_2SO_4 AND TEMPERATURE

Acid type	Time, h	Yield of hydrolysates, g amino acid - N $100g^{-1}$		Decomposition of amino acid, mg NH_4^+- N $100g^{-1}$	
		PKM	JC	PKM	JC
HCl	6	53.11c	69.58c	5.00b	6.00b
	12	88.33b	92.28b	6.70b	8.00b
	18	90.91b	98.82a	10.00b	12.00b
	24	97.48a	100.00a	20.00b	20.00b
	30	100.00a	100.00a	110.00a	132.00a
Mean[1+]		85.966	92.136	30.34	35.60
H_2SO_4	6	59.33r	69.97r	8.00r	12.00r
	12	78.61q	92.61q	10.12r	12.80r
	18	97.06p	98.49p	12.80r	16.00r
	24	99.04p	99.06p	115.00q	58.92q
	30	100.00p	100.00p	145.00p	156.00p
Mean[2+]		86.808	91.826	58.184	51.14

* Mean values in a column within an acid / temperature group followed by different letters (i.e. a - d for HCl group; p - t for H_2SO_4 group) differ significantly at $p>0.05$

[1+] Mean values for the HCl / θ group;[2+] mean values for the H_2SO_4/ group. Optimum concentration of HCl is 8 and 6 moles dm^{-3} for PKM and JC respectively and H_2SO_4 is 10 and 8 moles dm^{-3} for PKM and JC respectively under both yield and decomposition. Optimum temperature of HCl and H_2SO_4 is 110°C for PKM and 95°C for JC under both yield and decomposition.

destruction using 6 moles dm^{-3} HCl at 95°C for 18 h or with 8 moles dm^{-3} H_2SO_4 at 95°C for 18 h. Optimum yield was obtained from hydrolysing palm kernel meal protein with 8 moles dm^{-3} HCl at 110°C for 24 h or with 10 moles dm^{-3} H_2SO_4 at 110°C for 18 h. The choice of the acid depends on the availability and cost.

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